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令和２年度
茶学総合研究センター
実績報告書

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茶学総合研究センター　実績報告書　目次

１．研究実績

課題名

（１）緑茶の機能性及び疫学に関する研究

1）緑茶の機能性発現メカニズムの研究

研究の目的：

緑茶には、がん、メタボリックシンドローム、肥満、心臓血管病，糖尿病、炎症性疾患、微生物感染症、神経変性疾患などいろいろな病気に対する予防、治療効果があることが示されてきた。これらの保健効果には、主に緑茶カテキンであるエピガロカテキンガレート(EGCG)が関わっていることが明らかになってきた。しかし、ヒトに対する効果については疫学研究によって効果があるとする論文が多くあるが、効果がないとする論文も散見される。また、作用メカニズムに関しては多くのデータが蓄積しているが、まだ不明な点も多い。本研究では、抗がん作用に関して、発表された論文を基に最新の知見をまとめるとともに、コーヒーおよびその主要ポリフェノールであるクロロゲン酸（CGA）の抗がん作用との比較を行い、また作用メカニズムについての考察を行うことを目的とした。

主な研究成果：

ヒトのがんに関する研究では、いくつかのがんで抗がん作用が認められるものの結論を出せるような結果は示されていない。緑茶摂取に関しては食道がんおよび婦人科系がん、またコーヒー摂取については、膀胱がんおよび肺がんで、むしろがんリスクが高くなる可能性が高いことが2018年までに報告されている。2018-2020年に発表された疫学調査研究では、お茶ががんリスクを低減したとする結果は、膀胱がん、脳腫瘍、直腸がん、グリオーマ、肺がん、非ホジキンリンパ腫、非メラノーマ性皮膚がんで認められ、コーヒーでは、脳腫瘍、直腸がん、肝がん、グリオーマ、肺がん、非メラノーマ性皮膚がんで認められている。一方、コーヒーでは、膀胱がん、肺がん、小児急性ミエロイドで、むしろリスク上昇が認められた。また、お茶、コーヒー摂取にがんリスク低減効果はなかったとする研究は、お茶で15件、コーヒーで12件報告されている。こうしたヒト研究における相反する結果は、摂取量、飲用時の温度、喫煙、アルコール摂取、遺伝的要因、などの補正が不十分であるためと考えられる。また、コーヒーやほうじ茶では、焙煎時に生成するアクリルアミドの混入なども結果を左右する可能性があり、さらに、腸内細菌叢も大きな影響を与えると考えられるが、これらを補正することは難しい。結果として、高度にデザインされたヒト介入試験が必要と思われる。

動物実験では、ほとんどの研究で、緑茶やコーヒーの抗がん作用が認められており、EGCGやCGAの作用機作もかなり明らかになっている。それによると、EGCGやCGAは共通するところが多く、その中でも活性酸素種（ROS）が関与する機構が注目される。両者ともそのROS消去作用により、抗がん作用を発揮する可能性がある（図１）。また、両者ともROS産生を促進する作用もあり、その場合は、図２の作用機作が考えられる。このROSの消去、産生促進という相反する作用がどういう条件下で区別されるのかは、まだ明らかになっておらず、今後の研究で解明することが必要である。

（担当：茶学総合研究センター 伊勢村護、中村順行）

主要な成果

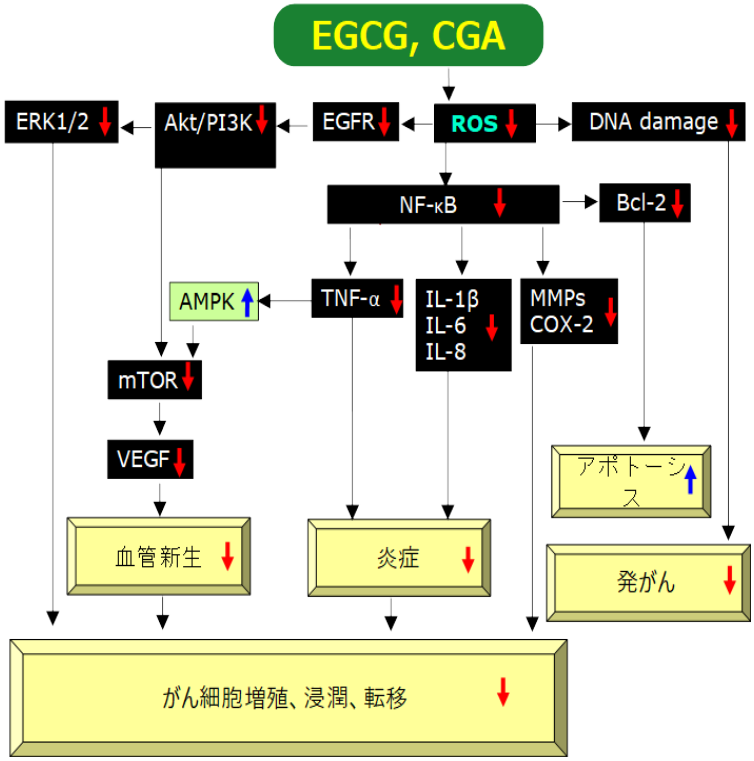


図１　EGCG、CGA の ROS 消去作用を介した抗がん作用メカニズム

（ボックス内の↓は阻害,抑制、↑は促進,発現上昇を示す。）
Sumio Hayakawa et al. Anti-Cancer Effects of Green Tea Epigallocatchin-3-Gallate and Coffee Chlorogenic Acid. Molecules, 25(19), 4553 (2020). 一部改変

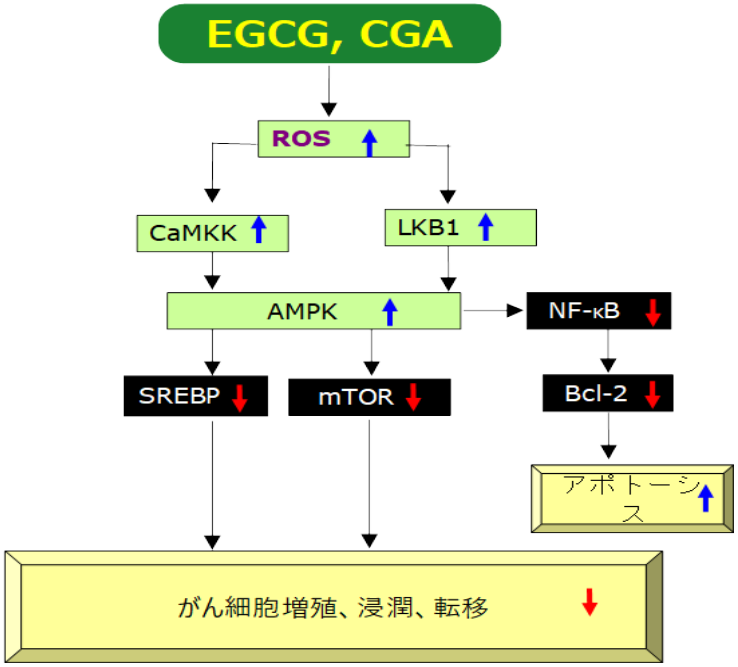


図２　EGCG、CGA の ROS 産生促進作用を介した抗がん作用メカニズム

（ボックス内の↓は阻害,抑制、↑は促進,発現上昇を示す。）
Sumio Hayakawa et al. Anti-Cancer Effects of Green Tea Epigallocatchin-3-Gallate and Coffee Chlorogenic Acid. Molecules, 25(19), 4553 (2020). 一部改変

論文発表

Sumio Hayakawa, Tomokazu Ohishi, Noriyuki Miyoshi, Yumiko Oishi, Yoriyuki Nakamura, Mamoru Isemura. Anti-Cancer Effects of Green Tea Epigallocatchin-3-Gallate and Coffee Chlorogenic Acid. Molecules, 25(19), 4553 (2020).

課題名：

（１）緑茶の機能性及び疫学に関する研究
2）緑茶カテキンによる脳機能の低下抑制の機構解明と寿命への影響

研究の目的：

緑茶の摂取が認知機能の低下予防に効果があることが、最近の研究において確かめられつつある。本研究では緑茶カテキンが脳機能および寿命に及ぼす影響を明らかにするため、緑茶カテキン摂取量について検討した。またカテキン摂取時に生ずる脳内の遺伝子発現変化について解析することにより、緑茶カテキンによる脳機能の低下抑制の機構解明をめざした。

研究の手法：

1) 緑茶カテキン摂取による寿命ならびに脳機能との影響を明らかにするため、加齢に伴い脳機能の低下が認められる老化促進モデルマウス SAMP10 を用いた。カテキン 1mg/kg はヒト（60kg)が緑茶１杯を飲む量に相当すると考えられることから、緑茶カテキンの摂取量を 1, 5, 10, 15, 30, 60mg/kg の用量で検討した。
2) 緑茶カテキンを１ヶ月間摂取していたマウスの海馬で、どのような遺伝子に変化が生じているか検討するとともに、加齢に伴う発現変化を検討した。

主な研究成果：

1) ステップスルー装置を用いた受動回避試験によるマウスの学習能の評価においては、緑茶カテキン 1mg/kg 以上を摂取していた SAMP10 マウスにおいて改善が認められ、15mg/kg のカテキンを摂取していたマウスで最も改善が認められた(図 1)。長期記憶能や空間作業記憶は 60mg/kg のカテキンを摂取していたマウスで有意な改善が認められた。
2) 寿命は、緑茶カテキンを 1mg/kg 以上を摂取していた SAMP10 マウスにおいて延伸が認められ、1mg/kg を摂取していたマウスが最も平均生存期間(MST)が長く、次いで 5～30 mg/kg を摂取していたマウスであった（表１)。
3) 緑茶カテキン(60mg/kg)を１ヶ月間摂取していた 2 月齢の SAMP10 の海馬で発現が有意に増加していた遺伝子をトランスクリプトーム解析により検索した結果、Egr2, Nr4a, Fos, Egr1, Npas4, Cyr61 等の最初期遺伝子の発現が高まっていることが明らかとなった。これら遺伝子について定量 PCR 解析により 6 および 12 月齢との発現変化の比較を行った結果、特に 2 月齢の時点で発現が高まっていたことが見出された(図２)。

これらのことから緑茶カテキンを毎日 1mg/kg 以上摂取することにより、加齢に伴う脳機能の低下が抑制され、寿命も延長されることが示された。緑茶カテキン摂取により、脳においてシナプスや神経回路の長期可塑的変化への関与が示唆されている最初期遺伝子の発現が高まっていたことから、緑茶カテキンは海馬神経の可塑性を高め、脳機能の低下を抑制していると考えられた。これらの成果は下記の雑誌に掲載された。

Unno K, Pervin M, Taguchi K, Konishi T, Nakamura N: Green Tea Catechins Trigger Immediate-Early Genes in the Hippocampus and Prevent Cognitive Decline and Lifespan Shortening.
Molecules 25, E1474 (2020)

今後の展望：

緑茶カテキンが加齢に伴う脳機能の低下抑制や寿命の延長を引き起こしていることが動物実験で確かめられたことから、緑茶を毎日１杯以上摂取することによりヒトにおいても同様の効果が期待されることが示唆され、疫学調査の結果を支持するデータとなった。今後、ヒト試験等への研究の更なる展開が期待される。

（担当：海野けい子、パービン・モニラ、田口今日子、中村順行）

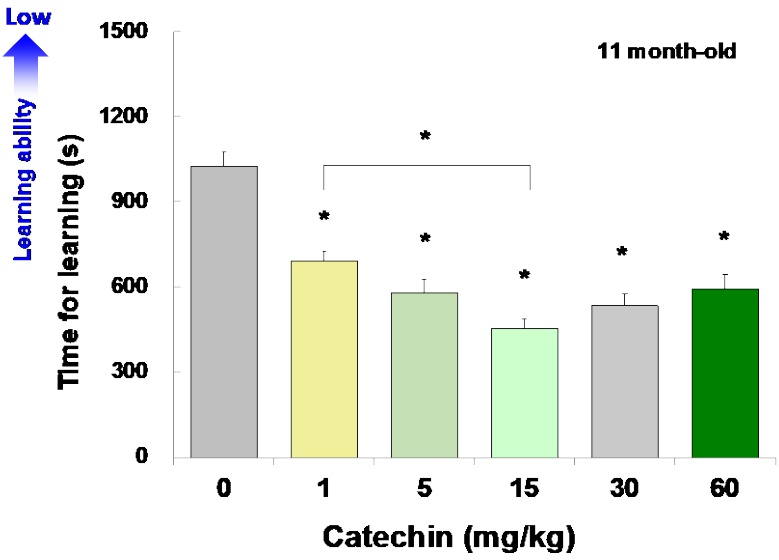


図 1. 緑茶カテキン摂取によるマウスの学習能低下抑制作用

表 1 緑茶カテキン摂取によるマウスの寿命の変化

GT-catechin (mg/kg)	MST (months)		*p* value
	month	Ratio	
0	10.8	1.00	-
1	17.2	1.59	0.027*
5	15.3	1.42	0.272
15	15.3	1.42	0.082
30	15.3	1.42	0.364
60	13.6	1.26	0.880

(*p*-value is based on Log-rank test)

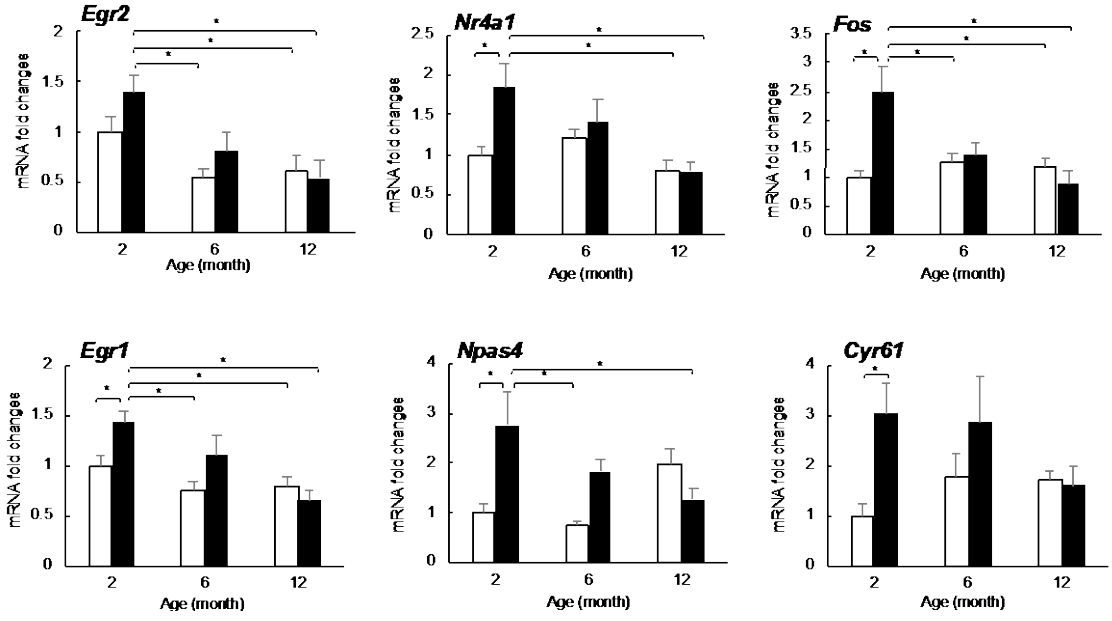


図 2 海馬における最初期遺伝子の発現変化　□コントロール、■カテキン

課題名：

（１）緑茶の機能性及び疫学に関する研究

3）白葉茶における抗うつ作用

研究の目的：

現代は多くの人が何らかのストレスを抱えたり、うつ状態に陥ったりしていることから、ストレス対策の重要性が指摘されている。これまでに抹茶のストレス軽減効果について動物実験およびヒト臨床研究が行われ、ストレス軽減効果が期待される抹茶は、テアニン(T)およびアルギニン(A)に対するカフェイン(C)およびエピガロカテキンガレート(EGCG, E)の割合(CE/TA比)が２以下である必要があることを明らかとしてきた。一方、茶樹を２週間ほど完全に遮光して作製された「白葉茶」では一般の煎茶に比べてアミノ酸量が 6~7 倍に増加し、一般煎茶に比べテアニン量が高いことから、白葉茶でも抹茶と同様にストレスを軽減できるか検討した。

研究の手法：

1) 20 代の参加者に、大学での生活と、学外に実習に行った時に毎日白葉茶３ｇを 500mL の水で浸出し飲んでもらった。この時、起床時および実習終了時に唾液アミラーゼ活性の変化ならびに不安感を示す STAI 値をストレスの指標として評価した。
2) 実験動物を用い CE/TA 比を変化させて、テアニン、アルギニン、カフェインおよび EGCG を摂取させ、副腎の肥大抑制効果を指標にストレス軽減効果を調べた。
3) 老化促進モデルマウス SAMP10 に白葉茶抽出液と同じ成分を摂取させ、尾懸垂試験による不動時間を指標にうつ様行動への影響を検討した。

主な研究成果：

1) 白葉茶の CE/TA 比は 1.12 であり、一般煎茶は 4.47 であった。白葉茶ではテアニンは量的には増加していたが、一般煎茶に比べ全アミノ酸に占める割合はむしろ低下し、一方アルギニンやグルタミン、アスパラギン、アスパラギン酸等の割合が増加していた（図１）。
2) 白葉茶を摂取した場合 STAI 値が低下する傾向が見られたが、有意ではなかった。起床時の唾液アミラーゼ活性、主観的なストレス、体調、達成感、睡眠時間などには、一般煎茶を摂取していた場合と違いは認められなかった。
3) 実験動物を用いたストレス軽減効果の検討で、茶浸出液としてテアニン、アルギニン、カフェインおよび EGCG を摂取させた場合は CE/TA 比が 1.12 では効果がなく、0.42 では効果が見られることが示された（図２）。
4) 白葉茶を摂取していた SAMP10 マウスでは、うつ様行動が改善された（図３）。

以上より、白葉茶は CE/TA 比が２以下であったがストレス軽減効果は認められなかった。これまでのデータと合わせて考えると、茶浸出液では抹茶と異なり CE/TA 比が 0.5 以下である必要があることが動物実験で確かめられた。一方白葉茶を摂取していた場合は、うつ様行動を改善する効果があることが動物実験で確かめられた。これらの成果は下記の雑誌に掲載された。

Unno K, Furushima D, Nomura Y, Yamada H, Iguchi K, Taguchi K, Suzuki T, Ozeki M, Nakamura Y. Antidepressant Effect of Shaded White Leaf Tea Containing High Levels of Caffeine and Amino Acids. Molecules. 2020, 25, E3550.

今後の展望：

白葉茶にはストレス軽減効果は認められなかったが、「うつ」を改善できる可能性が示唆された。今後うつ改善効果を評価できる簡便な実験系を構築するとともに、うつ改善に関与する緑茶成分とその作用機構が明らかとなることが期待される。

（担当：海野けい子、田口今日子、中村順行）

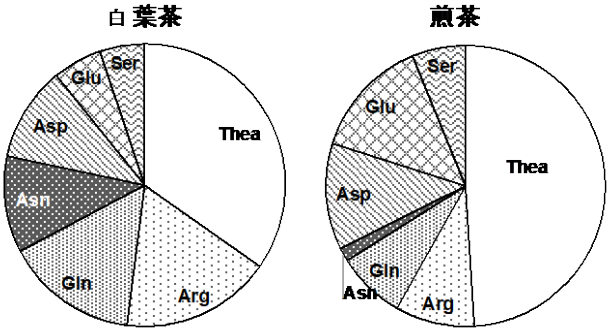


図１　白葉茶と煎茶におけるアミノ酸の割合

Thea, テアニン; Arg, アルギニン; Gln, グルタミン; Asn, アスパラギン; Asp,アスパラギン酸; グルタミン酸; Ser, セリン

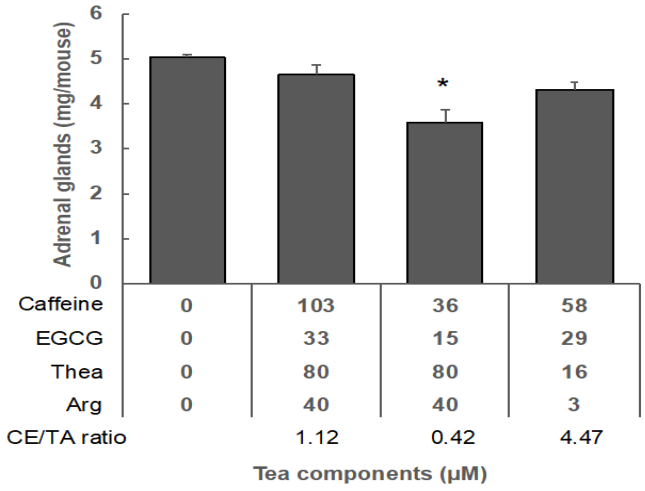


図２　白葉茶および煎茶成分のストレス軽減作用

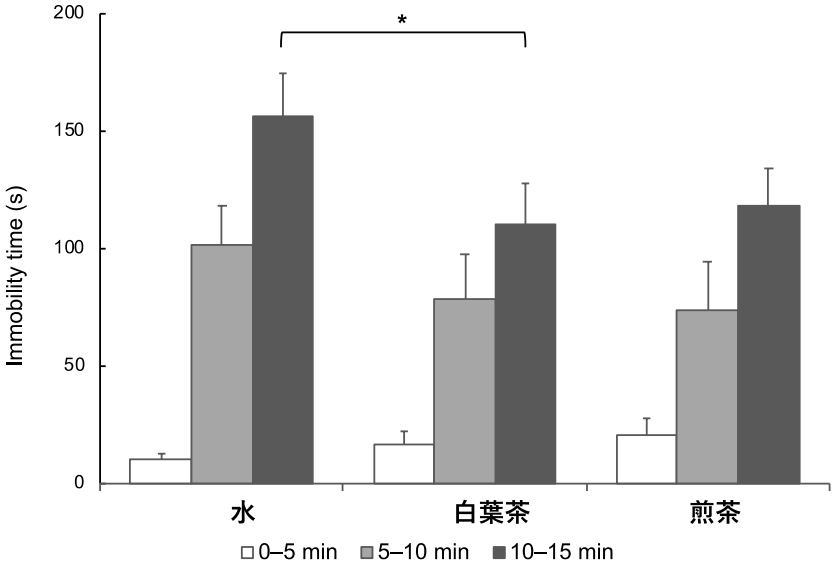


図３　白葉茶および煎茶の抗うつ作用

課題名：

（１）緑茶の機能性及び疫学に関する研究

4）ストレス負荷時における脳の代謝変化とテアニンの作用

研究の目的：

これまでの研究でテアニンがストレスを軽減する作用があることを明らかにしてきたが、ストレスに対する感受性には個人差があることも知られている。そこで、ストレス時における脳内でのテアニンの作用を明らかにするために、ストレス感受性が高い老化促進モデルマウス（SAMP10）と、通常の ddY マウスを用い、ストレス負荷により脳萎縮が最も顕著となる、ストレス負荷１ヶ月の時点での脳内代謝の変化を比較し、テアニンの作用機構について検討した。

研究の手法：

1) SAMP10 を通常の群飼育を行う群と対面飼育によりストレスを負荷する群に分け、更にテアニン（6 mg/kg）を飲水として摂取する群と、通常の水を摂取する群に分け、１ヶ月間のストレス負荷を行なった。海馬について、主要な代謝物を超高速液体クロマトグラフ-MS/MS システムにより分析した。対照として一般的な ddY マウスを用いた。
2) 顕著に変化した代謝物について、関連遺伝子の発現変化を定量的 PCR 解析により調べた。

主な研究成果：

1) ストレスを負荷した SAMP10 の海馬では「うつ」に関連することが報告されているキヌレニンが、ddY に比べ有意に高まっていた（図１）。その一因として、トリプトファンからキヌレニンへの代謝に関与するインドールアミン-2,3-ジオキシゲナーゼ(IDO)の活性がストレス負荷により高まり、テアニン摂取により低下していることが見出された(図２)。
2) 一方、抗うつ作用が報告されているカルノシンは ddY に比べ SAMP10 で低かったが、テアニン摂取により高まっていた(図１)。
3) ストレスを負荷した SAMP10 の海馬では、ヒスタミンの発現レベルが有意に高まっていたが、テアニン摂取により低下していた(図１)。ヒスタミンは様々なストレスによりその放出が促進され、海馬の興奮性にも強く作用する物質であることから、その重要性が示唆された。
4) アルギニンの代謝産物であるオルニチンには抗ストレス作用があることが報告されている。テアニンを摂取していた SAMP10 ではオルニチン量が高まっており(図１)、その一因としてアルギニンからオルニチンへの代謝に関与するアルギナーゼの発現がテアニン摂取により高まっていることが見出された(図２)。

以上より、ストレス感受性が高い脳においては、「うつ」や興奮に関与する脳内代謝物が増加していたが、テアニン摂取によりそれらの変化が抑制されていることが明らかとなった(図３)。この成果は下記の雑誌に掲載された。

Unno K, Muguruma Y, Inoue K, Konishi T, Taguchi K, Hasegawa-Ishii S, Shimada A, Nakamura Y. Theanine, Antistress Amino Acid in Tea Leaves, Causes Hippocampal Metabolic Changes and Antidepressant Effects in Stress-Loaded Mice. Int J Mol Sci. 22, E193 (2020)

今後の展望：

本研究成果は、ストレス感受性の違いは脳内における代謝の違いが一因となっていること、テアニンの摂取がそれら変化を改善していること示唆しており、ストレス感受性が高い人での脳の健康維持のために、テアニンが果たす役割の重要性を示すものである。

（担当：海野けい子、田口今日子、中村順行）

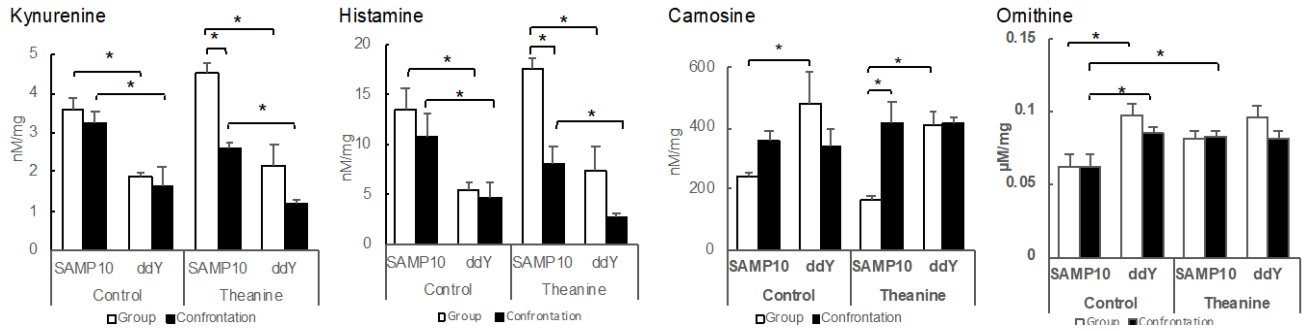


図１.群飼育および対面飼育 SAMP10 ならびに ddY マウスの海馬における代謝物量の変化

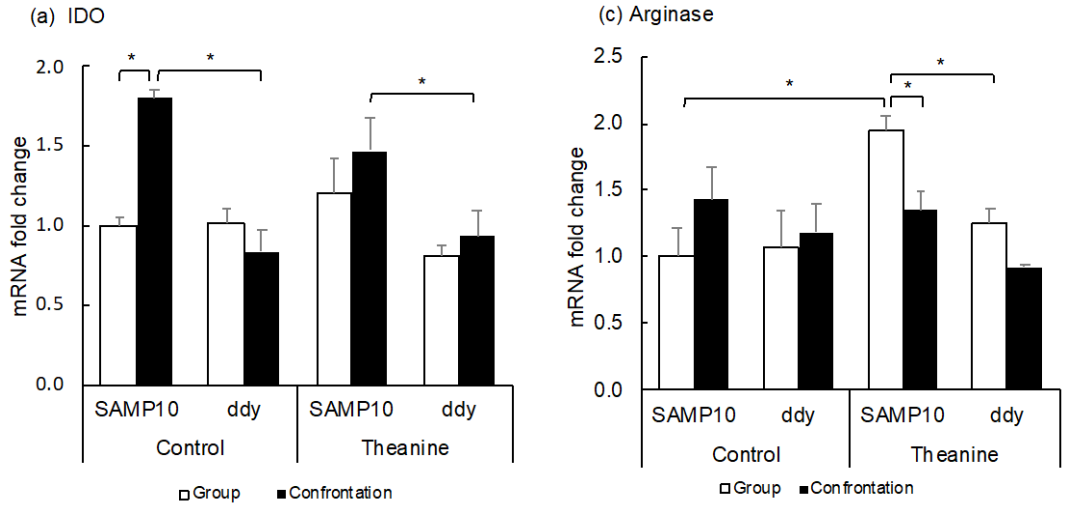


図 2. SAMP10 および ddY マウスの海馬における遺伝子発現変化

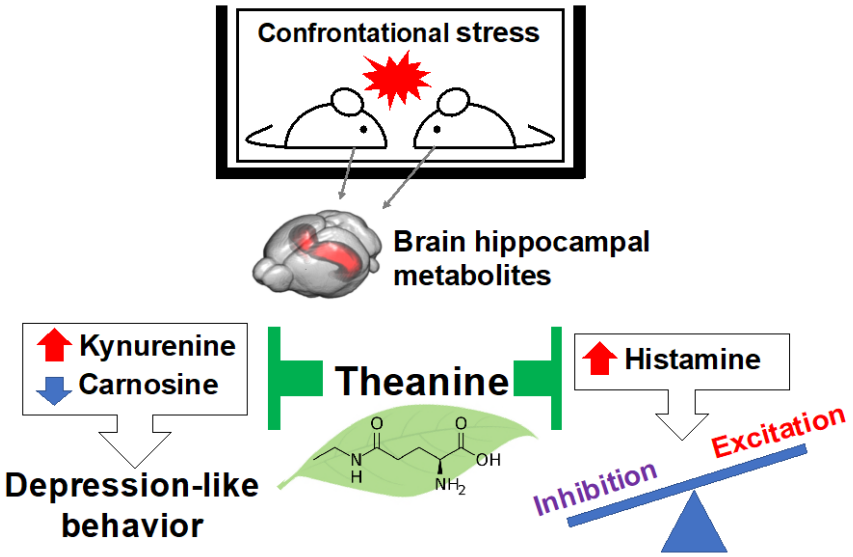


図３．ストレス負荷時における脳内代謝物の変化とテアニンの作用

課題名：

（１）緑茶の機能性及び疫学に関する研究

5）アルギニンの抗ストレス作用とその作用機構

研究の目的：

これまでの研究で、緑茶中の主要なアミノ酸であるテアニンが優れた抗ストレス作用を示すことを明らかにしてきた。その中で、テアニンに次いで高級緑茶に豊富に含まれるアミノ酸のアルギニンが、テアニンと同等あるいはそれ以上の優れた抗ストレス作用を示すことを見出してきた。そこでアルギニンについて老化促進モデルマウス（SAMP10）を用い抗ストレス作用を評価するとともに、その作用機構について検討した。

研究の手法：

1） SAMP10 を通常の群飼育を行う群と対面飼育によりストレスを負荷する群に分け、更にアルギニン（3 mg/kg）を飲水として摂取する群と、通常の水を摂取する群に分け、９月齢時に学習能およびうつ様行動を評価した。その後寿命への影響を測定した。
2） 対面飼育 3 日目のマウスについて、大脳皮質における酸化傷害の程度を比較するとともに、海馬における遺伝子発現の変化を調べた。

主な研究成果：

1） 群飼育および対面飼育の SAMP10 において、アルギニンの摂取により加齢に伴う学習能の低下が抑制されていた（図１）。また尾懸垂試験によるうつ様行動の評価でも、アルギニン摂取により改善が認められた(図１)。
2） 群飼育に比べ対面飼育のマウスでは平均生存期間（MST）が短縮していたが、アルギニンを摂取していた群では有意に MST が延長していた（図２）。
3） 対面飼育 3 日目の SAMP10 の脳では酸化傷害が高まっていたが、アルギニンを摂取していた群では有意に抑制されていた(図３)。
4） 対面飼育 3 日目の SAMP10 の脳では、ストレス応答や興奮毒性による神経細胞死に関連する Nr4a1, Arc, Cyr61 などの遺伝子発現が増加していたが、アルギニン摂取群ではそれが抑制されていた。一方ミトコンドリア機能とニューロンの生存維持に関与する Hba-a2, Hbb-b2 の遺伝子発現が、アルギニンを摂取群で増加した(図４)。

以上より、アルギニンがストレス負荷による酸化傷害を軽減し脳内のミトコンドリア機能を高めることにより、ストレス軽減効果を及ぼしていることが示唆された。この成果は下記の雑誌に掲載された。

Pervin M, Unno K, Konishi T, Nakamura Y. L-Arginine Exerts Excellent Anti-Stress Effects on Stress-Induced Shortened Lifespan, Cognitive Decline and Depression. Int J Mol Sci. 22, 508 (2021).

今後の展望：

本研究成果は、テアニンと同様にアルギニンがストレスを軽減する効果があり、その作用はテアニンとは異なる作用機構によることが明らかとなったことから、テアニンとアルギニンが相加的に作用することの重要性が示された。ストレス軽減による脳の健康維持において、緑茶摂取の意義が更に明らかになることが期待される。

（担当：パービン・モニラ、海野けい子、中村順行）

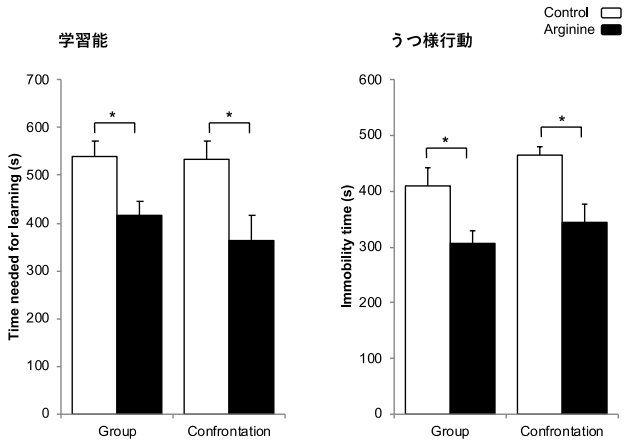
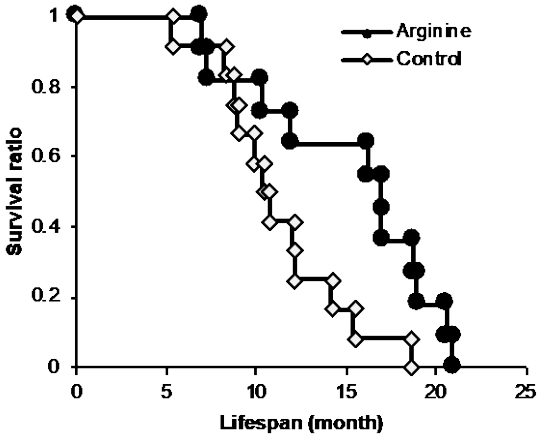


図１．ストレス負荷ならびにアルギニン摂取による学習能、うつ様行動への影響



Median survival time (MST)

Housing	Treatment	Median survival (month)	
Confrontation	Control	10.5	*
Confrontation	Arginine	16.6	
Group	Control	13.4	
Group	Arginine	11.2	

図２．ストレス負荷ならびにアルギニン摂取による寿命への影響

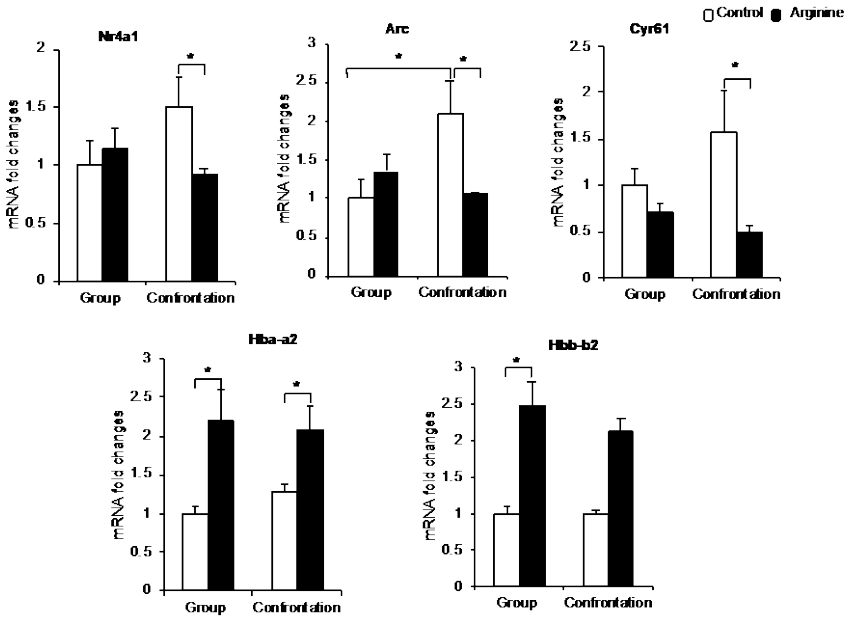


図３．ストレス負荷ならびにアルギニン摂取による海馬における遺伝子発現変化

課題名：

（１）緑茶の機能性及び疫学に関する研究

6）*Lactococcus lactis* subsp. *cremoris* を用いた後発酵茶の作製とその特徴

研究の目的：

機能性食品として伝統的な発酵食品が注目されている。本研究では、高品質で機能性を有する乳酸発酵茶の開発をするために、様々な機能性を有する細胞外多糖（EPS）を産生する乳酸菌、*Lactococcus lactis subsp. Cremoris* FC-4 *(L. cremoris)* を用いて後発酵茶を作製した。また、その成分分析と抗酸化活性を評価し機能性の検討を行った。

研究の手法

オートクレーブで滅菌した乾燥茶葉に、1.7×10^8 個/mL の乳酸菌(*Lactococcus lactis subsp. Cremoris* FC-4)を植菌後、混和して 25℃の暗所で 2 週間発酵させた。1 週間ごとにサンプリングし、-30℃に保存し実験に使用した。各サンプルは凍結乾燥処理し、粉末 1ｇに 100ｍＬの蒸留水を加えて 70℃、0.5 時間加熱処理し抽出液を得た。さらに、遠心分離により上清を採取しマイクロフィルターで濾過した溶液を成分分析のための試料とした。成分分析には HPLC と LC/MS を用いた。

抗酸化活性の測定には,後発酵茶抽出液 100μＬを安定なラジカルである DPPH（2,2-diphenyl -1-picrylhydrazyl）溶液 1ｍＬに加え,反応後の DPPH の量を吸光度測定（波長 520 nm）によって求め DPPH ラジカルの消去率を抗酸化活性とした

主な研究成果

1） 発酵に伴い、発酵に特徴的な pH の急激な減少は見られなかったが、発酵茶葉中の葉酸の減少およびアスコルビン酸の減少が確認されたことから *L. cremoris* による発酵の進行は遅いことがわかった　（Ｔａｂｌｅ1）。

2）発酵 14 日間では主要成分カテキン、アミノ酸（テアニンを含む）、カフェインは分解されずに保持されていた（Fig.1）。また、カテキン類の組成割合とアミノ酸の組成割合に変化はなかった（Fig.2）。これらの結果から主要成分は、*L. cremoris* による乳酸発酵の影響を受けずに保持されることが明らかとなった。

3）作製した後発酵茶は、発酵の影響を受けることなく高い抗酸化活性を示した（Fig.3）。

今後の展望

本研究で作製した後発酵茶は、主要成分を維持し高い抗酸化活性を示すことから、様々な病態や疾患に有効な働きをすることが期待される。今後は、生体機能性について検討したい。

（担当：食品栄養科学部　助教　斎藤貴江子）

主要な成果

Table 1. Change in pH, ascorbic acid and folic acid contents during fermentation

	Days of fermentation		
	0	2	7
pH	5.48	5.48	5.45
Total ascorbic acid (mg/100g)	33	11	4
Folic acid (mg/100g)	0.6	0.46	0.41

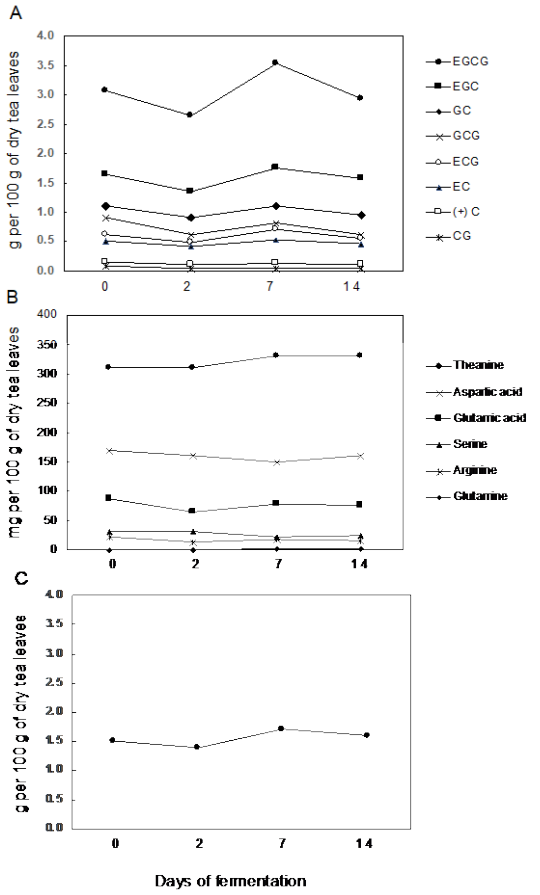


Fig. 1. Changes in catechin, amino acids and caffeine in the extract of fermented tea during fermentation: (A) Catechin, (B) Amino acids, (C) Caffeine

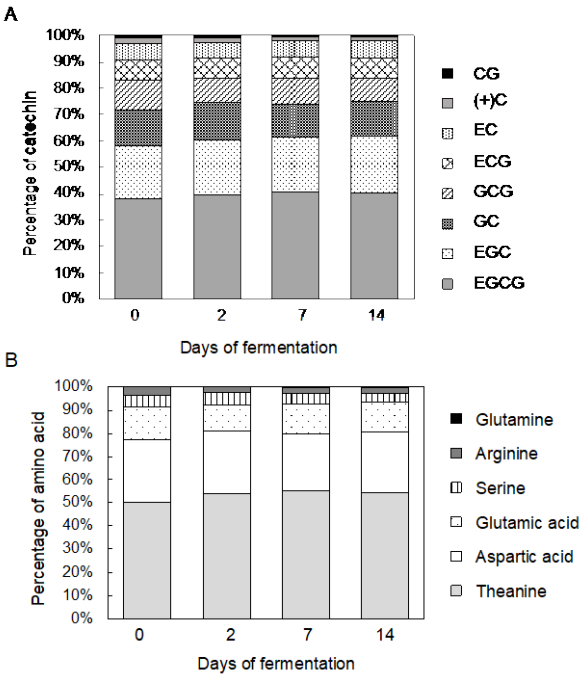


Fig. 2. Changes in the proportion of catechins and amino acids in the extract of fermented tea during fermentation: (A) Catechins, (B) Amino acids

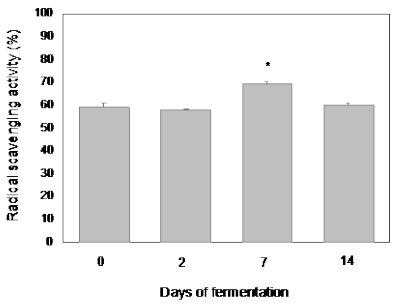


Fig.3. Change in antioxidant activity of the fermented tea during fermentation.The antioxidant activity was determined as DPPH radical scavenging activity of the tea extract. Data shown are mean±SEM (n=3), *p<0.05

課題名：
（2）茶学教育と人材育成
1）セミナーの開催
① 経営能力向上セミナー・シンポジウムなどの開催

研究の目的：

茶学総合研究センターでは、セミナーの依頼や各種シンポジウムなどに積極的に参画することにより、幅広い立場から茶の魅力を伝え、需要拡大を促し、茶業振興に寄与するとともに、茶に係る経営能力向上を目指すことを目的としている。

研究の手法：

茶に関して依頼のあったセミナーなどについて、主催者と綿密に連携しながら効率的なセミナーを行う。また、各種シンポジウムなどにおいては茶学総合研究センターの立場を踏まえ、茶の幅広い魅力や奥深さを伝え、ひいては経営能力の向上に寄与するよう心掛けた。なお、セミナーの大部分はオンライン上でパワーポイントを用いて実施し、その資料などは茶学総合研究センターのホームページにPDF版としてアップした。

主な研究成果：

1) 本年度開催した経営能力の向上に関する主要なセミナーは、コロナ禍のため例年よりは少なく11回であった(表1)。
2) セミナーの大部分はオンラインでの開催としたが、内容は多岐にわたり輸出が拡大しつつある抹茶、需要が増加している紅茶やお茶の機能性研究の成果を分かりやすく伝達してほしいとの依頼や低迷化している茶業の今後の方向性や地域の活性化・ブランド化戦略などについての要望が多く、全てに対応した。
3) お茶の健康については、茶業関係者のみならず、総合食品講座受講生など幅広い対象者に茶の機能性を各々の立場に応じて紹介した。
4) また、本年度はお茶の機能性や輸出についての依頼も多く、経営・マーケティングに結びつくような一歩踏み込んだセミナーとした。
5) 本年度のセミナーはコロナ禍のため人数限定やオンラインでの開催も多かったが、参加者からは好評で、来年度はより積極的に対応したいと感じた。

今後の展望：

来年度も、経営能力向上セミナーを継続するとともに、できる限り多くの要望に対応したいと考えている。

（担当：茶学総合研究センター　中村順行）

主要な成果

表１　セミナーの開催時期とタイトル

令03.02	茶の審査法の変化
令03.01	次世代に展開する茶の魅力
令03.01	海外市場の特性と輸出戦略
令02.12	茶は養生の仙薬～その魅力と機能性
令02.12	お茶の文化で世界を巡る
令02.11	茶の抗酸化力
令02.11	食品素材としてのお茶
令02.10	世界の茶の生産と加工
令02.10	高級抹茶の輸出戦略
令02.08	茶の機能と多用途利用
令02.08	お茶の淹れ方

課題名：

（2）茶学教育と人材育成

1） セミナーの開催

② 外国人を対象とした茶学講座

～セイロン紅茶のブランド力の向上とマーケティング～

研究の目的：

紅茶はスリランカを代表する農産品であるが、スリランカ政府は輸出産業としての競争力強化のため、品質・生産性向上や加工工場の近代化などを目指すこととしている。その一環として、近赤外分光分析計による茶成分分析計の有用性を①品質向上（生葉）、②品質管理（荒茶）、③高付加価値化（荒茶）段階で実証し、セイロン紅茶のブランド力の向上とマーケティング戦略を図ることを目的とした。

研究の手法：

セイロン紅茶のブランド力の向上とマーケティング戦略を図るためのセミナーを開催する。

主な研究成果：

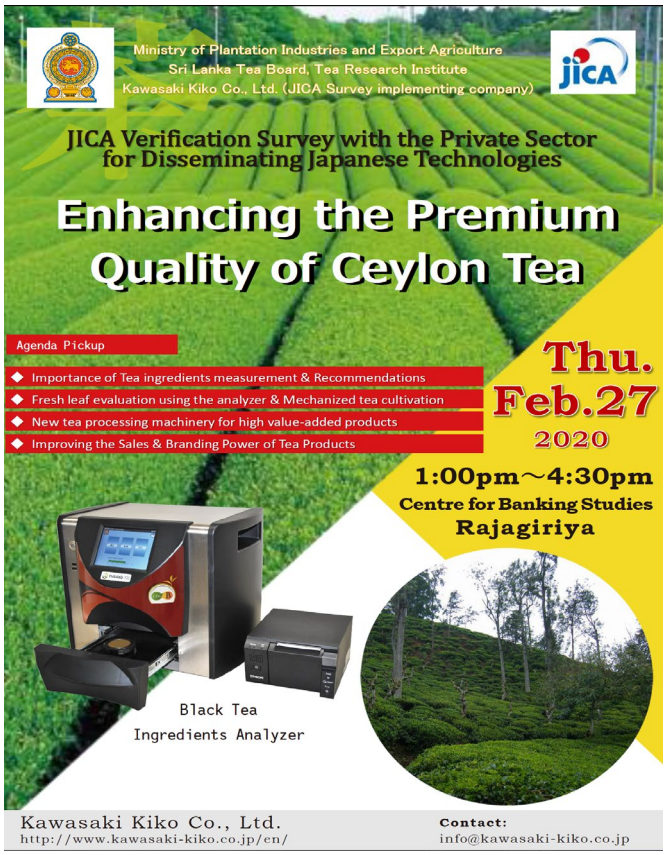
1) スリランカにおいて、茶は同国を代表する農産品である。茶の生産は 2017 年度、世界第 4 位となる年間約 307,720 トンであり、うち約 288,980 トンを輸出しており 、その輸出額は、同国輸出額全体の約 13%、農業分野の中では約 55%を占める。
2) スリランカ茶業の更なる発展を目指し、国際的な茶価が低迷し、労賃が上昇するなか、セイロン紅茶のブランド力の強化とマーケティング、さらには低コスト栽培方法について「Enhancement of Ceylon tea brand power and marketing」と題して講演した。
3) その概要は、「世界のお茶の現状」「セイロン紅茶の現状」「セイロン紅茶における主要な課題」「セイロン紅茶のブランド力の向上とマーケティング」「セイロン紅茶の低コスト生産に向けて」である。
4) なかでも、セイロン紅茶のブランド力の向上とマーケティングについては、ブランド力を向上するために、・プレミアムライオンロゴの創設、・セイロン紅茶の品質・紅茶の機能性 PR について提言した。
5) また、マーケティングについては、・輸出先により需要が異なるため、輸出先別マーケティング戦略や最近の世界的な話題の茶種などについて話題提供した。

今後の展望：

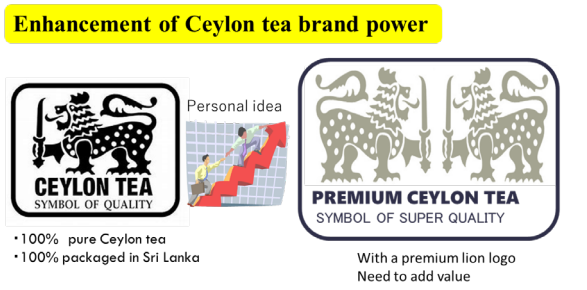
スリランカへの渡航は 2 月だったために、コロナの影響も少なかったが、それ以降は全く交流が途絶え、わずかにオンラインでの最低限の確認にとどまっている。コロナ禍が沈静化したら、再度セイロン紅茶のブランド力向上に対する交流を促進したいと考えている。

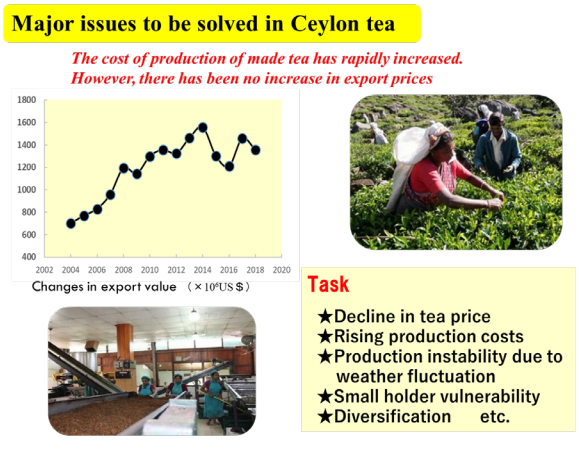
（担当：茶学総合研究センター　中村順行）

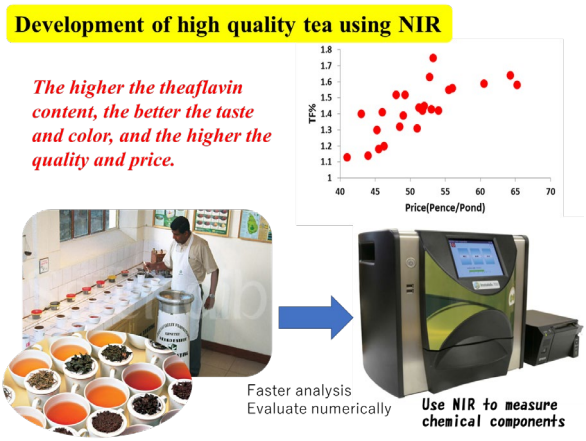
主要な成果



セミナー会場風景







課題名：
（２）茶学教育と人材育成
　2）人材の育成
　　① 茶学入門

研究の目的：

静岡県は全国有数の茶葉、飲料、加工食品の生産県であり、本学を含めた複数の大学や国公立の試験研究機関、民間企業において茶関連の食品、医薬品、化成品の研究が活発に行われている。したがって本学の学生はそれらを就職先として考えることも多い。また、静岡県においても世界緑茶協会や「茶の都」づくりの推進、さらには本学内にも「茶学総合研究センター」の開設など、茶を Keyword とした取組も多い。このような背景のもと、本学においても「茶学入門」をしずおか学のひとつの選択科目として行うことにより、学部を問わず茶に関する広範な知識と教養を身につけることを目的とする。

研究の手法：

当科目は茶について、歴史、文化、経済、生産、貿易、栽培、種類、加工、味、香り、生理、機能、効能など広範な項目にわたり、それぞれの専門家が分かりやすく講義を行う。

本年度はすべてオンラインで実施した。

主な研究成果：

1) 茶学入門の本年度の受講生は２３６名であり、社会人受講生はオンラインのため募集しなかった。
2) 本科目は選択科目のため、一年生の履修者が多く全体の８０％程度を占めていた（表 2)。これは、茶学入門が定着し、先輩から新入生に「履修したほうが良いおすすめの全学部共通科目」として紹介されていることも要因の一つであろうかと思われる。
3) 全履修者のうち単位修得者は９０％以上であったが、不修得者の大部分は欠席日数が多いためである。なお、本年度はオンラインのため、出席率が高かった。
4) 講義は、茶に関して全般にわたるものであり、その道の専門家により行われる(表 1)ため、非常に好評であり、茶の幅広い魅力を感じたり、何気なく飲んでいたお茶を見直すきっかけとなる学生も多く見られた。
5) さらに、茶に関する興味を深くする学生も多く、今後の研究の端緒になる可能性や、コーヒーなどからお茶に飲用を変えたなどとの意見も見られた。
6) また、他県出身者の多くは静岡県立大で静岡の特産物である茶を学べたことの意義は大きく、今後も継続してほしいとの要望が多かった。

今後の展望：

来年度も、茶学入門は継続するとともに、ぜひとも対面で行いたいと感じるとともに、社会人聴講生を多く受け入れたいと考えている。

（担当：茶学総合研究センター　中村順行）

主要な成果

表１　令和２年度　度茶学入門　講義期日と科目名

回数	月日	担当者	科目名
1	10月 1日	中村　順行	ガイダンス、世界の茶の生産加工
2	10月 8日	中村　羊一郎	茶の歴史
3	10月15日	吉野　亜湖	茶の文化
4	10月22日	川木　順平	茶の生産現場から ～多彩な品種と新しいお茶～
5	10月29日	岩崎　邦彦	茶のマーケティング
6	11月 5日	伊勢村　護	茶の疫学的研究成果より～ヒトへの貢献～
7	11月12日	太田　奈月	茶の香り
8	11月19日	中村　充 松島　章恵	茶の種類と美味しい淹れ方
9	11月26日	海野　けい子	茶の主要成分（テアニン、カフェイン）の生理機能
10	12月　3日	時光　一郎	茶のカテキンを活かした最新の商品開発
11	12月10日	齋藤　貴江子	茶樹を特徴づける化学成分とその代謝
12	12月17日	佐野　満昭	茶の幅広い魅力と機能
13	1月14日	小林　栄人	茶の都　しずおか　づくり
14	1月21日	ステファン･ダントン	世界に広まる日本茶の現状と課題
15	1月28日	中村　順行	次世代に展開する茶の魅力

表２　令和2年度茶学入門　受講者数

	1年	2年	3年	4年	その他
薬学部	45	1			
食品栄養科学部	28	1			
国際関係学部	25	20	5	3	
経営情報	36	17	1	1	
看護学部	52		1		
合計	186	39	7	4	

課題名：

（2）茶学教育と人材育成

2）人材の育成

② 県立大学以外の学生などを対象としたお茶講座

研究の目的：

茶学総合研究センターでは県立大学以外の学生を対象として、茶に対する知識と教養を高め、茶の幅広い魅力を知り､ひいては茶の愛飲者､茶の都しずおかのサポーターになっていただき、茶業振興に寄与することを目的とし、分かりやすさをモットーとした茶業講座を開設する。

研究の手法：

様々な分野の学生に対して、茶の知識量や興味に応じて、分かりやすく茶の歴史、文化、生産、種類、加工、味、香り、生理、機能、効能など広範な項目にわたり、茶の魅力をパワーポイントなどを用いて発信する。特に、大学コンソーシアムのなかで「ふじのくに学（茶学)」を開講することにより、参画する大学から多くの学生を受け入れる。本年度はコロナ禍のため初日は対面としたが、以後はオンライン講座とした。

主な研究成果：

1) 日本茶に関して､植物としての茶から茶葉に加工され､消費者に届くまでの過程を知るとともに、 国内外の様々なお茶をめぐる情勢について学び、これからのお茶の可能性を考えることをねらいに静岡県大学コンソーシアムでは「ふじのくに学（茶学)」を開講している。
2) この講座は大学間の単位互換授業として大好評である。本年はコロナ禍のため定員を 25 名としたが、数多くの応募があった。
3) 講義は､県立大の教員及び各分野の専門家が各々の分野に分かれて担当するが､初日を除いてすべてオンライン開催となり、グループ学習などは行いにくかった。
4) 実習についてもオンラインで「お茶のおいしい淹れ方」「急須がなくても楽しくお茶を入れる方法」「ホットプレートを用いた簡単なお茶の作り方」などを行ったが、どこまで伝わったのかは非常にわかりにくい部分もある。
5) また、昨年度はグループ学習として、集団討議の方法や KJ 法などについて指導するとともに、グループ内での結果のとりまとめ方、さらには発表方法についてもレクチャーし、これまで散漫になっていたグループ学習を有意義なものとしたが、本年度はオンラインのため相互の意見交換が精いっぱいだった。

今後の展望：

今後、茶の魅力を静岡県立大学のみならず、他大学とも連携しながらより幅広い学生に対して発信していくために、大学コンソーシアムの更なる利用や世界緑茶協会などとの連携を強化したいと考えている。

（担当：茶学総合研究センター　中村順行）

主要な成果

令和２年度 公益社団法人ふじのくに地域・大学コンソーシアム
短期集中講義「ふじのくに学（お茶）」

９ 月 ７ 日（月）
＜概論・茶文化＞
② ガイダンス
②講義「茶学概論」：静岡県立大学食品栄養科学部特任教授 中村順行氏
③講義「ふじのくに茶の都ミュージアムの役割とお茶の振興」：ふじのくに茶の都ミュージアム 副館長兼学芸課長 白井満氏
③ ふじのくに茶の都ミュージアム館内見学・抹茶体験 ふじのくに茶の都ミュージアム
９ 月 ８ 日（火）
＜お茶の生産・加工＞
① 講義「平地のお茶の生産・加工」：静岡県立農林環境専門職大学短期大学部准教授 中野敬之氏
② 講義「川根の茶業と生活・文化について」：つちや農園 土屋和明氏
③ ホットプレートでのお茶・紅茶作り ：静岡県立大学食品栄養科学部特任教授 中村順行氏
④ お茶の淹れ方講座 ：静岡県立大学 中村順行氏、亀岡葉子
９ 月 ９ 日（水）
＜お茶の生産・加工 / 流通・経営＞
① 講義（ONLINE）「静岡茶の流通～過去から現在、そして未来へ～」：静岡県立大学グローバル地域センター特任助教 粟倉大輔氏
②講義「お茶の歴史と文化」 ：静岡大学非常勤講師 吉野亜湖氏
③講義「お茶の価値を高めるマーケティング」 ：公益財団法人するが企画観光局 CMO 片桐優氏
④ 静岡市街のお茶関連商品提供店舗の散策時間 引率：公益財団法人するが企画観光局
９ 月 10 日（木）
＜マーケティング / 総論＞
①講義「茶の機能と多用途利用」：静岡県立大学食品栄養科学部特任教授 中村順行氏
② 講義（ONLINE）「外国人から見た日本茶」：株式会社おちゃらか代表 ステファン・ダントン氏
③グループワーク「静岡の茶業が活性化するための展開の仕方」：静岡県立大学食品栄養科学部特任教授 中村順行氏
③ 全体総括 ：静岡県立大学食品栄養科学部特任教授 中村順行氏

課題名：
（２）茶学教育と人材育成
　2）人材の育成
　　③ 学生に美味しいお茶をプロジェクトによる茶の提供

研究の目的：

静岡県は全国有数の茶葉、飲料、加工食品の生産県であり、本学を含めた複数の大学や国公立の試験研究機関、民間企業において茶関連の食品、医薬品、化成品の研究が活発に行われている。したがって本学の学生はそれらを就職先として考えることも多い。静岡の県立大学において多彩なお茶を飲む機会や学ぶ機会を提供することで、茶の持つ幅広く、奥深い魅力や静岡の風土の素晴らしさについての理解を深めるとともに、お茶の知識や愛飲者になってもらうことを目的とする。

研究の手法：

学生に美味しいお茶をプロジェクトを介し様々な機会をとらえ学生にお茶の飲んでいただくとともにお茶に関する情報発信を行う。

主な研究成果：

1) これまで、食堂や茶学入門、イベント時などを捉え、コロナ禍に対応した方法で学生にお茶を提供してきた。
2) 3～4 月には、ティバッグ数種類を準備し食事時に自由にお茶を愉しんでいただいた(下食堂)。利用者は 100～300 人/日。
3) 6 月には、企業からの協賛品であるお茶ゼリーとティバッグ 300 人分をクラブ学生に提供
4) 9～10 月にはコロナ禍を避けるためコップにティーバッグを入れての呈茶(下食堂)。各日ごとに限定緑茶３５カップ、紅茶３５カップ程度をおき自由に呈茶可能とした。
5) 10～11 月には試験的にティーサーバーによる冷茶を提供(下食堂)。大好評で毎日昼食時間の前半には 18ℓが空になる。
6) 10 月には、食べ物カフェに訪れた 50 人に紅茶の配布
7) 11 月の静岡市お茶の日(11 月 1 日)イベント前後 1 週間にパンフとともにティバッグの配布。50 個/日×10 日間
1) 11 月からは短大においてもティーサーバーによる呈茶を開始。非常に好評。
2) しずおか学「茶学入門」受講生 230 人余に紅茶と緑茶の配布。美味しい淹れ方も講義の中で指導した。

今後の展望：

現状、コロナ禍のためなかなか幅広い呈茶が行えないが、地道に活動するとともに、post コロナでは Free Tea Café なども再開したいと考えている。

（担当：茶学総合研究センター　中村順行）

主要な成果

コロナ対策のため

速やかに試飲し、

各様の香味をお楽しみください

皆さんが日常的に飲んでいる、紅茶/緑茶/烏龍茶は全て同じツバキ科の常緑樹である“カメリアシネンシス”というお茶の木から作られている事をご存じですか？それぞれ味も水色も香りも違いますが、これは葉を摘み取った後の発酵の度合いによって生まれているのです。その中でも高い香りが特徴の紅茶は、世界中で一番飲まれている“お茶”です。

世界三大紅茶は、ダージリン（インド）・キーマン（中国）・ウバ（スリランカ）ですが、紅茶は、品種やそれぞれの国の気候風土で味や香りが異なります。

最近ブームの国産紅茶（和紅茶）は、温暖な気候の鹿児島や静岡で多く作られています。海外の紅茶に比べて渋みが少なくすっきりとし、甘い香りで食事に合わせやすいのが特徴です。季節のフルーツや炭酸、生姜やシナモン等のスパイスでアレンジをして、自分が好きな味を見つけて愉しむのもお勧めです。

また、温かい紅茶は血流を良くし体を温める作用があります。ウイルスに負けない免疫力の高い体作りのためにも、“紅茶のある生活”を愉しみたいですね。（静岡県立大学 茶学総合研究センター）

【静岡県立大学　茶学総合研究センター】

大学内の茶に関する研究情報を一元化するとともに、産学民官と連携して茶を総合的に科学し、茶業振興に寄与することをめざし、茶の栽培加工から機能性、販売、経営手法まで総合的に科学する静岡県立大学食品栄養環境科学研究院附属のセンターです。

静岡市地域福祉共生センター「みなくる」
静岡市駿河区南八幡町3-1　南部図書館2階
TEL:054-201-9010　FAX:054-201-9020
MAIL:mina.ccrc@u-shizuoka-ken.ac.jp

共生事業受託：静岡県立大学
（「ふじのくに」みらい共育センター）

静岡のお茶で元気に！

静岡県産 和紅茶

～おいしい飲み方と
ミニ情報～

課題名：

（２）茶学教育と人材育成

3）機能性情報などの発信

①UC デービス校における機能性情報の発信

研究の目的：

米国内で静岡茶の機能性を訴求し、米国市場の潜在的需要を喚起し、静岡茶の輸出につなげるため、UC デービス校が主催するシンポジウムに参画し、機能性研究成果を発表することにより、産学官一体で、米国内で機能性を生かした静岡茶の認知度向上と販路拡大を図ることを目的とする。

研究の手法：

カルフォルニア大学デービス校において開催された GTI シンポジウムにおいて、講演発表を行う。なお、本年はコロナ禍のため、オンラインでの参加とする。

主な研究成果：

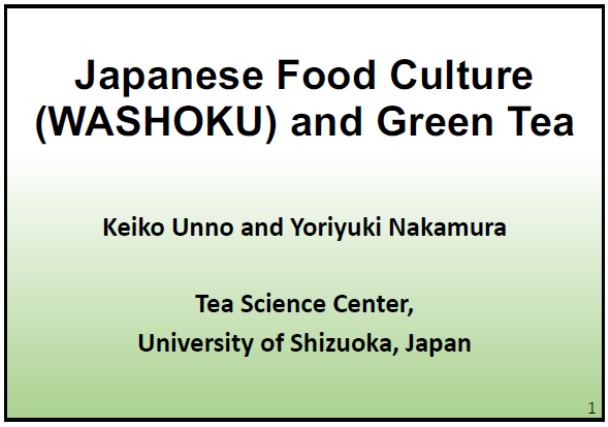
1) グローバルティーイニシアチブ第６回コロキウムが１月 21 日にオンラインで､延べ 822 人、ピーク時 422 人の参加者のもと開催された。
2) 第５回 GTISympo「Great Debate:TEA & WINE」が１月 16 日～17 日に開催され、米国の茶関連企業経営者、米国以外の国の茶関係者、研究者、学生、メディア等、延べ 500 名ほど参画した。
3) 主催者である Burnett 教授によるあいさつで、GTI の柱の一つである茶の教育については、茶の教育のためのシアターを作り、そこで台湾、日本、中国、ケニアのお茶について学べるようにしたい、とのことがあった。
4) 海野先生により、和食と緑茶の機能性について、カテキン、テアニンによる認知機能の向上効果と、同効果の発揮のためには低カフェイン化や水出しが有効であること、抗ストレス効果や認知機能向上効果を抹茶が発揮するための条件として、テアニン含量が十分に高いこと、日本産の抹茶の多くはこの条件を満たしていること、等が講演された。
5) 質疑応答の中で、「緑茶の機能性成分は日本産以外の緑茶にも含まれているのか？」との質問があり、テアニンについては、煎茶など他の緑茶に比べ抹茶に多く含まれている。これは日本でなされている被覆栽培によって顕著に含有量が高まる。これによりリラックスや睡眠品質の改善効果が得られる。」と西川により回答された。
6) 来年度の「第７回 GTI コロキウム」は 2022 年１月 13 日に開催されることが予告され、そのテーマは「 Tea and “Tea”」であり、Camellia sinensis から作られるお茶とどくだみ茶、麦茶、マテ茶などのようなお茶ではないお茶について行われるとのこと。

今後の展望：

今後さらに静岡茶の輸出拡大のため、デービス校との連携を活用して継続して静岡茶の認知度向上と販路拡大に向けた機能性情報の発信を行う。

（担当：茶学総合研究センター　海野けい子、中村順行）

主要な成果



UCDAVIS
COLLEGE OF LETTERS AND SCIENCE

Dr. Alexander F. Day, Associate Professor, History; Chair, East Asian Studies, Occidental College, Los Angeles
Socialist Tea Heritage: Selling China's Largest Tea County

Q&A Student Moderator, **Analizabeth Ramirez**, Psychology, Managerial Economics, Spanish, UCD 2021

Lunch Break

1:00 PM SESSION 3 UC Davis Students Talk about Tea
Introduced by **Dr. Joseph Sorensen**, East Asian Languages and Cultures

Xinyi (Cassie) Zhang, Food Science and Technology Major; President, Global Tea Club, University of California, Davis
Report on the Global Tea Club at UC Davis

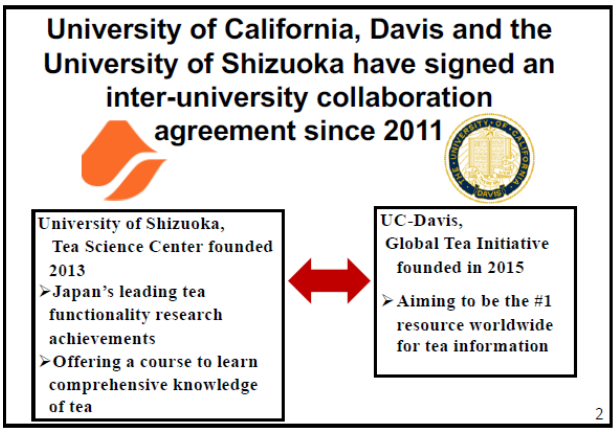
Dr. Frances E. Dolan, Distinguished Professor, English, University of California, Davis; Ben Fong, Doctoral Student, Comparative Literature, UC Davis; Grace Hayes, Doctoral Student, English, UC Davis; Mikhaila Redovian, Doctoral Student, English; Himali Thakur, Doctoral Student, English
Embracing Tea in Restoration England

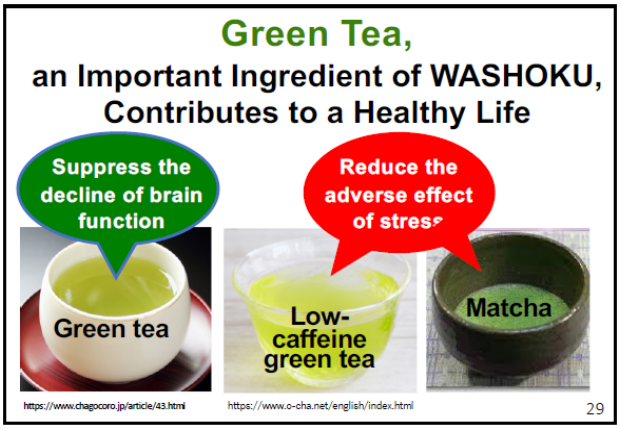
Q&A Student Moderator, **Gabrielle Tirsell**, Economics, UCD 2024

2:00 PM SESSION 4 Talking about Legendary Teas, New Teas, and Spiritual Beliefs
Introduced by **Dr. Shermain Hardesty,** Extension Economist Emerita, Agricultural and Resource Economics

Dr. Keiko Unno, Visiting Associate Professor, Tea Science Center, University of Shizuoka, Japan
Japanese Food Culture (WASHOKU) and Green Tea

https://globaltea.ucdavis.edu/





課題名：

（２）茶学教育と人材育成

3）機能性情報などの発信

②静岡茶の魅力の発信

研究の目的：

静岡県内の海外輸出を考えている茶業者(生産者、流通業者)へ茶の魅力や日本茶の特徴、機能性などの情報を提供し、産学官一体で静岡茶の魅力を訴求し、輸出者による米国内での認知向上につなげるためのセミナーを行う。

研究の手法：

本年はコロナ禍のため、「オンラインによる茶の機能性成分を活かした輸出戦略を考える‼」と題して、3回シリーズでセミナーを開催する。

主な研究成果：

1) 11月下旬から12月上旬にかけて3回にわたり、すべてオンラインによるセミナーを開催した。
2) 参加者は、各回50名弱であり、輸出関係茶業者、茶関連機械メーカー、行政者、海外サポートデスクなど多彩な参加者だった。
3) セミナーは3回にわたり実施した。
4) 第1回目：「茶の機能性成分の変異」「茶の主要成分の機能性～カテキン～」
5) 第2回目：「他国産と日本産茶の機能性成分から見た差別性」「茶の主要成分の機能性～カフェイン、テアニン」
6) 第3回目：「機能性成分を活かしたマーケティング」「国内外で市販されている抹茶や白葉茶の機能性の評価」
7) 各回、講演40分×２回＋質疑応答40分（各間隔５分間程度の休憩をはさむ予定）で行い、質問については、チャット機能からご投稿を受けた。
8) 質問内容は多岐にわたり、「カテキンと鉄分の関係、お茶と貧血」「GABAの機能性」「アルミニウム含量について」「機能性表示について」などなど多くの質問があった。
9) また、別日にオンラインによる個別相談会も開催し、4件の相談があった。
10) さらに、2月21～22日開催のJapanese Food Expo 2021の会場においてJapanese foods WASHOKU and the health benefits of Japanese green teaについて動画配信した。

今後の展望：

茶学総合研究センターでは、今後も茶の魅力を向上させるための情報について様々な方法で今後とも発信していく予定である

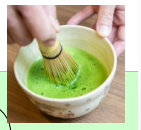
（担当：茶学総合研究センター　海野けい子、中村順行）

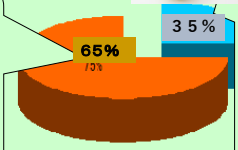
主要な成果

オンラインによる茶の機能性成分を
活かした輸出戦略を考える‼

3回シリーズでセミナー開催

第1回　茶の機能性成分の変異





不溶性成分
- ☆**食物繊維**20～30%)；便秘予防、大腸がん予防、心疾患予防
- ☆**たんぱく質**24%)；栄養
- ☆**βカロテン**(20mg%)；抗酸化、抗がん、抗糖尿、抗心疾患、免疫活性
- ☆**ビタミンE**(25～70mg%)；抗酸化、抗がん、免疫活性
- ☆**クロロフィル**0.80%)；がん予防、抗突然変異、抗腫瘍、免疫活性

水溶性成分
- ★**カテキン類**(10～18%)；抗酸化、抗菌、抗がん、生活習慣病予防、消臭、抗アレルギーなど
- ★**カフェイン**(3～4%)；眠気防止、強心、二日酔い防止
- ☆**フラボノール**0.6～0.7%)；抗酸化、抗がん、免疫活性
- ☆**ビタミンC**(200mg%)；抗酸化、免疫活性
- ☆**ビタミンB**(1.4mg%)；抗酸化、口内炎予防
- ☆**サポニン**(0.1%)；抗喘息、抗菌、血圧効果
- ★**テアニン**(0.6～2%)；リラックス、血圧降下作用などなど

茶はカテキンを始め多くの特異的な成分を含有し、それぞれ機能性をもつため、その機能性を活かした商品も数多く開発されている

第2回
他国産と日本産茶の機能性成分から見た差別性

国内外で多くの緑茶が市販されているが、輸出をさらに強化するためには他国産との違いを認識する必要がある。また、静岡産抹茶と特質も併せて紹介する

1. 国内外で市販される抹茶の販売状況
2. 抹茶が輸出を牽引
3. 国内外で市販される抹茶の粒度
4. 国内外で市販される抹茶の測色特性
5. 国内外で市販される抹茶の化学成分特性
6. 国内外で市販される抹茶の香り特性
7. 抹茶の定義は？

茶学総合研究センター
中村順行

第3回
機能性成分を活かしたマーケティング

海外輸出をさらに強化するために茶の機能性を活かしたマーケティングを考える

1. 輸出の現状
2. 消費嗜好特性
3. 購買特性
4. 国産抹茶の特質
5. 輸出国の特徴
6. 販売戦略

茶学総合研究センター
中村　順行

課題名：

（３）茶葉及び茶飲料の嗜好特性の解明
1）抹茶の市場特性の解明

研究の目的：

高級抹茶の輸出を促進するため、輸出の現状と輸出国の嗜好、消費、購買特性などを明らかにし、輸出戦略のマニュアル化の基礎資料とすることを目的とした。

研究の手法：

各種統計資料や主に米国と台湾における実態調査やアンケート調査により嗜好、消費、購買特性などを明らかにする。

主な研究成果：

1) 抹茶の生産量の推移：近年、抹茶は飲用のみならず加工用など多用途に利用され拡大基調が続いている。
2) 輸出の推移：全体の輸出量 5,108t、148 億円(2019)のうち粉末茶が 60%程度を占め、日本茶輸出を牽引し、アメリカと台湾で輸出量の 56%、金額の 55%と大半を占める。
3) 嗜好性:アメリカや台湾において抹茶を選ぶ際には、香味や価格が重視される。なかでも、抹茶は「香味に優れる」「すべてを摂取可能」「健康に良い」などとともに「色が綺麗」「多用途利用ができる」「文化的」などの利点を持ち、高い嗜好性を有している。
4) 消費特性:抹茶の飲用法としては、水や湯に溶いて飲む人が約半数程度と多く、牛乳などの他の飲料に混ぜて飲む人も 30%以上あり、これらの人は、「とても美味しい」「美味しい」と評価していた。日本茶の飲用場所は欧米諸国では概して自宅が多いが、一方アセアン諸国では日本食チエーン店で飲む機会が多い。このことは欧米諸国向きにはB to C が中心となるが、アセアン諸国向きにはB to B の商品群が必要となる。
5) 購買特性:抹茶は欧米諸国ではスーパーや茶専門店で購入することが多いが、シンガポール、タイ、マレーシアなどのアセアン諸国では圧倒的にスーパーで購入する比率が高い。高価格帯(5,000 円以上/100g)茶と低価格帯(1,000 円以下/100g)の抹茶とのトレンドサーチでは、高価格帯では「Ceremonial、 Japanese、 Premium、 Pure」などが、低価格帯では「Power、 Green、 Powder、USDA」が、重要なキーワードとして解析され、価格の違いによる商品への期待感の違いが明らかであった。

今後の展望：

これまでのデータをまとめ、日本産高級抹茶の販売戦略を構築し、マニュアル化し輸出関係者に流布する予定である。

（担当：茶学総合研究センター　中村順行）

主要な成果

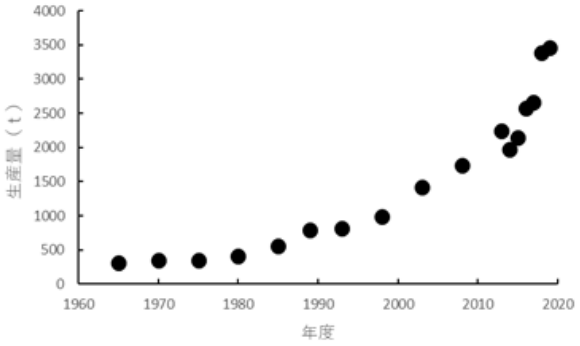


図１　てん茶生産量の推移

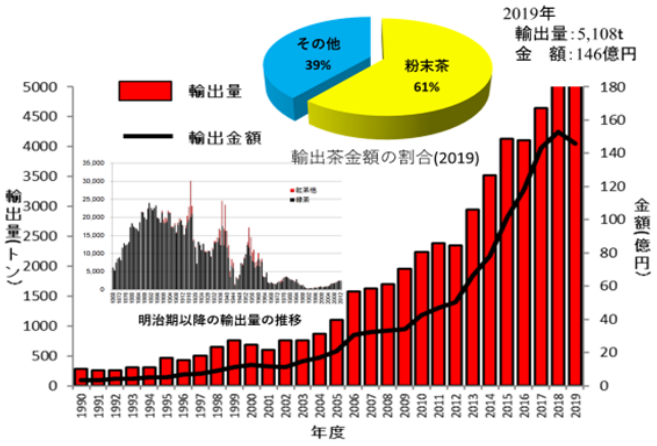


図2　　日本茶の輸出量と金額の推移

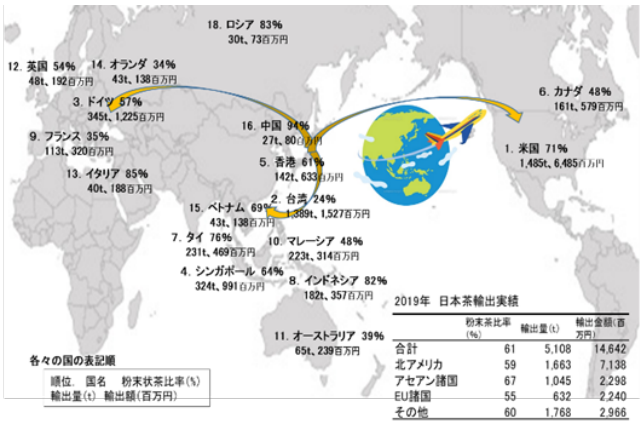


図３　世界各国への日本茶の輸出状況(2019)

図４　国内外で市販されている抹茶

表1　各国への日本茶の輸出概要

項　目	茶消費量	一人当たり消費量	日本茶輸出量	日本茶輸出金額	輸出kg当り価格	粉末茶比率
	千トン	kg	トン	百万円	円	%
アメリカ	129	0.40	1,485	6,485	4,368	71
カナダ	17	0.47	162	579	3,580	42
ロシア	253	0.89	30	73	2,429	89
イギリス	110	1.67	48	192	3,993	57
ドイツ	31	0.80	346	1,225	3,543	43
フランス	14	0.21	113	320	2,833	33
イタリア	7	0.12	40	188	4,679	83
オーストラリア	11	0.44	65	239	3,687	46
シンガポール	—	—	324	991	3,058	52
台湾	37	1.37	1,389	1,527	1,100	21
香港	11	1.51	143	633	4,430	46
タイ	—	—	231	469	2,027	73
ベトナム	—	—	60	105	1,742	92
マレーシア	25	0.79	223	314	1,410	35
中国	1,956	1.42	27	80	2,982	92
資料統計年度	2015～2017平均	2015～2017平均	2019	2019	2019	2020.1～8

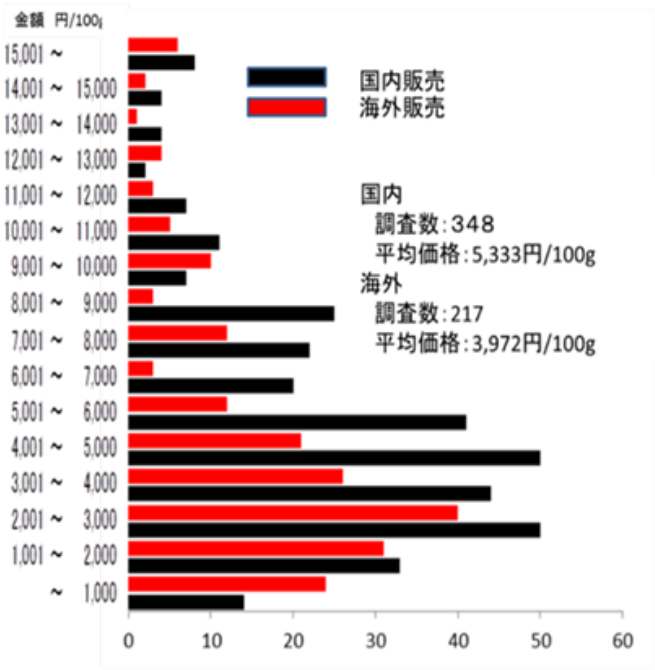


図５　国内外で市販される抹茶の金額比率各国へ

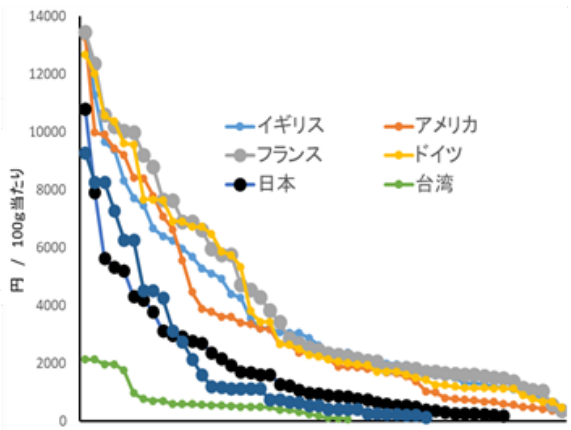


図６　各国で市販されている抹茶価格

表２　各国のネットで市販されている抹茶価格

国名	抹茶のみ		混合抹茶		平均価格(円/100g)		
	点数	比率(%)	点数	比率(%)	全体	上位10点	下位10点
イギリス	44	88.0	6	12.0	4,266	9,812	1,185
アメリカ	44	88.0	6	12.0	3,636	9,586	609
フランス	41	82.0	9	18.0	4,795	11,412	1,346
ドイツ	45	90.0	5	10.0	4,459	10,808	1,050
台湾	8	26.7	22	73.3	787	1,445	295
シンガポール	32	84.2	6	15.8	2,532	6,850	362
日本	34	77.3	10	22.7	2,042	5,330	304

※ 各々の国のアマゾンネットで市販されているMatchaあるいは抹茶の掲出順位別50点(台湾、シンガポールは掲出されたもの全て)程度を調査対象とした。

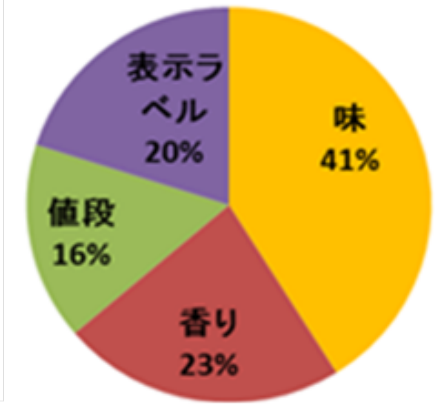


図８　抹茶の見分け方

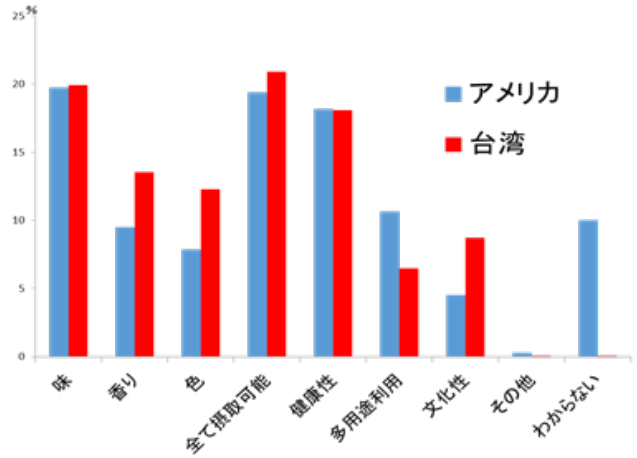


図７　抹茶の優位性

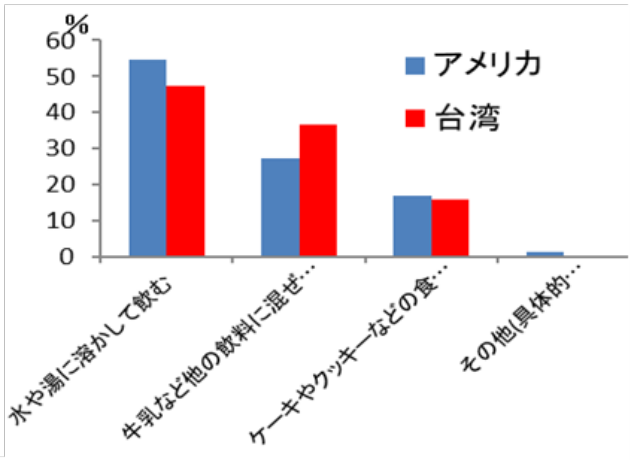


図９　抹茶の飲用方法

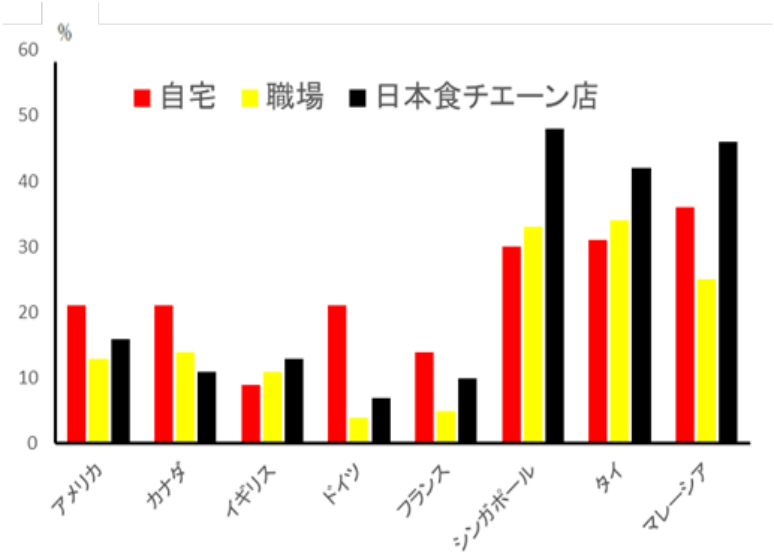


図１０　日本茶の飲用場所

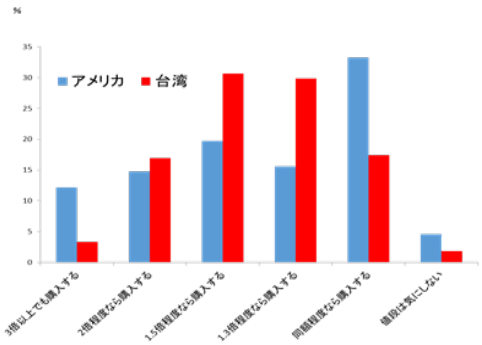


図１２　外国産抹茶に対する日本抹茶の購入

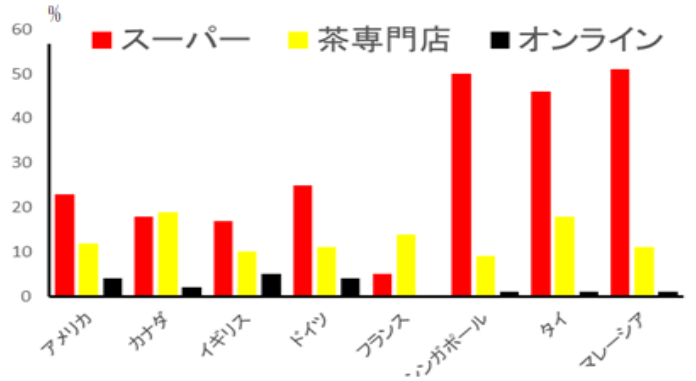


図１１　日本茶の購入場所

表３　抹茶関連商品の購入期待値段

	米国	台湾
	円	円
抹茶のみ(円/100g)	4,739	538
抹茶ラテ	599	310
抹茶アイス	631	267

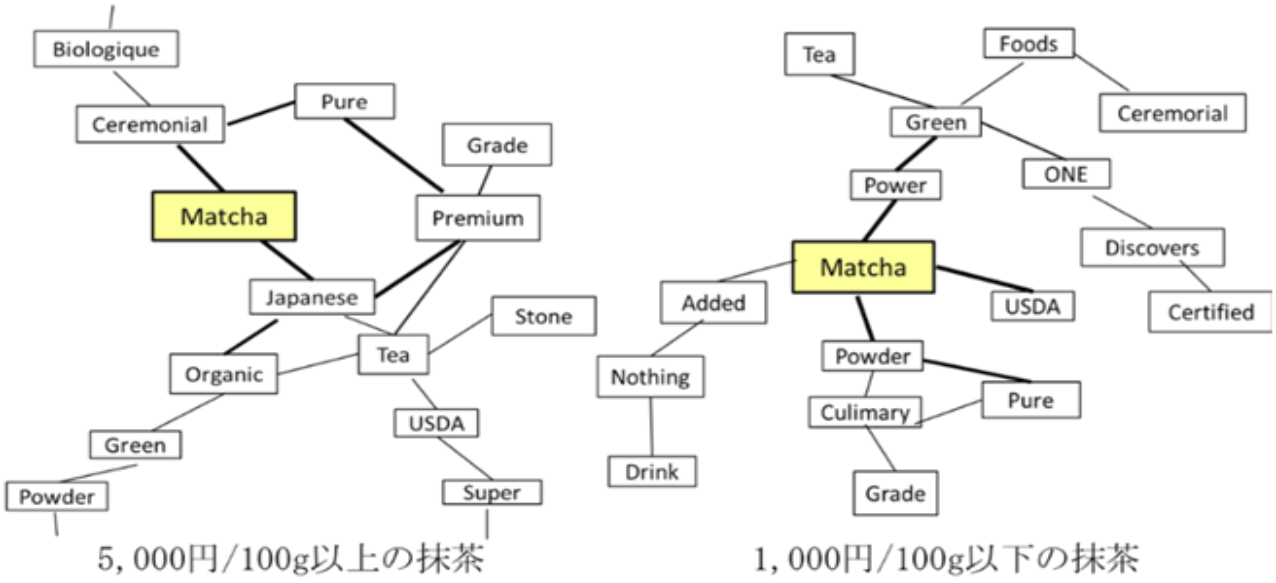


図１３　価格の異なる抹茶のトレンドサーチ解析

課題名：

（３）茶葉及び茶飲料の嗜好特性の解明
2）抹茶の科学的特性

研究の目的：

高級抹茶の輸出を促進するため、国内外で市販されている抹茶の粒度分布特性、測色特性、化学成分特性を明らかにし、輸出戦略のマニュアル化の基礎資料とすることを目的とした。

研究の手法：

国内外から購入した日本産抹茶 76 点、海外で市販されている日本産 40 点、外国産 17 点の計 133 点の抹茶の粒度分布を HORIBA 製の Laser Scattering Particle Size Distribution Analyzer LA-95 で､測色特性は KONICA MINORUTA 製の分光測色計 CM-5 Spectrophotometer で、化学成分は HPLC を用いて分析した。

主な研究成果：

1）国内外で市販される抹茶の粒度分布特性

国内で購入した抹茶の 55%程度はタイプ A の山型であり、残りの 30%程度はタイプ B の裾広がり型であった。タイプ D の二山型も若干みられたが、このタイプの抹茶は価格も安いものが多かった。一方、海外で購入した抹茶では、国内で市販されている抹茶特性とも異なり、日本産抹茶でさえタイプ A で 25%、タイプ D で 38%だった。また、外国産抹茶ではタイプ A は 10%、タイプ D では 63%と二山型が多かった。

2）測色特性

国内外で市販されている抹茶の金額と表色系の測色値について、金額が高くなるほど明度(L)、彩度(C*)、色相角度(h)が高くなる傾向がみられた。一方、a*値についてはマイナス値が大きくなり、緑色が強くなる傾向がみられた。h 値は 4,000 円/100g 以上の価格帯のものでは、大部分が 110 以上の数値を示し、低価格帯(1,000 円/100g)以下のものでは 105 以下のものが多く、価格が安価なほど h 値が低かった。また、a*値(数値が小さいほど緑色、高いほど赤色)は価格が高くなるほど低くマイナス 15 以下を示したが、低価格帯ではマイナス 10 以のものも多くみられた。

3）化学成分特性

抹茶は煎茶に比較しアミノ酸含量が高く、カテキン含量は低いが、葉色は濃緑色となりクロロフィル含量は高まる。133 点の化学成分でもテアニン含量および EGCG/EGC 比率は、市販価格が高くなるほど高くなる傾向が見られた。

今後の展望：

これまでのデータをまとめ、日本産高級抹茶の販売戦略を構築し、マニュアル化し輸出関係者に流布する予定である。

（担当：茶学総合研究センター　中村順行）

主要な成果

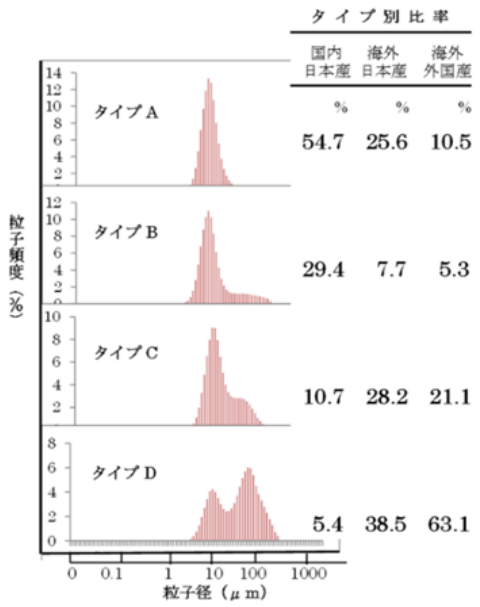


図１　国内外から購入した抹茶のタイプ別比率

表１　国内外から購入した抹茶のタイプ別価格と粒径の大きさ

タイプ	国内市販品		海外市販品			
	日本産		日本産		外国産	
	100g当たり価格(円)	平均径（μm）	100g当たり価格(円)	平均径（μm）	100g当たり価格(円)	平均径（μm）
A	6,902	15.5	5,801	16.5	1,569	16.7
B	4,010	23.8	2,515	23.7	3,780	19.2
C	2,698	26.2	4,178	30.1	1,241	30.8
D	1,572	52.6	3,461	47.9	1,599	38.8

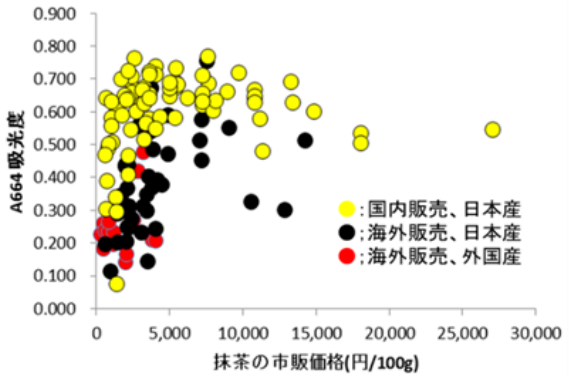


図２　国内外から購入した抹茶 A_{664} 吸光度

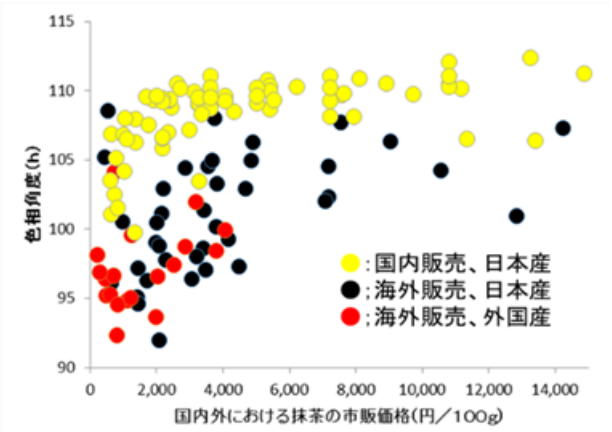


図３　国内外から購入した抹茶の色相(ｈ)角度

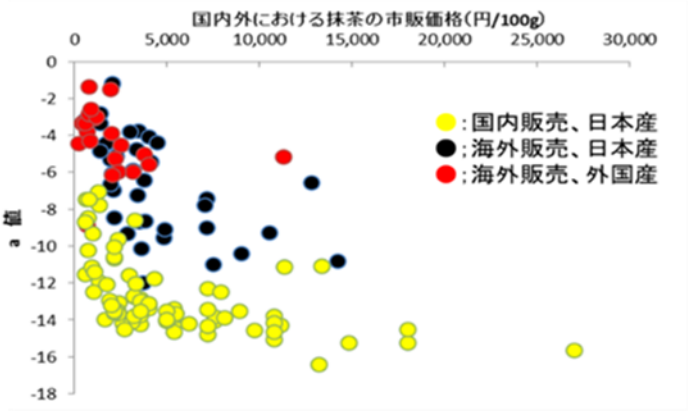


図４　国内外から購入した抹茶のａ値

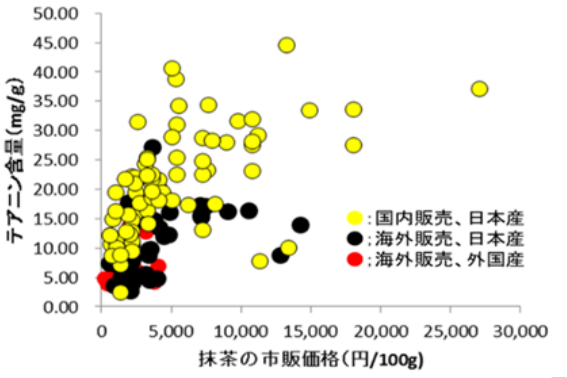


図５　国内外から購入した抹茶のテアニン含量

課題名：

（３）茶葉及び茶飲料の嗜好特性の解明
3）抹茶の輸出国別特性と輸出戦略

研究の目的：

高級抹茶の輸出を促進するため、国内外で市販されている抹茶の粒度分布特性、測色特性、化学成分特性を明らかにし、輸出戦略のマニュアル化の基礎資料とすることを目的とした。

研究の手法：

各種統計資料やアンケート調査により国別の特性を調査するとともに SWOT 解析などを行うことにより市場を大きく分類し、市場ごとの輸出戦略を構築する。

主な研究成果：

1）輸出国別特性

抹茶が輸出される国は 70 ヵ国以上と多いが、アメリカ、台湾、ドイツ、シンガポールなど主要な 8 ヵ国で 80%以上を占める。全体的には、健康志向への高まりとともに、日本食ブームが定着化しつつあり、付随して抹茶の飲食材への活用も増加している。

2）SWOT 解析

日本産高級抹茶の強みは「高品質、高付加価値」「高度な生産技術と衛生管理」などが上げられる。一方、弱みとしては中国産などに対して価格が高いこと。有機栽培抹茶の供給力不足。さらにマーケット情報が不足し、情報発信力が弱いことなどが上げられる。

3）抹茶市場の分類

①定着市場

定着市場には北米、香港のように比較的輸出単価の高い国と台湾、タイ、ベトナムのように輸出単価の安い国があり、高い国においては更なるブランディングの強化と需要拡大が重要となる。価格の安い国に対しては飲食加工用抹茶の低コスト生産を行う必要がある。

ブランディングを強化するためには「抹茶品質の差別化による優位性の保持」「機能性の付与」「本物志向への対応」「情報の発信」などが重要である。

② 制約市場

制約市場としては、特に EU 諸国は抹茶への意識も高く輸入単価も高いが、残留農薬規制が厳しく有機栽培抹茶が好まれる。

③ 有望市場

有望市場としては、日本茶への関心を急速に高め今後の更なる進展が期待されるシンガポール、ロシア、香港、台湾などがある。ネット環境も整っているため、EC を活用した販売戦略も重要になってくるものと思われる。

④ インバウンド市場

インバウンドはコロナ禍のため現状では途絶えているが、今後回復し、確実に「モノ」から「コト」消費に移行する傾向があるため、その対応への準備が必要である。

今後の展望：

これまでのデータをまとめ、日本産高級抹茶の販売戦略を構築し、マニュアル化し輸出関係者に流布する予定である。

（担当：茶学総合研究センター　中村順行）

成果の概要

表１　主要な輸出対象国別の特徴

対象国・地　域	特　　性
米国	・輸入量も多く、比較的高価な抹茶が使用され、知名度も高い ・健康イメージ。中国産抹茶も多く、飲食素材としての広がりも多い
カナダ	・比較的輸出単価の高いものの需要が多い ・日本茶に対して好意的で、茶専門店での購入が多い
イギリス	・輸出単価も高く、徐々に抹茶も浸透中 ・残留農薬規制が厳しい
ドイツ	・輸出単価も高く、抹茶市場も大きい ・自宅飲用比率が高く、残留農薬規制も厳しい
フランス	・日本茶は堅調な伸びを示しているが、抹茶比率は比較的小さい ・日本産の愛好者も多く、抹茶の文化性に着目
イタリア	・輸出単価も高く、抹茶の輸出比率が高い。 ・現状での茶消費量は少なく、今後の伸びが期待される
ロシア	・茶消費量は多く、抹茶への興味も増大化 ・抹茶は比較的安価なものが好まれ、Net 環境は整備されている
シンガポール	・需要量は多い。比較的安価。自宅での飲用が多く、知名度も高い。 ・輸出障壁は比較的低く、Net 環境は整っている
香港	・市場は大きく、抹茶単価も比較的高い ・輸出障壁も比較的低く、日本茶への興味も高い
台湾	・市場は非常に大きいが、抹茶単価は低い ・日本茶、抹茶の知名度高く、和食とのセットや文化性で評価されている
タイ	・抹茶需要が増加し、市場は堅調に伸び、関心も高い ・抹茶単価は低く、食材への活用が多い。Net 環境は整備されている
ベトナム	・茶の生産国でもあるが、日本茶への関心が高い ・抹茶需要も徐々に浸透しているが、その単価は低い
マレーシア	・日本茶需要は顕著な高まりを見せているが、抹茶単価は低い ・お茶はスーパーでの購入も多く、健康イメージが高い
中国	・輸出障壁が非常に高いが、日本産抹茶市場はある ・中国産抹茶との差別性が重要
オーストラリア	・日本茶需要は多いが、抹茶単価はやや低い ・在住日本人も多く、日本食レストランも多く今後の伸びが期待される

図３　世界の EC 市場規模の推移[9)]

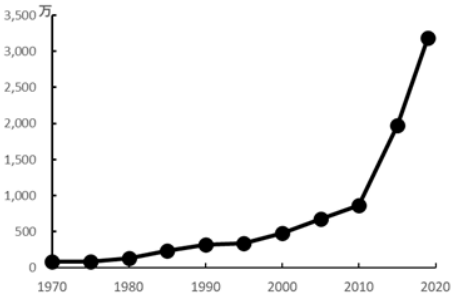


図４ インバウンド来日者数の推移[25)]

強み	機会、チャンス
・安心、安全 ・高品質、高付加価値 ・典型的な日本ブランド ・機能性研究の進展 ・高度な生産技術と衛生管理 ・歴史、文化性が高い ・健康イメージが高い ・日本食レストランの浸透 ・日本茶の認知度が高い	・健康ブーム、緑茶＝体に良いイメージ ・アジア諸国の経済発展 ・日系企業の海外展開 ・飲食材など他用途利用増加 ・インバウンド客の増加 ・和食ブーム ・日本ブランド信頼度高い ・消費者の関心の高さ ・高所得者層に人気
弱み	**脅威**
・情報発信力が弱い ・他国産に比較して価格が高い ・他国産との差別化要因が不明確 ・抹茶生産力が低い ・有機栽培抹茶の供給不足 ・マーケット情報不足 ・後継者不足	・他国産の低コスト生産抹茶の増加 ・EU の農薬規制（残留農薬基準の規制強化） ・東日本大震災の危険イメージ ・輸出相手国の情勢不安 ・国際標準化への出遅れ ・模倣品(にせブランド)への対応の遅れ ・日本産抹茶の供給力不足

図１　抹茶輸出に係る SWOT 解析

表２　抹茶市場の分類とその概略

分類	概略		該当国
定着市場	日本茶の浸透度が高く、輸出制約も比較的小さく実績の高い国	輸出単価の高い国	米国、香港、カナダ、オーストラリア、マカオ
		輸出単価の安い国	台湾、タイ
制約市場	日本茶への認知度、要望は比較的高いが、輸出障壁が高い国	残留農薬規制	ドイツ、フランス、イギリス、イタリアなどEU諸国
		ハラール	インドネシア、マレーシア、
有望市場	日本茶や抹茶への関心を急速に高め、今後の伸びが期待される国		シンガポール、ベトナム、インドネシア、ロシアなど

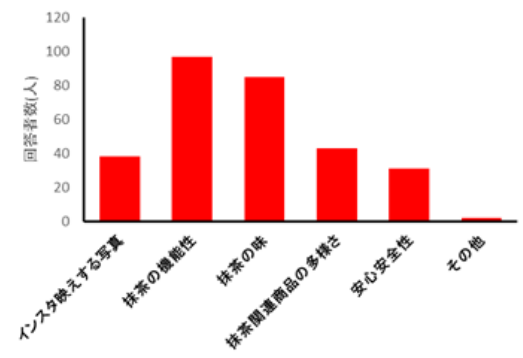


図５ インバウンドに聞いた抹茶の PR 方法

課題名：
（4）茶の高付加価値化とマーケティング
1）カフェインレス茶の需要予測

研究の目的：

最近、カフェインレス茶に対するニーズは高いが、希求度の高い消費者層や需要については明らかになっていない。そこで、各種統計資料やアンケートなどによりカフェインレス茶の需要を把握することを目的とする。

研究の手法：

各種統計資料やアンケート調査によりカフェインレス茶の需要予測を行う。

主な研究成果：

3）最近、カフェインによる各様の悪影響が懸念され、国によっては最大摂取量を定めている国もあるし、日本のように基準値が設定されていない国においても健康リスクにより妊婦さんや乳幼児ではカフェイン入り飲料が避けられる傾向にある。

4）カフェインレス飲料への需要の高まりは世界的な傾向ではあるが、そのニーズは機能面からの要望が生じることも多い。

5）カフェインレス茶に対する希求度は高所得者層で高く、また妊婦さんや夜眠れなくなる人、夜間の頻尿防止を考える人などで高い。また、アンケート調査でも「覚醒作用の低下」「良質な睡眠」「夜間の頻尿防止」などが期待され、今後の需要も台湾では 70%程度、アメリカでは 50%程度が高まるとしている。

6）日本では、まだ比率的に少ないもののカフェインレス、デカフェに対するニーズも高く、新商品が次々に開発され、今後の需要は拡大してくるものと考えられる。そのようななか、ターゲットとなるのは、まずはカフェインリスクを危惧する妊婦さんや小さな子供を持つ親、良質な睡眠や夜間の頻尿防止を望む老人などが上げられる。

7）アンケート調査では、高所得者層になるほどカフェインレス茶を望む傾向が認められた。さらに、「良質な睡眠」「夜間の頻尿防止」などを望む人は就寝前にカフェインレス茶を飲用する傾向が高いことが明らかにされている。

8）これらのことから、カフェインレス茶の需要は今後妊婦さんや小さな子供のいる親、良質な睡眠や夜間の頻尿防止などを望む人を中心としながらさらに高まるものと考えられるため、ターゲットをしっかりと絞り込み、機能面を訴求しやすいようなマーケット戦略が重要になるものと考えられる。

今後の展望：

これまでのデータをまとめ、カフェインレス茶の需要予測を公開し、ターゲットを絞ったカフェインレス茶に対する研究や商品開発につなげていく予定である。

（担当：茶学総合研究センター　中村順行）

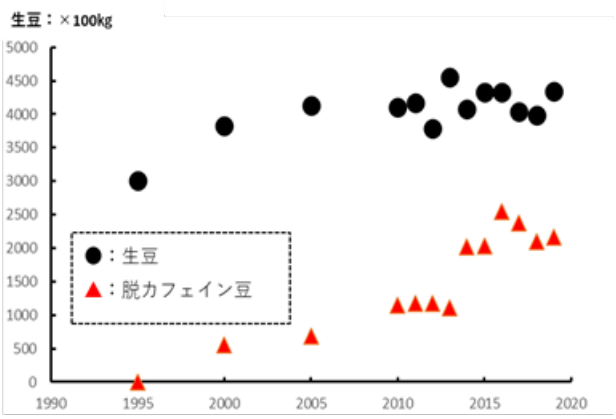


図1　国内におけるコーヒー生豆と脱カフェイン豆」の輸入の推移

写真1　国内で市販されているカフェインを低減化した茶商品

表1　各国におけるカフェインの最大摂取量

悪影響のない最大摂取量			飲料換算	機関名
妊婦		300mg／日		世界保健機関　(WHO)
		200mg／日		欧州食品安全機関　(EFSA)
		300mg／日	コーヒー　マグカップ2杯（237ml／杯）	カナダ保険者
		300mg／日		オーストリア保険・食品安全局（AGES）
		200mg／日		英国食品基準庁（ESA）
授乳中の女性		200mg／日　注1		欧州食品安全機関　(EFSA)
健康な子供及び青少年	子供、青少年	3mg／kg体重／日		欧州食品安全機関　(EFSA)
	子供（4～6歳）	45mg／日		
	子供（7～9歳）	62.5mg／日		カナダ保険者
	子供（10～12歳）	85mg／日		
	13歳以上の青少年	2.5mg／kg体重／日		
	子供（5～12歳）	95mg／日（3mg／kg体重／日）	コーラ2缶	欧州・ニュージーランド
健康な成人		400mg／日（3mg／kg体重／1回　注2）		欧州食品安全機関　(EFSA)
		400mg／日	コーヒー　マグカップ3杯（237ml／杯）	カナダ保険者
		400mg／日	・3～5カップ／日 ・カフェインとエナジードリンクを一緒に摂取すべきでない	米国保健福祉省（DHHS）及び農務省（USDA）
		210mg／日	インスタントコーヒー約3カップ	欧州・ニュージーランド

注1）　乳児に健康リスクは生じない
注2）　1回当たり摂取量約3mg/kg体重以下（例：体重70kgの成人で、約200mg以下）であれば急性毒性の懸念は生じない

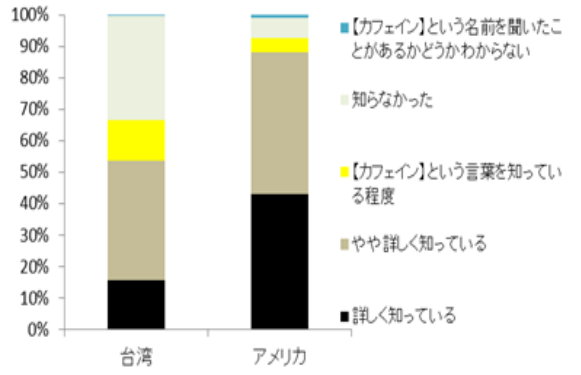


図2　カフェインに対する知識

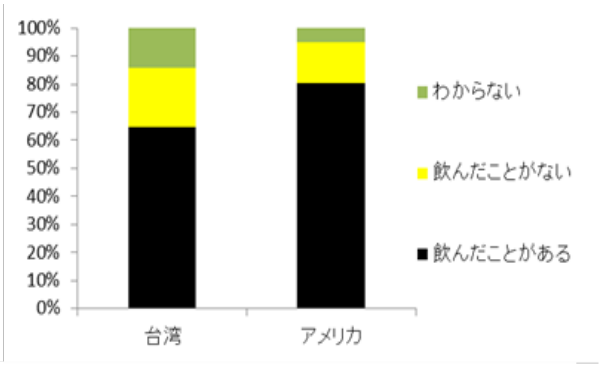


図3 カフェインレス飲料の飲用経験

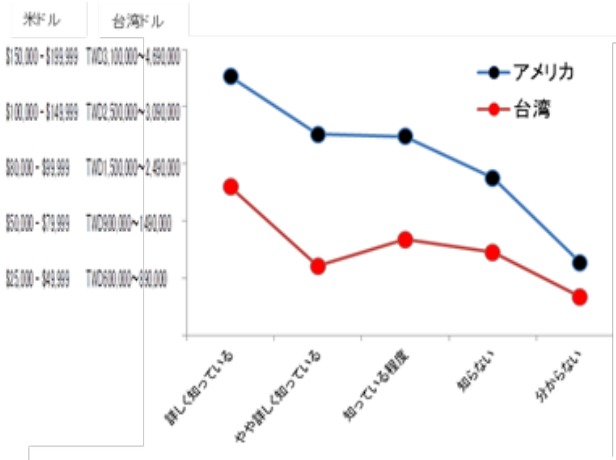


図4 カフェインの知識と収益との関係

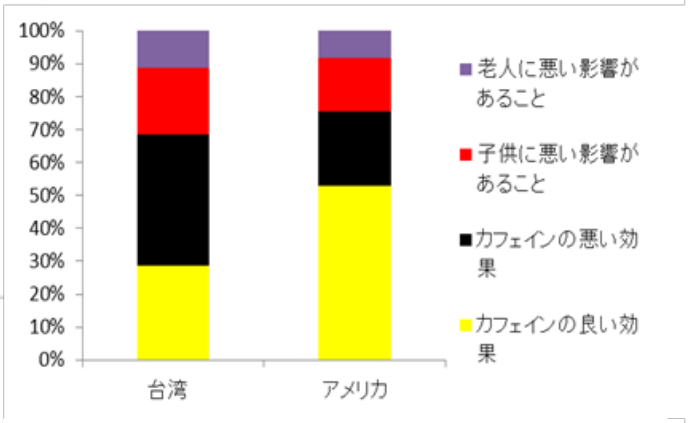


図5 カフェイン飲料選択時に気にする点

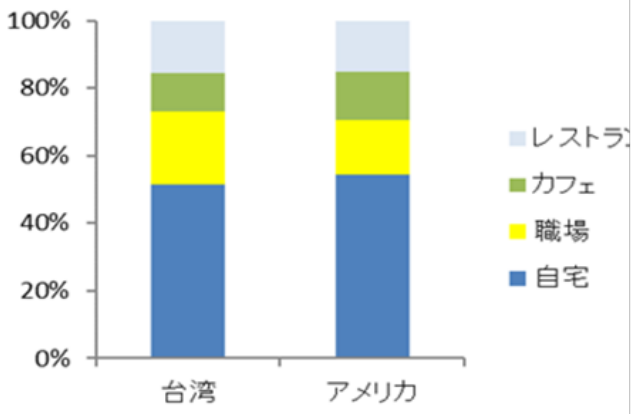


図6 カフェインレス茶の飲用場所

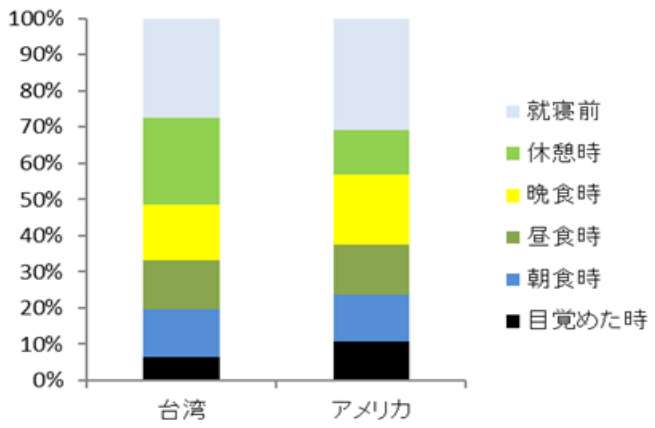


図7 カフェインレス茶の飲用時間帯

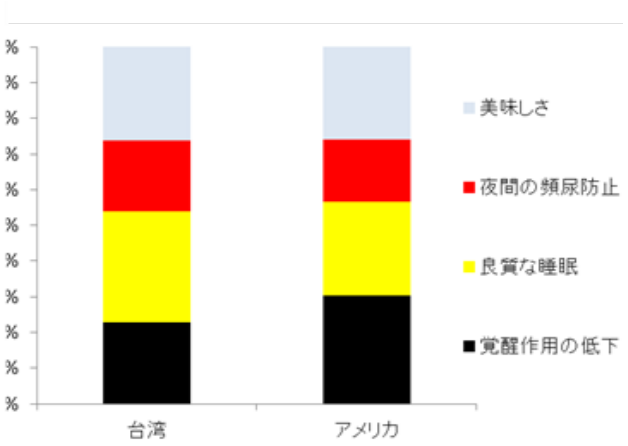


図8 新カフェインレス緑茶への期待点

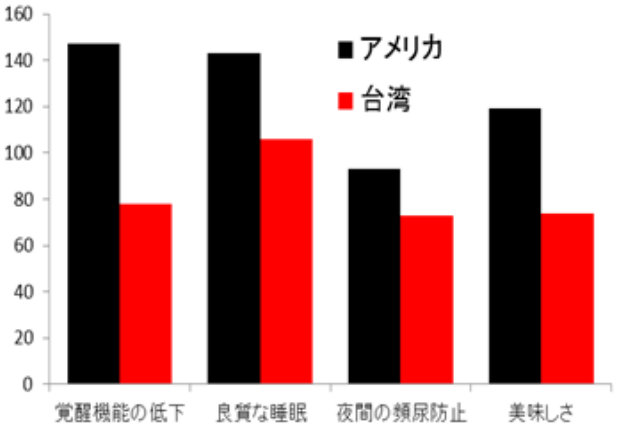


図9 機能性着目者のカフェインレス茶に対する期待

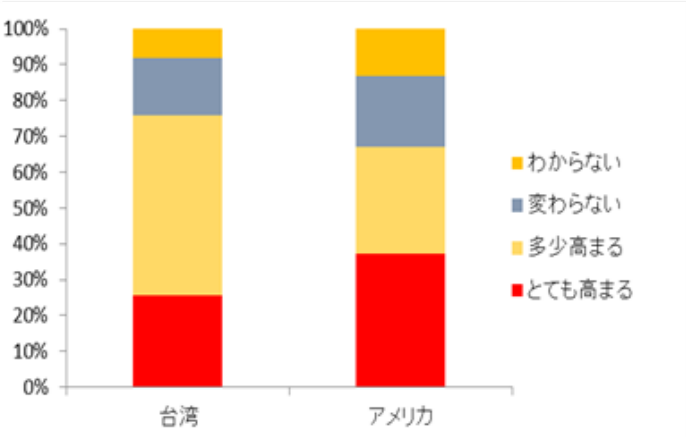


図10　夜間の頻尿防止に対するカフェインレス茶の今後の需要との関係

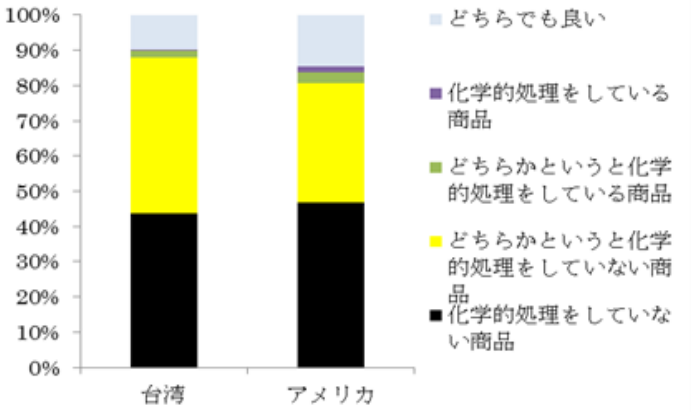


図11　ナチュラルカフェインレス飲料の選択志向

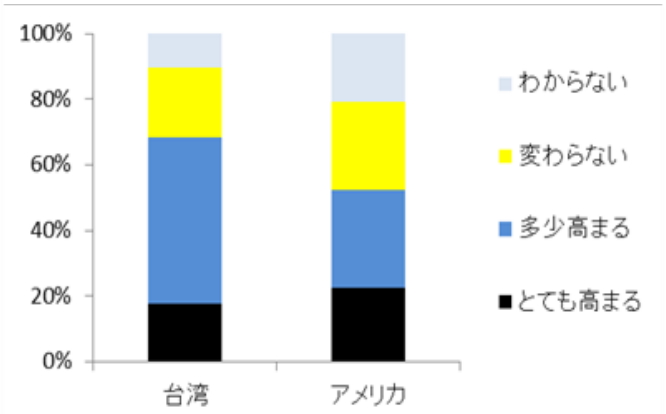


図12　カフェインレス飲料の需要予測

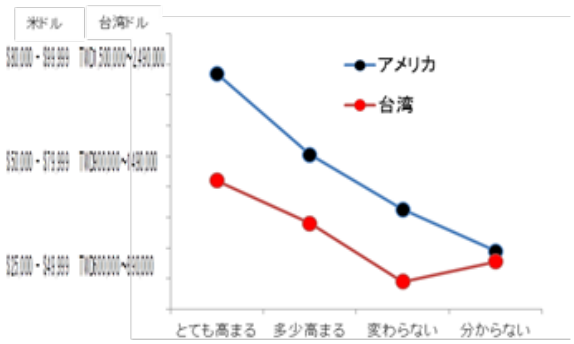


図13　カフェインレス茶の需要予測と収入との関係

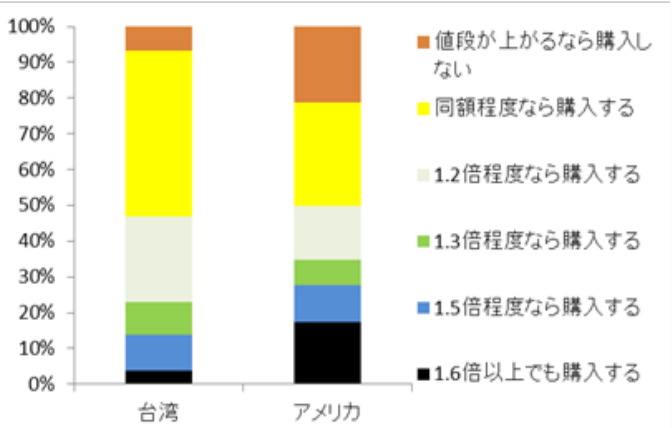


図14　新カフェインレス緑茶の購入希望価格

２．主要な発表論文など

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High Levels of Major Components and Antioxidant Activity of Fermented Tea Treated with *Lactococcus lactis subsp. cremoris*

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Authors' contributions

This work was carried out in collaboration between both authors. Author KS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author YN managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Tea is a popular drink all over the world and has been attracting attention for its beneficial health effects. We developed a fermented tea by processing it with an exopolysaccharides-producing lactic acid bacterium, *Lactococcus lactis subsp. cremoris*, in order to manufacture high-quality tea with a physiological function. *Lactococcus lactis subsp. cremoris*was added to tea leaves (*Camellia sinensis)* and fermented for two weeks. To examine the progress of fermentation, we determined the change in pH as well as the contents of ascorbic acid and folic acid in the extract of leaves. Decreases in ascorbic and folic acids were identified, but pH only slightly changed during fermentation, showing a slower development of fermentation with lactic acid bacteria. Furthermore, we analyzed the extract's components, such as catechins, amino acids, including theanine as the major amino acid, and caffeine. Although there were some fluctuations in contents, no significant change was seen over a period of two weeks. Fermentation had no effect on the degradation of these components, suggesting that they may be relatively stable. To investigate a potential physiological function, antioxidant activity was measured using 1,1-Diphenyl-2-picryl-hydrazyl,

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(DPPH). Consequently, the results showed that the activity of the extracts was unaffected by fermentation until the seventh day, when it began to increase. Our results suggested that the fermented tea developed in this study, which maintained its key components of catechins, theanine and caffeine, exhibit a physiological function as a processed tea and a novel food material.

Keywords: Fermented tea; Camellia sinensis; lactic acid bacteria; antioxidant activity; Exopolysaccharides (EPS); catechin; theanine.

1. INTRODUCTION

Tea (*Camellia sinensis*) has been regarded and used as a medicine since ancient times. Moreover, its health benefits have recently been demonstrated scientifically, and thus it is consumed worldwide [1]. Various types of teas can be manufactured as products (e.g. drink and food material) depending on how they are processed after harvesting. Green tea is non-oxidized leaves (generally called non-fermented tea), while oolong tea (generally called semi-fermented tea) is partially oxidized and black tea (generally called fermented tea) is fully oxidized by polyphenol oxidase and peroxidase in the manufacturing process. Although their characteristics and ingredients are slightly different, they are all popular and exhibit their own individual physiological function [1]. In addition to these teas, others are fermented by microorganisms. Among those, the most famous is Pu-erh tea, also known as post-fermented tea or dark tea,which originally came from Yunnan, China. In its production, it undergoes microbial fermentation with bacteria such as Aspergillus or Rhizopus [2,3]. Throughout the world, various fermented teas are made using microorganisms such as yeast, fungus or lactic acid bacteria (LAB), with each having its own characteristics [4,5]. These fermented teas should be considered as traditional foods. However, their contents are often unbalanced, and their quality is typically not uniform because they may not be made with a single microorganism, or they may be made by a method that relies on specific experience or tradition. One Japanese fermented tea, Awaban-cha, is fermented with LAB, a microorganism popular for its health benefits. It is also, however, fermented by several microorganisms in addition to the main LAB [4].

Recently, we developed a fermented tea using only an LAB that is resistant to catechins, *Lactobacillus plantarum*, which is derived from a plant. However, its components, including the catechins, decomposed during fermentation, while its antioxidant activity was maintained [5]. To develop a novel fermented tea with high quality and health benefits, we focused on using *Lactococcus lactis subsp. Cremoris* (*L. cremoris*), which was originally isolated from yoghurt traditionally produced in Scandinavian countries [6,7]. During its growth and metabolism, *L. cremoris* produces exopolysaccharide (EPS), a water-soluble long-chain polysaccharide that exhibits physiological effects such as antibacterial, anti-mutagenic, and antitumor activity as well as immune regulation, cholesterol lowering, and regulation of gastrointestinal function [8,9]. Moreover, EPS protects cells from environmental stress through the effects of water retention, osmotic pressure resistance, and antimicrobial resistance [10]. In this study, we employ *L. cremoris* to develop fermented tea and determine its main component. In addition, its antioxidant activity is discussed.

2. MATERIALS AND METHODS

2.1 Chemicals and Materials

The chemicals used in this experiment were purchased from Sigma-Aldrich, Mo., USA. Authentic reagents (Wako Pure Chemicals Industries, Ltd., Japan) were obtained to determine the concentrations of the main components of the tea using an automatic amino acid analyzer and high-performance liquid chromatography (HPLC, Agilent 1100, Agilent Technologies, Palo Alto, Calif., USA). Freshly plucked tea leaves (*Camellia sinensis* L. cv.'Yabukita') were washed and dried at 50°C for 24 hours.

2.2 Production of Fermented Tea

After autoclaved, 100 g of dried tea leaves were mixed with 100 mL of distilled water (DW), and was added an LAB, *Lactococcuslactis subsp. cremoris* CF−4 (1.7×10^8 cells/mL) (Konno Co., Ltd., Akita, Japan).Then, the mixture was packed into an anaerobic airtight container and fermented at 25°C for 2 weeks under a shaded condition [5]. At 0, 2, 7, and 14 days, the mixture was stored at -30°C for analysis.

2.3 Preparation of Fermented Tea Extract

The fermented tea samples were dried using a vacuum freeze dryer (FD-80, EYLA, Tokyo, Japan), finally, the water content of the sample was less than 1%, and then milled for 30 seconds to make a powder. Next, 1 g of the powder was added to 100 mL DW, which was heated and kept at 70°C for 1 hour to make an extract. The extract was spun down, and the supernatant was collected and then filtered with a 0.4-µm membrane for use in the following experiments [5].

2.4 Analysis of pH, Ascorbic Acid and Folic Acid

The extract of fermented tea leaves was measured by pH meter (Horiba, Ltd., Japan). To determine ascorbic acid content, the tea extract was pretreated and applied to high-performance liquid chromatography (HPLC) adopting a silica column (4.6 i.d. x 100 mm, 5 µm, Tokyo Chemical Industry Co. Ltd., Tokyo, Japan). The mobile phase for the detection was ethyl acetate:n-hexane:aceticacid:DW (60:40: 5:0.5) at a flow rate of 1.0 mL/min at 40°C. Each peak was identified by comparing the UV-Vis spectral characteristics at 495 nm and retention times with those of a commercial standard.A microbiological assay was conducted for analysis of folic acid using *Lactobacillus rhamnosus* ATCC 7469 [11–13]. The microbiological method was adopted from AOAC method given Official Status by AOAC (Method 992.05, 2002) and AACC (AACC Method 86-47).

2.5 Analysis of Catechins, Amino Acids and Caffeine

We analyzed catechins, amino acids and caffeine as previously described [5]. Briefly, Catechins and caffeine were analyzed using HPLC (Agilent 1100, Agilent Technologies, Palo Alto, Calif., USA) equipped with a C18 column (4.6 i.d. x 150 mm, 5 µm, Tokyo Chemical Industry Co. Ltd., Tokyo, Japan). The HPLC column was maintained at 30°C in an oven. The mobile phase for the detection was 0.1 M sodium dihydrogen phosphate buffer:acetonitrile (87:13) at a flow rate of 1.0 mL/min. The reagents were purchased from Sigma-Aldrich (St. Louis, Mo., USA), and HPLC-grade reagents were used for the analysis. Each peak was identified by comparing the UV-Vis spectral characteristics and retention times with those of commercial standards. The concentration of amino acids in the extract was analyzed using an L-8500 automatic amino acid analyzer (Hitachi Co. Ltd., Tokyo, Japan), which is a dedicated instrument for ion-exchange chromatography via the method of post-column derivatization using ninhydrin reagents that contain sodium borohydrate and propylene glycol monomethyl. The analytical column was a Hitachi HPLC Packed column (ion-exchange resin, 4.6 mm i.d., 60 mm length, 3 µm particle size). Throughout the elution program, the flow rate for buffer solutions was 0.35 mL/min. The flow rate for ninhydrin solution was 0.30 mL/min. All buffers were purchased from Wako Pure Chemicals Industries, Ltd., Japan, as a whole package. Detection was by spectrophotometry at 570 and 440 nm with the ninhydrin reaction.

2.6 Determination of Antioxidant Activity

The stable free radical DPPH (1,1-Diphenyl-2-picryl-hydrazyl, Sigma-Aldrich, St. Louis, MO, USA) was used to estimate the antioxidant activity of the fermented tea. 1.5-ml aliquot of DPPH solution (0.1 mM, in 95% ethanol) was mixed with 100 µL of tea extract. Standard green tea extract (*Camellia sinensis* L. cv.'Yabukita') was used as a control. The mixture was shaken vigorously and left to stand for 20 min at room temperature. The absorbance at 517 nm of the DPPH solution was measured using a spectrophotometer (Bio-Spec, Shimadzu, Kyoto, Japan). The antioxidant activity was determined as DPPH radical scavenging activity, which was calculated using the following equation:

Scavenging activity (%) = [1- (absorbance of sample/absorbance of control)] ×100

2.7 Statistical Analysis

Data were expressed as the mean ± standard error of the mean (SEM). Statistical analysis was performed using Student's *t*-test and one-way analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1 Fermented State

Generally, the progress of fermentation by an LAB involves a significant decrease in pH due to the production of lactic acid [14]. In this study, the pH did not dramatically decrease over 14 days as shown Fig. 1. However, through typical fermentation processes the amount of ascorbic acid has dramatically decreased [15,16]. Since it

was not reported that *L. cremoris* consumes ascorbic acid to grow, the result showed that the fermentation might proceed without extreme changes in pH. In addition to the ascorbic acid degradation, folic acid clearly decreased for 14 days in Table 1. Most LABs consume folic acid as a growth factor. Therefore, a decrease in folic acid indicates the growth of LAB.

The results in Table 1 showed that the fermentation with *L. cremoris* progressed slowly, at least for 14 days. Mild fermentation may involve a property of EPS, which is the product of *L. cremoris*. It has been reported that the EPS production is related to fermentation conditions (pH, temperature, etc.) and it also depends on bacteria [17]. The pH of yogurt prepared with *L. cremoris* is moderate, leading to a soft taste [18]. Again, EPS has very good characteristics that are useful for LAB as well as human health [19]. The function of *L. cremoris* with EPS requires further study, but *L. cremoris* may exhibit a unique action during fermentation.

3.2 Stability of Main Components

Analysis of the important components ofthe extract such as caffeine, catechins, and amino acids, including theanine, were performed using samples on 2, 7, and 14 days (Fig. 1). These components exhibit excellent taste in palatability as well as having health benefits. Among these components, catechins account for more than 10% in tea leaves, which gives tea its astringency, theanine is around 2%, which provides a delicious taste (called *umami*), and caffeine is around 3%, which supplies bitterness.

Since catechins are the most abundant component in tea leaves, they play an important role with their many physiological functions [1]. The main catechins are (-) Epigallocatechingallate (EGCG), (-) Epigallocatechin (EGC), (-) Gallocatechin (GC), (-) Gallocatechingallate (GCG), (-) Epicatechingallate (ECG), (-) Epicatechin (EC), (+) Catechin (C), and (-) Catechingallate (CG). In addition, there are other various small amounts of catechin derivatives. EGCG is found in the highest amounts in tea leaves. Fig. 1 indicated the change in each catechin content during fermentation for 14 days. These catechins decreased slightly on day 2 and increased on day 7, but no significant change was seen on day 14. Previous studies have reported that catechin degrades to a smaller molecule by fermentation [5,20–22]. In particular, it has been reported that within a catechin's structure, the gallate groups were most easily eliminated [23]. EGCG and ECG decompose into EGC and EC, respectively, in the early stages of fermentation. Consequently, the composition ratio of each catechin changed; for example, the amount of EC was greater than that of EGCG. However, in this study, no obvious degradation was observed in any catechin, and, moreover, there was no dramatic decrease in EGCG, which was the most likely to decompose. Fig. 2.(A) showed the proportion of each catechin remained almost the same for 14 days, indicating that the catechins contained in the extract are extremely stable. The increase in EGCG on day 7 seems to be due to other factors, and further investigation is needed; however, EGCG was maintained in the content without any reduction in fermentation for 14 days, showing that fermented tea may exhibit a similar physiological function to that of standard green tea. EGCG is the most powerful molecule in tea catechins, and it exhibits antimutagenic, anticancer, antiarteriosclerotic, antibacterial, and antiatherosclerotic effects [1]. Consequently, fermented tea rich in EGCG appears to be beneficial for human health.

Tea contains a large amount of amino acids that express themselves in delicious flavor, contributing significantly to the good taste of tea. The amount of amino acids fluctuated slightly, but not dramatically, over 14 days, showing that each amino acid was relatively stable under fermentation [Fig. 1.(B)]. In addition, the proportion of each amino acid remained nearly the same over the 14 days [Fig. 2.(B)]. In particular, theanine, consisting of more than 50% of the amino acids in tea leaves, was quite stable. Theanine (γ-ethylamide-L-glutamic acid) is an extremely rare amino acid in nature, and it has psychoactive properties because it is readily absorbed and permeates the blood-brain barrier to function in the brain [24]. This function leads to reduced mental and physical stress and improved cognition [25–29]. These results suggest that this fermented tea has the effect of improving brain function.

Caffeine did not significantly change except for a slight increase on day 7, thus keeping relatively stable for 14 days as shown in Fig. 1(C). This stability of caffeine was consistent with the results of previous studies [5]. While caffeine has some side effects, it was reported to enhance the physiological function of catechins through synergistic effects [30,31] and to improve cognition and boost one's mood in combination

with theanine [32]. The caffeine contained in this fermented tea might play a similar role to that of standard green tea in supplying a physiological function.

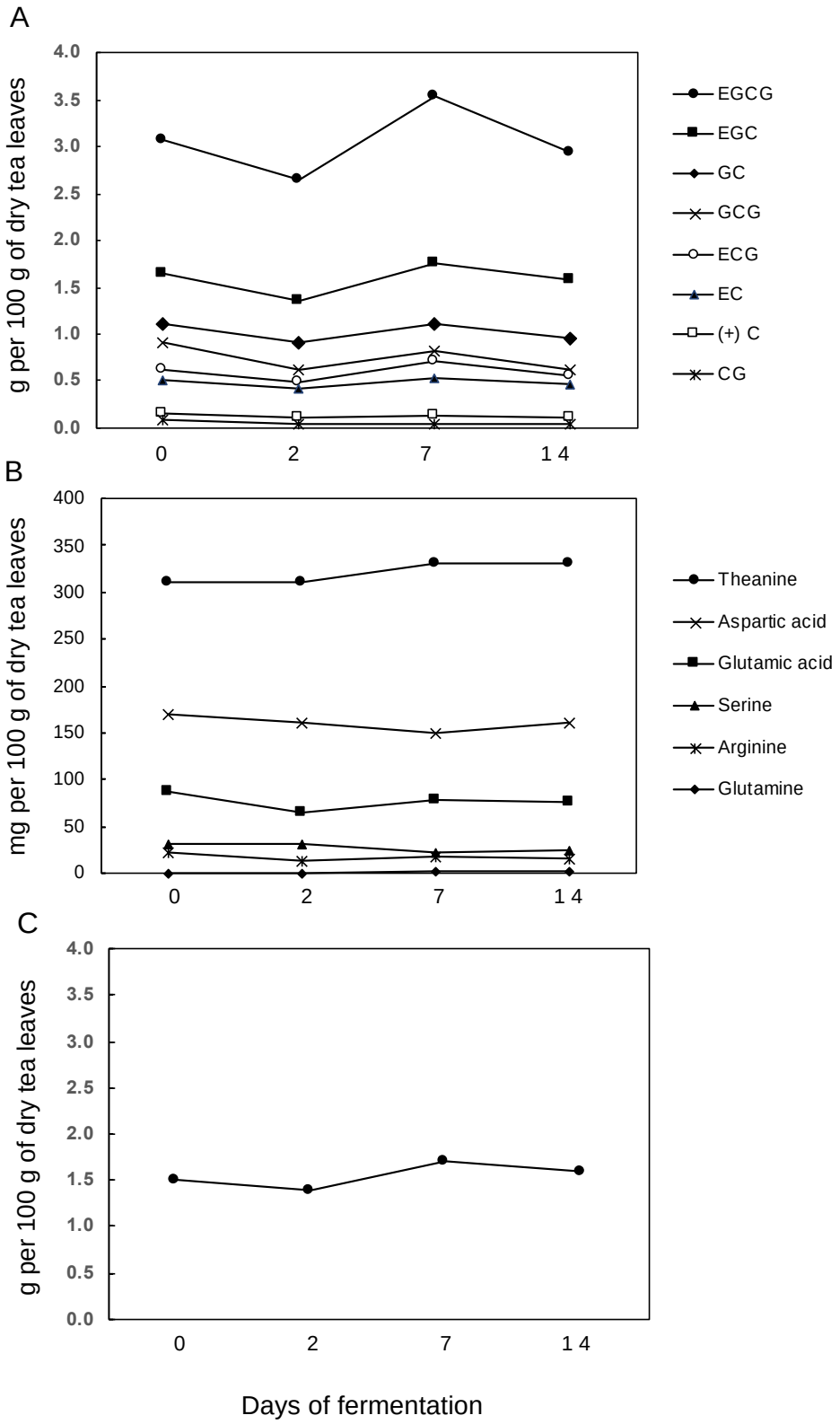

Fig. 1. Changes in catechin, amino acids and caffeine in the extract of fermented tea during fermentation: (A) Catechin, (B) Amino acids, (C) Caffeine

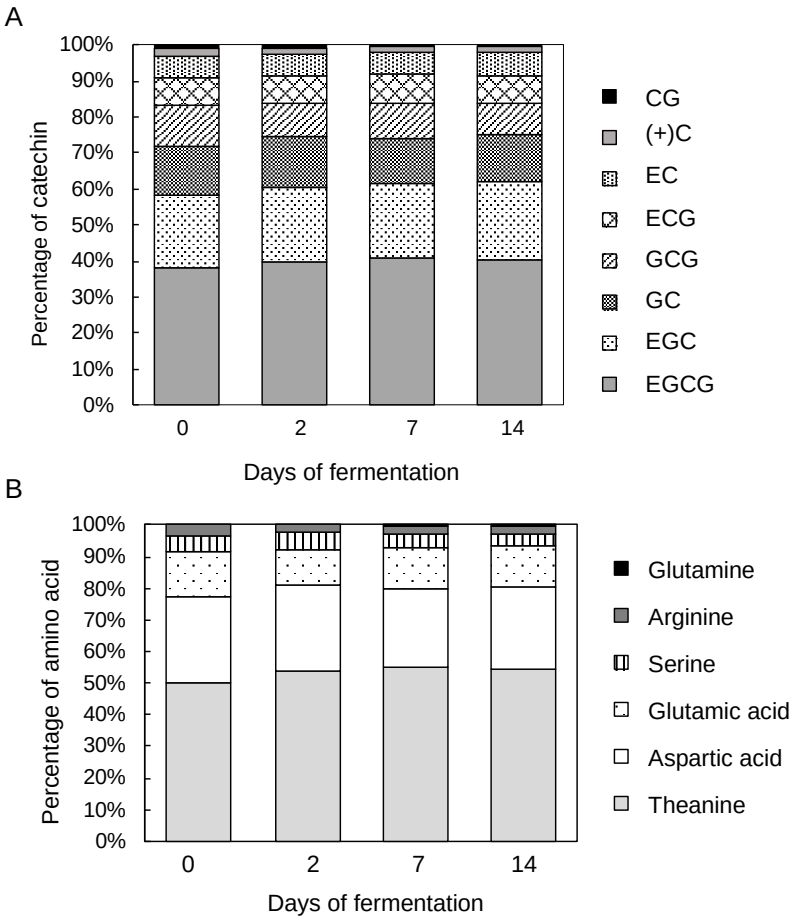

Fig. 2. Changes in the proportion of catechins and amino acids in the extract of fermented tea during fermentation: (A) Catechins, (B) Amino acids

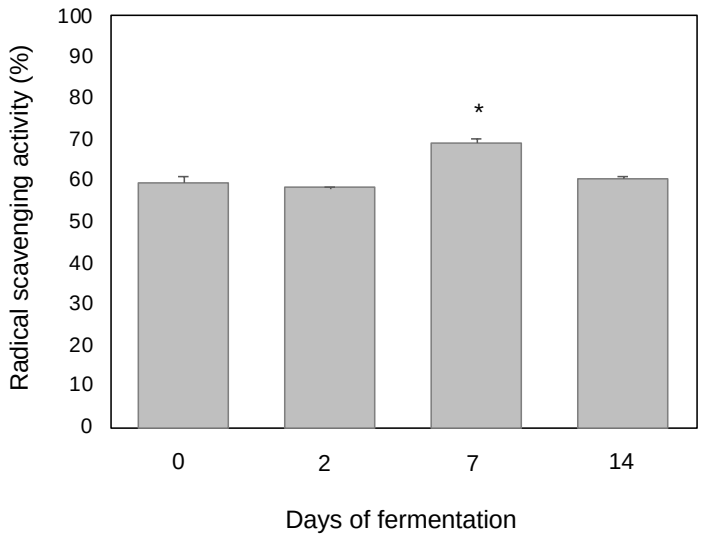

Fig. 3. Change in antioxidant activity of the fermented tea during fermentation. The antioxidant activity was determined as DPPH radical scavenging activity of the tea extract. Data shown are mean±SEM (n=3), *p<0.05

Table 1. Change in pH, ascorbic acid and folic acid contents during fermentation

	Days of fermentation			
	0	**2**	**7**	**14**
pH	5.48	5.48	5.45	5.43
Total ascorbic acid (mg/100 g)	33	11	4	2
Folic acid (mg/100 g)	0.6	0.46	0.41	0.36

3.3 Effect of Fermentation on Antioxidant Activity of the Fermented Tea

We determined the antioxidant activity of the fermented tea's extract using a DPPH method that was accurate and convenient (Fig. 3). Consequently, this activity did not decrease during fermentation. On the contrary, a significant increase was seen on day 7. It is well known that catechins exhibit a strong antioxidant activity. The catechins were not decomposed by fermentation, including the most effective molecule, EGCG, which also increased on day 7 [Fig. 1.(A)]. The antioxidant activity on day 7 may be deeply involved in the performance of EGCG. There was no dramatic decrease in pH for 14 days (Table 1), which may lead to stable antioxidant activity because, generally, antioxidant activity is inhibited by lower pH [33]. Although the mechanism of EGCG contributing to the increase on day 7 is unclear, this phenomenon is definitely interesting for the antioxidant activity of fermented tea. *L. cremoris* could play an important role in increasing other antioxidants and suppressing degradation, since microbial fermentation has provided special qualities and special active compounds that possess powerful antioxidant activities [34,35]. In this study, we employed a characteristic LAB, *L. cremoris*, that produces EPS and that might affect a antioxidant activity. Since there is no report on the effect of EPS derived from LAB on fermented tea but only on yogurt, further research is needed to identify the mechanism that activates antioxidant or the relevant effective molecules.

4. CONCLUSION

We developed fermented tea using *L. cremoris*, a unique lactic acid bacterium that produces EPS. This tea's fermentation progressed slowly. Its main components, i.e. catechins, theanine, and caffeine, were extremely stable and did not dramatically degrade over 14 days. The tea's antioxidant activity was also maintained stably, and a significant increase in antioxidant activity was seen on day 7 of fermentation. This fermented tea prepared with *L. cremoris* may have many unique functions and multiple beneficial effects on human health, in addition to the function provided by standard tea leaves.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 2
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Honeybees (*Apis mellifera*) Produce Honey from Flowers of Tea Plants (*Camellia sinensis*)

Kieko Saito[1,2*], Rieko Nagahashi[3], Masahiko Ikeda[3] and Yoriyuki Nakamura[2]

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ABSTRACT

We obtained honey from the blooming flowers of tea plants (*Camellia sinensis L.*) pollinated by honeybees (*Apis mellifera L.*). Functional amino acids, theanine, which is a unique ingredient to tea, was determined using reversed-phase chromatography. We also determined the main ingredients: caffeine and catechins. The obtained honey contained theanine, which shows that it was derived from tea flowers. The theanine concentration of the nectar of the tea flowers exceeded that of the honey. Caffeine was detected (but no catechins) in both the honey and the nectar of the tea flowers. Our results refute the previously held view that tea nectar is toxic to honeybees. Our new finding reveals that it is possible to obtain honey from the nectar of tea flowers. The obtained honey and the nectar of tea flowers contained a very rare amino acid, theanine, indicating that the honey was derived from tea flowers. Furthermore, the nectar of tea flower contained the best caffeine concentration that activated the brain function of honeybees to produce the honey.

Keywords: Tea; Camellia sinensis; theanine; flower; honey.

1. INTRODUCTION

Green tea (*Camellia sinensis L.*) leaves provide beneficial effects for human health, and the functions of the main components of their leaves have been widely studied [1]. Recently several physiological functions (e.g. antioxidant, antimicrobial, immunomodulatory and antitumor activities) of tea flowers have been reported [2-5], and the flowers have received attention as a natural healthy material for food and cosmetics. The health-promoting effects of green tea are mainly attributed to its polyphenol content [6], particularly flavanols and flavonols, which represent 30% of fresh leaf dry weight [7]. It is not well known that the fragrant tea flowers have sweet nectar. The tea nectar may be attractive to honeybees. One study of bee pollen collected from the flowers of tea plants suggests that honeybees like the pollen of tea (*Camellia sinensis L.*) [8]. However, the honey from tea flowers has not been studied, even though in autumn, many tea fields are filled with blooming flowers in almost all the tea production areas around the world. The most utilized part of the tea plant is the leaves. Thus, less attention has been paid to tea flowers. Since the application of asexual propagation to tea plants, tea flowers have become a “waste resource”, competing with tea leaves for water and nutrients. To promote the yield and quality of tea leaves, some chemicals, such as ethephon and α-naphthalene acetic acid, have been used to suppress tea plant blossoming [9], Sharma et al. reported that tea nectar exhibited toxicity to honeybees (*Apis mellifera L.*) [10]. Healthy broods and larvae were fed the nectar of tea flowers in the laboratory and were killed. Sharma’s report discouraged beekeepers from harvesting the honey of tea

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flowers whose nectar might have been toxic to physiologically immature broods and larvae, even though they could eat the nectar by themselves. Some other workers also reported toxic nature of the *Camellia sinensis* nectar [11,12,13].

It remains unclear whether tea nectar is toxic to honeybees. In this study, we took actual tea honey from the flowers to investigate whether the honeybees collected tea nectar to produce honey. To

determine whether the honey was derived from tea flowers, theanine (γ-ethylamide-L-glutamic acid), which is a specific amino acid of tea plants [14-17]. Furthermore, we investigated the concentration of catechin and caffeine, which are the main ingredients in tea plants. We also analyzed the theanine, the catechin, and the caffeine of the tea nectar to compare them with the obtained honey.

2. MATERIALS AND METHODS

2.1 Beekeeping

We used honeybees (*Apis mellifera L.*) to obtain honey from tea flowers according to Japan's beekeeping association's manual [18]. The honey was collected from September to November 2013 around tea fields. Samples were obtained from individual beehive cells with pipettes.

2.2 Plant Materials

Tea plants (*Camellia sinensis L.*) were cultured in hydroponics to obtain the nectar of tea flowers in quality and quantity [19]. The plants were cultured in a nutrient solution under controlled condition for several months until the tea flowers bloomed [20]. The nectar of the tea flowers was carefully collected with pipettes at the bottom of pistil just after blooming and kept at 4°C until it was used.

2.3 Analytical Reversed-phase High-performance Liquid Chromatography (HPLC)

We determined the theanine, catechin, and caffeine content of the honey or nectar using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, Calif.) that was equipped with a C18 column (4.6 i.d. x 150 mm, 5 μm, Tokyo Chemical Industry Co. Ltd., Tokyo, Japan) [20]. The HPLC column was maintained at 30°C in an oven. The mobile phase for the detection was 0.1 M NaH_2PO_4 buffer/acetonitrile (87:13) at a flow rate of 1.0 ml/min.

Each peak was identified by comparing the UV-Vis spectral characteristics and retention times with those from commercial standards supplied by Wako Pure Chemicals Industry, Ltd., Japan.

2.4 Statistical Analysis

Data are expressed as mean ± standard deviation. Analyses were performed using Student's *t*-test (Microsoft Excel Version 14.5.2) for comparison between honey and nectar.

3. RESULTS AND DISCUSSION

We collected actual honey from tea flowers that contained theanine, which is a very rare amino acid and ingredient of green tea that has only been found in several camellia species and one mushroom,

Xerocomus badius [21,22]. Bees normally continue flying in a 3 km area to collect flower nectar, although during this experiment, there were no plants with theanine in the vast area around the beehives. Theanine was detected from the honey collected in our experiment, and the nectar of the flowers also included theanine, indicating that it was actually derived from the tea flowers. Honeybees, especially, *Apis mellifera L.,* tend to collect the nectar of a single species of flower, such as acacia and lotus. We placed beehives in the middle of a vast expanse of a tea field, so the honeybees could collect the nectar of tea flowers. Recently, Wright et al. [23] reported that caffeine appears to have a secondary advantage that attracts honeybees and enhances their long-term memory [24], which suggests that honeybees learn to seek the nectar of flowers that possess caffeine. They also argued that 0.1 mM (0.019 mg/mL) of caffeine activated the brains of honeybees, supporting the data of Table 1 where the tea nectar included about 0.02 mg/mL of caffeine. Such definite evidence suggests that honeybees collect nectar from tea plants. Caffeine tastes bitter to mammals and is toxic and repellent to pollinators at high doses; however, tea nectar, which includes a low dose of caffeine, attracts honeybees to it. Even though Sharma et al. demonstrated the toxicity of tea nectar, they failed to experimentally show that it affected adult honeybees; it only affected the broods and larvae. In addition, their nectar was derived from pollen collected by adult honeybees [10]. The tea nectar obtained in this study did not include catechins (Table 1), but the pollen included catechins (0.5 mg/g) and caffeine (0.345 mg/g) [25], where the LD_{50} values for a rat (oral) are 2 g/kg and 192 mg/kg, respectively [26]. Catechins and caffeine in tea pollen are probably nontoxic for mammals. However, their LD_{50} values in honeybees are unclear because no data exists for them. Catechins and/or the

caffeine of the pollen may affect honeybees, especially broods, larvae, and immature bees, even though the tea nectar did not include catechins. Recent reports suggest that such agricultural chemicals as pesticides, herbicides, and fungicide pollute pollen and nectar and kill honeybees [2732]. In this study, after obtaining honey from tea flowers, we conclude that the nectar of tea flowers is attractive to honeybee, but not toxic. Our new finding, which presents significant information on the relationship of honeybees (*Apis mellifera L.*) and tea flowers, might activate tea and beekeeping industry, leading to develop the production of honey from tea nectar. Moreover, the honey from tea flower might be a novel honey with additional function.

Table 1. Concentration of main ingredients of the tea nectar and the obtained honey

	Theanine (mg/mL)	Catechins (mg/mL)	Caffeine (mg/mL)
Honey	0.0747±0.0177 (n=6)	ND	0.00657±0.0032 (n=6)
Nectar	0.0990±0.0616 (n=4)	ND	0.023±0.00675* (n=4)

*ND; Not Detected. *Significantly different (p=<0.005; nectar vs. honey)*

4. CONCLUSION

In this study, we showed honeybees produced honey from flowers of tea plants. The obtained honey and the nectar of tea flowers contained a very rare amino acid, theanine, indicating that the honey was derived from tea flowers. Furthermore, the nectar of tea flower contained the best caffeine concentration that activated the brain function of honeybees to produce the honey.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 5

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Development and Properties of Green Tea with Reduced Caffeine

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ABSTRACT

Caffeine is one of the main components of green tea and has side effects such as sleeplessness. Senior citizens, children, and pregnant woman should avoid tea despite its known beneficial effects. In this study, we developed green tea with reduced caffeine content (low caffeine tea) as a palatable tea that can be offered to everyone. To reduce the tea's caffeine content, we subjected the plucked tea leaves to a hot-water spray process, and successfully produced a low caffeine tea infusion with 30% the caffeine content. The concentrations of other main components, such as catechins and theanine, in the low caffeine tea infusion did not differ from the control. Further, the physiological function of the tea was assessed; the anti-oxidative activity was investigated using a stable free radical and the antilipase activity using an artificial substrate. There were no significant differences between the infusions of low caffeine tea and green tea in anti-oxidative and anti-lipase activities. The results showed that our developed low caffeine tea could be an attractive high quality tea with health benefits for everyone.

Keywords: Camellia sinensis; green tea; reduced caffeine; anti-oxidative activity; anti-lipase activity.

1. INTRODUCTION

Many kinds of tea are produced and consumed worldwide. Tea types, based on processing or harvested leaf development are black (fermented), green (non-fermented) and oolong (semifermented). These major tea types differ in how tea is produced and processed according to the different processes of drying and fermentation that determine its chemical composition [1]. One reason for tea's popularity is that it exhibits various physiological functions, such as improvement of brain function as well as anticancer, anti-obesity, antiallergic and antioxidative activities [2-4]. Green tea (*Camellia sinensis (L.) Kuntze*) contains catechins (8-20%), caffeine (2-4%) and theanine (1-8%) as the main components, with each component imparting a distinct taste [5]. However, caffeine exhibits some side effects, including sleeplessness. Senior citizens, children, and pregnant woman should avoid tea despite its known beneficial effects. Several kinds of decaffeinated green tea have been produced [6] and some have been commercially available. McKay and Blumberg [7] reported a per capita mean consumption of tea in the world of 120 mL/day. Approximately 76 –78% of the tea produced and consumed is black tea, 20 –22% is green tea and less than 2% is oolong tea [8]. However, these products were not popular with consumers because of their altered taste, attributable to the decrease in main ingredients during the manufacturing process, as well as the high cost. As an effective way to remove caffeine from tea leaves, Tsushida and Murai reported that fresh green tea leaves were steamed with boiling water for a

few minutes prior to primary rolling [9]. Hot-water treatment is a simple and economically efficient method to decrease the caffeine content in tea leaves without chemical toxicity. 'Benifuuki' and 'Benihomare' green teas, which exhibit anti-allergic activity, were soaked in hotwater to reduce the caffeine content, and it was demonstrated that the anti-allergic compound was maintained in the processed tea leaves [10,11]. Thus, hot-water treatment might not decrease the physiological function of tea leaves. The maximum caffeine levels are always limited to 4 mg g^{-1} for leaf teas and 10 mg g^{-1} for instant teas [12].

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In this study, a green tea with reduced caffeine content (low caffeine tea) was manufactured using a hot-water spray process. Further, the main components of the low caffeine tea infusion as well as its anti-oxidative and anti-lipase activities were determined in an effort to elucidate its health benefits.

2. MATERIALS AND METHODS

2.1 Reagent

The reagents used in this experiment were purchased from Sigma-Aldrich (St. Louis, MO, USA), and high performance liquid chromatography (HPLC) grade reagents were used for the HPLC analysis.

2.2 Low Caffeine Tea Manufacturing Process

Fresh tea leaves (*Camellia sinensis (L.) Kuntze*) were plucked and automatically sprayed with hot water (95°C, 180 seconds) to reduce the caffeine content of tea leaves [13,14]. A tea processing machine with regulated temperature and shower time and possessing high-performance efficiency and stability was used (Terada Co. Ltd. Shizuoka, Japan). After centrifugal dehydration at 3000 rpm for 1 min, the green tea was prepared through a standard manufacturing process.

2.3 Preparation of Tea Leaf Infusions

Three grams of tea leaves (green tea and low caffeine tea) were infused in 100mL of tap water for 0.5, 1, 2 and 6 hours at room temperature. The infusion was centrifuged for 5 min at 3000 rpm and the supernatant was filtered (0.45 μm filter, Millipore, Merck kGaA, Darmstadt, Germany).

2.4 Determination of Caffeine, Catechin and Theanine Contents

To determine the caffeine, catechin and theanine contents, the tea leaf infusions were applied to a reversed-phase high-performance liquid chromatography (Agilent 1100 series HPLC system, Agilent Technologies, Santa Clara, CA, USA) equipped with a reverse phase C18 column (3 μm particle size, 150 x 4.6 mm i.d.; Shiseido, Kyoto, Japan). The HPLC column was maintained at 30°C in an oven. For detection of compounds, 0.1 M NaH2PO4 buffer/acetonitrile was employed at 87:13 for caffeine and catechin, and 87:5 for theanine as the mobile phase at a flow rate of 1.0 ml/min. Individual peaks were identified by comparing their UV-Vis spectral characteristics and retention times with those of

commercial standards supplied by Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). Green tea leaves treated without hot water were used as the control.

2.5 Determination of Anti-oxidative Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich) as a stable free radical was used to determine the anti-oxidative activity of the tea infusions. A 1.5-ml aliquot of DPPH solution (0.1 mM, in 95% ethanol) was mixed with 100 μL of tea infusion. The mixture was shaken vigorously and left to stand for 20 min at room temperature. The absorbance at 517 nm of the DPPH solution was measured using a spectrophotometer (Bio Spec, Shimadzu, Kyoto, Japan). The radical scavenging activity was measured as a decrease in the absorbance of DPPH, indicating anti-oxidative activity, and was calculated using the following equation:

Scavenging activity (%) = [1- (absorbance of sample/absorbance of control)] × 100

2.6 Inhibition of Lipase Activity

Lipase inhibitory activity was determined in the infusions in order to estimate its anti-obesity effect. 4-methylumbelliferyl oleate (4-MUO) was used as a substrate to measure the pancreatic lipase inhibitory activity. The sample solution (25 μL of 3 h infusion) was added to 50 μL of 0.1 mM 4-MUO solution dissolved in a buffer consisting of 66 mM Tris–HCl (pH 7.4), 7 mM NaCl, 3 mM CaCl2, and 2 mM dimethyl sulfoxide (DMSO). These were mixed in a 96-well microplate, and then 25 μL of lipase solution (50 U/mL) was added to initiate the enzyme reaction. After incubation at 37°C for 60 min, the reaction was stopped with 50 μL of 0.1 mM citric acid, and the amount of 4-methylumbelliferone (4-MU) released by lipase was measured using a fluorometric microplate reader (Varioskan, Fisher Scientific, MA, USA) at λex 355 nm and λem 460 nm.

3. RESULTS

We manufactured a high quality low caffeine tea with health benefits for everyone. First, we determined the caffeine, catechin and theanine contents of the low caffeine tea and green tea (control) infusions at various infusion times (Fig.1). The concentrations of each component in both the low caffeine tea and green tea infusions were increased in an infusion time-dependent manner. The caffeine in the low caffeine tea was infused slowly, and the concentration was extremely low compared to the green tea, i.e., the level was decreased to less than one-third that of green tea at 6 h (Fig. 1A). The caffeine content differed significantly between all of the low caffeine and green tea samples.

The concentrations of catechin and theanine were also increased in an infusion time-dependent manner; moreover, there were no significant differences between the low caffeine tea and green tea, except at the 1 h infusion time (Fig. 1B, C). In other words, the catechin content of the 6 h infusion was very similar between the low caffeine tea and the green tea. Catechins mainly include epicatechin gallate (ECG), epigallocatechin gallate (EGCG), epicatechin (EC), catechin (C), and epigallocatechin (EGC). Among catechins, the most highly infused were EGC, followed by EC, EGCG, C, ECG in both the low caffeine tea and the green tea, and there was no difference in the rank order of catechins between the two groups (Fig. 1B). The analysis of theanine revealed the same trend as for catechins, and there were no significant differences between the low caffeine tea and the green tea at the 0.5, 3 and 6 h infusions (Fig. 1C). The results showed that the low caffeine infusion had reduced caffeine content; however, both catechin and theanine levels, as the main components, were maintained. Next, we determined the physiological function of the low caffeine tea. The 3 h infusion was used as the

sample in this experiment, in reference to the result of Fig. 1. Fig. 2 shows the anti-oxidative activity of the low caffeine tea infusion in comparison to the green tea. The stable free radical DPPH was used to determine the radical scavenging activity of the sample. Anti-oxidative activity was indicated by a decrease in DPPH absorbance. Anti-oxidative activity was increased up to 1 h and was maintained at the same level until 6 h; further, the activities of the low caffeine tea and green tea did not significantly differ.

We also determined the anti-obesity function of the low caffeine tea by assessing lipase activity (Table 1). Inhibition of lipase activity did not significantly differ between the low caffeine tea infusions and the green tea infusions.

4. DISCUSSION

As the popularity of green tea has increased recently, caffeine-free green tea options are also being marketed. Taking into account physiological function and taste, we produced a green tea with reduced caffeine content instead of a caffeine-free beverage, and succeeded in reducing the caffeine content by 70%. While caffeine has some side effects, it was reported to enhance the physiological function of catechins through synergistic effects [15-17]. In addition, the combination of L-theanine and caffeine improves brain function in humans [18,19]. It has also been reported that caffeine is necessary for the characteristic taste of tea [20]. Therefore, by reducing the caffeine of green tea instead of completely removing it, the taste and physiological function are maintained, enabling the production of a high quality green tea. The complete removal of caffeine negatively impacts the taste of tea, necessitating the addition of chemicals to improve the quality and taste, and this is a serious issue for tea as a functional food and beverage. We treated fresh tea leaves with a hot water process (95°C, 180 seconds) to produce low caffeine tea; the physiological property of caffeine allows it to be easily eluted by hot water [21]. This is a safe and stable processing method that does not necessitate contamination by chemical substances and resins. From the viewpoint of functionality and taste, it is very important that catechin and theanine levels are maintained as the major components besides caffeine. The total amount of catechins was not reduced in the 6 h infusion compared with the standard green tea beverage, although EGCG, which is the most abundant catechin in tea leaves, was not highly contained in the low caffeine tea infusion. This result is in agreement with a report that, due to their physical properties, EGC is easily dissolved in cold water, while EGCG is difficult to elute [22,21].

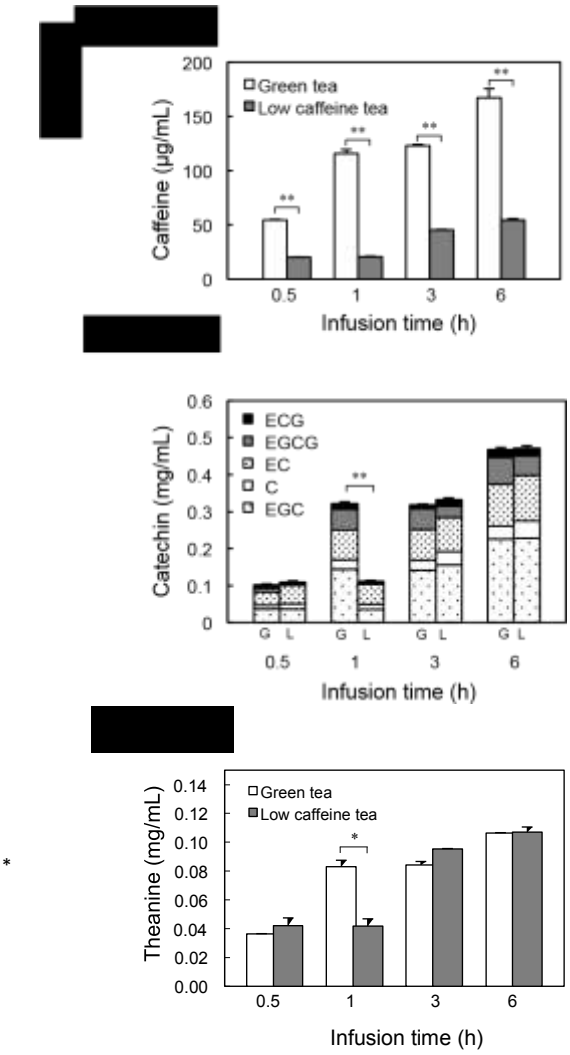

Fig. 1 Quantitative determination of the main components in low caffeine tea and green tea

*Asterisk (*) indicates statistical significance compared with green tea at the same infusion time. G, green tea; L, low caffeine tea. Each bar shows the mean ± SD (n=3, **p<0.005, *p<0.05).*

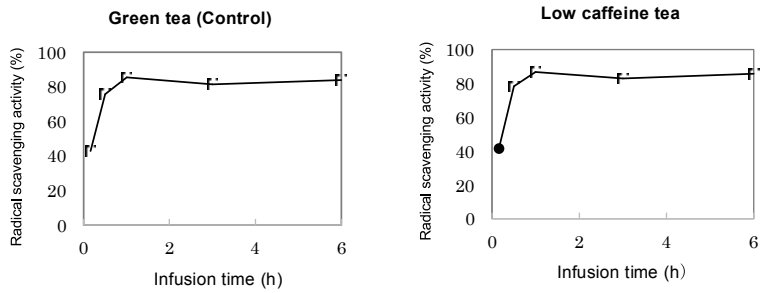

Fig. 2. Comparison of anti-oxidative activity in green tea and low caffeine tea

Table 1. Inhibitory effect of low caffeine tea and green tea on lipase activity

Infusion time (h)	Inhibition (%) of green tea (control)	Inhibition (%) of low caffeine tea	Significance*
0.5	82.06±0.285 (n=3)	83.12±0.393 (n=3)	n.s.
1	86.93±0.377 (n=3)	84.34±0.938 (n=3)	n.s.
3	87.03±0.277 (n=3)	88.19±0.198 (n=3)	n.s.
6	87.67±0.320 (n=3)	88.26±0.025 (n=3)	n.s.

*Low caffeine tea was compared to each control. n.s.: not significant.
Inhibition (%) is normalized to activity in the absence of inhibitor.

Both catechin and theanine levels were much lower in the low caffeine tea than the green tea only for the 1 h infusion, while no differences were seen for the 3 or 6 h infusions. The manufacturing process might have an effect on the elution of compounds from tea leaves, resulting in the significant difference for the 1 h infusion only. Besides, there appeared to be no differences between the low caffeine tea and the green tea in the contents of catechins and theanine. Moreover, the low caffeine tea exhibited the same level of anti-oxidative activity as the green tea at any infusion time, even with the decrease in EGCG as the most abundant anti-oxidant in tea leaves [23]. EGC, which exhibited relatively high anti-oxidative activity, is easily infused in cold water and might be responsible for the antioxidative activity instead of EGCG.

In regards to the inhibitory effect of low caffeine tea on lipase activity, despite the decrease in caffeine content, the low caffeine tea exhibited the same level of lipase activity as the green tea. The role of caffeine in this function is not clear; however, the lipase inhibitory effect might be enhanced by the synergistic interaction between catechin and theanine.

The low caffeine tea with the high-quality components produced in this study is suitable for consumption by everyone, even those avoiding caffeine, and also exhibits the functions of antioxidative and lipase inhibitory activities.

5. CONCLUSION

We reduced the caffeine content of green tea infusion by 70% to avoid the side effect of caffeine using a hot-water spray process. However, both catechin and theanine levels, as the main components, were maintained. The low caffeine tea exhibited the functions of antioxidative and lipase inhibitory activities at the same level as green tea. We developed more reasonable and high-quality low caffeine tea than ever.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Chapter 7

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Roots of Hydroponically Grown Tea (*Camellia sinensis*) Plants as a Source of a Unique Amino Acid, Theanine

Kieko Saito[4,5*] and Yoriyuki Nakamura[2]

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ABSTRACT

The beneficial effects of green tea are well documented. However, most research has reported the effects of green tea brewed solely from leaves or leaf extracts. We focused on tea roots and developed a hydroponic system to explore the effect on roots that biosynthesize one of the rarest functional amino acids, theanine. The level of theanine in tea roots was much higher than in leaves, which was analyzed using HPLC. Moreover, a higher level of theanine was detected in white rootlets than in lignified roots. Thus, tea roots cultured hydroponically in a controlled environment might be considered a natural drug containing theanine, which could lead to synergistic effects with other ingredients of the root. This novel medicinal material from the roots demonstrates a significant medical function for tea that extends beyond its leaves.

Keywords: Tea; Camellia sinensis; theanine; roots; hydroponics.

1. INTRODUCTION

Green tea (Camellia sinensis) leaves are used to make a well-known beverage with beneficial effects on health, and the functions of the main leaf components have been widely studied [1]. Theanine (γ-ethylamide-L-glutamic acid), one of the rarest amino acids and an ingredient of green tea (also found in Camellia genus, *C. assamica*, *C. taliensis*, *C. irrawadiensis, C. furfuracea*), and has not been found in any other plantand has only been found in one mushroom, *Xerocomus badius* [2,3]. Recently, the biosynthesis of theanine in two species belonging to the genus Schima (*S. wallichii* and *S. mertensiana*) was also investigated [4]. The current research has shown that theanine has psychoactive properties, because it is readily absorbed and permeates the blood-brain barrier to function in the brain [5-9], leading to reduced mental and physical stress, improved cognition, and boosting of mood in a manner that is synergistic to caffeine [10-12].
Thus, tea leaves containing theanine, which can exhibit preventive or ameliorating effects on brain dysfunction, have begun to attract attention in our aging and stressed society. Though

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theanine is synthesized from glutamic acid and ethylamine by γ-L-glutamylethylamide ligase in the roots, and accumulates in leaves through stems [13], the roots have not been extensively studied. Detailed quantitative analysis of roots cultivated in soil is complicated by the presence of a lignified taproot with very fine lateral roots that are intricately shaped. In addition, it appears that lignified taproots contain less theanine than leaves. We therefore employed a modified hydroponic culture system to examine whether the roots of tea plants could be used as a potential source of theanine. We analyzed the root theanine content and assessed the potential application of tea roots as a medicine for improving human physiological function.

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2. MATERIALS AND METHODS

2.1 Hydroponic Culture of Tea Plants

In this experiment, we used tea plant (Camellia sinensis var. Yabukita) cuttings that had been grown in soil until roots were established for approximately 1-2 months in order to conveniently obtain young plants with roots (Fig. 1B). The plants with fresh roots were moved to plastic pots and cultured in a nutrient solution with continuous aeration under controlled conditions in a Biotron incubator (Nihonika, Japan) [14]. Day/night temperatures were kept at 25/18°C, photosynthetic photon flux density (PPFD) at the plants was 40.0 μmol m-2 s-1 during the 12 h day period, and the relative humidity was about 60%. The nutrient solution was changed once a week. The roots were shaded and cultured for several months to supply materials for this experiment.

2.2 Determination of Theanine

To determine the concentration of theanine, actively growing white roots were washed with distilled water, dried in a drying oven at 50°C overnight, homogenized with three times the volume of 3% sulfosalicylic acid solution using an ultrasonic homogenizer, and then centrifuged at 12,000 g for 10 min. The concentration of the amino acids in the filtered supernatant was analyzed using an L-8500 automatic amino acid analyzer (Hitachi Co. Ltd., Tokyo, Japan).

3. RESULTS AND DISCUSSION

We employed hydroponics to allow quantitation of the content of theanine in the roots of tea. Fig. 1A shows the appearance of a representative plant cultured hydroponically for one month after transplanting from soil, and thenthe plants were grown for six months to obtain a large amount of fine whiteroots (Fig. 1B). The yield of roots of the tea plant produced depends entirely on the growth (data not shown).

Tea roots cultivated hydroponically were ideally suited for the analysis and biosynthesis of theanine; the white rootlets contained 12 g theanine per 100 g dry weight of roots, a value three times higher than that of lignified taproots cultivated hydroponically (Table 1); for comparison, the typical theanine content of leaves from plants cultivated in soil is about 1-2 g/100 g.

The various biosynthesized substances obtained by hydroponic cultivation (e.g. saccharides, flavonoids) were present at lower amounts than in plants cultivated in soil due to the effect of PPFD on photosynthesis in leaves (data not shown). In the presence of sunshine or other light, theanine is converted to other compounds, such as catechins, so high PPFD inhibits the accumulation of theanine in leaves [13,14]. In addition, only a trace amount of theanine was detected in roots cultivated in soil, indicating that roots cultivated in soil are not a suitable source of theanine. However, hydroponically cultivated tea roots could contain higher amounts of theanine. In addition, the composition and amount of amino acids contained in the roots are different from those in leaves [15], suggesting that tea root might be a medicine or remedy effective in treating a disease or part of the body.

Generally, high-quality green tea is cultured in the shade so that it will accumulate theanine, which has a pleasant flavor; shade inhibits the decomposition of theanine. However, this procedure leads to only 2% theanine in dried leaves, which is inefficient for collection of theanine and is not industrially practical. Accordingly, a chemical means of synthesis was developed as a method for industrial production of theanine in large quantities [16]. However, the yield of this organic synthesis is low, and the operation is complicated by the need for separation and purification of theanine from a mixture of unreacted materials and byproducts. In addition, recently a synthetic method of theanine using bacteria was developed, which has now become an important source of theanine [17-20]. However, the product obtained by this method is not the genuine theanine from Camellia genus. In this study, our findings suggested that hydroponic culture could be employed as an alternative method to obtain large amounts of theanine, albeit not in high purity. However, tea roots may offer a new type of drug based not only on the function of theanine but also possible synergy with other tea root components, which might offer benefits as a Chinese herbal medicine.

Consequently, hydroponics makes it possible to control environmental conditions during growth of tea plants. We have already succeeded in rooting cuttings of tea plants in a nutrient solution only. Therefore, it is likely that this approach to cultivation will facilitate the extraction of theanine from the roots.

Recent demand for theanine has increased due to its use as a food additive for enhancing flavor and as a supplement for supporting human health, especially mental health [5-9]. Unno demonstrated that theanine exhibit the stress-reducing function in humans and animals [21-24].

Indeed, we propose that the roots of tea plants, which, may attenuate brain dysfunction.

Further study using animals will likely reveal the effects of tea roots on the brain and other organs [25]. Tea roots hydroponically cultivated, which include phytochemicals might be a novel material for our health.

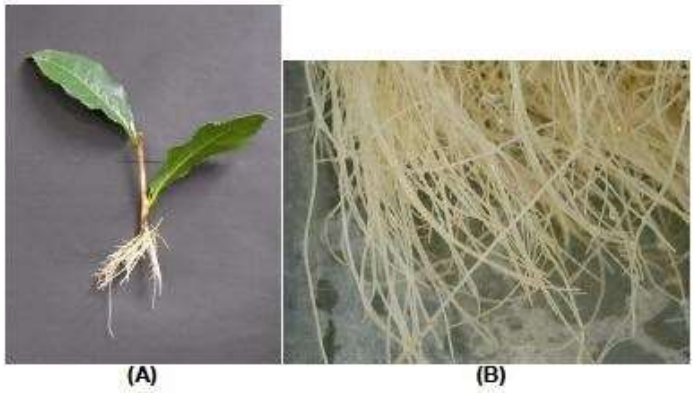

Fig. 1. Tea roots cultivated hydroponically
(A) Tea plant one month after transplanting from soil
(B) Actively growing tips of the roots after six months

Table 1. Concentration of theanine produced by different cultivation systems

Sample	Cultivation	Conc. (g/100g)[a]
Leaves	Soil	1.30 ± 0.61[b]
Leaves	Hydroponics	1.45 ± 0.26
Lignified taproots	Hydroponics	3.33 ± 1.15
Fine white roots	Hydroponics	9.8 ± 1.75

[a]*Values represent the means ± SEM (n=3).*
[b]*Max amount when plants were shaded and cultivated.*

4. CONCLUSION

We determined high amounts of theanine from tea roots, especially fine white roots, which was hydroponically cultivated under a controlled environment, and suggested tea roots.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Article

Mutation in Sodium-Glucose Cotransporter 2 Results in Down-Regulation of Amyloid Beta (A4) Precursor-Like Protein 1 in Young Age, Which May Lead to Poor Memory Retention in Old Age

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Abstract: Senescence-accelerated mouse prone 10 (SAMP10) exhibits cerebral atrophy and depression-like behavior. A line of SAMP10 with spontaneous mutation in the *Slc5a2* gene encoding the sodium-glucose cotransporter (SGLT) 2 was named SAMP10/TaSlc-*Slc5a2*^{slc} (SAMP10-ΔSglt2) and was identified as a renal diabetes model. In contrast, a line of SAMP10 with no mutation in SGLT2 (SAMP10/TaIdrSlc, SAMP10(+)) was recently established under a specific pathogen-free condition. Here, we examined the mutation effect in *SGLT2* on brain function and longevity. No differences were found in the survival curve, depression-like behavior, and age-related brain atrophy between SAMP10-ΔSglt2 and SAMP10(+). However, memory retention was lower in SAMP10-ΔSglt2 mice than SAMP10(+). Amyloid beta (A4) precursor-like protein 1 (*Aplp1*) expression was significantly lower in the hippocampus of SAMP10-ΔSGLT2 than in SAMP10(+) at 2 months of age, but was similar at 12 months of age. CaM kinase-like vesicle association (*Camkv*) expression was remarkably lower in SAMP10(+). These genes have been reported to be involved in dendrite function. Amyloid precursor proteins have been reported to involve in maintaining homeostasis of glucose and insulin. These results suggest that mutation in *SGLT2* results in down-regulation of *Aplp1* in young age, which can lead to poor memory retention in old age.

Keywords: senescence-accelerated mouse prone 10; sodium-glucose cotransporter 2; amyloid beta (A4) precursor-like protein 1; memory retention; glucosuria

1. Introduction

Senescence-accelerated mice (SAMs) were developed by a group at Kyoto University in Japan [1]. Moreover, in 1981 it was reported that inbred senescence-prone (SAMP) strains were developed as models of accelerated senescence and senescence-resistant (SAMR) strains as the normal aging control [2]. Among SAMP strains, SAMP10 has characteristics of brain atrophy, cognitive decline, and depression-like behavior [3,4]. Therefore, SAMP10 mice have been used as a model of neurodegenerative disease similar to SAMP8 [5], which has been widely used as a model for Alzheimer's disease [6]. In 2005, SAMP10/TaSlc mice maintained under specific pathogen-free (SPF) conditions in Japan SLC (Hamamatsu-city, Shizuoka, Japan) [7] were discovered to excrete glucose in urine. In 2009, a deletion mutation was found in the sodium-glucose cotransporter 2 (*SGLT2*) of SAMP10/TaSlc. Although there were heterozygous mutant mice in the SAMP10/TaSlc line until around 2008, the line has had no heterozygous mice since 2010. The mutation site was identified in 2014 and we previously reported that SAMP10/TaSlc exhibits persistent glucosuria and lowered expression of Slc5a2 [8]. Based on DNA sequencing, we identified a nucleotide deletion in the *Slc5a2* gene of SAMP10/TaSlc. As the *Slc5a2* gene encodes SGLT2, we designated this strain as SAMP10/TaSlc-*Slc5a2*^{slc} (SAMP10-ΔSglt2). On the other hand, SAMP10/TaIdr mice, which had been bred at Aichi Prefectural Welfare Development Center since 1998, did not develop glucoseuria and had no mutation in the *Slc5a2* gene. Mutations in the *Slc5a2* gene were shown to occur spontaneously in SAMP10/TaSlc. Thereafter, the line of SAMP10/TaIdr was reestablished under SPF conditions in Japan SLC as SAMP10/TaIdrSlc (SAMP10(+)).

Using SAMP10/TaIdr, which has no mutation in SGLT2, Shimada et al. have reported that neuronal DNA damage [9], loss of synapse [10], impairment of proteasome activity [11], and microglial impairment [12] are involved in age-related neurodegeneration. On the other hand, we have demonstrated additional characteristics in SAMP10/TaSlc (i.e., in SAMP10-ΔSglt2), including increased superoxide generation [13], DNA oxidative damage [14], and a decrease in antioxidative enzymes [15]. We have also reported preventive effects of antioxidative agents such as green tea catechin, β-cryptoxanthin, green soybean extract, and sesamin on neurodegeneration in SAMP10-ΔSglt2 [16–21]. Despite these available data, it has not yet been confirmed whether the mutation of SGLT2 has no effect on age-related brain atrophy, lowered learning and memory abilities, and depressive behavior.

In recent years, an increased number of diabetic patients has become a major problem [22]. Based on the specific expression of SGLT2 in the kidney, SGLT2 inhibitors have been demonstrated to be effective for the treatment of patients with type 2 diabetes. Other than the appearance of sugar in urine, there is no particular problem for patients with renal diabetes. However, SGLT2 has recently been shown to express alfa cells in the pancreas [23], as well as in choroid plexus epithelial cells and ependymal cells in the brain [24]. These results suggest that there might be new cautions regarding the use of SGLT2 inhibitors.

In this study, we compared the characteristics between SAMP10-ΔSglt2 and SAMP10(+) and examined the effect of mutation of SGLT2 on cognitive function, brain atrophy, and longevity. As a result, it was found that SAMP10-ΔSglt2 mice had lower memory retention than SAMP10(+) mice. We investigated whether or not Sglt2 mutations affected gene expression in the brain. Using SAMP10-ΔSglt2 mice, studying the relationship between age-related cognitive decline and glucose homeostasis could be a new strategy for understanding diabetes.

2. Results

2.1. Characteristics of SAMP10-ΔSglt2, SAMP10(+) and SAMR1

Although the median survival time (MST) of SAMR1 was 710.5 days, herein the time was 432 days in SAMP10(+) and 408 day in SAMP10-ΔSglt2. The lifespan of SAMP10 in both lines—SAMP10-ΔSglt2 and SAMP10(+)—was almost the same ($p = 0.5506$) and was significantly ($p < 0.0001$) shorter than that of SAMR1 (Figure 1A). SAMP10-ΔSglt2 body weight was lower than SAMP10(+) up to eight months

old. The weight of SAMP10 in both lines was significantly lower than SAMR1 after five months of age, and their weight decreased after 10 months of age (Figure 1B).

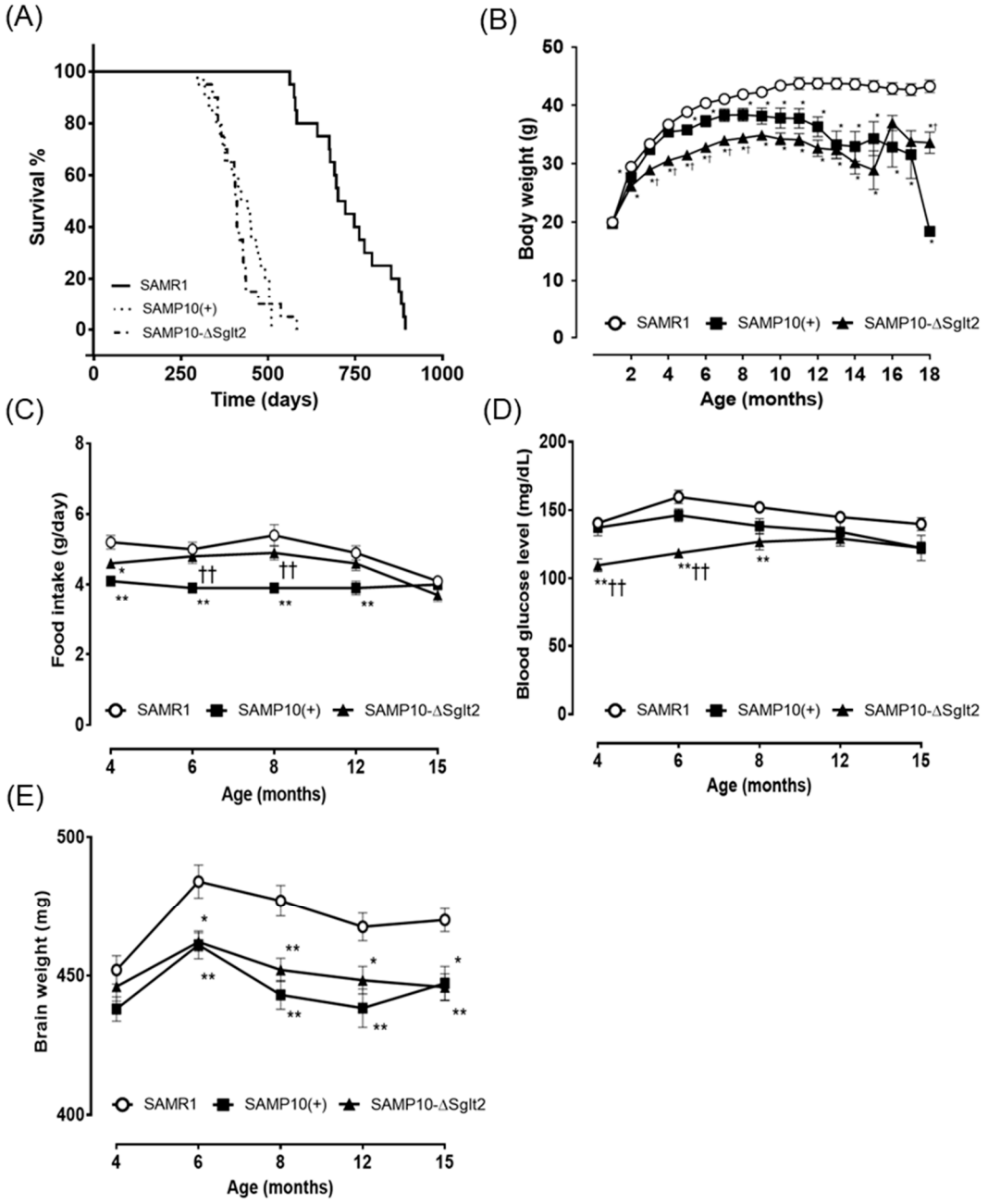

Figure 1. Survival curves (**A**) and body weight (**B**) in male senescence-accelerated mouse prone10 (SAMP10)/TaSlc mice (SAMP10-ΔSglt2), SAMP10/TaIdrSlc (SAMP10(+)) mice, and SAMR1/TaSlc (SAMR1) (n = 20 in each group). Data are expressed as mean ± standard error of the mean (SEM) in (**B**). Age-related change in food intake (**C**), blood glucose levels (**D**), and age-related change in brain weight (**E**) in male SAMP10-ΔSglt2, SAMP10(+), and SAMR1 at 4, 6, 8, 12, and 15 months of age. SAMR1 mice: n = 10; SAMP10(+) mice: n = 7–10; SAMP10-ΔSglt2 mice: n = 8–10. Data are expressed as mean ± SEM. * $p < 0.05$ and ** $p < 0.01$ versus SAMR1 mice; † $p < 0.05$ and †† $p < 0.01$ versus SAMP10(+).

Food intake in SAMP10(+) was significantly lower than in SAMP10-ΔSglt2 and SAMR1. By contrast, there was no significant difference in food intake between SAMP10-ΔSglt2 and SAMR1 except at four months of age (Figure 1C). The blood glucose level of SAMP10-ΔSglt2 was significantly lower than in SAMP10(+) and SAMR1 at four and six months of age, but no significant difference was

observed after 12 months of age (Figure 1D). The brain weight of three lines postnatally increased up to six months of age and, thereafter, slightly decreased throughout their lifespans. The brain weights in SAMP10-ΔSglt2 and SAMP10(+) were lower than in SAMR1 after six months of age and no significant difference was observed throughout the lifespan between SAMP10-ΔSglt2 and SAMP10(+) (Figure 1E). Age-related brain atrophy was quite similar between SAMP10-ΔSglt2 and SAMP10(+). Urinary glucose was consistently above 500 mg/dL in mice older than 2 months of age when measured with test strip for clinical examination. There was no effect of aging on urinary glucose [8].

2.2. Memory Retention and Depression-Like Behavior

Passive avoidance test was used to study the learning and memory of the animals. In the acquisition trial, three lines showed short response latencies and no significant difference was observed within each line. In the retention test conducted 24 h after the acquisition trial, there was no significant difference between SAMP10(+) and SAMR1. On the other hand, SAMP10-ΔSglt2 had significantly shorter retention latencies compared with SAMP10(+) and SAMR1 (Figure 2A), indicating that SAMP10-ΔSglt2 showed lower memory retention than SAMP10(+).

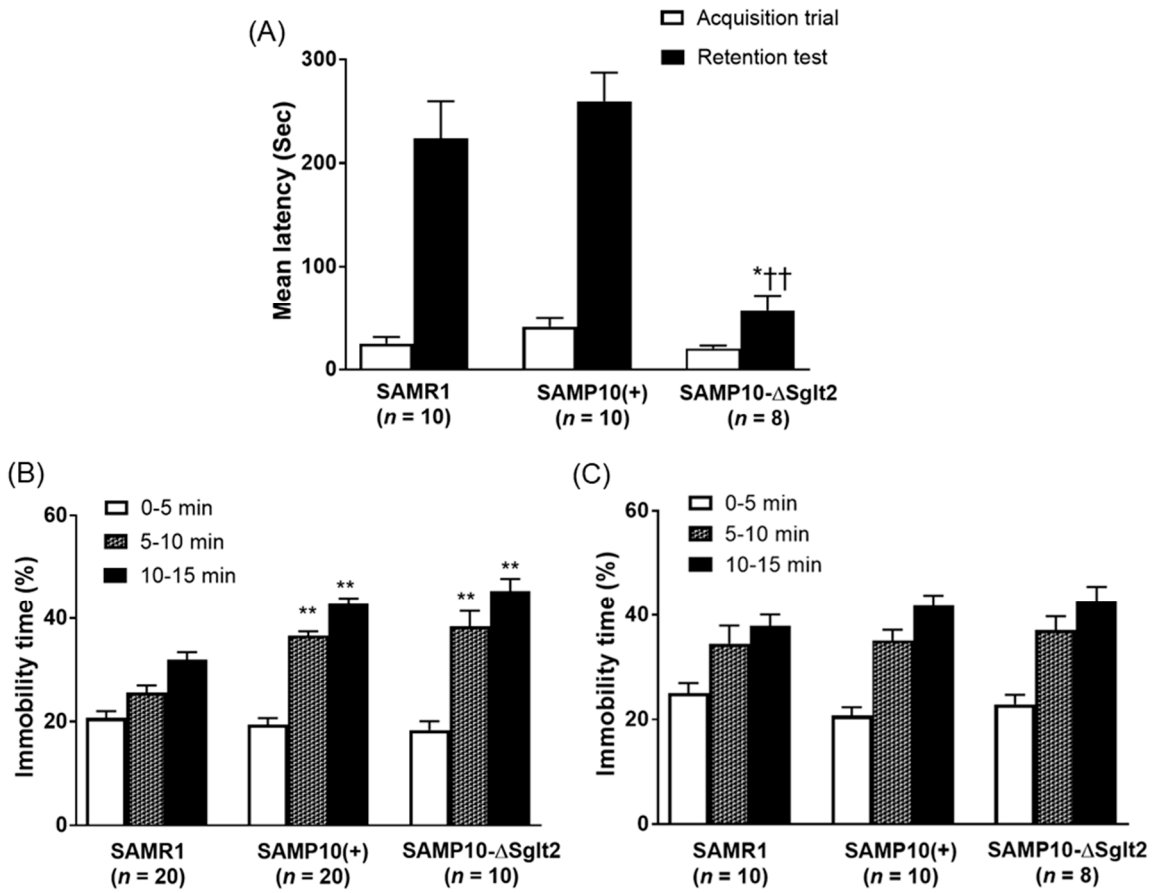

Figure 2. Passive avoidance response at 12 months of age in male SAMP10-ΔSglt2, SAMP10(+) and SAMR1 (**A**). Tail suspension at 4 months of age (**B**) and 12 months of age (**C**) in male SAMP10-ΔSglt2, SAMP10(+) and SAMR1. Data are expressed as mean ± SEM. * $p < 0.05$ and ** $p < 0.01$ versus SAMR1; †† $p < 0.01$ versus SAMP10(+).

SAMP10-ΔSglt2 and SAMP10(+) showed a marked increase in immobility as compared with SAMR1 at four months of age (Figure 2B). In contrast, no significant difference was found in immobility among each strain at 12 months of age (Figure 2C). Behavioral responses between SAMP10-ΔSglt2 and SAMP10(+) was quite similar at 4 and 12 months of age, confirming both lines exhibited significant behavioral depression even at young age of tail suspension.

2.3. Transcriptome and the Levels of Gene Expression

The hippocampus of two-month-old mice of SAMP10-ΔSglt2 and SAMP10(+) was used for analysis. Transcriptome analysis was performed at this age when no morphological changes were observed yet. DNA microarray data were obtained using high-density oligonucleotide microarrays. The top 10 genes that were significantly up- and down-regulated in SAMP10-ΔSglt2 and SAMP10(+) are listed in Table 1. The amyloid beta (A4) precursor-like protein 1 (*Aplp1*) was significantly down-regulated. Aplp1 was essential for proper synapse maintenance [25] and increased neurogenesis [26]. Cysteine rich protein 61 (Cyr61) was needed for dendritic arborization of hippocampal neurons [27] and the expression level was regulated by methylation [28]. On the other hand, CaM kinase-like vesicle-associated (Camkv) was up-regulated in SAMP10-ΔSglt2. The kinase is reported to be required for dendritic spine maintenance [29,30]. Zinc finger protein of the cerebellum 1 (Zic1) is reported to have function in maintaining neural precursor cells in an undifferentiated state [31]. Protein kinase C, delta (Prkcd) has been implicated in regulating hypothalamic glucose homeostasis [32].

Table 1. Down- and up-regulated genes in the hippocampus of SAMP10-ΔSGLT2 compared with SAMP10(+) at 2 months of age.

	Symbol	Full Name	ΔZ	*p*
Down-Regulated	Aplp1	amyloid beta (A4) precursor-like protein 1	−1.1688	6.77×10^{-48}
	Olfr716	olfactory receptor 716	−0.4277	0.0013
	Trav14-1	T cell receptor alpha variable 14-1	−0.5237	0.0031
	Cyr61	cysteine rich protein 61	−0.2115	0.0004
	Ifna12	interferon alpha 12	−0.3784	0.0004
	Sult2a2	sulfotransferase family 2A, dehydroepiandrosterone (DHEA)-preferring, member 2	−0.3743	0.0072
	Pth	parathyroid hormone	−0.2515	0.0014
	LOC100043315	uncharacterized LOC100043315	−0.2768	0.0087
	Rpl28-ps4	ribosomal protein L28, pseudogene 4	−0.2998	0.0024
	Prl2c1	Prolactin family 2, subfamily c, member 1	−0.2691	0.0082
Up-Regulated	Camkv	CaM kinase-like vesicle-associated	1.5327	6.73×10^{-47}
	Mir148b	microRNA 148b	0.4986	0.0003
	Vmn1r177	vomeronasal 1 receptor 177	0.3498	0.0078
	Zic1	zinc finger protein of the cerebellum 1	0.3551	2.67×10^{-16}
	LOC434035	immunoglobulin kappa-chain VK-1	0.3064	0.0069
	Prkcd	protein kinase C, delta	0.3021	1.93×10^{-12}
	Aspn	asporin	0.2295	0.0052
	Vmn1r8	vomeronasal 1 receptor 8	0.3163	0.0053
	Tcf7l2	transcription factor 7 like 2, T cell specific, HMG box	0.2341	0.0002
	Calb2	calbindin 2	0.2799	4.73×10^{-8}
		ΔZ = expression level (SAMP10-ΔSglt2 − SAMP10(+))		

2.4. Effect of Sglt2 Mutation on Gene Expression in Hippocampus

The expression levels of Aplp1, Cyr61, and Camkv were examined by quantitative real-time reverse transcription PCR (qRT-PCR). The degree of gene expression in the hippocampus of SAMP10-ΔSglt2

was compared with SAMP10(+) and SAMR1, and at 2 and 11–12 months of age to compare whether the changes in the younger ages persist into old age. At two months, the level of Aplp1 was significantly lower in SAMP10-ΔSglt2 than SAMP10(+) and SAMR1. However, the level of SAMP10-ΔSglt2 increased drastically to levels similar to SAMP10(+) at 11–12 months of age (Figure 3). On the other hand, the level of Camkv was significantly lower in SAMP10(+) than SAMP10-ΔSglt2 and SAMR1 at both 2 and 11–12 months. The level of Cyr61 tended to be higher in both SAMP10 than SAMR1 at both 2 and 11–12 months, but there was no difference between SAMP10-ΔSglt2 and SAMP10(+). Individual differences affected the transcriptome data of Cyr61 because the analysis was done using each two samples. Differences in gene methylation may be a cause of individual differences in the expression level of Cyr61 in SAMP10 [28].

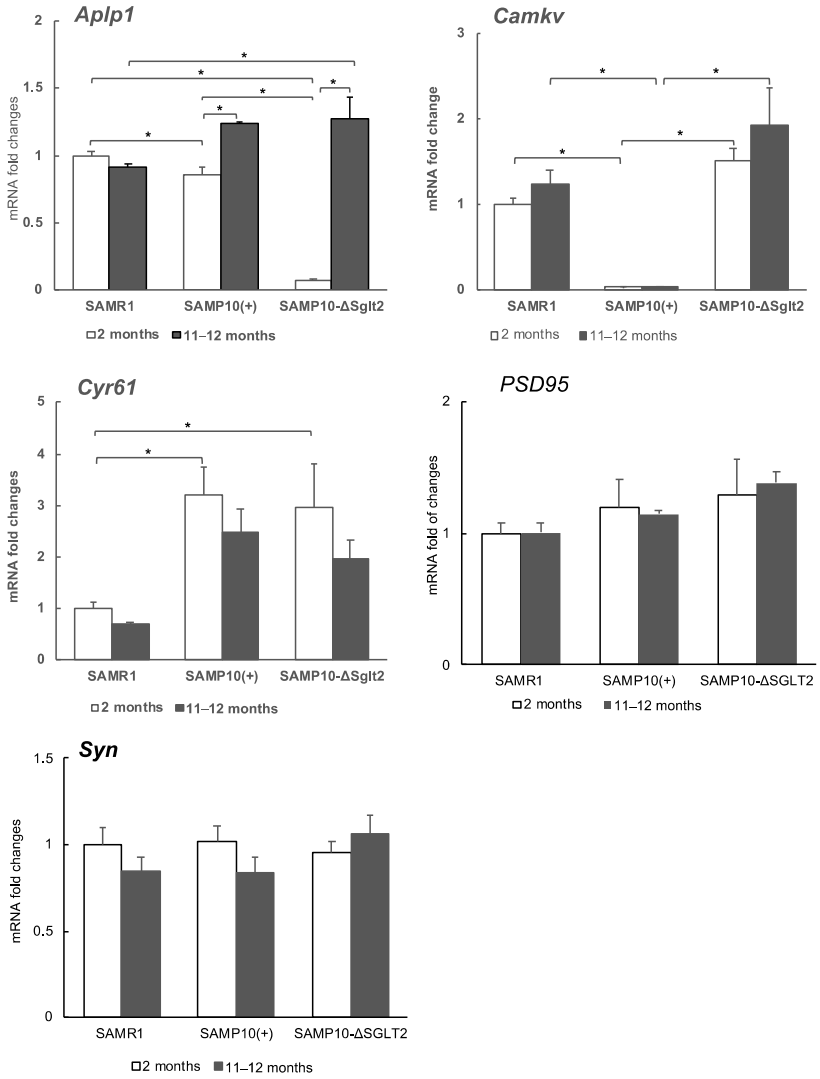

Figure 3. Expression of genes in hippocampi of in male SAMP10-ΔSglt2, SAMP10(+) and SAMR1. The levels of Aplp1, Camkv, Cyr61, PSD95, and Syn were measured at 2 and 11–12 months of age (n = 4–6, * $p < 0.05$).

Since differences in memory retention were observed in both lines of SAMP10, we compared the expression levels of synaptophysin (Syn) and postsynaptic density 95 (PSD95) as synapse-related proteins. These levels were not significantly different among SAMR1, SAMP10-ΔSGLT2, and SAMP10(+) at both 2 and 11–12 months.

3. Discussion

Lines of SAMP10, SAMP10(+) and SAMP10-ΔSglt2 were found to exhibit similar shortened lifespan, age-related brain atrophy, and depression-like behavior. However, there was a difference in memory retention between SAMP10(+) and SAMP10-ΔSglt2. Originally, SAMP10 had age-related decreased memory retention [5], but newly established SPF grade SAMP10 (SAMP10(+)) had a high memory retention ability, similar to SAMR1. The gene background or epigenetic modification of SAMP10(+) may be different from the original SAMP10 (SAMP10/TaIdr). On the other hand, SAMP10-ΔSglt2 showed reduced memory retention ability while aging. The cause of such a difference in memory retention ability was unknown. SAMP10-ΔSglt2 mice had lower body weight and blood glucose levels than SAMP10(+), despite a higher food intake than SAMP10(+). Slight but long-lasting low levels of blood glucose can have some disadvantageous effects on cognitive function. Hypoglycemia has been reported to reduce cognitive function [33,34]. Recently, SGLT2 was reported to be expressed in choroid plexus epithelium epithelial cells and ependymal cells [24], which suggests that glucose uptake from the cerebrospinal fluid to the brain may be reduced. This can be a reason for poor performance during the memory retention test.

Aplp1 and Aplp2 are members of the amyloid precursor protein (APP), which is the source of the neurotoxic amyloid beta (Aβ) peptide involved in Alzheimer's disease (AD). Although all APP family members have a role in synapse formation and synaptic plasticity, Aplp1 is reported to be especially essential for synapse maintenance [35]. In addition, as a novel function for the APP family, *APP* and *Aplp2* expression has been reported to modulate plasma insulin, glucose concentration, and body weight [25]. Aplp1 may be involved in glucose metabolism as a member of the APP family. Since Aplp1 plays an important role in synapse formation, it is easily predicted that a significant decrease in expression at an early age has an important effect. Despite the increased food intake in SAMP10-ΔSglt2, the blood glucose level was lower in SAMP10-ΔSglt2 than SAMP10(+) at a young age. The altered expression of *Aplp1* with age may be involved in changes in blood glucose levels and body weight. SAMP10-ΔSglt2 is a model of renal diabetes. It is possible to easily put mice in a hypoglycemic state by controlling the food. It also serves as a model for long-term use of SGLT2 inhibitors. In addition, SAMP10-ΔSglt2 may be a useful model for studying the role of Aplp1 in cognition and glucose homeostasis.

We have previously reported that the expression of *Aplp1* was suppressed in aged SAMP10-ΔSglt2 ingested with the green soybean extract. At that time, the decline of cognitive function and Aβ accumulation were suppressed [19]. High expression of *Aplp1* increased Aβ accumulation. However, similar levels of *Aplp1* in both lines of aged SAMP10 (Figure 3) suggested that low level of *Aplp1* at young age was more important for aging-related cognitive decline than Aβ accumulation. It is currently unknown why *Aplp1* expression changes significantly with age. Some abnormality may be occurring in the metabolism or gene expression control of APPs, including *Aplp1*.

Camkv is reported to be an important synaptic protein in maintaining dendritic spines because the knockdown in hippocampal CA1 impairs synaptic transmission and plasticity [29]. Low expression of *Camkv* in SAMP10(+) may be a problem because a precise regulation of Camkv for activity-dependent synthesis and post-translational phosphorylation is critical for dendritic spine maintenance. The level in SAMP10-ΔSGLT2 tended to be higher than SAMR1. The mutation of SGLT2 may cause abnormal regulation of *Camkv*, resulting in high expression and abnormal maintenance of dendrite. The *Camkv* gene is reported to be one of the more promising loci for post-traumatic stress disorder [36]. SAMP10(+) may be a useful PTSD model showing decreased *Camkv* expression.

Camkv phosphorylated by cyclin-dependent kinase 5 causes activation of RhoA, resulting in a loss of dendrite spines [30]. Tight regulation of RhoA activity is crucial for maintaining dendritic spines. The difference between RhoA activity and the expression of *Camkv* and *SGLT2* mutations in SAMP10 strain still need to be investigated. The reason why *Cyr61* increased in both SAMP10 lines is also a potential topic for future study. Pre- and post-synaptic markers, *Syn* and *PSD95*, did not show

any difference in mRNA expression between the two lines of SAMP10, but their protein levels need to be investigated.

A detailed research on morphological changes of dendrite in SAMP10 has already been conducted by Shimada et al. [37]. Since SAMP10-ΔSglt2 and SAMP10(+) showed similar brain atrophy (Figure 1E), both lines are expected to show similar morphological changes. However, detailed studies of dendritic morphological changes will be conducted in the near future.

In conclusion, we found that the mutation of *SGLT2* results in down-regulation of *Aplp1* during young age, which can lead to poor memory retention in old age. On the other hand, *Camkv* was up-regulated. In the future, it will be necessary to clarify the significance of SGLT2 expression in the choroid layer in brain and in pancreatic alpha cells, as well as to carefully observe the effect of SGLT2 inhibitors on brain function.

4. Materials and Methods

4.1. Animals

Male SAMP10/TaSlc (SAMP10-ΔSglt2), SAMP10/TaIdrSlc (SAMP10(+)), and SAMR1/TaSlc (SAMR1) obtained from Japan SLC (Shizuoka, Japan) were bred under SPF conditions in a temperature- and humidity-controlled room with a 12/12-h light/dark schedule (24 ± 1 °C; 45–65% humidity; light period, 08.00–20.00 h). A normal diet (MR-A1; Nosan Corporation, Kanagawa, Japan) and tap water were available ad libitum. Male SAMR1 mice as control mice have normal longevity and a similar genetic background to SAMP10 mice. At the start of the longitudinal study, four-week-old male mice were selected and housed alone per cage, preventing fights. All mice were inspected at least once a day. All study procedures were reviewed and approved by Japan SLC Animal Care and Use Committee and University of Shizuoka Laboratory Animal Care Advisory Committee (approval No. 195241, 9 January 2020). They were in accordance with the guidelines of the US National Institutes of Health for the care and use of laboratory animals.

4.2. Measurements of Physiological Parameters, Glucose Levels, and Brain Weight

Mice were weighed, food intake was calculated, and blood glucose levels were measured using a blood glucose meter and test tips (Bayer Yakuhin, Ltd., Osaka, Japan). Measurements of blood glucose level were done from 2 pm to 4 pm at a fixed time. After decapitation, the brain was weighed at 4, 6, 8, 12, and 15 months of age.

4.3. Measurements of Behavioral Task

Learning and memory abilities were assessed by acquisition trials and retention tests, respectively, using a passive avoidance system. The passive avoidance response procedure was described in a previous paper [38], wherein a two-compartment step-through passive avoidance apparatus SGS-002 (Muromachi Kikai Co., Ltd., Tokyo, Japan) was used. A 0.5 mA foot shock was applied to the floor grid for 3 s.

Depression-like behavior was assessed as immobility time by the tail suspension test. Each mouse was suspended by the tail for 15 min using a tail suspension apparatus BS-TS2 (Brain Science. Idea. Co., Ltd., Osaka, Japan) and the amount of movement was automatically recorded. Tasks at different ages were done using different groups of mice.

4.4. Measurement of DNA Microarray and Principal Component Analyses

Each mouse was used at two months of age. An RNeasy Mini Kit (74104, Qiagen, Valencia, CA, USA) was used for extraction of total RNA from the hippocampus. To synthesize biotinylated cRNA, total RNA was processed using one-cycle target labeling and control reagents (Affymetrix, Santa Clara, CA, USA), and hybridized to a Total RNA Mouse Gene 1.0 ST Array (Affymetrix) with three biological

repeats per group. Raw data that were parametrically normalized [39] were statistically tested by two-way ANOVA [40] at $p < 0.001$.

4.5. Quantitative Real-Time Reverse Transcription PCR (qRT-PCR)

The hippocampus of mice aged 2 and 11–12 months was used for this analysis. qRT-PCR analysis was performed using the PowerUp™ SYBR™ Green Master Mix (A25742, Applied Biosystems Japan Ltd., Tokyo, Japan) and automated sequence detection systems (StepOne, Applied Biosystems Japan Ltd., Tokyo, Japan). Relative gene expression was measured by previously validated primers for *Aplp1* [41], *Camkv* [29], *Cyr61* [42], *Syn* and *PSD95* [43] genes. cDNA derived from transcripts encoding β-actin was used as the internal control.

4.6. Statistical Analyses

Data are expressed as means ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism version 7.0 (GraphPad Software Inc, San Diego, CA, USA). Survival data were analyzed by the log-rank (Mantel–Cox) test and Kaplan–Meier survival curves. The passive avoidance response was compared by one-way analysis of variance (ANOVA) followed by the Kruskal–Wallis test. Other parameters ware analyzed by ANOVA and followed by the Tukey–Kramer method, where $p < 0.05$ was considered statistically significant.

5. Conclusions

We found that mutations in *SGLT2* cause down-regulation of *Aplp1* during young age but not old age for SAMP10-ΔSglt2 mice. Since *Aplp1* is essential for synaptic maintenance, the reduced expression may lead to reduced memory retention in old age. On the other hand, *Camkv* was low in SAMP10(+) and slightly higher in SAMP10-ΔSglt2 than SAMR1. Since precise regulation of Camkv is important for maintaining dendritic spines, altered expression of *Camkv* may be associated with depressive behavior. Summarized data is shown in Table 2.

Table 2. Characterization of SAMP10-ΔSglt2 and SAMP10(+) compared to SAMR1.

Mouse Line	SAMR1	SAMP10-ΔSglt2	SAMP10(+)
Lifespan	Long	Short	Short
Cerebral atrophy	−	+	+
Depression	−	+	+
Mutation in SGLT2	−	+	−
Glucose in urine	−	+	−
Glucose in blood	Normal	Low in young	Normal
Memory retention	High	Low in aged	High
Aplp1 in the hippocampus	Normal	Low in young	Normal in young
Camkv in the hippocampus	Normal	Slightly high	Low

Author Contributions: Conceptualization, K.U. and T.H.; methodology, K.U. and Y.T.; software, Y.T.; validation, T.K. (Tomokazu Konishi); formal analysis, Y.T. and K.T.; investigation, M.S., A.M., T.K. (Takumi Kurotaki) and M.P.; resources, Y.T.; writing—original draft preparation, K.U. and Y.T.; writing—review and editing, A.S. and S.H.-I.; supervision, Y.N.; funding acquisition, T.H. and S.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

SAMP10	Senescence-accelerated mouse prone 10
SAMP10-ΔSglt2	SAMP10/TaSlc-*Slc5a2*^{slc}, SAMP10 with mutation in *SGLT2*
SAMP10(+)	SAMP10/TaIdrSlc, SAMP10 without mutation
SAMR1	SAMR1/TaSlc, senescence-resistant strain
SGLT2	sodium-glucose cotransporter 2
Aplp1	amyloid beta (A4) precursor-like protein 1
Camkv	CaM kinase-like vesicle-associated
Cyr61	Cysteine rich protein 61
PSD95	Postsynaptic density 95
Syn	Synaptophysin

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Improvement of Learning and Memory in Senescence-Accelerated Mice by S-Allylcysteine in Mature Garlic Extract

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Abstract: S-allylcysteine (SAC), a major thioallyl compound contained in mature garlic extract (MGE), is known to be a neuroactive compound. This study was designed to investigate the effects of SAC on primary cultured hippocampal neurons and cognitively impaired senescence-accelerated mice prone 10 (SAMP10). Treatment of these neurons with MGE or SAC significantly increased the total neurite length and number of dendrites. SAMP10 mice fed MGE or SAC showed a significant improvement in memory dysfunction in pharmacological behavioral analyses. The decrease of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, *N*-methyl-D-aspartate (NMDA) receptor, and phosphorylated α-calcium/calmodulin-dependent protein kinase II (CaMKII) in the hippocampal tissue of SAMP10 mice fed MGE or SAC was significantly suppressed, especially in the MGE-fed group. These findings suggest that SAC positively contributes to learning and memory formation, having a beneficial effect on brain function. In addition, multiple components (aside from SAC) contained in MGE could be useful for improving cognitive function by acting as neurotrophic factors.

Keywords: S-allylcysteine (SAC); mature garlic extract (MGE); hippocampal neuron; senescence-accelerated mice; memory; cognitive function

1. Introduction

In recent years, the consumption of certain foods by aged individuals for the purpose of promoting and maintaining brain function has attracted much attention, particularly in terms of improving the accuracy of memory and judgment. Above all, garlic (*Allium sativum* L. *Liliaceae*) has been widely used as a food and medicine for thousands of years [1,2]. Garlic contains S-allylcysteine (SAC), which is considered to be useful for memory improvement. Mature garlic extract (MGE) made from garlic that has been aged at a low temperature contains more SAC than aged-garlic extract (AGE) made from common black garlic. In addition, MGE contains cycloalliin, which is useful for increasing fibrinolytic activity and preventing hyperlipidemia [3,4], and γ-glutamyl-S-allylcysteine, which contributes to hypotensive effects through angiotensin converting enzyme inhibitory and vasodilating activities [5,6].

SAC, which is an organosulfur compound in garlic, has a high bioavailability of 98.2% (rat, 50 mg/kg, p.o.) [7]. Therefore, by reaching the systemic circulation and passing through the blood–brain barrier [8], SAC can have various effects on the brain. SAC has been confirmed to cause a significant increase in the formation of branching per axon as well as a survival-promoting effect on

primary cultured hippocampal neurons [9,10]. Since such neurotrophic factors improve learning and reduce memory impairment [11], SAC is considered to be a beneficial component for brain function. Also, SAC has been shown to have a selectively neuroprotective effect by reducing cell death caused by endoplasmic reticulum stress induced by amyloid β (Aβ) and tunicamycin [12–14]. In addition, it has been found that SAC inhibits Aβ fibrillation, destabilizes preformed Aβ fibrils [15], and reduces hyperphosphorylation of the tau protein, which induces neurofibrillary tangles and Aβ deposition [16]. Therefore, SAC is expected to be applied for the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.

Senescence-accelerated mice (SAM) have been established as a model for studying human aging and age-related disorders. There are several senescence-prone inbred strains (SAMP) and senescence-resistant inbred strains (SAMR). SAMP mice have a short lifespan and exhibit many characteristic age-dependent pathologies at an early age [17,18]. Among these strains, the SAMP10 mouse strain was established by Shimada and colleagues [19,20]. The age-related morphological changes seen in the SAMP10 brain, such as the retraction of dendritic arbors, a decrease in the density of the dendritic spine [21], a loss of synapses [22], and impairment in learning and memory [23–26], are more consistent with observations on the aging human brain than those on the brain of mice with Alzheimer's disease. Several behavioral tests of brain function using SAMP10 and SAMR1 mice have been widely used to study the effects of food materials on the prevention of brain senescence, and materials such as garlic [27,28] and green tea [29–31] have been found to improve learning memory impairment and suppress brain atrophy.

Regarding the physiological function of SAC and AGE components, the research focus has been on the antioxidative effects while the neurotrophic factor is poorly understood. Although there have been many reports that AGE and SAC have useful effects on neuronal morphological changes and learning behavior [9,10,27,28], the detailed mechanism of how SAC affects memory-related receptors, such as the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor and the *N*-methyl-D-aspartate (NMDA) receptor, in the hippocampus is not clear. In addition, few reports have examined the effects of long-term intake of these substances on learning and memory. In this study, we examined the efficacy of MGE, but not AGE, and SAC for increasing the total neurite length and the number of dendrites in primary cultured embryonic mouse hippocampal neurons. Next, we analyzed learning and memory-formation-related behavioral experiments and protein expression levels in the hippocampal tissue of SAMP10 mice that were continuously fed a diet containing MGE or SAC for a period of 10 months (from ages 2 to 11 months). Our findings demonstrate strong evidence that MGE and SAC possess potential neurotrophic properties and also preserve learning and memory functions to maintain young brain function.

2. Materials and Methods

2.1. Animals and Preparation of Matured Garlic Extract

C57BL/6J mice were obtained from Charles River Laboratory Japan (Yokohama, Japan).

Male SAMP10/TaSlc (SAMP10) and male SAMR1/TaSlc (SAMR1) mice were obtained from Japan SLC (Shizuoka, Japan) at 4 weeks of age. Mice were housed under a standard 12 h light/dark cycle (light phase 9:00–21:00) at a constant temperature of 22 ± 1 °C with food and water provided ad libitum throughout the experiments.

SAMP10 and SAMR1 mice were fed a diet (CE-2; Clea, Tokyo, Japan) containing MGE or SAC (Tokyo Kasei, Tokyo, Japan) starting at 2 months of age. MGE was manufactured by extracting the water-soluble fraction of garlic supplied by Takko Kawamura Agri Service Inc. (Aomori, Japan).

In this study, a diet with a low concentration of MGE, i.e., 0.20% of the diet (*w*/*w*) (L-MGE) and one with a high concentration of MGE, i.e., 1.0% of the diet (*w*/*w*) (H-MGE) were prepared. In addition, a diet containing the same amount of SAC as in H-MGE was prepared. In a previous report, a diet containing 2% AGE or 0.002–0.004% SAC was used [27,32]. Based on that, we set

the amount of SAC to 0.002% (=20 mg/kg diet) and the amount of H-MGE to 1.0%. L-MGE was set to 0.2% to investigate the concentration-dependent effect. Since mice consume 150 g of diet/kg (body weight)/day [33], L- and H-MGE were consumed at 0.30 g and 1.5 g MGE/kg (body weight)/day, respectively. As a result of quantifying the amount of SAC by high performance liquid chromatography (HPLC) (Shimadzu, Tokyo, Japan), 1.0 g of MGE contained 2.0 mg of SAC. Therefore, the L-MGE- and H-MGE-fed groups consumed 0.60 and 3.0 mg of SAC per day, respectively.

2.2. Cell Culture

Primary cultured hippocampal neurons were prepared from C57BL/6J mice on gestational days 15–16, as described previously with minor modifications [34]. The embryonic hippocampus was digested with 0.25% trypsin and 0.1 mg/mL DNase for 10 min at 37 °C and then gently pipetted to mechanically dissociate the cells. Neurons were seeded on poly-D-lysine-coated cell disks (Sumitomo Bakelite, Tokyo, Japan) in neural basal medium containing B-27 and GlutaMax supplement (Thermo Fisher Scientific, Waltham, MA, USA), and the cultures were started on day 0 in vitro (DIV 0). Culture medium was exchanged for fresh medium every 3–4 days. MGE or SAC was added to the culture medium along with 1 μM cytosine β-D-arabinofuranoside on DIV 2. At DIV 4 (48 h) and 5 (72 h), the total neurite length and number of dendrites treated with MGE or SAC were measured by immunofluorescence staining.

2.3. Immunofluorescence and Image Quantification

Hippocampal neurons on poly-D-lysine-coated cell disks were fixed with formaldehyde for 10 min and then incubated with blocking buffer (PBS with 10% goat serum and 1% BSA) for 1 h at room temperature on DIV 4 and 5. Anti-MAP2 antibody (dendrite marker, Abcam, Cambridge, UK) was added to the disks at 1:1000 dilution in Can Get Signal Solution B (Toyobo, Osaka, Japan), which were then incubated for 1 h at room temperature. After 2 washings with TBS-T solution, each for 10 min, goat-rabbit IgG antibody coupled to Alexa fluor 568 (Thermo Fisher Scientific, Waltham, MA, USA) at 1:200 dilution in Can Get Signal Solution B was added, and the cells were incubated for 30 min at room temperature under shaded conditions. After 3 washings with TBS-T solution for 10 min each time, nuclear DNA was stained with Hoechst 33342 (Dojindo, Kumamoto, Japan) at 1:1000 dilution in distilled water. After another 3 washings for 10 min each time, the cell disks were mounted on glass slides. Images were acquired with a fluorescence microscope (Olympus, Tokyo, Japan). Images of MAP2-positive cells obtained by immunofluorescence staining were transformed using an IN Cell Translator (GE Healthcare, Buckinghamshire, UK), and quantification of total neurite length and number of dendrites per neuron was performed with an IN Cell Analyzer Workstation (GE Healthcare, Buckinghamshire, UK).

2.4. Behavioral Experiments

SAMP10 mice were randomly divided into four groups (n = 18 mice per group). The mice were fed a CE-2 diet containing MGE or SAC for a period of 10 months (from ages 2 to 11 months). One of the SAMP10 groups and the SAMR1 group (n = 12) were fed the CE-2 diet without MGE or SAC and acted as control mice for the behavioral experiments. Six months after the start of breeding, additional 4-week-old SAMP10 mice were purchased as Young-SAMP10 mice to allow us to observe age-related declines in brain function. Learning and memory ability were measured by performing the Y-maze, step-through passive avoidance, and novel object recognition tests on animals at 11–12 (Old-SAMP10 and Old-SAMR1) or 5–6 (Young-SAMP10) months of age. The mice were sacrificed at the age of 12 (Old-SAMP10 and Old-SAMR1) or 6 (Young-SAMP10) months, and tests were carried out to obtain the hippocampal tissue. The samples were immediately frozen at −80 °C. All protocols for animal procedures were approved by the University of Shizuoka Laboratory Animal Care Advisory Committee (approval No. 166197) in accordance with the Internal Regulations on Animal Experiments

at the University of Shizuoka, which are based on the Law for the Humane Treatment and Management of Animals (Law No. 105, 1 October 1973).

2.4.1. Working Memory

Spontaneous alternations and exploratory behavior in the Y-maze were used as hippocampal-based tasks to assess working memory. Immediate working memory was evaluated by recording spontaneous alternations during a single session in the Y-maze [35,36]. The Y-maze apparatus was made of black plastic with three arms (40 cm × 15 cm × 35 cm), each extending at 120° from a central platform. Each mouse was placed on the end of one arm and allowed to move freely through the maze during an 8-min session, and the number of arm entries was counted. Each series of arm entries was visually recorded, and an arm entry was defined as when the hind paws of the mouse were completely within the arm. The number of alternations was defined as the number of combinations (i.e., abc, bca, triplets) of entrances into the three different arms in succession and was considered to reflect the working memory capacity. The percentage of spontaneous alternations (%) was calculated from the following formula and used as an index of short-term memory:

$$\text{Alternation (\%)} = (\text{number of alternations})/(\text{total arm entries} - 2) \times 100.$$

2.4.2. Memory Acquisition Test and Retention Test

A step-through passive avoidance task was carried out according to the protocol method reported earlier [29]. This test was based on the fact that mice prefer dark places. The apparatus was connected to a light chamber and a dark chamber with a door between them. The mice in the test were initially placed in the light chamber. When a mouse entered the dark chamber, the door was closed, and an electric foot-shock was delivered at 0.05 mA for 1 s (Muromachi Kikai Co. Ltd., Tokyo, Japan). The mouse was then gently removed and replaced in the bright room. One minute later, the door was opened, and the time taken for the mouse to enter the dark chamber was measured. The trial was terminated when the mouse remained in the light chamber for 300 s without entering the dark room, and this was repeated five times until the mouse had satisfied the acquisition criterion. In such multiple-trial passive avoidance tests, the number of trials required for the mouse to satisfy the acquisition criterion is usually regarded as an index of memory acquisition. The total time spent in the light chamber during each trial was deducted from 300 s and was considered the time needed for learning. The time taken for each trial was totaled—the shorter the learning time, the higher the learning ability.

One month later, the mice were assessed again to see whether they remained in the light chamber. The number of mice remaining in the light chamber for 300 s was used as the acquisition criterion for long-term memory.

2.4.3. Novel Object Recognition Test

This task was performed on days 1–5 according to a previously described protocol with some modifications [37,38]. The novel object recognition test was based on the characteristic of a preference for a novel object. The task was divided into three different sessions (habituation, training, and retention). In the habituation session, each mouse was individually placed in an open box (30 cm × 30 cm × 35 cm height) without objects for three consecutive days and allowed to explore for 10 min each day. Secondly, a training session was performed on the day 4. Two novel objects (X and Y) were placed in the open box, and the mice were allowed to explore the objects freely for 10 min. The total time spent exploring an object, which was calculated as the total time that a mouse directed their nose toward an object at a distance of <1 cm and/or touched the object with their nose, was assessed manually for 10 min using two stopwatches. Thirdly, a retention session was performed on day 5. The mice were allowed to explore an open field for 10 min in the presence of two objects of different shapes and colors, i.e., the familiar object X and a novel object, Z.

The time spent exploring each object was recorded as before. Then, the exploration time for each object in the training (X or Y) and retention (X or Z) sessions was evaluated against the total exploring time. Cognitive function was evaluated by exploratory preferences obtained from the time ratio for each object, e.g., X or Y/(X + Y) × 100 (%) in the training session, and X or Z/(X + Z) × 100 (%) in the retention session. An exploratory preference index of 50% corresponds to chance, and a significantly higher exploratory preference index reflects good recognition memory.

2.4.4. Western Immunoblotting

At the end of the behavioral experiments, the hippocampus was removed, placed on an ice-cold plate, immediately frozen, and stored −80 °C. Hippocampus protein extracts were obtained by homogenization of the hippocampal tissue in Passive Lysis Buffer (Promega, Madison, WI, USA) supplemented with proteinase and phosphatase inhibitors. The homogenate was centrifuged at 13,000× *g* for 20 min to obtain a supernatant, which was then subjected to protein estimation (Bradford assay), and a defined volume of the supernatant containing a fixed amount of protein was analyzed by Western immunoblotting.

For preparation of the tissue lysates for Western blotting, a Laemmli buffer (Bio-Rad Laboratories, Hercules, CA, USA) was added. Prior to electrophoresis, samples were denatured at 95 °C for 6 min. An equal amount of total protein (20 µg) from tissue homogenate was loaded onto a 7.5% mini-gel (Mini-PROTEAN TGX Precast Gel, Bio-Rad Laboratories, Hercules, CA, USA) along with a molecular weight marker (Bio-Rad Laboratories, Hercules, CA, USA). Protein bands on the separating gel were transferred to a polyvinylidene difluoride membrane (Trans-Blot Turbo Mini PVDF, 0.2 µm, Bio-Rad Laboratories, Hercules, CA, USA) in accordance with the manufacturer's instructions. The membranes were then blocked for 1 h in blocking buffer (PVDF Blocking Reagent for Can Get Signal, Toyobo, Osaka, Japan) at room temperature and incubated in solution 1 (Can Get Signal Solution, Immunoreaction Enhancer Solution for primary antibody, Toyobo, Osaka, Japan) and the primary antibodies AMPA receptor GluR1 subunit (anti-GluR1; molecular weight (MW), 102 kDa; 1:1000 dilution; Abcam, Cambridge, UK), anti-GluR1 phosphorylated at serine 831 (anti-pGluR1; MW, 106 kDa; 1:1000 dilution; Abcam, Cambridge, UK), NMDA receptor 2B subunit (anti-NR2B; MW, 166 kDa; 1:1000 dilution; Abcam, Cambridge, UK), anti-NR2B phosphorylated at tyrosine 1472 (anti-pNR2B; MW, 180 kDa; 1:1000 dilution; Merck, Darmstadt, Germany), and anti-α-calcium/calmodulin-dependent protein kinase II phosphorylated at threonine 286 (anti-pCaMKII; MW, 50 kDa; 1:1000 dilution; Cell Signaling Technology, Danvers, MA, USA) overnight at 4 °C. Membranes were washed with Tris-buffered saline with 0.1% Tween-20 (TBS-T) and then incubated in solution 2 (Can Get Signal Solution, Immunoreaction Enhancer Solution for secondary antibody, Toyobo, Osaka, Japan) and the secondary antibody (HRP-Linked Anti-IgG, 1:10,000 dilution, GE Healthcare, Buckinghamshire, UK) for 1 h at room temperature. After the membrane had been washed with TBS-T, the relative amounts of bound antibodies were detected with a chemiluminescent substrate (ECL, GE Healthcare, Buckinghamshire, UK). The specific bands were scanned and quantified with ChemiDoc XRS+ and ImageLab software (Bio-Rad Laboratories, Hercules, CA, USA).

2.5. Flowchart

The summarized method is provided in the following flowchart (Figure 1).

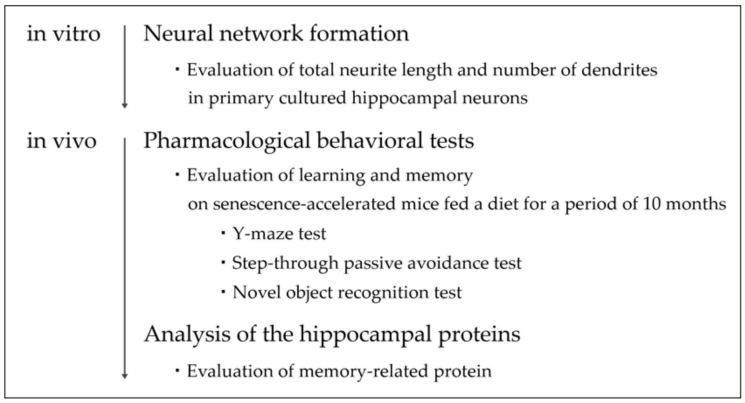

Figure 1. Flowchart of the study.

2.6. Statistical Analyses

The results were analyzed using JMP 8 (SAS Institute Inc., Cary, NC, USA). The data were collected from at least three independent experiments and are expressed as the mean ± standard error of the mean (SEM). For all results, assuming a Gaussian distribution, data were analyzed by one-way analysis of variance (ANOVA). To perform multiple comparisons, Dunnett's test or the Tukey–Kramer test were used for post-hoc analysis after ANOVA. $p < 0.05$ was considered statistically significant. The levels of statistical significance are indicated as follows: *; $p < 0.05$, **; $p < 0.01$.

3. Results

3.1. Effects of MGE on the Total Neurite Length and Number of Dendrites in Primary Cultured Hippocampal Neurons

We first examined whether MGE and SAC induce increases in the total neurite length and number of dendrites in hippocampal neurons. The total neurite length and number of dendrites per neuron at 0 h (DIV2) were 212.5 ± 6.08 μm (Table 1) and 3.47 ± 0.127 (Table 2), respectively. A typical image of a MAP2-positive neuron treated with 50 μg of MGE is shown in Figure 2. Primary cultured hippocampal neurons treated with MGE at 48 and 72 h showed significant increases in the total neurite length and number of dendrites, and the neurons increased in a concentration-dependent manner at 48 h. In the case of treating with SAC, the neurite length and number of dendrites increased significantly; however, no concentration dependency was found. The concentration of SAC that showed the maximum effect was 10 ng/mL at 72 h. These results suggest that SAC and the multiple other components contained in MGE have the ability to synergistically enhance the effect of early neurite outgrowth.

Table 1. Effects of increasing the total neurite length with mature garlic extract (MGE) and S-allylcysteine (SAC) on the morphology of primary cultured hippocampal neurons.

	Concentration	48 h	72 h
		Total Neurite Length, μm	Total Neurite Length, μm
MGE (μg/mL)	0	278.9 ± 7.24	362.9 ± 8.60
	5	306.7 ± 9.04 *	419.3 ± 9.53 **
	50	323.5 ± 8.77 **	430.7 ± 9.40 **
	500	392.8 ± 8.18 **	435.2 ± 9.30 **
SAC (ng/mL)	0	259.1 ± 6.85	342.9 ± 8.15
	10	300.3 ± 8.04 **	445.8 ± 12.9 **
	100	294.8 ± 7.08 **	433.5 ± 11.3 **
	1000	303.8 ± 7.56 **	398.1 ± 7.56 **

0 h: 212.5 ± 6.08 μm. The data are presented as the mean ± SEM (n = 102–144 from 3–5 independent experiments). *; $p < 0.05$, **; $p < 0.01$ compared with the concentration at 0 h at each time point (one-way ANOVA and Dunnett's post-hoc test).

Table 2. Effects of increasing the number of dendrites with MGE and SAC on the morphology of primary cultured hippocampal neurons.

	Concentration	48 h	72 h
		Number of Dendrites	Number of Dendrites
MGE (μg/mL)	0	5.29 ± 0.137	5.29 ± 0.186
	5	5.86 ± 0.176 *	7.16 ± 0.217 **
	50	6.06 ± 0.166 **	6.88 ± 0.196 **
	500	6.81 ± 0.207 **	7.92 ± 0.226 **
SAC (ng/mL)	0	5.34 ± 0.190	5.32 ± 0.148
	10	6.19 ± 0.166 **	7.35 ± 0.242 **
	100	6.20 ± 0.163 **	6.76 ± 0.203 **
	1000	5.88 ± 0.133	6.46 ± 0.180 **

0 h: 3.47 ± 0.127. The data are presented as the mean ± SEM (n = 102–144 from 3–5 independent experiments). *; $p < 0.05$, **; $p < 0.01$ compared with the concentration at 0 h at each time point (one-way ANOVA and Dunnett's post-hoc test).

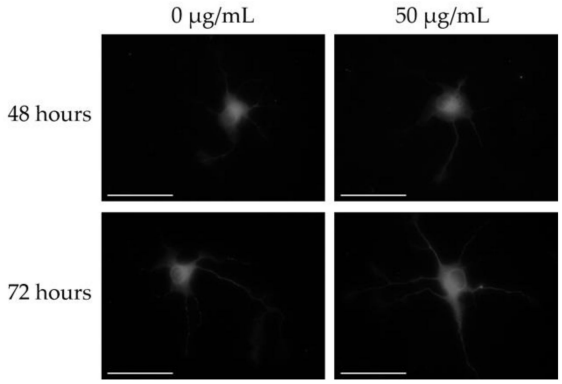

Figure 2. Images of MAP2-positive primary cultured hippocampal neurons treated with MGE. The neurons were treated with 0 (control) or 50 μg of MGE for 48 (day in vitro (DIV) 4) or 72 h (DIV 5). These images of MAP2-positive cells obtained by immunofluorescence staining were subjected to image conversion using the IN Cell Translator. Bars = 100 μm.

3.2. Y-Maze Test

The Y-maze test was performed to investigate the effects of MGE and SAC on the improvement of the spatial working memory, which is a kind of short-term memory. As shown in Figure 3A, old SAMP10 mice showed significantly reduced spontaneous alternations compared with old SAMR1 and young SAMP10 mice (F (2, 33) = 17.33, $p = 7.17 \times 10^{-6}$). In old SAMP10 mice, the number of alternations in the MGE- and SAC-fed groups significantly increased compared with that of the control group (F (3, 57) = 23.99, $p = 3.62 \times 10^{-10}$; Figure 3B). As shown in Figure 3C, young SAMP10 mice showed a high value for total arm entries (F (2, 33) = 40.35, $p = 1.37 \times 10^{-9}$), although there was no significant difference in the total arm entries in old SAMR1 and old SAMP10 mice. On the other hand, there was no significant difference in the total arm entries among the SAMP10 groups (F (3, 57) = 5.868, $p = 1.46 \times 10^{-3}$; Figure 3D). These results suggest that the neural circuit for memory formation in old SAMP10 mice is stimulated by MGE and SAC intake and has a positive effect on recovery from the decline in short-term memory ability. In addition, only young SAMP10 mice showed a high rate of spontaneous locomotor activity, which was calculated from the number of arm entries.

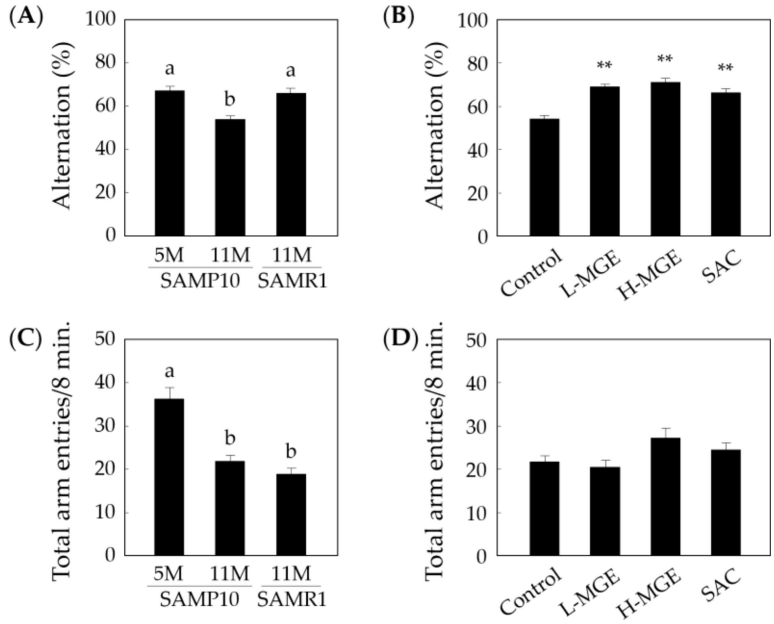

Figure 3. Effects of MGE and SAC on working memory in senescence-accelerated mice (SAM). The behavior of SAMP10 (5 and 11 months old) and SAMR1 (11 months old) was observed for 8 min in a Y-maze. The ratios of alternation (%, (**A**,**B**)) and total arm entries (**C**,**D**) were measured. (**B**,**D**) show the results for 11-month-old SAMP10. The L- and H-MGE groups consumed 0.20% and 1.0% of MGE in the diet (*w*/*w*), respectively. The SAC and H-MGE groups consumed the same amount of SAC. Each value represents the mean ± SEM (n = 8–17). ^{a,b}; $p < 0.05$ (One-way ANOVA and Tukey–Kramer post-hoc test), **; $p < 0.01$ compared with the control group (One-way ANOVA and Dunnett's post-hoc test). SAMP: senescence-prone inbred strains; SAMR: senescence-resistant inbred strains.

3.3. Step-Through Passive Avoidance Test

The time taken to learn not to enter the dark chamber was recorded using a step-through passive avoidance task, in which a shorter learning time implied a higher learning ability. As shown in Figure 4A, the learning time of old SAMP10 mice fed a normal diet was significantly longer than that of young SAMP10 and old SAMR1 mice (F (2, 33) = 18.38, $p = 4.32 \times 10^{-6}$). In the old SAMP10 mice fed MGE or SAC, the learning times were significantly shortened (F (3, 56) = 25.99, $p = 1.15 \times 10^{-10}$; Figure 4B), equivalent to the learning ability of young SAMP10 mice. These results suggest that MGE and SAC contribute to learning acquirement and efficiency in old SAMP10 mice.

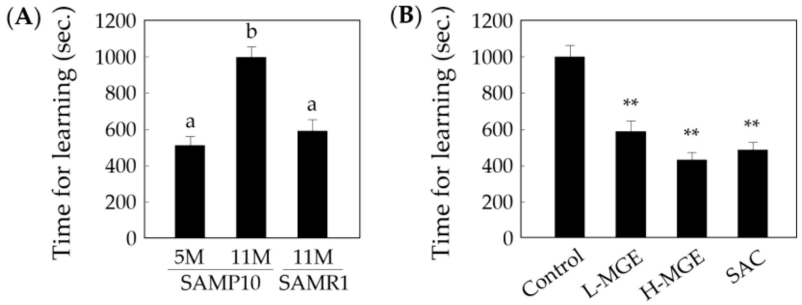

Figure 4. Effects of MGE and SAC on learning in SAM mice. (**A**) The learning time of SAMP10 (5 and 11 months old) and SAMR1 (11 months old) was examined using a step-through test system. The time needed for acquisition (**A**,**B**) of the avoidance response was measured. (**B**) Results for 11-month-old SAMP10 mice. The L- and H-MGE groups consumed 0.20% and 1.0% of MGE in the diet (*w*/*w*), respectively. The SAC and H-MGE groups consumed the same amount of SAC. Each value represents the mean ± SEM (n = 8–16). ^{a,b}; $p < 0.05$ (One-way ANOVA and Tukey–Kramer post-hoc test), **; $p < 0.01$ compared with the control group (One-way ANOVA and Dunnett's post-hoc test).

The memory retention test showing the avoidance response was assessed at 1 month after the acquisition test. The number of mice that stayed in the light chamber for at least 300 s was only measured once. As shown in Table 3, all of the young SAMP10 mice succeeded at remembering the avoidance response (8/8), whereas only 46.7% (7/15) of the old SAMP10 mice remained in the light chamber. An increased number of old SAMP10 mice in the MGE- or SAC-fed groups satisfied the acquisition criteria, exceeding the success rate of the old SAMR1 mice (Table 4). These findings suggest that long-term spatial memory retention is possibly due to intake of MGE and SAC.

Table 3. Effects of MGE and SAC on the memory of the avoidance response in the retention test for SAMP10 (6 and 12 months old) and SAMR1 (12 months old) mice.

Mice	Age	Number of Animals Success (Left) Failure (Right)		Memory Retention (%)
SAMP10	6 M (young)	8	0	100
SAMP10	12 M (old)	7	8	46.7
SAMR1	12 M (old)	7	4	63.6

Table 4. Effects of MGE and SAC on the memory of the avoidance response in the retention test for 12-month-old SAMP10 mice.

Mice	Diet	Number of Animals Success (Left) Failure (Right)		Memory Retention (%)
SAMP10	Control	7	8	46.7
SAMP10	L-MGE	9	4	69.2
SAMP10	H-MGE	10	3	76.9
SAMP10	SAC	11	4	73.3

3.4. Novel Object Recognition Test

The non-spatial memory ability was also evaluated in the novel object recognition test. During the training session, there was no biased exploratory preference in all groups (Figure 5A,B). When the retention session was performed 24 h after the training session, old SAMP10 mice fed a normal diet did not change their level of exploratory preference, whereas young SAMP10 and SAMR1 mice showed significantly increased preference for novel object C ($F(2, 31) = 9.299$, $p = 6.86 \times 10^{-4}$; Figure 5C). SAMP10 mice in the MGE- and SAC-fed groups also showed a significantly increased exploratory preference (F (3, 52) = 6.788, $p = 6.00 \times 10^{-4}$; Figure 5D), indicating that a diet containing MGE and SAC supports non-spatial memory formation in SAMP10 mice. These results suggest that the diets containing these ingredients reduced age-related memory decline and enhanced long-term non-spatial memory retention. In addition, the changes in exploratory preference did not show a concentration-dependent characteristic in the MGE-fed group.

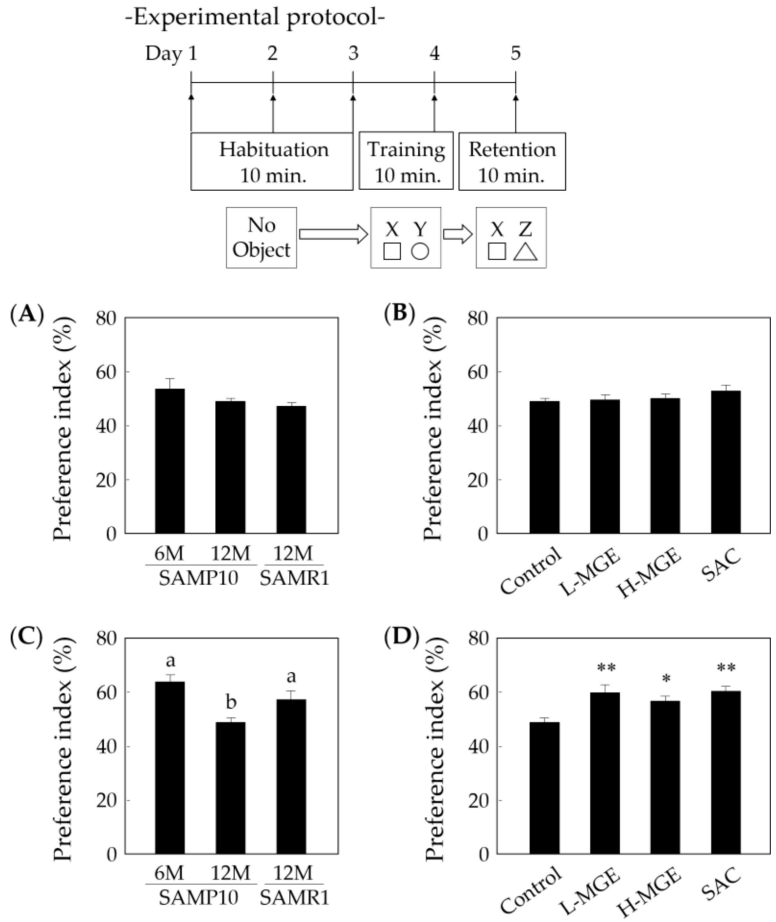

Figure 5. Effects of MGE and SAC on non-spatial memory in SAM mice. The exploratory preference (%) of SAMP10 (6 and 12 months old) and SAMR1 (12 months old) was observed in the novel object recognition test. The time spent exploring two objects was recorded for 10 min in the training sessions (**A**,**B**) and retention sessions (**C**,**D**). (**B**,**D**) show the results for 12-month-old SAMP10 mice. The L- and H-MGE groups consumed 0.20% and 1.0% of MGE in the diet (*w*/*w*), respectively. The SAC and H-MGE groups consumed the same amount of SAC. Each value is presented as the mean ± SEM (n = 8–15). ^{a,b}; $p < 0.05$ (One-way ANOVA and Tukey–Kramer post-hoc test), *; $p < 0.05$, **; $p < 0.01$ compared with the control (One-way ANOVA and Dunnett's post-hoc test).

3.5. Western Immunoblotting

Several studies have indicated that tyrosine phosphorylation of NR2B and serine phosphorylation of GluR1 can regulate the activity of NMDA and AMPA receptors in neurons and that these phosphorylation reactions are involved in learning and memory formation [39,40]. It is also well known that CaMKII-dependent signaling in neurons is involved in survival, brain development, learning, and memory formation [40–44]. Therefore, the relationship between protein expression and learning and memory formation was investigated using hippocampal tissue for Western immunoblotting after completion of the behavioral experiments (Figure 6A). The protein expression levels of learning and memory-related receptors and the phosphorylation levels (GluR1, pGluR1, NR2B, pNR2B, and pCaMKII) were reduced in old SAMP10 mice compared with the expression and phosphorylation levels in young SAMP10 mice (Figure 6B). Old SAMP10 mice fed H-MGE showed significant suppression of the decrease in the expression levels of all proteins (Figure 6C). On the other hand, the SAC-fed group only showed suppression of the decrease in the expression of GluR1 and pCaMKII, although no concentration-dependent actions of MGE and no correlation between the H-MGE and SAC groups were observed. These results suggest that SAC and the multiple other components contained in MGE

have the ability to increase protein expression, suggesting that they work to maintain and enhance learning and memory functions.

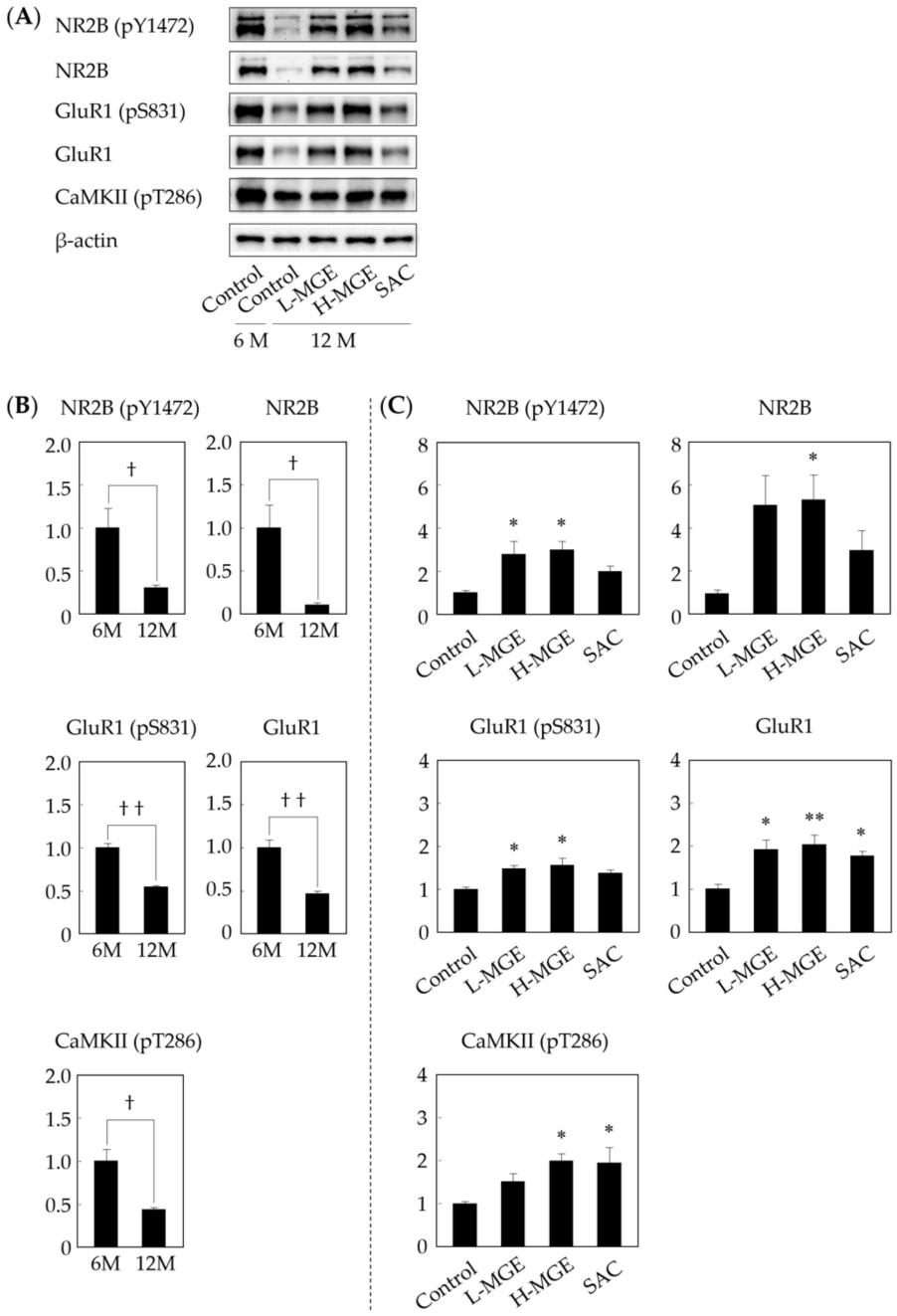

Figure 6. Effects of MGE and SAC on hippocampal proteins in SAMP10 mice. The molecular mechanism of memory was investigated by Western immunoblotting of hippocampal tissue after completion of the behavioral experiments (**A**). Protein levels for 6- and 12-months-old SAMP10 mice (**B**) and 12-months-old SAMP10 mice (**C**) are shown. Each value is presented as the mean ± SEM (n = 4). †; $p < 0.05$, ††; $p < 0.01$ (Student's t-test), *; $p < 0.05$, **; $p < 0.01$ compared with the control group (One-way ANOVA and Dunnett's post-hoc test).

4. Discussion

The essence of the brain is the processing of external information and subsequent plastic regulation of neuronal function. Neurons form networks through synaptic structures and communicate with each other through neurotransmitters and synaptic receptors [45]. In this study, treatment of primary

cultured hippocampal neurons with MGE or SAC significantly increased the total neurite length and number of dendrites. SAC showed a certain neurotrophic effect and further activated neurons through a synergistic effect with multiple other components contained in MGE. We suggest that the neurotrophic effects of MGE and SAC are strongly involved in the enhancement of transmission efficiency and information-processing ability by neural network formation. Furthermore, we propose that the increase in the number of dendrites plays a role in the ability of the brain to receive a lot of information. Thus, although SAC showed the maximum effect on the neurons at low concentrations, these data (Tables 1 and 2) alone do not suggest that it has a useful effect on learning and memory.

Therefore, pharmacological behavioral tests were performed to investigate the inhibitory effects on memory dysfunction of feeding a diet containing MGE or SAC to SAMP10 and SAMR1 mice. The Y-maze is considered to be a hippocampus-dependent memory test, since it evaluates spatial working memory, an index of short-term memory, through assessing the continuous selection of arms [46,47]. In addition, it has the advantage of providing a measure of locomotor activity of mice by counting the number of arm entries [48]. The step-through passive avoidance test is closely related to the amygdala-dependent memory, since it is used as an index of long-term memory to evaluate avoidance behavior against an aversive stimulus (electric foot-shock) that has been experienced once [49]. In addition, the novel object recognition test evaluates long-term memory through the recognition of unique non-spatial information of novel objects by utilizing a characteristic of rodents, i.e., the preference for novelty. In a previous study, this test was related to olfactory-cortex-dependent memory, which is considered to be one of the major pathways of neural information related to episodic memory [50]. It has been suggested that the hippocampus reactivates specific memory representations of the olfactory cortex and amygdala during memory retrieval [51], and, in particular, the amygdala and hippocampus collect information from related cortical areas and are deeply involved in the processes of cognition, memory formation, and emotional expression [52]. In all pharmacological behavioral tests performed, SAMP10 mice fed MGE or SAC showed significantly reduced learning and memory dysfunction and significant improvements in learning and short- and long-term memory formation. In addition, since the locomotor activity obtained from the number of arm entries did not differ significantly in any group, there were no differences in the amount of exercise, exploratory behavior, or motivation. Short-term memory formation requires the activity of existing ionotropic receptors and kinases in neuronal cells, and memory consolidation from short- to long-term memory requires the induction of de novo protein synthesis in the brain after learning [53,54]. Therefore, it is suggested that, by improving the above-mentioned process, MGE and SAC enhance learning activity and memory consolidation.

It is known that synaptic transmission efficiency is not constant and changes following exposure to a stimulus, a phenomenon called synaptic plasticity [55]. The long-term memory circuit is formed by the induction of long-term potentiation (LTP), which causes a long-term increase in transmission efficiency at neuronal synapses and requires activation of NMDA-type receptors and induction of AMPA-type receptor expression [38,39,56,57]. The AMPA-type receptor has four subunits, GluR1–4 [58], and the regulation of AMPA-type receptor expression is one of the important mechanisms underlying synaptic plasticity. Similarly, the NMDA-type receptor is composed of NR1 and NR2 subunits, and the NR2 subunit has four subtypes, NR2A–2D. LTP is induced by the activation of CaMKII caused by Ca^{2+} influx from NMDA receptors. Activated CaMKII phosphorylates GluR1 at serine 831, increases the channel conductance states, and is involved in LTP induction [43,59]. LTP is thought to be induced by an increase in an excitatory postsynaptic current (EPSC) and synaptic plasticity when phosphorylated GluR1 is recruited onto the postsynaptic membrane to increase the synaptic transmission efficiency [38,39,56,60]. Thus, the AMPA-type receptor containing GluR1 must be expressed on synaptic membranes for memory formation, and the activation of the NMDA-type receptor that stimulates them is essential for this process. However, there have been numerous reports of decreases in AMPA- and NMDA-type receptor expressions in the hippocampus of SAMP10 and aged rodents, suggesting an association with an age-related decline in learning ability [61–66]. It has also been reported that one of the earliest biological

manifestations of Alzheimer's dementia is a decrease in AMPA-type receptors and impaired synaptic plasticity [67,68], and a reduction of autophosphorylation of CaMKII at threonine 286 in the frontal cortex and hippocampus of Alzheimer's disease brains is a key contributor to synaptic dysfunction, neurodegeneration, and memory impairment [44,69]. We analyzed the hippocampal proteins of SAMP10 after the behavioral experiments and found that the expression levels of GluR1, NR2B, and phosphorylated CaMKII, which are involved in learning and memory abilities, were significantly increased in the MGE-fed group of SAMP10 mice. On the other hand, the SAC-fed group of SAMP10 mice showed an increase in the expression of proteins, except for NR2B. Although there is some room for memory consideration regarding the involvement of transcription factors such as the cyclic AMP response element binding protein (CREB), which is essential for the process of memory consolidation, the results of behavioral experiments and memory-related protein expression in this study suggest the importance of GluR1 and phosphorylated CaMKII in maintaining learning and memory functions. In addition, when memory is consolidated, synaptic changes occur in excitatory neurons that use glutamate as a neurotransmitter. AMPA- and NMDA- type glutamate receptors, which play important roles in the memory processes that occur in the postsynaptic membrane, are thought to mediate some of these changes [60,70].

Although the detailed mechanism of how SAC affects learning memory is not clear, we speculate that SAC might produce useful changes on the action of AMPA- and NMDA-type receptors in the postsynaptic membranes and on the mechanism of memory formation. MGE was also found to contribute to equal or better maintenance of postsynaptic function. As with cultured hippocampal neurons, this phenomenon is considered to be a synergistic effect of the multiple other components contained in MGE. Our findings indicate that MGE and SAC are possibly involved in the regulation of synaptic plasticity through mechanisms that promote hippocampal neuronal differentiation, regulate the synaptic microenvironment, and suppress a decrease in memory-related proteins.

5. Conclusions

We suggest that MGE and SAC positively contribute to learning, memory formation, and the maintenance of young brain function. The results of this study were obtained using SAMP10 mice, which showed morphological changes similar to those in humans with mild memory and cognitive impairments during aging. Thus, MGE and SAC could be applied in foods to improve the accuracy of memory and judgment.

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Theanine, Antistress Amino Acid in Tea Leaves, Causes Hippocampal Metabolic Changes and Antidepressant Effects in Stress-Loaded Mice

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Abstract: By comprehensively measuring changes in metabolites in the hippocampus of stress-loaded mice, we investigated the reasons for stress vulnerability and the effect of theanine, i.e., an abundant amino acid in tea leaves, on the metabolism. Stress sensitivity was higher in senescence-accelerated mouse prone 10 (SAMP10) mice than in normal ddY mice when these mice were loaded with stress on the basis of territorial consciousness in males. Group housing was used as the low-stress condition reference. Among the statistically altered metabolites, depression-related kynurenine and excitability-related histamine were significantly higher in SAMP10 mice than in ddY mice. In contrast, carnosine, which has antidepressant-like activity, and ornithine, which has antistress effects, were significantly lower in SAMP10 mice than in ddY mice. The ingestion of theanine, an excellent antistress amino acid, modulated the levels of kynurenine, histamine, and carnosine only in the stress-loaded SAMP10 mice and not in the group-housing mice. Depression-like behavior was suppressed in mice that had ingested theanine only under stress loading. Taken together, changes in these metabolites, such as kynurenine, histamine, carnosine, and ornithine, were suggested to be associated with the stress vulnerability and depression-like behavior of stressed SAMP10 mice. It was also shown that theanine action appears in the metabolism of mice only under stress loading.

Keywords: carnosine; histamine; kynurenine; ornithine; SAMP10; stress; theanine

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1. Introduction

Under similar circumstances, the stress response is heterogeneous in both humans and experimental animals. There is a difference in susceptibility to stress, that is, some people develop stress-related disorders such as depression and post-traumatic stress disorder, while others do not. To elucidate the difference in stress susceptibility, senescence-accelerated mouse prone 10 (SAMP10) mice are suitable as a stress-vulnerable model. SAMP10 mice are known to display age-related characteristic brain atrophy and depression-like behavior [1,2]. In addition, when SAMP10 mice were loaded with stress on the basis of territorial consciousness in males, stressed SAMP10 mice showed accelerated brain atrophy, cognitive dysfunction, and lifespan shortening [3]. The stress was based on territorial consciousness as follows: After two male mice were housed in a partitioned cage for one month to establish territorial consciousness (single housing), the partition was removed to expose the mice to psychosocial confrontational stress, and the two mice subsequently cohabited in the same cage (confrontational housing). Volumetric brain changes induced by psychosocial

stress have been observed one month after confrontational housing in SAMP10 mice, and atrophy continued thereafter [4]. Cerebral atrophy has also been observed in ddY mice, a strain that ages normally, but this was temporary. Significant adrenal hypertrophy, a typical stress response, has been observed at least one week after confrontational housing in ddY mice [5], and therefore it was considered that SAMP10 and ddY mice felt similar psychosocial stress by confrontational housing. However, stress due to confrontational housing may not last long in ddY mice. We investigated changes in gene expression in the hippocampus on the third day of stress loading, and clarified the difference between SAMP10 and ddY mice, that is, the expression levels of neuronal PAS domain protein 4 (*Npas4*) and lipocalin 2 (*Lcn2*) were involved in the brain atrophy and stress vulnerability of SAMP10 ice, and the changed expression of *Npas4* and *Lcn2* was prevented by theanine ingestion [4]. Theanine is a nonprotein amino acid that exists almost exclusively in tea (*Camellia sinensis* L.) leaves. Theanine intake suppresses psychosocial stress [6]. Indeed, adrenal hypertrophy, a typical stress response, has been significantly suppressed in mice that have ingested theanine even under stress loading [3,5]. In addition, brain atrophy due to chronic stress has been significantly suppressed in SAMP10 and ddY mice that ingested theanine [3]. Npas4 is a transcription factor that plays a role in the development of inhibitory synapses [7]. Lcn2, which is primarily secreted by reactive astrocytes, directly induces neuronal damage and amplifies neurotoxic inflammation under many brain conditions [8]. These early changes in gene expression are considered to be one reason for the stress vulnerability of SAMP10 mice. There is a need to investigate how these changes in gene expression subsequently affect brain metabolism. We focused on the metabolites that have been used to evaluate the diversity of amine-mediated metabolic patterns and pathways that are a confirmed diagnosis based on the pathophysiology of the brain in Alzheimer's disease [9].

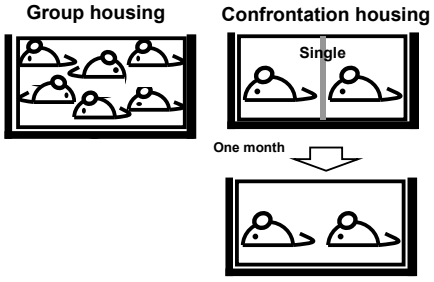
The hippocampus is a stress-vulnerable tissue in the brain. Neurogenesis in the hippocampus occurs throughout the life of a wide range of animal species, and it could be associated with hippocampus-dependent learning and memory [10–12]. However, restrained chronic stress has been shown to significantly decrease hippocampal volume and impair hippocampal neurogenesis in mice [13]. Hippocampal neurogenesis reportedly plays an important role in the regulation of the inhibitory circuitry of the hippocampus [14]. In addition, the maintenance of a balance between inhibitory and excitatory elements in the brain is believed to be important for synaptic plasticity and cognitive function [15,16], and the regulation of inhibitory neuronal activation may be especially important in the hippocampus during chronic stress [17–20]. Thus, we focused on the hippocampus in both SAMP10 and ddY mice that were housed confrontationally. Group-housing mice were used as a model for low-stress conditions.

To examine the reason for the different stress-sensitivity levels between SAMP10 and ddY mice, brain metabolites have been compared between SAMP10 and ddY mice. In this study, hippocampal metabolites were analyzed by comparing confrontational and group-housing SAMP10 and ddY mice that ingested theanine water or control water. From the obtained results, some factors related to depressive behavior were found. Therefore, the effects of confrontational housing and theanine ingestion on depression-like behavior of SAMP10 mice were examined. In addition, the expression of several enzymes involved in the synthesis of these metabolites was measured.

2. Results

2.1. Effects of Theanine Ingestion on Brain Metabolites in Senescence-Accelerated Mouse Prone 10 (SAMP10) and ddY Mice Stressed by Confrontational Housing

The SAMP10 and ddY mice were divided into two groups, i.e., confrontational and group-housing, respectively. These mice were further divided into two groups that ingested theanine or control water. For confrontational housing, two mice were housed in a partitioned cage for one month to establish territorial consciousness (single housing). Then, the partition was removed to expose the mice to confrontational stress, and the two mice subsequently cohabited in the same cage (confrontational housing) for one month (Figure 1).



Mouse strain	SAMP10	ddY
Control (water)	Group	Group
	Confrontation	Confrontation
Theanine (6 mg/kg)	Group	Group
	Confrontation	Confrontation

Figure 1. Experimental protocol.

The effects of theanine intake on metabolites in the hippocampus of mice stressed by confrontational housing were analyzed by principal component (PC) analysis [21]. The group-housing mice were used as the reference of a low-stress condition. Both PC1 and PC2 displayed differences between SAMP10 and ddY mice, and they both indicated the difference of housing conditions and theanine treatment (Table 1). Metabolites were analyzed in both SAMP10 and ddY mice together. Thirty-eight metabolites that were positive in ANOVA were analyzed by PCA (Table 2). The PC for samples presented characteristics of the groups on axes of PC1 and PC2. First, PC1 detected the difference between SAMP10 and ddY mice (Table 1). On both axes, conditions and treatment were separated differently wfor SAMP10 and ddY mice, which showed that condition and treatment had different effects on ddY and SAMP10 mice. On the one hand, in ddY mice, there was a significant difference in conditions on the PC1 axis, and there was not much change on the PC2 axis. On the other hand, the SAMP10 mice required theanine, as well as a confrontation to increase PC1, but the direction of change was the same as that of the ddY mice. This combination also showed a significant difference on the PC2 axis.

PC for metabolites is inextricably linked to PC for samples in a mathematical sense. Confrontation alone decreases in ddY mice, but in SAMP10, the substances that needed more theanine were kynurenine (Kyn) and histamine. In addition, it was 5-methoxytryptamine that decreased only in SAMP10 mice with the combination of confrontation and theanine (Table 2).

Some metabolites with negative PC1 scores tend to be higher in SAMP10 mice (Table 2 and Figure 2). The levels of Kyn in the group-housing SAMP10 mice were significantly higher than those of the ddY mice that had ingested theanine or not (Figure 2a). Similarly, the levels of histamine, 5-methoxytryptamine, 2,4-diamonobutyric acid, and histidinol were higher in the SAMP10 mice than in ddY mice (Figure 2b–e). However, these levels were significantly lower in the SAMP10 mice that had ingested theanine under confrontational housing than in those under group housing. Kyn and 5-metoxytryptamine are tryptophan (Trp) metabolites, which both present strong signals in PCA (Table 2).

In contrast, guanosine monophosphate (GMP), which showed a positive PC1, showed significantly higher values for ddY mice in group and confrontational housing than those of the SAMP10 mice, when theanine had not been ingested (Figure 2f). The level of carnosine was significantly higher in the ddY mice than in the SAMP10 mice with and without theanine intake (Figure 2g). Putrescine (Put) was significantly higher in the ddY mice than theSAMP10 mice for the confrontational-housing mice that ingested no theanine (Figure 2h). Ornithine (Orn) was significantly higher in the ddY mice than in the SAMP10 mice for the group and confrontational housing mice that had ingested no theanine (Figure 2i). In addition, theanine ingestion increased the level of Orn in SAMP10 (Figure 2i). Adenosine was significantly higher in the ddY mice than in the SAMP10 mice for the group and confrontational housing mice that had ingested theanine or not (Figure 2j).

Table 1. Data of principal component analysis of metabolites in senescence-accelerated mouse prone 10 (SAMP10) and ddY mice.

Mouse	Condition	Treatment	PC1	PC2	PC3	PC4
SAMP10	Group	Control	–0.07338	0.03785	–0.00872	–0.00107
			–0.03254	–0.00421	–0.02051	–0.00247
			–0.07107	0.03476	–0.00459	–0.00835
			–0.03904	–0.00196	–0.00954	–0.01278
		Theanine	–0.07666	0.02389	0.00652	0.01317
			–0.06840	0.02850	–0.00088	0.00442
			–0.07974	0.02936	0.01287	0.00789
	Confrontation	Control	–0.04038	0.00882	0.01114	–0.02924
			–0.04848	–0.01130	0.00611	–0.01154
			–0.06143	0.00294	0.01720	0.00465
			–0.00513	–0.06122	0.00394	0.00318
		Theanine	0.00952	–0.10804	–0.01076	0.00681
			–0.00350	–0.05210	0.01452	0.02649
			0.01278	–0.11432	–0.00996	–0.01817
			–0.02355	–0.03912	0.00415	–0.00177
ddY	Group	Control	0.01670	0.00225	–0.01376	–0.02015
			0.01147	0.00520	–0.01819	–0.00116
			0.02528	0.00516	–0.01773	0.01430
		Theanine	–0.00682	0.00349	0.00519	0.01326
			–0.04108	–0.01144	–0.00352	0.02319
			0.02797	0.00704	0.00463	0.00567
			0.17347	0.04502	0.02486	0.01496
	Confrontation	Control	0.09426	0.02753	–0.00925	0.01045
			0.04019	0.00548	–0.02618	–0.01805
			0.09584	0.02586	0.01472	0.00620
			–0.01268	–0.03017	–0.02188	0.02948
		Theanine	0.07307	0.01096	0.02558	–0.03502
			0.06694	0.01848	0.00251	0.00074
			0.04413	0.00230	–0.02052	–0.02141
			0.14107	0.03713	0.02662	0.01047

Note, mice were housed in groups of six per cage. After establishing territorial consciousness by single housing for one month, then, confrontational housing was carried out for one month. These mice ingested theanine or control water. The color gradient in each column indicates the level of metabolites. The darker the red, the higher than the overall average, and the darker the blue the lower than the overall average.

Table 2. Statistically different metabolites in the hippocampus of SAMP10 and ddY mice.

Metaborites	PC1	PC2
Kynurenine	−0.12390	0.02227
Histamine	−0.06985	0.00491
5-Methoxytryptamine	−0.03622	−0.08937
2.4-Diaminobutyric.acid	−0.03085	−0.00003
Histidinol	−0.02561	0.00316
5-Aminovaleric.acid	−0.02370	−0.00610
Cadaverine	−0.02067	−0.00384
Diacetyl spermidine	−0.01859	0.00364
3-Methoxyanthranilate	−0.01750	−0.00783
Leucine	−0.00513	0.00335
Methionine	−0.00364	0.00280
Proline	−0.00277	0.00327
Creatinine	−0.00269	−0.00184
2-Aminoadipate	−0.00263	−0.00433
Aspartic acid	−0.00229	−0.00076
Arginine	−0.00213	0.00345
Cystine	−0.00201	0.00122
2-Aminobutyric.acid	−0.00160	0.00033
Valine	−0.00124	0.00227
Phenylalanine	−0.00119	0.00302
Alanine	−0.00084	0.00084
β-Alanine	−0.00064	0.00208
N^G-monomethyl-arginine	−0.00028	−0.00849
Isoleucine	−0.00019	0.00310
Glutamine	−0.00008	−0.00027
Histidine	0.00037	0.00187
Hypoxanthine	0.00103	0.00440
Glutamic acid	0.00132	−0.00123
Spermidine	0.00143	−0.00015
Glutathione reduced	0.00146	−0.00238
Serine	0.00162	−0.00050
CMP	0.00262	0.00253
Adenine	0.00368	−0.01675
Adenosine	0.00482	−0.00904
Ornithine	0.00487	−0.00004
Putrescine	0.00668	−0.01895
Carnosine	0.00901	0.00522
GMP	0.01038	−0.00718

GMP, guanosine monophosphate and CMP, cytidine monophosphate. The color gradient in each column indicates the level of metabolites. The darker the red, the higher than the overall average, and the darker the blue the lower than the overall average.

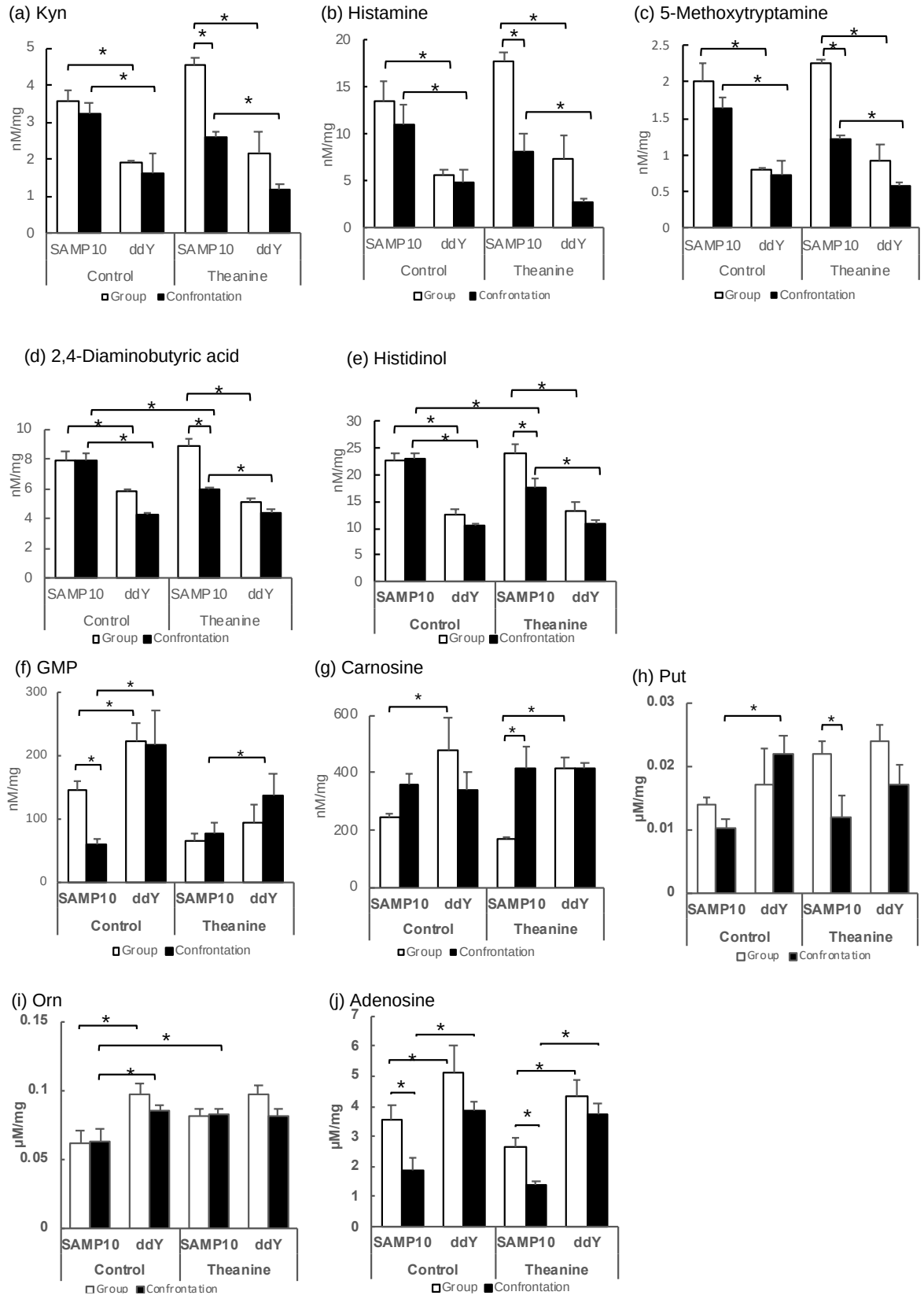


Figure 2. Metabolite levels in the hippocampus of SAMP10 and ddY mice; (**a**) Kyn, (**b**) histamine, (**c**) 5-methoxytryptamine, (**d**) 2,4-diaminobutyric acid, (**e**) histidinol, (**f**) GMP, (**g**) carnosine, (**h**) Put, (**i**) Orn, and (**j**) adenosine. Mice were housed confrontationally for one month after single housing for one month (closed column). Group-housed mice were kept in a group of six for two months (open column). Mice ingested theanine (20 μg/mL, 6 mg/kg) or water (control) for two months (n = 3–4, *, $p < 0.05$).

2.2. Effect of Theanine Ingestion on Depression-Like Behavior

To evaluate depression-like behavior in mice, the tail suspension test is used widely for preclinical screening of antidepressants. Mice are considered to present immobility as depression-like behavior after they are subjected to inescapable stress and failure in efforts to free themselves. As a result of determining the depressive behavior of the SAMP10 mice by the tail-suspension test, the effect of theanine intake was not observed in the group-housing mice, but immobility time was reduced significantly by theanine intake in mice under confrontational housing (Figure 3). Similarly, in ddY mice, theanine intake significantly reduced immobility time under confrontational housing. Reduced immobility time meant that depression-like behavior was improved.

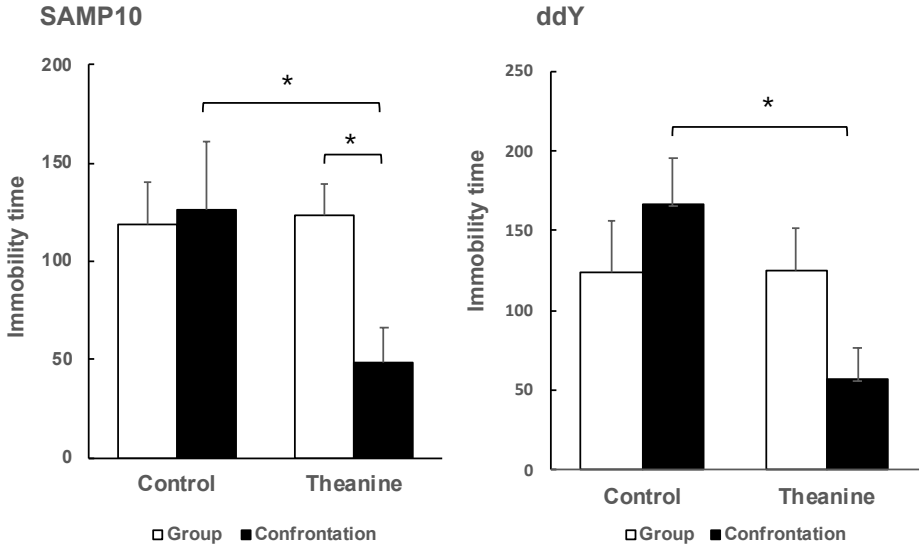


Figure 3. Effect of theanine intake on depression-like behavior of SAMP10 and ddY under group or confrontational housing. Mice were housed confrontationally for one month after single housing for one month (closed column). Group-housed mice were kept in a group of four for two months (open column). Mice ingested theanine (20 μg/mL, 6 mg/kg) or water (control) for two months ($n = 4$, *, $p < 0.05$).

It was not possible to compare SAMP10 and ddY mice because the optimal observation time depended on the mouse strain, but it was possible to compare the effects of confrontational housing on depression-like behavior within the same strain. There was no significant difference between group and confrontational housing in the control mice, and the presence or absence of theanine intake had no effect on immobility time. However, immobility time was shortened only in mice that ingested theanine under confrontational housing.

2.3. Effect of Theanine Ingestion on the Levels of Indoleamine/Tryptophan-2,3-dioxygenase, Arginase and Histidine Decarboxylase

Since Kyn is produced from Trp via indoleamine/tryptophan-2,3-dioxygenase (IDO, TDO), these expression levels in the hippocampus were compared between SAMP10 and ddY mice (Figure 4a,b). The expression of IDO was significantly higher in the control SAMP10 mice under confrontational housing than that of the group-housing SAMP10 mice and that of the control ddY mice under confrontational housing. The IDO levels were also significantly higher in the SAMP10 mice than in ddY mice that ingested theanine under confrontational housing. The level of TDO in the control SAMP10 mice was lower than the control ddY mice. The levels of TDO were not changed significantly in the SAMP10 and ddY mice when they ingested theanine. These results indicated that the expression of IDO was increased in the SAMP10 mice but not in the ddY mice under confrontational housing.

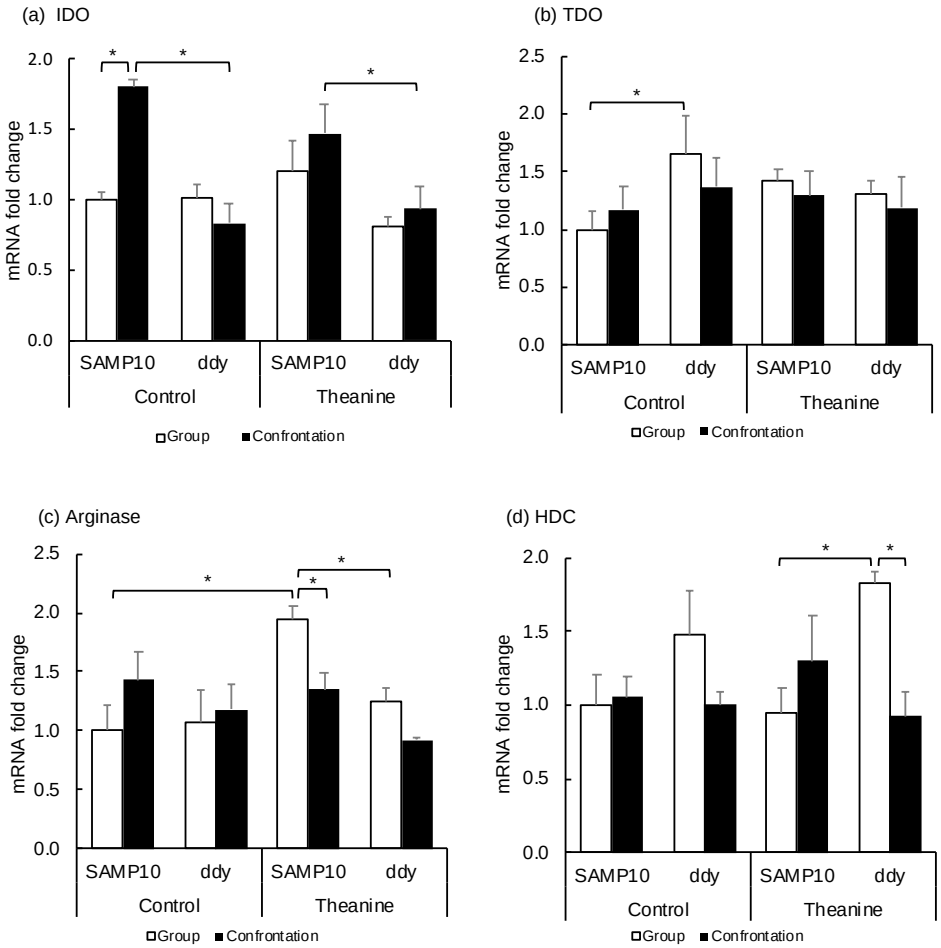


Figure 4. Effect of theanine intake on mRNA expression of SAMP10 and ddY mice under group or confrontational housing. (**a**) Indoleamine-2,3-dioxygenase (IDO); (**b**) Tryptophan-2,3-dioxygenase (TDO); (**c**) Arginase; (**d**) Histidine decarboxylase (HDC). Mice were housed confrontationally for one month after single housing for one month (closed column). Group-housed mice were kept in a group of four for two months (open column). Mice ingested theanine (20 μg/mL, 6 mg/kg) or water (control) for two months ($n = 4$, *, $p < 0.05$).

Ornithine is produced from arginine via arginase. The expression level in the hippocampus was compared (Figure 4c) and the expression level was significantly increased in SAMP10 mice that ingested theanine, but was lowered by confrontational housing. The levels of arginase in ddY mice were not changed.

Histamine is synthesized from histidine via histidine decarboxylase (HDC), in which mRNA is expressed exclusively in the posterior hypothalamus. The expression levels in the hypothalamus were compared (Figure 4d). The expression of HDC in the hypothalamus was higher in ddY mice that ingested theanine under group housing than in the SAMP10 mice. The level in ddY mice was significantly suppressed by confrontational housing.

3. Discussion

Changes in hippocampal metabolites were compared among mice with different stress susceptibilities. The levels of Kyn, histamine, 5-metoxytryptamine, 2,4-diaminobutyric acid, and histidinol were significantly higher in the SAMP10 mice than in the ddY mice. However, these levels were suppressed in the SAMP10 mice that had ingested theanine under confrontational housing. The Kyn level did not decrease in the group-housing SAMP10 mice that had ingested theanine, suggesting that theanine began to act only when some metabolism changes occurred due to stress loading. Kyn has been reported to increase in chronically stressed rats [22]. In addition, the Kyn pathway plays a key role in

depression-like behavior in mice [23,24]. Kyn levels were lower in the ddY mice than in the SAMP10 mice, but even lower when the ddY mice ingested theanine under confrontational housing. Similarly, shortening of immobility time was observed in both the SAMP10 and ddY mice that ingested theanine under confrontational housing.

Kyn is produced from Trp via IDO/TDO and TDO is an enzyme present in the liver [25]. The effects of stress loading and theanine ingestion on IDO and TDO expression levels were investigated. The level of IDO was significantly higher in the SAMP10 mice than in the ddY mice under confrontational housing, while the levels were not changed among the SAMP10 and ddY mice under group housing with or without theanine ingestion (Figure 4a). The high expression level of IDO in the SAMP10 mice under confrontational housing may contribute to the high amount of Kyn in the SAMP10 mice. However, the increased level of Kyn level in the group-housing SAMP10 mice that ingested theanine could not be explained by the expression levels of IDO and TDO alone.

Carnosine presents at a high concentration in the brain and has been reported to have antidepressant-like activity [26,27]. Carnosine levels were significantly lower in the SAMP10 mice than in the ddY mice, but the levels were significantly increased in the SAMP10 mice that had ingested theanine under confrontational housing. Depression-like behavior was suppressed in the SAMP10 and ddY mice that had ingested theanine under confrontational housing, where Kyn was suppressed, and carnosine was increased. The suppression of depression-like behavior only in mice that ingested theanine under confrontational housing may be explained by the changes in Kyn and carnosine. Carnosine is synthesized from β-alanine and histidine. In the SAMP10 mice, β-alanine and histidine tended to increase with theanine intake (Supplementary Table S1).

Theanine has been reported to suppress the depression-like behavior in mice and rats using the tail suspension test, forced swimming test, and elevated plus maze test [28–30]. Ogawa et al. [29] measured amino acid levels in cerebrospinal fluid and found that glutamate and methionine were increased in mice that ingested theanine. Shen et al. [30] measured monoamine levels in the limbic-cortical-striatal-pallidal-thalamic circuit in stressed mice. We showed the data of methionine and glutamate in the hippocampus (Supplementary Table S1), but these results could not be compared due to different experimental conditions.

Npas4 regulates the formation and maintenance of inhibitory synapses in response to excitatory synaptic activity [7,31]. On the basis of our previous data [4], we focused on the levels of excitatory and inhibitory neurotransmitters, glutamate and γ-aminobutyric acid (GABA), but significant change was not observed (Supplementary Table S1).

Histamine has strong effects on the excitability in the hippocampus, and histamine release is enhanced by a variety of stressors [32]. The synthesis of histamine is under the control of inhibitory H_3 autoreceptors located on histamine neurons [33]. However, theanine intake significantly suppressed histamine levels in the SAMP10 mice under confrontational housing, suggesting that the histaminergic system was an important target for theanine. In addition, histamine is strongly suggested to have a pivotal role in the regulation of sleep and wakefulness via H_1 or H_3 receptor [33]. Theanine has been suggested to improve sleep quality based on actigraph-based sleep studies [34] and based on the Pittsburgh Sleep Quality index [35], but the mechanism remains unclear. The high histamine levels in the SAMP10 mice could not be explained by HDC expression levels, as HDC expression in the hypothalamus was higher in the ddY mice than in the group reared SAMP10 mice. Further research is needed on the effects of theanine on histaminergic nerves.

Histidinol is dehydrogenated to histidine, suggesting that elevated histidinol levels in the SAMP10 mice may be partly involved in elevated histamine levels. In addition, 2,4-diaminobutyric acid has been reported to inhibit neuronal GABA transport [36]; GABA is a main inhibitory transporter. GABA levels did not differ at all in these mice (Supplementary Table S1), but increased levels of histamine, histidinol, and 2,4-diaminobutyric acid may make the balance between excitability and inhibitory states predominantly excitatory. It

has been reported that longevity is dynamically regulated by the excitatory–inhibitory balance of neural circuits [37]. Increased excitation may have contributed to the shortened lifespan of confrontational-housing SAMP10 mice [3].

5-Methoxytryptamine is an agonist of serotonin receptors. Although increased levels of 5-methoxytryptamine may enhance the stress-related adaptive behavioral responses [38], its role is currently unknown.

Put and Orn are metabolites of arginine (Arg), a semi-essential amino acid that is metabolized to form a number of bioactive molecules such as nitric oxide (NO) [39]. Arg is hydrolyzed by arginase to Orn, and Orn becomes Put by ornithine decarboxylase. Polyamines containing Put, spermidine, and spermine are essential for normal cellular function such as neurogenesis and aging [40]. Orn was significantly increased with theanine intake in the SAMP10 mice under confrontational housing and tended to be increased under group housing. In the SAMP10 mice, it was suggested that Orn synthesis was increased by increased expression of arginase by theanine ingestion. Orn has been reported to have an antistress effect [41]. In addition, the antistress effect of Arg has been confirmed in mice [42]. While the level of Orn was lower than that of Arg in the hippocampus, the increase in Orn with theanine intake may be important.

Adenosine is present in all nervous cells containing neurons and glia, and it modulates to lead to the homeostatic coordination of brain function [43]. Adenosine activates membrane-located G-protein coupled receptors. The A1 receptor is an inhibitory receptor coupled with Gi/o proteins, and the A2A receptor is an excitatory receptor coupled with Gs proteins [44]. It has been reported that chronic stress altered adenosine metabolism in a zebrafish brain [45]. However, the expression levels of adenosine and Put were further reduced by the ingestion of theanine under confrontational-housing conditions in the SAMP10 mice. These results suggest that changes in adenosine and Put are not important in the antidepressant action of theanine in the SAMP10 mice. The levels of GMP were lower in the SAMP10 mice than in the ddY mice, but the metabolic role in stress sensitivity is currently unknown.

Summarizing the above, when hippocampal metabolites were compared between the SAMP10 and ddY mice that were stressed for one month, Kyn and histamine were higher in the SAMP10 mice than in the ddY mice. On the one hand, their expression was suppressed in the SAMP10 mice that had ingested theanine under confrontational housing, on the other hand, the expression of carnosine increased in SAMP10 mice that had ingested theanine during stress loading. In addition, Orn was increased in the SAMP10 mice through increased expression of arginase by ingestion of theanine. These metabolic changes were well correlated with the improvement in depression-like behavior in SAMP10 mice with theanine intake under chronical stress.

Actually, theanine has been reported to have beneficial effects on depressive disorder, anxiety, sleep disorders, and cognitive decline in patients with major depressive disorder, and on stress-related symptoms and cognitive functions in healthy adults [35,46]. The results of our study are considered to be important clues for elucidating how theanine acts in the brain to ameliorate stress-related symptoms.

This study has some limitations. First, the brain metabolites examined in this study mainly focused on 60 metabolites, which were amine-mediated metabolites based on the pathophysiology of the brain in Alzheimer's disease. The second was that the amount of neurotransmitters actually released, such as glutamate and GABA, was not always parallel to the amount of hippocampal metabolites, making it impossible to directly assess the balance between excitation and inhibition. The third was that the evaluation of the stress-loaded period was only one month based on the stress period in which the degree of brain atrophy was most prominently observed after stress loading. However, it is necessary to consider different stress loading periods in the future.

4. Materials and Methods

4.1. Animals and Theanine Preparation

Four-week-old male SAMP10 (SAMP10-ΔSglt2) and ddY (Slc:ddY) mice were purchased from Japan SLC Co. Ltd. (Shizuoka, Japan) and kept in conventional conditions in a temperature- and humidity-controlled room with a 12–12 h light–dark cycle (light period 08.00–20.00, temperature 23 ± 1 °C, relative humidity of 55 ± 5%). Mice were fed a normal diet (CE-2; Clea Co. Ltd., Tokyo, Japan) and water ad libitum. All experimental protocols were approved by the University of Shizuoka Laboratory Animal Care Advisory Committee (approval no. 136068 and 195241) and were in accordance with the guidelines of the U.S. National Institutes of Health for the Care and Use of Laboratory Animals.

L-Theanine (suntheanine; Taiyo Kagaku Co. Ltd., Yokkaichi, Japan) was used at 20 μg/mL normal tap water, according to previous data [3,5]. Mice consumed a theanine solution ad libitum. The theanine solution was freshly prepared twice a week. The mouse intake of theanine was equivalent to 6 mg/kg.

4.2. Housing Conditions for Stress Experiments

Four-week-old mice were housed in groups of six per cage for five days to habituate them to novel conditions. Then, mice were divided into two groups, namely, confrontational and group housing, according to a previously described method (Figure 3) [3]. In brief, for confrontational housing, a standard polycarbonate cage was divided into two identical subunits by a stainless steel partition. Two mice were housed in the partitioned cage for one month to establish territorial consciousness (single housing). These mice were further divided into two groups that ingested theanine or control water. Then, the partition was removed to expose the mice to confrontational stress, and the two mice subsequently cohabited in the same cage for one month (confrontational housing). Mice were classified as follows: mice that ingested theanine under confrontational housing, mice that ingested control water under confrontational housing, mice that ingested theanine under group housing, and mice that ingested control water under group housing. The cages were placed in a styrofoam box (width 30 cm, length 40 cm, and height 15 cm) in order to avoid visual social contact between cages.

4.3. Measurement of Metabolites by Ultrahigh Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS)

The metabolite analytes and internal standards (stable isotope) were obtained from Wako Pure Chemical Co. (Osaka, Japan), Kanto Chemicals (Tokyo, Japan), Tokyo Kasei Co., Ltd. (Tokyo, Japan), Sigma-Aldrich (Buchs, Switzerland), and Cambridge Isotope Laboratories (Andover, USA). In addition, other regents, such as derivatization and mobile phase, were obtained from Wako Pure Chemical Co. (Osaka, Japan). In this study, we used an ACQUITY ultraperformance liquid chromatography system (UPLC-H class; Waters, MA, USA) coupled to a Xevo TQD triple quadrupole mass spectrometer equipped with an electrospray ionization source and positive mode. Reversed-phase analysis was performed using an ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 × 150 mm; Waters) at 50 °C. An injection volume of 5 μL was used, and the total run time of analysis was 10 min using a mobile phase based on 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Detailed information is shown in a previous report [47].

Mice were anesthetized with isoflurane, and blood was removed from the jugular vein. The brain was carefully dissected, and the hippocampus was immediately frozen. Three or four mice in each group were used for analysis. The brain-tissue sample (ca. 20 mg) was added to 1 mL of water/methanol (3:7, *v*/*v*) and internal standards (20 μL). The extraction solution with two zirconia beads (3.0 mm) in the tubes was homogenized for 10 min by a Shake Master and centrifuged at 15,000 rpm for 5 min at 4 °C for deproteinization. Then, the supernatant (500 μL) was transferred to 100 μL 0.1 M $NaHCO_3$ (pH 9.0) and derivatized with an equal volume of 40 mM 9-fluorenylmethyl chloroform for 10 min at room temperature. Derivatization was halted by adding 1% formic acid (100 μL). Then, the

solution was removed using a centrifugal evaporator, and residue was dissolved in 100 µL of 0.1% formic acid in water/acetonitrile (1:1, *v*/*v*). Solutions were vortexed, and 5 µL of each was analyzed by UHPLC–MS/MS.

4.4. Principal Component Analysis

Principal component analysis (PCA) was performed on the metabolites to compare the effects of theanine intake on controls under group or confrontational housing. To reduce the effects of individual variability among samples, PCA axes were estimated on a matrix of each group's sample means and applied to all data [48].

4.5. Tail-Suspension Test

To investigate behavioral depression, the mice were individually suspended by their tails at a height of 30 cm using a clip for tail suspension (MSC2007; YTS Yamashita-Giken, Tokushima, Japan). Immobility behavior was observed for 15 min, as described previously [5,49]. Mice were considered to be immobile only when they hung passively and were completely motionless. The immobility time for the final five minutes was compared between SAMP10 mice under group or confrontational housing to examine the effect of theanine intake. Observation was similarly performed for five minutes in ddY mice. Since the optimal observation time differed depending on the mouse strain, the SAMP10 and ddY mice could not be compared, but the effect of housing condition on depression-like behavior within the same strain could be compared.

4.6. Quantitative Real-Time Reverse Transcription PCR (qRT-PCR)

The mice were anesthetized with isoflurane and blood was removed from the jugular vein. The brain was carefully dissected, and the hippocampus and hypothalamus were immediately frozen. The brain sample was homogenized, and total RNA was isolated using a purification kit (NucleoSpin® RNA, 740955, TaKaRa Bio Inc., Shiga, Japan), in accordance with the manufacturer's protocol. The obtained RNA was converted to cDNA using the PrimeScript® RT Master Mix kit (RR036A, Takara Bio Inc., Shiga, Japan). Real-time quantitative RT-PCR analysis was performed using the PowerUp™ SYBR™ Green Master Mix (A25742, Applied Biosystems Japan Ltd., Tokyo, Japan) and automated sequence detection systems (StepOne, Applied Biosystems Japan Ltd., Tokyo, Japan). Relative gene expression was measured by previously validated primers for IDO and TDO [50], HDC [51], and arginase [52] genes. The primer sequences were mentioned in Table 3. cDNA derived from transcripts encoding β-actin was used as the internal control.

Table 3. Sequence of primers used in qRT-PCR analysis.

Gene	Forward Sequence	Reverse Sequence	Ref.
IDO	GGGCTTCTTCCTCGTCTCTC	TGGATACAGTGGGGATTGCT	[50]
TDO	TCCAGGGAGCACTGATGATA	CTGGAAAGGGACCTGGAATC	[50]
HDC	CGTGAATACTACCGAGCTAGAGG	ACTCGTTCAATGTCCCCAAAG	[51]
Arginase	CATGGGCAACCTGTGTCCTT	TCCTGGTACATCTGGGAACTTTC	[52]

4.7. Statistical Analyses

With SAMP10 or ddY, only substances with a *p*-value less than 0.05 in the housing condition or theanine treatment were selected and applied to PCA to reduce experimental noise. To cancel individual differences in the samples, the axes were calculated from the mean for each group and applied to all data [53]. Each substance log data were centered prior to PCA. Values of PCs were scaled [54]. Confidence intervals and significance of differences in means were estimated by using Fisher's least significant difference test.

5. Conclusions

Increased Kyn and decreased carnosine levels are associated with depression-like behavior in SAMP10 mice. An increased histamine level may be a reason for the shortened

lifespan of stress loaded SAMP10 mice. In addition, an increase in Orn due to theanine intake may have a role in stress reduction. Theanine was indicated to reduce stress vulnerability by correcting those metabolic alterations.

Supplementary Materials: Supplementary materials can be found at https://www.mdpi.com/1422-0067/22/1/193/s1. Table S1: Metabolite levels measured in the hippocampus of SAMP10 and ddY (nM/mg).

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and all experimental protocols were approved by the University of Shizuoka Laboratory Animal Care Advisory Committee (approval no. 136068 and 195241).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

Arg	Arginine
CMP	Cytidine monophosphate
GABA	γ-Aminobutyric acid
GMP	Guanosine monophosphate
HDC	Histidine decarboxylase
IDO	Indoleamine-2,3-dioxygenase
Kyn	Kynurenine
Lcn2	Lipocalin 2
NO	Nitric oxide
Npas4	Neuronal PAS domain protein 4
Orn	Ornithine
PC	Principal component
PCA	Principal component analysis
Put	Putrescine
SAMP10	Senescence-accelerated mice prone 10
Trp	Tryptophan
TDO	Tryptophan-2,3-dioxygenase
UHPLC-MS/MS	Ultra-high performance liquid chromatography-tandem mass spectrometry

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Green Tea Catechins Trigger Immediate-Early Genes in the Hippocampus and Prevent Cognitive Decline and Lifespan Shortening

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Abstract: Senescence-accelerated mouse prone 10 (SAMP10) mice, after ingesting green tea catechins (GT-catechin, 60 mg/kg), were found to have suppressed aging-related decline in brain function. The dose dependence of brain function on GT-catechin indicated that intake of 1 mg/kg or more suppressed cognitive decline and a shortened lifespan. Mice that ingested 1 mg/kg GT-catechin had the longest median survival, but the dose was less effective at suppressing cognitive decline. The optimal dose for improving memory acquisition was 60 mg/kg, and memory retention was higher in mice that ingested 30 mg/kg or more. To elucidate the mechanism by which cognitive decline is suppressed by GT-catechin, changes in gene expression in the hippocampus of SAMP10 mice one month after ingesting GT-catechin were analyzed. The results show that the expression of immediate-early genes such as nuclear receptor subfamily 4 (*Nr4a*), FBJ osteosarcoma oncogene (*Fos*), early growth response 1 (*Egr1*), neuronal PAS domain protein 4 (*Npas4*), and cysteine-rich protein 61 (*Cyr61*) was significantly increased. These results suggest that GT-catechin suppresses age-related cognitive decline via increased expression of immediate-early genes that are involved in long-term changes in plasticity of synapses and neuronal circuits.

Keywords: green tea catechin; cognitive function; immediate-early gene; lifespan; SAMP10

1. Introduction

Senescence-accelerated mouse prone 10 (SAMP10) mice that ingested green tea catechins (GT-catechin, 60 mg/kg) were found to have suppressed aging-related decline in brain function [1,2]. SAMP10 mice used in this study had characteristic accelerated senescence and age-related cognitive decline [3,4]. Since SAMP10 mice generate more superoxide anions, a reactive oxygen species (ROS), than SAMR1 mice, which have the same genetic background but a normal lifespan, oxidative damage is considered to be a factor that promotes brain aging [5]. In fact, mice that consumed GT-catechin at this dose had increased activity of glutathione peroxidase and reduced brain peroxidation [6,7]. However, ROS generation was not suppressed even in mice that consumed GT-catechin (unpublished data). GT-catechin may also suppress brain aging via another pathway other than the suppression of oxidative damage.

Since the daily concentration of GT-catechin ingested by Japanese people is about 0.3–0.6 mg/mL, for animal experiments a dose of about one-third that concentration was tried, considering the effects of species differences. When SAMP10 mice (average body weight 33 g) drank 10 mL/day of water containing GT-catechin (0.2 mg/mL), catechin intake was 60 mg/kg. GT-catechin contains

epigallocatechin gallate (EGCG) as the main catechin, followed by epigallocatechin (EGC). We found that EGCG is important for suppression of cognitive decline [7]. In addition, learning time was significantly shorter in mice that ingested EGCG for 5 months and more than in age-matched control mice, while the difference in starting age of EGCG ingestion had little effect on learning ability [8]. A shorter ingestion period of two or three months tended to suppress the decrease in learning ability [8]. It seems that catechin intake needs to be continued for a certain length of time.

Furthermore, we speculated that EGCG could be absorbed into the brain parenchyma via the blood–brain barrier, thereby promoting neuronal differentiation [7]. However, since the blood–brain barrier permeability of EGCG is about 4% and the concentration of EGCG in the brain is considerably lower than in the periphery, the effects of catechins may be different in the brain and in the periphery. In this study, we investigated the effects of GT-catechin on cognitive function and longevity at concentrations of 1–60 mg/kg.

The effects of flavonoids containing EGCG on lifespan in nematodes, flies, and mice have been reviewed [9]. Studies with mutant nematodes and flies gave us new information about genes regarding longevity regulated by catechins. Xiong et al. reported that EGCG promotes nematode longevity by inducing ROS production and triggering mitochondrial biosynthesis [10]. Mice have longer lifespans than nematodes and flies, and therefore take a longer time to study, but can provide important evidence to examine the effects of catechins on the human lifespan.

Regarding the effects of catechins on cognitive function, improvements have been reported in Alzheimer's disease model mice [11–14], Down syndrome model mice [15], high-fat and high-fructose diet ingesting mice [16], a rat model with reduced cerebral blood flow [17], mice with stress-induced cerebral dysfunction [18], a streptozotocin-induced model of dementia [19], and post-traumatic brain injury [20]. On the other hand, there are some reports that EGCG intake did not affect cognitive function, for example, in mice that had induced inflammation [21], in a Down syndrome mouse model [22], and in aged mice that were given beta-alanine [23]. Where does this difference come from? The antioxidative activity of catechins has been explored for their protective effects in a variety of systems [24]. In addition, neurogenesis in the adult hippocampus is critically involved in adult brain function, and EGCG has been reported to promote adult hippocampal neurogenesis [25]. Furthermore, it has been reported that EGCG plays an important role in the development of the nervous system, in forming connections between neurons [26]. Therefore, we comprehensively investigated gene expression changes in the hippocampus of mice ingesting catechins.

2. Results

2.1. Effect of GT-Catechin Ingestion on Lifespan

SAMP10 mice are susceptible to aging and have a shorter lifespan than SAMR1 and other mouse strains. Although the median survival time (MST) was 10.8 months under conventional conditions, it was significantly prolonged in mice that ingested more than 1 mg/kg of GT-catechin (Figure 1). The longest MST was observed in mice that ingested GT-catechin at 1 mg/kg (Table 1). This was 1.59 times longer than the MST of control mice.

MST was 15.3 months in mice that ingested GT-catechin at a concentration range of 5–30 mg/kg. This was 1.42 times longer than the MST of control mice. In mice that ingested GT-catechin at 60 mg/kg, MST was 1.26 times longer than that of control mice.

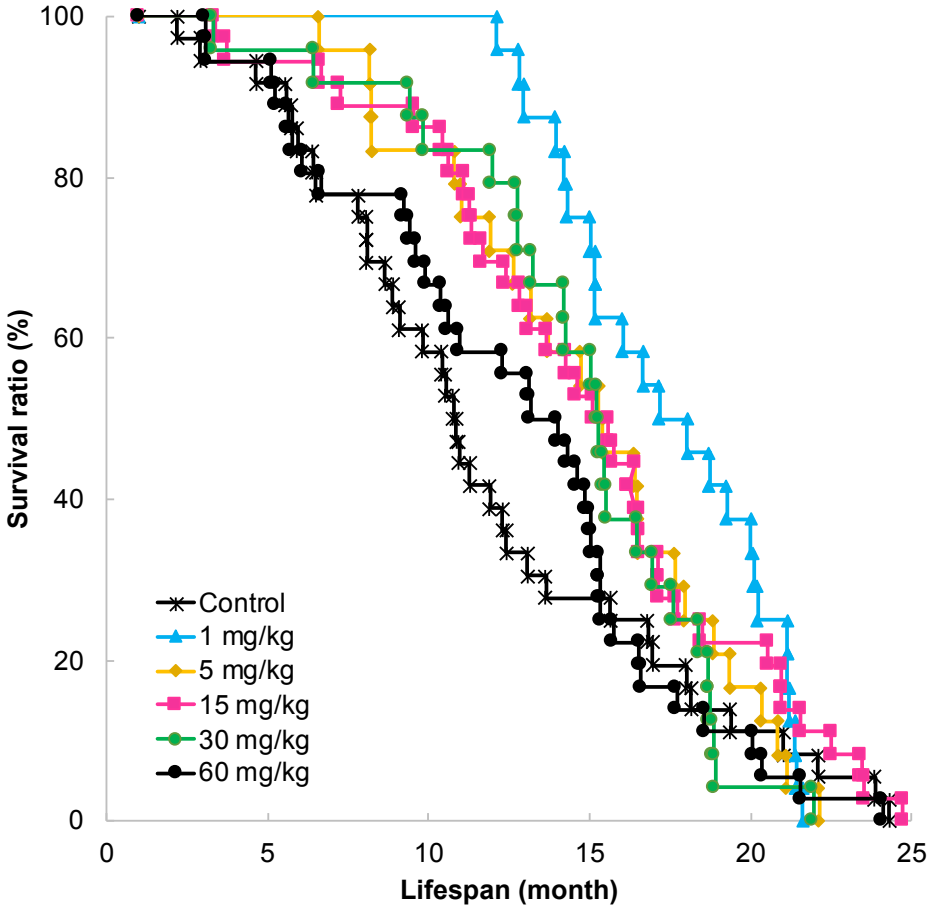

Figure 1. Effect of catechin ingestion on longevity of SAMP10 mice that consumed GT-catechin in water from 1 month of age. GT-catechin solution was freshly prepared twice a week. Three groups of 36 mice each consumed 0 (control), 15, and 60 mg/kg of GT-catechin. Another 3 groups of 24 mice each consumed 1, 5, and 30 mg/kg of GT-catechin.

Table 1. Effect of green tea catechin (GT-catechin) ingestion on median survival time (MST) of senescence-accelerated mouse prone 10 (SAMP10) mice.

GT-Catechin (mg/kg)	MST (Months)		*p*-Value
	Month	Ratio	
0	10.8	1.00	-
1	17.2	1.59	0.027 *
5	15.3	1.42	0.272
15	15.3	1.42	0.082
30	15.3	1.42	0.364
60	13.6	1.26	0.880

p-value is based on log-rank test. * $p < 0.05$.

2.2. Memory Acquisition, Memory Retention, and Working Memory

To evaluate memory acquisition, the time for learning not to enter a dark room was measured when mice were 11 months old using a step-through passive avoidance task. A longer learning time implies lower learning ability. The dose-dependency of GT-catechin was examined. Learning time was significantly shorter in mice that ingested 1 mg/kg or more of GT-catechin than in control mice that ingested no GT-catechin (Figure 2). The time for learning was shortest in mice that ingested 15 mg/kg of GT-catechin.

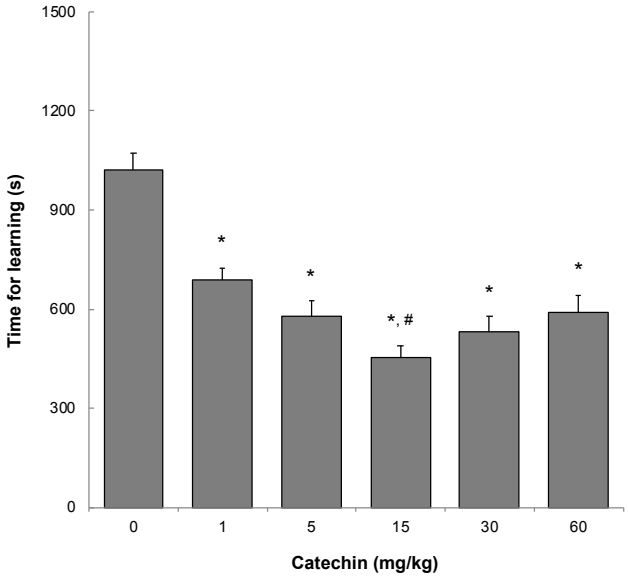

Figure 2. Effect of GT-catechin ingestion on learning ability of SAMP10 mice. A step-through passive avoidance task was carried out using 11-month-old mice. When a mouse entered a dark chamber from a light chamber, the door was closed and an electric foot-shock was delivered at 50 μA for 1 s. Acquisition of the avoidance response was judged as successful if the mouse remained in the light chamber for 300 s. The trial was repeated until the mouse satisfied the acquisition criterion within five trials. This result from successive trials was summed for each mouse to give a measure of the time required for learning not to enter the light chamber (i.e., learning time) (n = 16–24; * $p < 0.05$ to control, # $p < 0.05$ to mice that ingested 1 mg/kg).

Memory retention ability was measured at 12 months of age. To evaluate memory retention, mice were examined by the step-through passive avoidance task 1 month later. Long-term memory was better in mice that ingested a higher concentration of GT-catechins. The ratio of mice that consumed 30 mg/kg of GT-catechin tended to be higher compared to control mice ($p = 0.060$), and the ratio of mice that consumed 60 mg/kg of GT-catechin was significantly higher compared to control mice ($p = 0.024$) (Table 2).

Table 2. Effect of catechin ingestion on long-term memory of 12-month-old SAMP10 mice.

	Ratio	*p*-Value
0	0.567	—
1	0.583	0.533
5	0.720	0.186
15	0.737	0.185
30	0.800	0.060
60	0.833	0.024 *

One month after the step-through passive avoidance task, the same test was performed on the mice. If a mouse was able to stay in the light chamber for 300 s, then memory was determined to have been retained. The ratio represents memory-retained mice/tested mice (n = 19–36; *, $p < 0.05$).

The working memory of 11-month-old SAMP10 mice was examined using a Y-maze. This test is based on the natural instinct of a mouse to investigate a new area rather than one that it has just searched. This spontaneous alternative behavior is an index of working memory. When the searching behavior of the mice was observed, no effect was found in mice that ingested less than 5 mg/kg of GT-catechin. However, the spontaneous alternative ratio increased in a dose-dependent manner in mice that ingested more than 15 mg/kg of GT-catechin. A significant effect was observed in mice that ingested 30 mg/kg or more of GT-catechin (Figure 3).

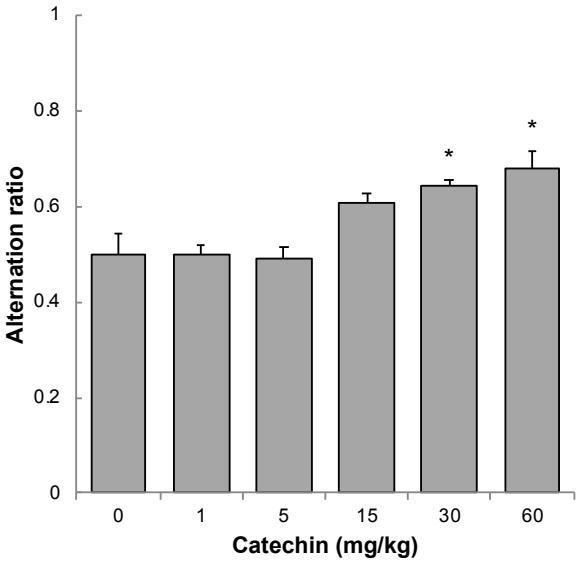

Figure 3. Effect of GT-catechin ingestion on working memory of 11-month-old SAMP10 mice. Searching behavior was observed in a Y-maze. The number of occasions in which spontaneous alternation behavior was observed was counted and the ratio of alternation was calculated as follows: (number of arm entries showing spontaneous alternation)/(total number of arm entries − 2) (n = 18–21; * $p < 0.05$)

2.3. Transcriptome

The hippocampi of two-month-old mice that ingested GT-catechin for one month were used for analysis. DNA microarray data of GT-catechin, which were obtained using high-density oligonucleotide microarrays, showed 605 positive genes based on two-way ANOVA ($p < 0.001$). The top 20 genes that were significantly upregulated following the ingestion of GT-catechin are listed in Table 3. Growth hormone (Gh) is synthesized in the central nervous system and is critical for neural development [27]. Among these 20 genes, early growth response 2 (Egr2), activity regulated cytoskeletal-associated protein (Arc), nuclear receptor subfamily 4, groupA, member 1 (Nr4a1), FBJ osteosarcoma oncogene (Fos), neuronal PAS domain protein 4 (Npas4), early growth response 1 (Egr1), and cysteine rich protein 61 (Cyr61) are immediate-early genes (IEGs) [28–30]. Dual specifificity phosphatase 1 (Dusp1) is reported to be an IEG in sensory neurons [31]. GTP-binding protein (Gem) promotes morphological differenciation in neurons [32]. Heat shock protein 1A (Hspa 1a) and heat shock protein 1 (Hspb1) are involved in stress and cell differentiation [33,34]. Cysteine-rich EGF-like domains 2 (Creld2) and stromal cell-derived factor 2-like 1 (Sdf2l1) play a role in endoplasmic reticulum (ER) homeostasis [35,36]. Period homolog 1 (Per1), a circadian clock gene, is critical for long-term memory formation [37].

2.4. Effect of GT-Catechin Intake on the IEG Levels

The increase in IEGs (*Egr2*, *Arc*, *Nr4a1*, *Fos*, *Npas4*, *Egr1i* and *Cyr61*) was confirmed by qRT-PCR. The degree of gene expression in the hippocampus and prefrontal cortex of mice that ingested GT-catechin for one, five, or 11 months was compared with control mice of the same age (two, six, and 12 months of age, respectively). The expression levels of IEGs (*Nr4a1*, *Fos*, *Npas4*, *Egr1*, and *Cyr61*) increased significantly in the hippocampi of two-month-old mice that ingested GT-catechin, but the difference was lower in six-month-old mice and was not observed in 12-month-old mice (Figure 4). *Egr2* was not significantly increased ($p = 0.08$) but tended to be higher in two-month-old mice that ingested GT-catechin compared to age-matched controls. *Arc* was not significantly higher than the age-matched controls. In the prefrontal cortex, effects of GT-catechin ingestion and aging on the expression of IEGs were not observed (data are not shown). Although the increase of *Gh* expression following catechin intake was interesting, there were large individual differences in the expression levels of *Gh* (data not shown).

Table 3. Upregulated genes in hippocampi of mice that ingested GT-catechin (60 mg/kg): top 20.

Symbol.	Full Name	ΔZ	*p*
Gh	growth hormone	0.5621	4.55×10^{-7}
Egr2	early growth response 2	0.3793	1.32×10^{-24}
Arc	activity regulated cytoskeletal-associated protein	0.2996	2.49×10^{-35}
Nr4a1	nuclear receptor subfamily 4, group A, member 1	0.2858	1.79×10^{-37}
Fos	FBJ osteosarcoma oncogene	0.2497	4.31×10^{-20}
Egr1	early growth response 1	0.2216	1.56×10^{-28}
Dusp1	dual specificity phosphatase 1	0.2123	1.07×10^{-23}
Gem	GTP binding protein (gene overexpressed in skeletal muscle)	0.1948	1.21×10^{-12}
Hspa1a	heat shock protein 1A	0.1916	1.28×10^{-11}
Rtl1	retrotransposon-like 1	0.1847	8.34×10^{-8}
Hspa1a	heat shock protein 1A	0.1827	8.76×10^{-22}
Hspb1	heat shock protein 1	0.1795	3.10×10^{-9}
Npas4	neuronal PAS domain protein 4	0.1735	7.10×10^{-12}
Cyr61	cysteine rich protein 61	0.1687	1.93×10^{-10}
Creld2	cysteine-rich with EGF-like domains 2	0.1674	1.34×10^{-14}
Per1	period homolog 1 (Drosophila)	0.1671	5.43×10^{-15}
Unc13c	unc-13 homolog C (C. elegans)	0.1559	4.13×10^{-5}
Hey2	hairy/enhancer-of-split related with YRPW motif 2	0.1551	1.21×10^{-9}
Agxt2l	alanine-glyoxylate aminotransferase 2-like 1	0.1526	0.000375
Sdf2l1	stromal cell-derived factor 2-like 1	0.1503	1.09×10^{-8}

ΔZ = expression level (catechin–control)

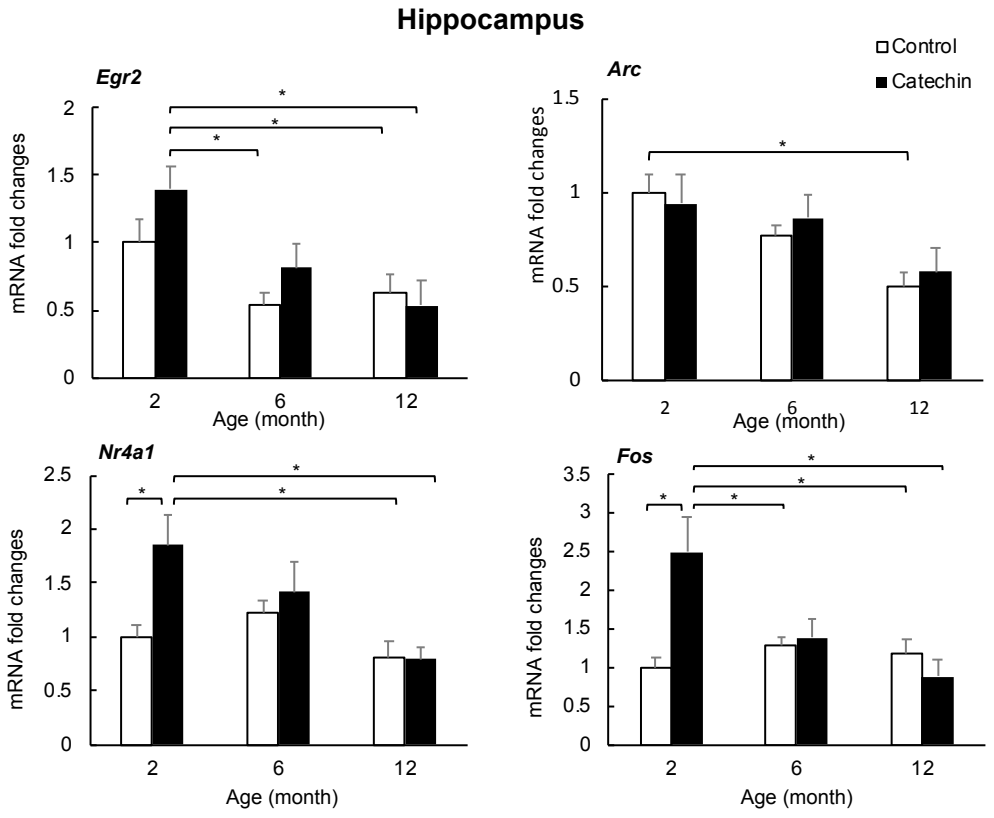

Figure 4. *Cont.*

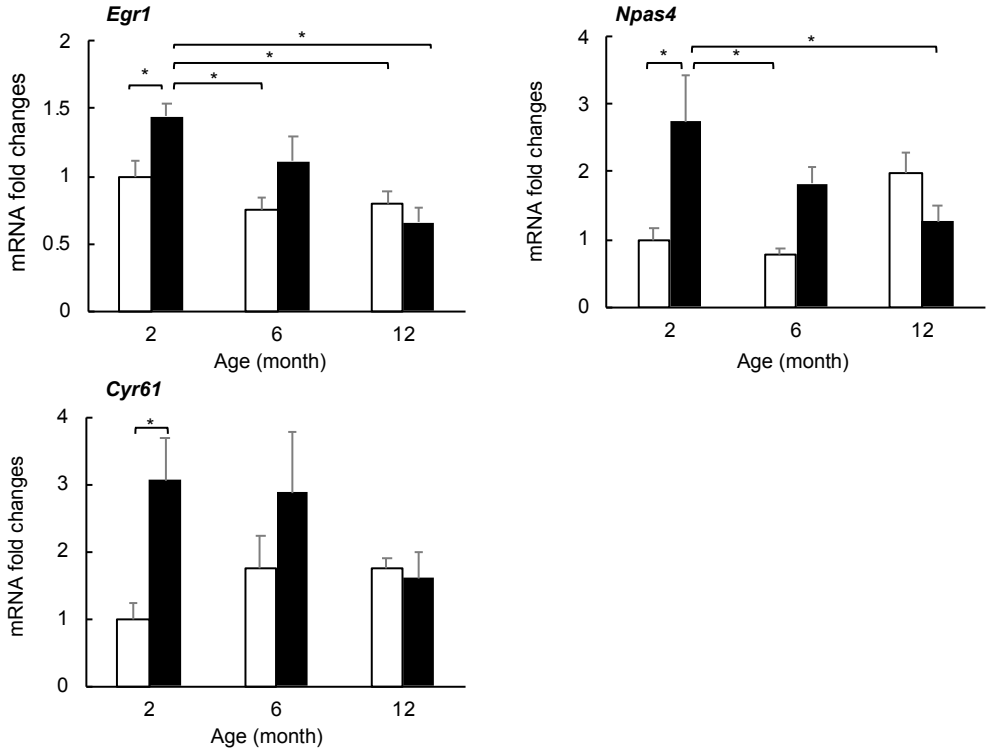

Figure 4. Expression of immediate-early genes (IEGs) in hippocampi of mice that ingested GT-catechin (60 mg/kg) and controls (n = 6, * $p < 0.05$).

3. Discussion

We examined how much GT-catechin intake is needed to prevent age-related cognitive decline. After examining the learning (memory acquisition) ability by a step-through passive avoidance task, the results showed that the intake of GT-catechin at 1 mg/kg or more showed a significant improvement, and that mice that consumed 15 mg/kg of GT-catechin had the best results (Figure 2). Long-term memory (memory retention) ability, as assessed by the step-through passive avoidance task performed one month later, increased with increasing concentrations of GT-catechin, with a significant improvement observed when 60 mg/kg of GT-catechin was consumed (Table 2). Working memory, as evaluated by the alternative ratio using a Y-maze, increased significantly when 30 mg/kg or more of GT-catechin was consumed (Figure 3). Taken together, these results suggest that GT-catechin intake of at least 1 mg/kg or more suppresses aging-related cognitive decline in SAMP10 mice.

MST increased significantly (1.6-fold) when 1 mg/kg of GT-catechin was consumed relative to the control group, and increased 1.3-fold when 60 mg/kg was consumed. High doses of GT-catechin are not required to suppress the shortening of longevity in SAMP10 mice. There was no change in maximum lifespan. Antioxidants can control autoxidation, reduce oxidative stress, and subsequently increase healthy longevity [38]. In fact, oxidative stress (the level of 8-oxodeoxyguanosine) was significantly lower in the liver and kidney of mice that consumed GT-catechin than age-matched control mice (data not shown). However, the effects on longevity may not be fully explained by antioxidant activity alone, as mice that ingested 1 mg/kg GT-catechin rather than 60 mg/kg had a longer lifespan. Since catechin has both antioxidant and pro-oxidant properties [39], GT-catechin may promote mouse longevity as in nematodes, by inducing ROS production and triggering mitochondrial biosynthesis [10]. In addition, it has been suggested that 4%–5% of GT-catechin in the blood reaches the brain parenchyma via the blood–brain barrier [40]. Therefore, differences in brain and peripheral GT-catechin levels can have different effects on cognitive function and longevity in SAMP10 mice. Further investigation is needed to elucidate the mechanism of GT-catechin in terms of its effects on longevity.

In any case, this study revealed that intake of GT-catechin at a dose of 1 mg/kg or more per day tends to improve both age-related cognitive decline and lifespan shortening. Whether this dose is directly applicable to humans is not yet known. However, since GT-catechin in green tea eluate is 30–60 mg/100 mL, drinking at least 1–2 cups of green tea every day might benefit the suppression of aging-related cognitive decline and lifespan shortening. Indeed, some epidemiological studies have shown that consuming green tea daily reduces the risk of dementia [41], and frequent consumption of green tea (≤5 cups/day) reduces the risk of developing dementia [42]. A study of the relationship between green tea intake and the risk of mortality in Japanese men and women showed that the risk of mortality decreases as green tea consumption increases [43].

Next, the mechanism to suppress age-related cognitive decline in mice that ingested GT-catechin was examined. Studies using cultured neurons suggest that EGCG has a stronger effect on neurite outgrowth than EGC [7]. Neurons transmit information by expanding neurites and forming synapses, therefore, the effect of EGCG on neurite outgrowth is noteworthy. To clarify the target of GT-catechin in the brain, we examined changes in gene expression that occurred in the hippocampus of mice that ingested GT-catechin.

DNA microarray and qRT-PCR results indicate that the expression of some IEGs (*Nr4a1*, *Fos*, *Egr1*, *Npas4*, and *Cyr61*) was significantly increased in two-month-old mice that ingested GT-catechin for one month. Increased expression of three of these IEGs (Fos, Egr-1, and Npas4) plays a key role in long-term synaptic plasticity [44]. In addition, Npas4 is thought to regulate excitatory and inhibitory balance within circuits. Nr4a1 is a key component that regulates the density and distribution of spines and synapses [45], and has been reported to be involved in the suppression of age-related decline in brain function [46]. Furthermore, Cyr61 is needed for the dendritic arborization of hippocampal neurons [30]. Increased expression of these IEGs is considered to increase synaptic plasticity, leading to the maintenance and improvement of learning and memory ability. IEGs are endogenous genes whose expression is first induced in response to extracellular stimuli, and their expression is widely used as a marker of neural activity. Increased expression of IEGs was more pronounced in the hippocampus than in the prefrontal cortex, suggesting that it is important for hippocampal function. The transcription of many IEGs in neurons is initiated by calcium ion influx associated with synaptic activity and action potential [47]. It has also been reported that EGCG modulates calcium signals in hippocampal neurons [48,49]. These data suggest that EGCG incorporated into the hippocampus stimulates IEG expression by causing an increase in intracellular calcium ions in nerve cells.

In conclusion, GT-catechin, mainly EGCG, suppressed age-related cognitive decline and lifespan shortening. The expression of some IEGs was increased in the hippocampi of mice that ingested GT-catechin. The common factor that causes increased expression of these IEGs may be an increase in calcium ions in neurons triggered by EGCG.

4. Materials and Methods

4.1. Animals

Four-week-old male SAMP10/TaSlc (SAMP10) mice were purchased from Japan SLC Co. Ltd. (Shizuoka, Japan) and kept in conventional conditions in a temperature- and humidity-controlled room with a 12 h–12 h light–dark cycle (light period, 08:00–20:00; temperature, 23 ± 1 °C; relative humidity, 55 ± 5%). Mice were fed with a normal diet (CE-2; Clea Co. Ltd., Tokyo, Japan) and water ad libitum. Mice were housed in groups of 6 per cage. All experimental protocols were approved by the University of Shizuoka Laboratory Animal Care Advisory Committee (approval no. 136068) and were in accordance with the guidelines of the US National Institutes of Health for the care and use of laboratory animals.

4.2. Experimental Design

For the experiment, 180 mice were prepared and divided into 6 groups containing a control. Six mice were housed per cage. These mice were used for measurement of cognitive function and longevity. Three groups of 36 mice each consumed GT-catechin (Sunphenone BG; Taiyo Kagaku Co. Ltd., Yokkaichi, Japan) in water at concentrations of 0, 50, and 200 μg/mL from 1 month of age. These mice (body weight 30–35 g) drank about 10 mL of water daily. This intake of GT-catechin corresponds to 0, 15, and 60 mg/kg, respectively. Another 3 groups of 24 mice each consumed GT-catechin in water at concentrations of 3.3, 17, and 100 μg/mL from 1 month of age. These concentrations correspond to 1, 5, and 30 mg/kg, respectively. Mice consumed GT-catechin solution, which was freshly prepared twice a week. The amount of GT-catechin water consumed by mice was measured and the GT-catechin intake was calculated.

Another 48 mice were used for DNA microarray analysis and quantitative real-time reverse transcription PCR (qRT-PCR). The mice consumed GT-catechin in water at a concentration of 200 μg/mL (60 mg/kg). Sunphenone BG contains several catechins: 40.7 w/w% EGCG, 17.4 w/w% EGC, 12.3 w/w% ECG, 7.6 w/w% EC, 3.1 w/w% gallocatechin, 1.9 w/w% catechin, and 1.7 w/w% gallocatechin gallate. The remaining portion consists of some other catechins from green tea. Sunphenone BG does not contain caffeine.

4.3. Memory Acquisition and Retention Tests

A step-through passive avoidance task was carried out using 11-month-old mice as described previously [1,2]. In brief, when a mouse entered a dark chamber from a light chamber, the door was closed and an electric foot-shock was delivered at 50 μA for 1 s (SGS-003, Muromachi Kikai Co., Ltd., Tokyo, Japan). Acquisition of the avoidance response was judged as successful if the mouse remained in the light chamber for 300 s. The trial was repeated until the mouse satisfied the acquisition criterion within 5 trials. The time that a mouse could not stay in the light chamber, i.e., the time spent in the dark chamber in a 300 s trial, was recorded. The results from successive trials were summed for each mouse to give a measure of the time required to learn not to enter the light chamber (i.e., "learning time"). One month later, the same test was performed on the mice. If the mouse was able to stay in the light chamber for 300 s, retention of the avoidance response was determined to be successful. The ratio was obtained as memory-retained mice/tested mice.

4.4. Working Memory

Searching behavior was observed in a Y-maze (MYM-01M; Muromachi Kikai Co., Ltd.). The positions of arms that a mouse entered and the number of times it entered them was observed for 8 min, as described previously [1]. "Alternation behavior," i.e., entering the 3 different arms successively, is considered to reflect working memory capacity. The number of occasions on which spontaneous alternation behavior was observed was counted and the "ratio of alternation" was calculated as follows: (number of arm entries showing spontaneous alternation)/(total number of arm entries - 2).

4.5. Measurement of DNA Microarray and Principal Component Analysis

The mice were housed in groups of 6 for 1 month and ingested water containing green tea catechin or nothing (control). Every three mice 2 months of age were anesthetized with isoflurane and blood was removed from the jugular vein. The hippocampus was removed and frozen immediately. Total RNA was extracted from the hippocampus using an RNeasy Mini Kit (74104, Qiagen, Valencia, CA, USA). Total RNA was processed to synthesize biotinylated cRNA using One-Cycle Target Labeling and Control Reagents (Affymetrix, Santa Clara, CA, USA) and then hybridized to a Total RNA Mouse Gene 1.0 ST Array (Affymetrix), with 3 biological repeats per group. Raw data were parametrically normalized [50]

by using the SuperNORM data service (Skylight Biotech Inc., Akita, Japan). The significance of GT-catechin ingestion was statistically tested by two-way ANOVA [51] at $p < 0.001$.

To compare the effects of GT-catechin ingestion, we performed principal component analysis (PCA) [52] on ANOVA-positive genes [53]. To reduce the effects of individual variability among samples, the axes of PCA were estimated on a matrix of each group's sample means and applied to all data, which were centered using the sample means of control mice.

4.6. Quantitative Real-time Reverse Transcription PCR (qRT-PCR)

Mice 2, 6, and 12 months of age that ingested water containing GT-catechin (60 mg/kg) or not were used for this analysis. Mice were anesthetized with isoflurane and blood was removed from the jugular vein. The brain was carefully dissected and the hippocampus and prefrontal cortex were immediately frozen. The brain sample was homogenized, and total RNA was isolated using a purification kit (NucleoSpin®®® RNA, 740955, TaKaRa Bio Inc., Shiga, Japan) in accordance with the manufacturer's protocol. The obtained RNA was converted to cDNA using the PrimeScript®®® RT Master Mix kit (RR036A, Takara Bio Inc.). qRT-PCR analysis was performed using the PowerUp™ SYBR™ Green Master Mix (A25742, Applied Biosystems Japan Ltd., Tokyo, Japan) and automated sequence detection systems (StepOne, Applied Biosystems Japan Ltd.). Relative gene expression was measured by previously validated primers for *Egr2* [54], *Arc* [55], *Nr4a1* [56], *Fos* [57], *Egr1* [58], *Npas4* [59], and *Cyr61* [60] genes (Table 4). cDNA derived from transcripts encoding β-actin was used as the internal control.

Table 4. Primer sequences for qRT-PCR.

Gene	Forward Sequence (5′-3′)	Reverse Sequence (5′-3′)	Ref.
Egr2	CTACCCGGTGGAAGACCTC	AATGTTGATCATGCCATCTCC	[54]
Arc	ACGATCTGGCTTCCTCATTCTGCT	AGGTTCCCTCAGCATCTCTGCTTT	[55]
Nr4a1	CTGCCTTCCTGGAACTCTTCA	CGGGTTTAGATCGGTATGCC	[56]
Fos	AAGTAGTGCAGCCCGGAGTA	CCAGTCAAGAGCATCAGCAA	[57]
Egr1	CCTTCCAGTGTCGAATCTGCAT	ACAAATGTCACAGGCAAAAGGC	[58]
Npas4	AGCATTCCAGGCTCATCTGAA	GGCGAAGTAAGTCTTGGTAGGATT	[59]
Cyr61	CCCCCGGCTGGTGAAAGTC	ATGGGCGTGCAGAGGGTTGAAAAG	[60]

4.7. Statistical Analysis

Statistical analysis for cognition activity was performed using one-way ANOVA. Confidence intervals and significance of differences in means were estimated by using Tukey's honest significant difference method. Fisher's exact probability test was used for qRT-PCR. After calculating the survival rate by the Kaplan–Meier method, the difference in survival rate was tested by the log rank test.

5. Conclusions

This study reveals that intake of GT-catechin at a dose of 1 mg/kg or more per day tends to improve both age-related cognitive decline and lifespan shortening. GT-catechin, mainly EGCG, suppressed age-related cognitive decline via the increased expression of some IEGs in the hippocampus.

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Sample Availability: Samples of the compounds ..... are available from the authors.

molecules

MDPI

Article

Antidepressant Effect of Shaded White Leaf Tea Containing High Levels of Caffeine and Amino Acids

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Abstract: The young leaves of green tea become lighter in color than usual when protected from sunlight by a shading net for about two weeks while growing. These leaves are called "shaded white leaf tea" or SWLT. In the eluate of SWLT, the amount of amino acids (361 mg/L) was significantly higher than that in regular tea (53.5 mg/L). Since theanine and arginine, the first and second most abundant amino acids in SWLT, have significant antistress effects, we examined the antistress effect of SWLT on humans. SWLT or placebo green tea (3 g) was eluted with room-temperature water (500 mL). Participants consumed the tea for one week prior to pharmacy practice and continued for 10 days in the practice period. The state-trait anxiety inventory, an anxiety questionnaire, tended to be scored lower in the SWLT group than the placebo, but other stress markers showed no differences. The effect of the difference in SWLT components examined with mice showed that aspartic acid and asparagine, which are abundant in SWLT, counteracted the antistress effects of theanine and arginine. Large amounts of caffeine also interfered with SWLT's antistress effect. Thus, SWLT, which is high in caffeine and amino acids, suppressed depressant behavior in mice.

Keywords: antidepressant effect; antistress effect; asparagine; aspartate; caffeine; clinical study; green tea; salivary α-amylase; theanine

1. Introduction

Tea (*Camellia sinensis* (L.) Kuntze) shoots are rich in theanine, one of the umami components of tea. However, when the leaves are exposed to direct sunlight, theanine (L-theanine, Thea) is used for the synthesis of catechins and other compounds as a nitrogen source [1]. An increase in catechins and a decrease in theanine causes an increase in astringency. On the other hand, tea strains in which the green tea shoots turn yellowish-white are generally called white leaf tea. These are reported to be excellent in terms of aroma and taste according to sensory tests, because they have lower amounts of chlorophyll and catechins, and a higher amount of free amino acids, than regular green tea [2]. However, these cultivars have disadvantages, such as low yield and difficulties with propagation.

Therefore, a technique has been developed in which the green tea leaves of a general cultivar, such as "Yabukita," are turned yellowish-white by completely protecting them from light [3]. If the young leaves are protected from sunlight with a shading net for about two weeks, the decomposition of theanine is suppressed, resulting in green tea with an increased amount of amino acids compared to normal green tea [4]. Green tea prepared in this way is called "shaded white leaf tea," or SWLT. The amino acids in shoots with 100% shading are more than doubled as compared with the levels before their covering. Theanine (Thea), which is an amino acid that accounts for about half of the total free amino acids in tea leaves, increases 1.3-fold with shading. Arginine (Arg), the second most common amino acid, increases 2.5-fold with shading. The amount of serine (Ser) and asparagine (Asn) in the tea leaves is small, but increases by 3.8- and 16.9-fold, respectively, with shading [4].

Many studies have shown that Thea exhibits an excellent antistress effect [5–8]. In addition, arginine (Arg), which is the next most abundant amino acid after Thea, also exhibits an excellent antistress effect, similar to the effect theanine has on mice [9]. Therefore, in this study we investigated the antistress effect of SWLT, which is rich in Thea and Arg, in humans.

On the other hand, tea shoots have a high amount of caffeine. Previous studies have shown that the antistress effects of Thea and Arg are counteracted by caffeine and epigallocatechin gallate (EGCG) [9]. We made a low-caffeine green tea, and examined the antistress effect on humans. The results showed that stress was reduced in participants in their 20s, 40–50s, and 90s [10–12]. Matcha green tea is rich in Thea, Arg, and caffeine. When the green tea ingredients were mixed with powdered feed, as in the case of matcha green tea, animal experiments clearly showed that antistress effects were not observed if the molar ratio (of caffeine and EGCG/Thea and Arg, CE/TA) was 2 or more [13]. In addition, participants in their 20s showed reduced stress with matcha with a CE/TA ratio of 1.7, but not with matcha with a ratio of 10 [13]. These data indicate that the ratio of tea components, along with Thea content, is very important.

SWLT has a high Thea and Arg content and is below a CE/TA ratio of 2, so it is expected to show an antistress effect. According to a survey by the Japanese Ministry of Health, Labor and Welfare, about 60% of men and women experience significant stress at work. Green tea is the most common beverage that people drink daily in Japan, so it would be very meaningful if it was scientifically revealed that the intake of green tea helps to maintain good mental health. Therefore, we first examined whether SWLT can reduce stress, because of the high amounts of Thea and Arg in SWLT. However, we found that the stress-reducing effect of SWLT in students was about the same as a placebo green tea. Next, we investigated the reason for this unexpected result for SWLT using an animal psychosocial stress model, and based on this data, we examined the antidepressant effect of SWLT.

Depression is one of the most common psychiatric disorders, and stress is an important risk factor for depression [14]. Tea consumption is shown to reduce the risk of depression [15], and green tea catechins have been shown to decrease depressive syndromes in experimental animals [16]. Thea has been reported to have beneficial effects on depressive syndromes, anxiety, and sleep disturbance in patients with a major depressive disorder [17]. In addition, caffeine is suggested to be a therapeutic agent for motivational dysfunction in depression [18]. Therefore, we evaluated the antidepressant effect of SWLT containing high levels of caffeine and amino acids.

2. Results

2.1. Content of Theanine, Caffeine, and Catechins in SWLT and Placebo Green Tea

The content of amino acids in SWLT was much higher than in the placebo green tea in the eluate derived from steeping for 3 h (Table 1). The placebo green tea was a medium-grade common green tea. Caffeine was about two times higher in SWLT than in the placebo green tea. Although the contents of EGCG were similar between SWLT and the placebo green tea, the content of EGC was higher in the placebo green tea than SWLT.

Table 1. The contents of caffeine, catechins, and amino acids in the eluate of SWLT and placebo tea.

Tea	Caffeine (mg/L)	Catechins (mg/L)						
		EGCG	EGC	ECG	EC	GC	CG	(+)C
SWLT	209.8	150.4	135.2	24.6	41.0	5.0	2.8	3.4
Placebo	112.0	134.2	229.0	21.0	46.6	13.6	4.6	2.0
Tea	**Free amino acids (mg/L)**							
	Thea	**Arg**	**Gln**	**Asn**	**Asp**	**Glu**	**Ser**	**GABA**	**Total**
SWLT	140.2	69.9	51.7	33.8	33.5	19.3	12.6	0	361.0
Placebo	28.8	5.4	3.9	0.7	5.5	6.9	2.2	0	53.5

Shaded white leaf tea (SWLT) and placebo green tea (3 g) were steeped in 500 mL of room-temperature water for 3 h. EGCG, (−)-epigallocatechin gallate; EGC, (−)-epigallocatechin; ECG, (−)-epicatechin gallate; EC, (−)-epicatechin; CG, (−)-catechin gallate; (+) C, (+)-catechin; Thea, theanine; Arg, arginine; Gln, glutamine; Asn, asparagine; Asp, aspartic acid; Glu, glutamic acid; Ser, serine; GABA, γ-aminobutyric acid.

Since the participants ingested 500 mL of SWLT or placebo green tea, the participants that ingested SWLT consumed about 70 mg of Thea and 35 mg of Arg per day, and the participants who ingested the placebo tea consumed about 29 mg Thea and 5 mg of Arg per day. When the CE/TA ratio, which is an index of the antistress effect in the case of matcha, was also applied to the SWLT and placebo green tea, SWLT scored 1.12 and the placebo tea scored 4.47.

2.2. Levels of Salivary Amylase Activity (sAA), State-Trait Anxiety Inventory (STAI) Value, Physical Condition, Subjective Stress, Achievement Emotion, and Sleeping Time in Students

The level of sAA is usually low at the time of waking up but becomes high as a result of sympathetic excitement during the day [19,20]. In both groups, the level of post-practice sAA tended to be higher than the level of pre-practice sAA, but not significantly so. However, there was no significant difference in sAA levels between the SWLT and placebo tea groups in pre-practice and post-practice during routine daily life at the university and pharmacy practice (Table 2).

Table 2. Effect of SWLT intake on each test item.

Test item	Practice		SWLT	Placebo tea	*p*
sAA	University	Pre-practice	28 ± 4	24 ± 3	0.484
		Post-practice	45 ± 3	47 ± 3	0.645
	Pharmacy	Pre-practice	28 ± 4	22 ± 3	0.231
		Post-practice	46 ± 7	34 ± 5	0.292
STAI value		Before the pharmacy practice	45 ± 3	47 ± 3	0.645
		After the pharmacy practice	40 ± 3	46 ± 3	0.065
Physical condition (score 1–5)		University	2.5 ± 0.2	2.7 ± 0.1	0.482
		Pharmacy	2.6 ± 0.1	2.6 ± 0.1	0.828
Subjective stress (VAS: 0–10)		University	4.2 ± 0.3	4.6 ± 0.3	0.395
		Pharmacy	4.6 ± 0.4	4.6 ± 0.3	0.765
Achievement emotion (score 1–5)		Pharmacy	2.4 ± 0.2	2.5 ± 0.2	0.567
Sleeping time (h)		University	6.5 ± 0.1	6.4 ± 0.2	0.536
		Pharmacy	6.4 ± 0.2	6.5 ± 0.2	0.508

These data represent the mean ± standard error of the mean (SEM) (SWLT $n = 24$, placebo $n = 24$).

Next, the STAI values were examined to assess anxiety based on appraisal standards. The values were no different between the SWLT and placebo groups before the pharmacy practice. On the other hand, the values tended to be lower in the SWLT than in the placebo group after the pharmacy practice ($p = 0.065$). Physical condition was not different between the two groups during routine daily life at

the university and pharmacy practice. Subjective stress was evaluated by each participant at the end of daily practice using visual analogue scales (VAS: 0–10). The average score was not different between the two groups (Table 2). Sense of achievement was evaluated by participants as an ordinal scale at the end of the daily pharmacy practice. There was no difference between the average of both groups (Table 2). The average sleeping time was not different between the SWLT and placebo groups during routine daily life at the university and pharmacy practice.

2.3. Antistress Effects of SWLT in a Mouse Model of Psychosocial Stress

The relationship between the intake of tea components from SWLT and the suppression of stress was examined in a mouse model. SWLT has much higher amounts of free amino acids and a different composition ratio compared with placebo green tea. When compared in molar ratios, Thea accounted for about half of the total free amino acids in placebo green tea, while it was only one-third for SWLT. On the other hand, the proportion of Arg, Gln, Asn, and Asp increased (Figure 1). Therefore, the effect of these amino acids on the stress response of mice was examined.

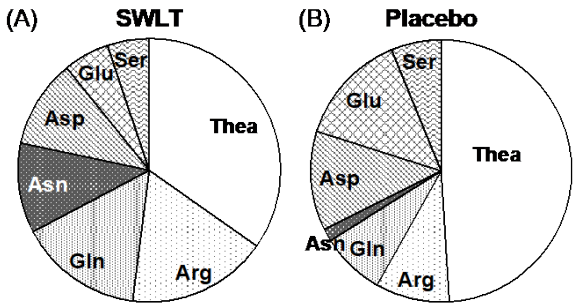

Figure 1. Molar ratio of free amino acids in SWLT and placebo green tea. (**A**) SWLT, (**B**) Placebo green tea.

Adrenal hypertrophy was observed in mice stressed by confrontational housing, but not in mice that ingested Thea under stressed conditions. When the mice ingested Arg and Gln with Thea, no hypertrophy was observed, but when the mice also ingested Asn and Asp with Thea, Arg, and Gln, the adrenal gland was significantly enlarged in mice under stressed conditions (Figure 2).

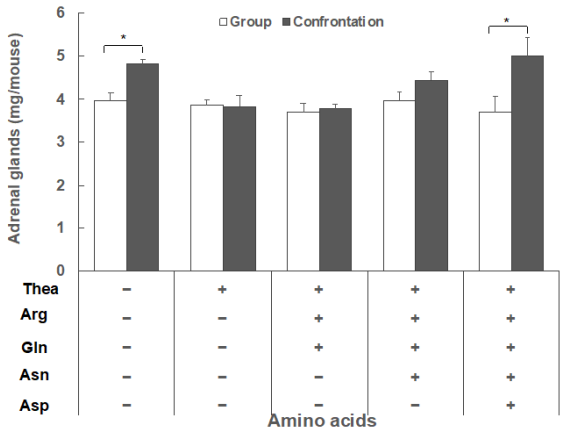

Figure 2. Effect of amino acid composition on the stress response in mice. Each amino acid concentration is the same in SWLT, as follows: Thea 140 mg/L, Arg 70 mg/L, Gln 52 mg/L, Asn 34 mg/L, and Asp 34 mg/L. Two mice were housed in a partitioned cage for six days (single housing). Then, the partition was removed and subsequently the two mice cohabited the same cage for one day (confrontational housing). Group housing mice were housed in groups of four. These mice ingested water containing amino acids ad libitum. Data are shown as mean ± SEM (n = 4–8, * $p < 0.05$).

Furthermore, the effects of caffeine and EGCG on Thea and Arg were also examined. Thea, Arg, caffeine and EGCG, in the same composition ratio as in SWLT or placebo tea, were added to the drinking water and allowed to be taken freely. As a result, adrenal hypertrophy was not sufficiently suppressed. The molar ratio of caffeine and EGCG to Thea and Arg (CE/TA) was 1.12 for SWLT and 4.47 for placebo tea. When caffeine and EGCG were reduced to a CE/TA ratio of 0.42, adrenal hypertrophy was significantly suppressed (Figure 3).

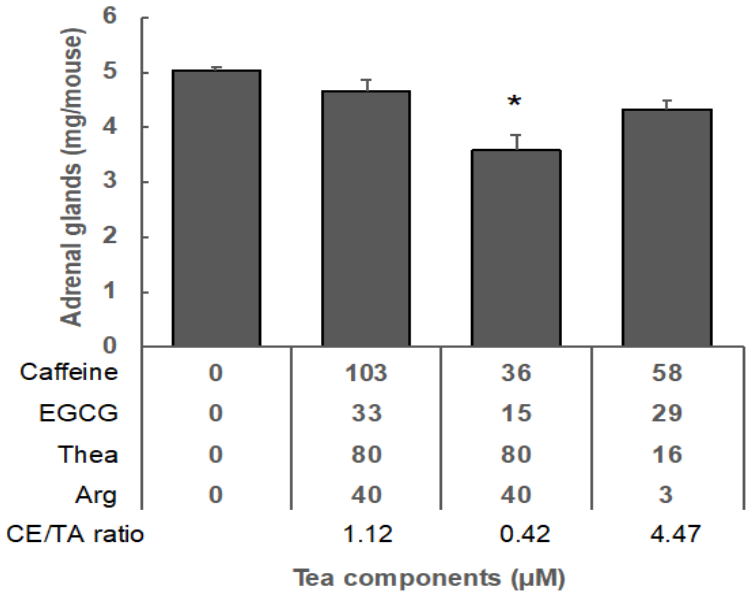

Figure 3. Effect of tea components on the stress response in mice under confrontational housing conditions. After single housing for six days, the mice were housed confrontationally for one day. These mice were separated into four groups, as follows: Group 1; control. Group 2; mice ingested water containing the same concentrations of caffeine (103 μM), EGCG (33 μM), Thea (80 μM) and Arg (40 μM) as SWLT (CE/TA = 1.12). Group 3; mice ingested water containing caffeine (36 μM), EGCG (15 μM), Thea (80 μM), and Arg (40 μM). The CE/TA ratio of this composition was 0.45. Group 4; mice ingested water containing the same concentrations of caffeine (58 μM), EGCG (29 μM), Thea (16 μM), and Arg (3 μM) as in placebo green tea (CE/TA = 4.47). Data are shown as mean ± SEM (n = 4, * $p < 0.05$).

It has been confirmed that similar results can be obtained at different concentrations if the CE/TA ratio is the same [9]. However, it was newly revealed that Asn and Asp act antagonistically to the antistress effect of Thea (Figure 2). Therefore, to clarify the role of Asp in the antistress effect of SWLT, the effect of the coexistence of Asp with caffeine, EGCG, Thea, and Arg was investigated. Even if Asp coexisted, adrenal hypertrophy was suppressed if the CE/TA ratio in the drinking water was 0.5 or less (Figure 4). The antagonistic effect of Asp on Thea and Arg was found to be weaker than that of caffeine and EGCG.

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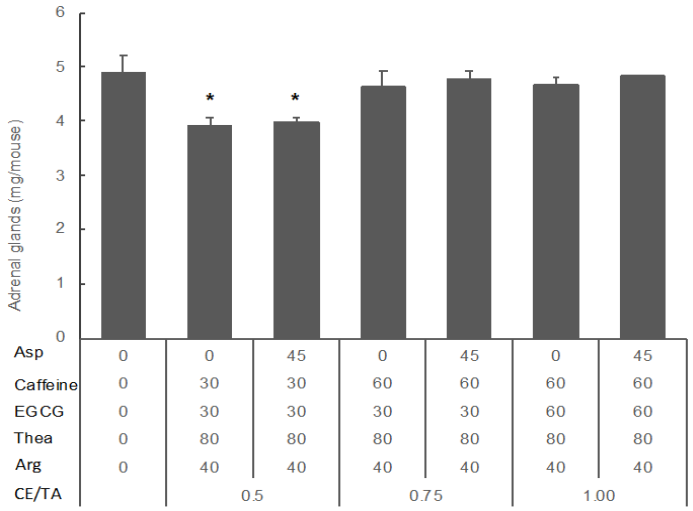

Figure 4. Effect of tea components on the stress response in mice under confrontational housing conditions. After single housing for six days, the mice were housed confrontationally for one day. These mice ingested water containing Asp (45 μM), caffeine (30 or 60 μM), EGCG (30 or 60 μM), Thea (80 μM), and Arg (40 μM). Data are shown as mean ± SEM (n = 3–4, * $p < 0.05$).

2.4. Antidepressant Effects of SWLT

Senescence-accelerated mouse prone 10 (SAMP10) mice are reported to exhibit depression-like behavior [21,22]. These mice were fed SWLT or placebo green tea as drinking water for one month and evaluated by the tail suspension test. A longer immobility time indicates stronger depressive behavior. The immobility behavior was significantly shorter in mice that ingested SWLT compared to the control mice (Figure 5). Depressive behavior tended to be suppressed similarly in mice that ingested placebo tea.

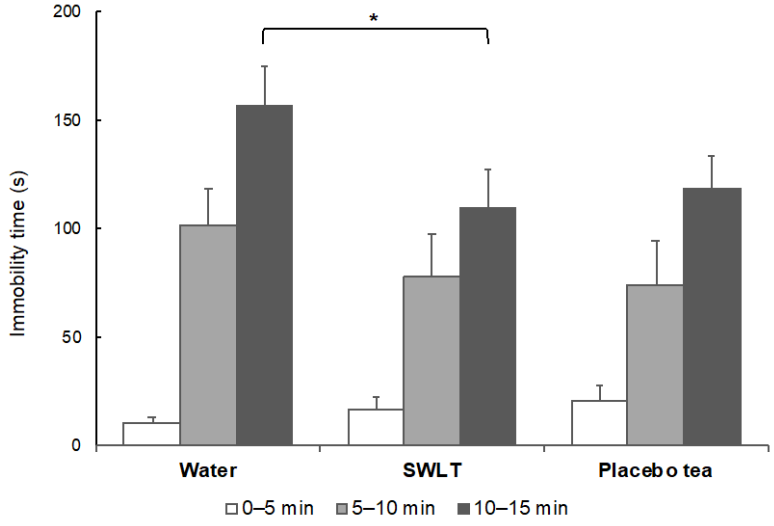

Figure 5. Effect of SWLT ingestion on depression-like behavior of SAMP10 mice. These mice ingested SWLT or placebo green tea as drinking water for one month and were evaluated using the tail suspension test. The control mice drank water. Data are shown as mean ± SEM (n = 5–6, * $p < 0.05$).

3. Discussion

Since it was clarified that the stress-reducing effect of Thea was increased by Arg and suppressed by caffeine and EGCG [9], the stress-reducing effect of green tea has been evaluated using green tea

with lowered caffeine, and matcha that is rich in Thea and caffeine [10–13]. Based on these results, it has been clarified that matcha tea with a CE/TA molar ratio of 2 or less has a stress-reducing effect [13]. As the SWLT used in this experiment contained a large amount of Thea and had a CE/TA ratio of 1.21, a stress-reducing effect was expected. In clinical studies, anxiety, as assessed by the STAI value, tended to be lower with intake of SWLT than the placebo (regular green tea), but the grade of stress measured by sAA or subjective stress was not different between the SWLT and placebo groups. These data suggested that the stress-reducing effect of SWLT was not particularly strong. Therefore, we conducted animal experiments in order to clarify why the stress-reducing effect of SWLT was not as strong as expected, despite very high levels of Thea and Arg.

Thea synthesized in the roots is transferred to the stems and leaves, and is then metabolized to polyphenols—mainly catechins—under light conditions [1]. However, in the dark, this metabolism is suppressed, resulting in Thea accumulation. On the other hand, strong shading causes growth suppression and metabolism changes in tea leaves. Therefore, shading the tea leaves increases the amino acid content [3] but causes a large change in the ratio of each amino acid. The proportion of Thea declined, while the proportions of Arg, Gln, Asn, and Ser increased significantly (Figure 1). The increase in these amino acids is thought to be due to degradation of the soluble protein [4]. Arg has an antistress effect, but Gln and Glu have no effect [9]. Since aspartate is known to act as an excitatory neurotransmitter, similar to Glu [23], we examined whether Asn and Asp, which are abundant in SWLT, affect the antistress activity of Thea. When amino acids were present in the proportions contained in the SWLT, the stress-reducing effect of Thea was not affected in the presence of Arg and Gln, but was canceled by the addition of Asn and Asp (Figure 2). This indicates that the high ratio of other amino acids to Thea, particularly Asn and Asp, is one reason for the low antistress effect of SWLT.

In matcha, a very fine powdered type of green tea, the typical stress response, adrenal hypertrophy, is suppressed in mice if the molar ratio of caffeine and EGCG to Thea and Arg (CE/TA) is 2 or less. In the case of infused green tea with water, the effect of reducing stress was observed in humans when the CE/TA ratio was 0.54 or less [10], but was not observed when the ratio was 0.9 or more [10]. The SWLT used in this study had a CE/TA ratio of 1.12, and when the CE/TA ratio was reduced to 0.45, a stress-reducing effect was observed in mice (Figure 3). From these results, it was clarified that, though the amount of theanine was high in the SWLT, Asp and caffeine were also high, so the expected stress-reduction effect could not be obtained. However, the antidepressant effect of SWLT was significantly higher than in the control. A similar effect was observed in mice that ingested placebo green tea. Tea consumption has been reported to be associated with an antidepressant effect [15]. The amount of caffeine in tea may be an important factor for the antidepressant effect [18]. As long-term stress causes anxiety and depression, the antidepressant effect of SWLT and green tea may be of value in stressful modern societies.

In addition, one of the causes of stress-induced depression may be an imbalance between the excitatory neurotransmitter Glu and the inhibitory neurotransmitter GABA [24]. Changes in Glu and GABA balance cause changes in neuronal excitability, synaptic plasticity, and normal central nervous system function [25]. Therefore, modulating the balance between Glu and GABA may improve stress-induced anxiety and depression.

In the hippocampus of mice that ingested Thea (6 mg/kg) in drinking water for two weeks, the level of Glu was significantly reduced, and, conversely, the level of GABA increased [26]. GABA is synthesized by the decarboxylation of Glu [27]. Altered glutamate decarboxylase (GAD) activity causes an altered GABA level and excitatory/inhibitory balance, but it is not yet known how Thea is involved in regulating GAD activity. However, we recently found that the expression level of the transcription factor neuronal PAS domain protein 4 (*Npas4*) increased in mice that ingested Thea [28]. Since Npas4 regulates the formation and maintenance of inhibitory synapses in response to excitatory synaptic activity, the increased expression of Npas4 by Thea suggests increased GABA release [29]. In addition, Thea inhibits Glu uptake from the Gln receptor, resulting in the inhibition of Glu release [29,30]. That is, when incorporated into the brain, Thea modulates the Glu/GABA balance. On the other hand, caffeine

increases the level of Glu in the brain, but not the GABA level [31], and EGCG facilitates Glu release [32]. In addition, EGCG has been reported to suppress overexpression of the GABA pathway and to inhibit GABA by modulating the $GABA_A$ receptor [33–35]. From these results, Thea, caffeine, and EGCG are considered to influence the Glu/GABA balance in the brain. Furthermore, Asp works as an excitatory neurotransmitter, while Arg reduces physical stress and anxiety through nitric oxide synthesis [36,37]. Arg has also been reported to have an antidepressant effect in rats through increased expression of brain-derived neurotrophic factor [38]. Taken together, these green tea components cause changes in the excitatory/inhibitory balance in the brain due to differences in their composition. That is, SWLT may have antidepressant effects rather than an antistress effect similar to that of regular green tea due to its high levels of caffeine and amino acids. Ketamine, a fast-acting antidepressant, is considered to play a crucial role in the glutamatergic system [39]. Thus, the composition balance of SWLT may have a major impact on the glutamatergic system. A further detailed study is required, including of the interactions between SWLT components.

4. Materials and Methods

4.1. Effect of SWLT Ingestion on Humans

Tea (*Camellia sinensis* (L.) *Kuntze*) leaves were collected in Shizuoka, Japan in May. About two weeks before harvesting, the leaves were protected from direct sunlight with a shading net [3]. Then, the tea leaves were made into green tea through the usual process. We termed this shaded white leaf tea, or SWLT. One tea bag of SWLT or placebo (normal sencha) tea (3 g of tea in a bag) was steeped in 500 mL of room-temperature water. Tap water was used in this experiment. The participants prepared SWLT or placebo tea every morning and ingested it by the evening. The tea bag was left in the water until the evening. Similarly, after each day's pharmacy practice, the participants drank these teas. For the measurement of the tea component in the eluate, tea leaves of SWLT (3 g) and placebo tea were steeped in 500 mL of room-temperature water for 3 h and stirred occasionally.

4.2. Measurement of the Tea Components by HPLC

The eluates of the SWLT and placebo tea were measured by HPLC, as described previously [9]. In brief, catechins and caffeine in the eluates were measured by HPLC (SCL-10Avp, Shimadzu, Japan; Develosil packed column ODS-HG-5, 150 × 4.6 mm, Nomura Chemical Co. Ltd., Seto, Japan) according to the method of Horie et al. [40]. Catechins and caffeine were measured at 280 nm. Free amino acids in the tea leaves were measured by HPLC as described above, using glycylglycine as an internal standard [41]. Amino acids were detected at an excitation wavelength of 340 nm and at an emission wavelength of 450 nm (RF-535 UV detector, Shimadzu, Japan).

4.3. Participants

Forty-eight healthy fifth-year students from the University of Shizuoka participated in the experiment, and were randomly divided into two groups by sex: SWLT (n = 24, 11 men and 13 women; average age 22.7 ± 1.1 y) and placebo (n = 24, 11 men and 13 women; average age 22.4 ± 0.9 y). They received SWLT or placebo tea bags in sealed envelopes. The participants were assigned to practice outside the university, in a hospital or a dispensing pharmacy, for 11 weeks. The first 10 days of the practice program were analyzed, because these days were assumed to be the most stressful. None of the participants reported having an acute or chronic disease, regular medication intake, or habitual smoking. They were instructed to mainly drink the test tea, and not to consume other theanine- and caffeine-rich beverages such as green tea, coffee, and black tea throughout the experiment. They could drink water freely, but they did not consume alcohol at night. The study was conducted in accordance with the Declaration of Helsinki and Ethical Guidelines for Medical and Health Research Involving Human Subjects (Public Notice of the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare, 2008). The study protocol was approved by the

Ethics Committee of the University of Shizuoka (No. 29-57). All the participants received verbal and written information about the study and signed an informed consent form before entering the study. This study was registered at the University Hospital Medical Information Network (UMIN) (registration ID no. UMIN 000031778). The study period was April to May 2018.

4.4. Procedure

This study was a group comparison design and the participants were randomly assigned to the SWLT or placebo tea groups. The participants did not know whether they were consuming SWLT or placebo tea, because they had no information about the SWLT's aroma and taste. The intake of SWLT or placebo tea was from one week prior to pharmacy practice and continued for 10 days into the practice period, for a total of 17 days. All the collected data were used for the analysis. To assess the anxiety of the participants, the state-trait anxiety inventory (STAI) test (Japanese STAI Form X-1, Sankyobo, Kyoto, Japan) was carried out before and after their pharmacy practice. A questionnaire that included feedback on physical condition, subjective stress, and sense of achievement was assigned for 10 days after each day's practice. The physical condition of participants was assessed on an ordinal scale (5, very good; 4, good; 3, normal; 2, somewhat poor; 1, bad). Subjective stress was evaluated using visual analogue scales (VAS: 0–10), from very relaxed to highly stressed. Sense of achievement was assigned an ordinal scale (5, complete; 4, better; 3, a little better; 2, a little worse; 1, much worse) [8]. Sleep hours were also recorded.

4.5. Measurement of Salivary Amylase Activity (sAA)

An oral cavity enzyme, sAA, is generally used a marker of stress. To assess the physiological stress response, sAA was measured using a colorimetric system (Nipro Co., Osaka, Japan), as described previously [42]. Saliva was collected twice a day, in the morning after waking up (prepractice) and in the evening after pharmacy practice (postpractice), for 10 days during the practice. To establish a baseline of sAA before the pharmacy practice, the sAA of participants was measured every morning and evening for seven days during routine daily life at the university. The measurement was carried out before pharmacy practice. Prior to sampling, participants washed their mouths with water. After saliva was collected for 30 s using a sampling tip, each participant measured their own sAA immediately every morning and evening for 17 days.

4.6. Animals and the Stress Experiment

Male ddY mice (Slc: ddY, four weeks old) were purchased from Japan SLC Co. Ltd. (Shizuoka, Japan) and kept under conventional conditions in a temperature and humidity-controlled environment with a 12/12 h light/dark cycle (light period, 8:00 a.m.–8:00 p.m.; temperature, 23 ± 1 °C; relative humidity, 55 ± 5%). The four-week-old mice were housed in groups of four in a cage for five days to allow them to adapt to cohabitation. The mice were fed a normal diet (CE-2; Clea Co. Ltd., Tokyo, Japan) and water ad libitum. All experimental protocols were approved by the University of Shizuoka Laboratory Animal Care Advisory Committee (approval No. 195241) and were in accordance with the guidelines of the U.S. National Institute of Health for the Care and Use of Laboratory Animals. The mice were then divided into two groups: the confrontational group and group housing, according to a previously described method [7]. To apply psychosocial stress to the mice, confrontational housing was established in a standard polycarbonate cage (16 × 27 cm) that was divided into two identical subunits by a stainless-steel partition, as described previously [7]. In brief, two male mice were housed in a partitioned cage for six days (single housing) to establish territorial consciousness. Then, the partition was removed to expose the mice to confrontational stress for 24 h (confrontational housing). Each cage was placed in a Styrofoam box (width 30 cm, length 40 cm, height 15 cm) to avoid visual social contact between cages. At the end of the 24 h of confrontational housing, the mice were sacrificed and the adrenal glands were weighed.

4.7. Ingestion of Tea Components by Mice

The effect of the tea components on the stress response was examined in mice (four mice/group, $n = 84$). Two mice were housed in a partitioned cage for six days (single housing). Then, the partition was removed and subsequently the two mice cohabited the same cage (confrontational housing). The mice consumed tea components in water ad libitum for seven days (single housing for six days and confrontational housing for one day). Mouse body weight was measured on the last day of the experiment. Group housing mice were used as a control. In SWLT, Gln, Asn and Asp were abundant compared to the placebo tea. Based on the amino acid concentration in the SWLT eluate (Table 1), the relationships among amino acids were examined. Each amino acid concentration was as follows: Thea 140 mg/L, Arg 70 mg/L, Gln 52 mg/L, Asn 34 mg/L, and Asp 34 mg/L. The stress-reducing effect on adrenal hypertrophy was compared among these groups as follows: Group 1, no amino acids; Group 2, Thea; Group 3, Thea, Arg, and Gln; Group 4, Thea, Arg, Gln, and Asn; Group 5, Thea, Arg, Gln, Asn, and Asp.

Next, the effects of caffeine and EGCG on the antistress effect of Thea and Arg were examined in mice under confrontational housing conditions. These mice were separated into four groups as follows: Group 1; control. Group 2; mice ingested water containing the same concentrations of caffeine (103 μM), EGCG (33 μM), Thea (80 μM) and Arg (40 μM) as SWLT (CE/TA = 1.12). Group 3; mice ingested water containing caffeine (36 μM), EGCG (15 μM), Thea (80 μM), and Arg (40 μM). The CE/TA ratio of this composition was 0.45. Group 4; mice ingested water containing the same concentrations of caffeine (58 μM), EGCG (29 μM), Thea (16 μM) and Arg (3 μM) as placebo tea (CE/TA = 4.47).

Furthermore, Asp was added, and the antagonistic effect of caffeine and EGCG against the antistress effect of Thea and Arg was investigated in mice under confrontational housing conditions. These mice were separated into seven groups, as follows: Group 1; control. Group 2; mice ingested water containing the same concentrations of caffeine (30 μM), EGCG (30 μM), Thea (80 μM) and Arg (40 μM) (CE/TA = 0.5). Group 3; Group 2 + Asp (90 μM). Group 4: mice ingested water containing caffeine (60 μM), EGCG (30 μM), Thea (80 μM), and Arg (40 μM) (CE/TA = 0.75). Group 5; Group 4 + Asp (90 μM). Group 6; mice ingested water containing the same concentrations of caffeine (60 μM), EGCG (60 μM), Thea (80 μM) and Arg (40 μM) (CE/TA = 1.0). Group 7; Group 6 + Asp (90 μM). The tea components used were as follows: L-theanine and EGCG (Taiyo Kagaku Co. Ltd., Yokkaichi, Japan), caffeine, Arg, Gln, Asp, and Asn (Wako Pure Chemical Co. Ltd., Osaka, Japan).

4.8. Tail Suspension Test

Male SAMP10/TaIdrSlc mice were purchased from Japan SLC (Shizuoka, Japan). The four-week-old mice were housed in a group of six in a cage. The mice were fed a normal diet (CE-2; Clea Co. Ltd., Tokyo, Japan) and water containing tea components of SWLT or placebo green tea ad libitum. The concentration of each component of SWLT or placebo green tea was matched to the intake per human body weight per day. These mice ingested SWLT or placebo green tea for one month. The tea components used, other than those mentioned in Section 4.7, were as follows: EGC and EC (Taiyo Kagaku Co. Ltd., Yokkaichi, Japan), Glu and Ser (Wako Pure Chemical Co. Ltd., Osaka, Japan). The control mice drank tap water. To investigate behavioral depression, the mice were individually suspended by their tails at a height of 30 cm using a clip for tail suspension (MSC2007; YTS Yamashita-Giken, Tokushima, Japan). The immobility behavior was observed for 15 min, as described previously [7].

4.9. Statistical Analysis

These results are expressed as the mean ± SEM. The influence of stress on sAA was evaluated using a one-way analysis of variance (ANOVA), followed by a Wilcoxon/Kruskal-Wallis post hoc test or Fisher's least significant difference test for multiple comparisons. In each analysis, a p-value < 0.05 was considered to be statistically significant.

5. Conclusions

The antistress effect of SWLY was compared with common green tea in a clinical study. However, the stress-reducing effect of SWLT in students was not high. Next, the effects on stress of caffeine and amino acids, which are abundant in SWLT, were examined in animal experiments. Furthermore, we observed the depression-like behavior of mice that ingested SWLT or common green tea. The results showed that SWLT, which has higher levels of caffeine and amino acids such as Thea, Arg, Gln, Asn, and Asp than common green tea, may have an antidepressant effect.

This study had some limitations. The participants were solely young students, so it is necessary to investigate participants of different ages. The students were tested under conditions of mild stress, but different stress conditions should be considered in the future.

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Sample Availability: Samples of the compounds are not available from the authors.

molecules

MDPI

Review

Anti-Cancer Effects of Green Tea Epigallocatchin-3-Gallate and Coffee Chlorogenic Acid

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Abstract: Tea and coffee are consumed worldwide and epidemiological and clinical studies have shown their health beneficial effects, including anti-cancer effects. Epigallocatechin gallate (EGCG) and chlorogenic acid (CGA) are the major components of green tea polyphenols and coffee polyphenols, respectively, and believed to be responsible for most of these effects. Although a large number of cell-based and animal experiments have provided convincing evidence to support the anti-cancer effects of green tea, coffee, EGCG, and CGA, human studies are still controversial and some studies have suggested even an increased risk for certain types of cancers such as esophageal and gynecological cancers with green tea consumption and bladder and lung cancers with coffee consumption. The reason for these inconsistent results may have been arisen from various confounding factors. Cell-based and animal studies have proposed several mechanisms whereby EGCG and CGA exert their anti-cancer effects. These components appear to share the common mechanisms, among which one related to reactive oxygen species is perhaps the most attractive. Meanwhile, EGCG and CGA have also different target molecules which might explain the site-specific differences of anti-cancer effects found in human studies. Further studies will be necessary to clarify what is the mechanism to cause such differences between green tea and coffee.

Keywords: cancer; tea; coffee; EGCG; chlorogenic acid; ROS; AMPK; NF-κB

1. Introduction

Green tea is produced by processing of leaves of the plant *Camellia sinensis* (Theaceae) and is popularly consumed worldwide. Green tea has been shown to have beneficial effects on human health such as anti-cancer, anti-obesity, anti-diabetic, anti-cardiovascular, anti-infectious and anti-neurodegenerative effects [1,2]. (−)-Epigallocatechin gallate (EGCG) is the most abundant catechin in green tea and believed to be mostly responsible for these biological effects (Figure 1). A cup of green tea typically brewed from 2.5 g of tea leaves contains 240–320 mg of catechins, of which EGCG accounts for 60–65% [3].

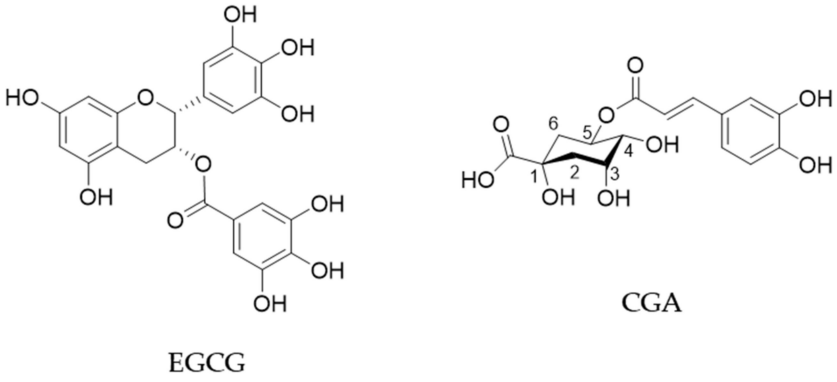

Figure 1. Chemical structures of EGCG and CGA.

Black tea is produced also from *C. sinensis* through enzymatic processing (so called fermentation) by intrinsic enzymes and microorganisms during which catechins can be polymerized to give catechin derivatives such as theaflavins and theasinensins [4]. Black tea has been shown to have physiological effects similar to those of green tea with lesser effects as compared with green tea due to its lower content of EGCG.

Coffee is also consumed worldwide and has various health effects. It contains about 2000 different chemicals and the major polyphenols are chlorogenic acid (CGA, Figure 1) and its derivatives which amount to about 3% *w/w* of roasted coffee powder [2,5]. A single cup of coffee may contain 20–675 mg of CGAs [6].

In this review, we discuss recent evidence from human studies to support the anti-cancer effects of consumption of green tea and coffee and mechanistic aspects of the actions of EGCG and CGA based on the results of cell-based and animal experiments. After the International Union of Pure and Applied Chemistry reversed the order of numbering of atoms on the quinic acid ring in 1976 and suggested the name 5-caffeoylquinic acid for chlorogenic acid instead of 3-caffeoylquinic acid [7,8], there has been some confusion in the nomenclature of chlorogenic acid. In this review, we use the term CGA according to the respective authors' description. Caffeine is contained abundantly in tea and coffee and may contribute to the anti-cancer effects of these beverages. However, cell-based and animal studies have shown that EGCG as well as CGA exert anti-cancer effects by themselves as shown below. Therefore, for the safe of clarity, the current review focuses on EGCG and CGA but excludes any discussion on caffeine, which has already been comprehensively reviewed [9–12].

2. Anti-Cancer Effects of Green Tea

2.1. Human Studies on Green Tea

Several epidemiological studies have shown the anti-cancer effects of consumption of tea. A survey in 2013 conducted by Yang and Hong of prospective cohort and case controlled studies which had been reported by 2008 revealed that green tea consumption showed risk-reduction in a total of 39 cases of breast, colon, esophagus, kidney/bladder, lung, ovary, pancreas, prostate, stomach cancers, whereas 46 cases showed no risk-reduction [1,13]. In the case of black tea, 28 and 92 cases showed risk-reduction and no risk-reduction, respectively, for these cancers [13]. These findings suggest that green and black teas have a preventive effect in some types of cancer.

When observational epidemiological studies were reviewed on over 1,100,000 participants from 46 cohort studies and 85 case-control studies [14], in three studies involving 52,479 participants, a lower overall cancer incidence (summary relative risk (RR) = 0.83, 95% confidence interval (CI) = 0.65–1.07) was found for the highest intake of green tea compared with the lowest consumption. For most of the site-specific cancers, a decreased RR was found by this comparison. However, results were conflicting,

since cohort studies in some cancer sites such as oesophageal, prostate and urinary tract cancer showed an increased RR. Table 1 added to show the effects of green tea on cancer, further explained in later text.

Table 1. Recent observational epidemiological studies on anti-cancer effects of green tea.

Cancer Type	Evaluation: Decrease (↓) or No Effect (+/−) in Cancer Risk	Hazard Risk (HR) or Odds Ratio (OR) or Relative Risk (RR) [Confidence Interval]	Note	Reference
Breast cancer	↓	HR = 0.82 [0.70–0.95] for ≥5 vs. 0 cups/day	Cohort study onwomen with family history of breast cancer	[15]
Breast cancer	↓	HR = 0.86 [0.75–0.99] for highest vs. lowest intake	Meta-analysis of 16 cohort and case-control studies	[16]
Breast cancer	↓	OR = 0.83 [0.72–0.96]	Meta-analysis of 14 case-control studies	[17]
Colorectal cancer	+/−		Cohort study on men and women	[18]
Colon cancer	↓	RR = 1.32 [0.90–1.94] for once/day vs. less than once/day RR = 0.76 [0.57–1.02] for 2–3 times/day RR = 0.78 [0.49–1.22] for ≥4 times/day	Cohort study on men	[18]
Head and neck squamous cell carcinoma	↓	OR = 0.29 [0.16–0.52] for <1 cup/day vs. no intake OR = 0.38 [0.17–0.86] for ≥1 cup/day vs. no intake	Case-control studyon men and women	[19]
Hematologic neoplasms	↓	HR = 0.65 [0.42–1.00] for ≤2 cups/day vs. no intake HR = 0.73 [0.47–1.13] for 3–4 cups/day vs. no intake HR = 0.63 [0.42–0.96] for ≥5 cups/day vs. no intake	Cohort studyon men and women	[20]
Total cancer	↓	HR = 0.89 [0.83–0.96] for 1–2 cups/day vs. <1 cup/day HR = 0.91 [0.85–0.98], for 3–4 cups/day vs. <1 cup/day	Meta-analysis on 8 cohort study on women	[21]

A recent review of 144 randomized controlled trials (RCTs) and case-control studies also provided evidence for beneficial effect of green tea in some cancer sites [14]. For example, the summary RR of prostate cancer in the green tea-supplemented participants was 0.50 (CI = 0.18–1.36) on the basis of three RCTs on 201 participants. However, the summary RR from 2 studies for gynecological cancer was 1.50 (CI = 0.41–5.48), indicating conflicting outcomes for some cancer sites.

In a recent survey of epidemiological studies reported from 2014 to 2018 on tea's anti-cancer effects, Xu et al. [22] found that 5 and 2 studies of total 11 studies showed favorable and unfavorable effects of tea consumption, respectively, while 4 studies gave no effect, indicating a difficulty in drawing any conclusion.

More recent PubMed data search for human studies published from 2019 to April 2020 provided several papers showing anti-cancer effects of green tea [15–21] (Table 1). For example, in a population-based prospective cohort study in which 13,957 men and 16,374 women participated, the multiple-adjusted colon cancer RR (0.78, CI = 0.49–1.22) of men consuming ≥4 times of green tea daily was lower than that of the <1 time consumers, although no significant associations between green tea consumption and colorectal cancer (CRC) risk were found in men and women [18]. However, the same search revealed that 3 studies for cervical, liver and stomach cancers did not show significant risk reduction by green tea consumption [23–25]. Thus, human studies found health benefits of green tea consumption in many cases, but it is also true that there are several conflicting results probably due to incomplete elimination of confounding factors.

Polyphenon® E is a standardized catechin preparation of green tea extract which was approved by the United States Food and Drug Administration in 2006 under the name of sinecatechins for the topical treatment of genital warts [26]. Its efficacy has been proven by several clinical studies as exemplified by a systematic review of three clinical trials in which Polyphenon® E treatments resulted in significantly higher rates of complete clearance of baseline and new warts compared with controls with very low recurrence rates [27]. Genital warts are caused by human papilloma viruses (HPVs) such as types 6, 11 and 16 [28].

In view of successful application to various types of viral agents, Polyphenon® E may be expected to be useful for the possible application to HPV-associated cancers such as cervical cancer and lymphocytic leukemia. A clinical trial in which 51 patients with HPV-infected cervical lesions were treated with Polyphenon® E ointment or capsules or both, resulted in an overall 69% response rate as compared with that of 10% in untreated groups [29].

In a phase II trial on 42 patients with asymptomatic, chronic lymphocytic leukemia, it caused a sustained reduction of ≥20% of the absolute lymphocyte count in 31% of patients and ≥50% reduction in palpable lymphadenopathy in 69% patients [30]. Thus, future clinical intervention studies with Polyphenon® E could lead to clear evidence for the anti-cancer effects of green tea.

2.2. Basic Research on Anti-Cancer Action of Green Tea and EGCG

A large number of cell-based and animal studies have provided evidence to support EGCG's anti-cancer effects. For example, Wang et al. [31] demonstrated that EGCG decreased the numbers of intestinal aberrant crypt foci and colorectal tumors in rats treated with dimethylhydrazine. In a review article, Aggarwal et al. [32] summarized the results of 30 cell-based and 26 murine studies. Also, a comprehensive review by Gan et al. [33] summarized 63 cell-based studies reported in 2001–2015 and 21 animal studies reported in 2007–2015 which demonstrated the anti-cancer effects of EGCG. These authors suggested that these anti-cancer effects may be not due to EGCG itself but to its intracellular metabolites in view of EGCG's low bioavailability.

These basic studies have also proposed mechanisms under which EGCG exerts these effects [1–5]. This review focuses mechanisms related to anti-oxidant and pro-oxidant effects, anti-inflammatory effects, anti-angiogenic effects, induction of apoptosis, modulation of epigenetic pathways and EGCG's binding to cancer-related proteins which have been reviewed in many articles [26,34–39].

2.3. Mechanisms for Anti-Cancer Effects of EGCG

2.3.1. Anti-Oxidant and Pro-Oxidant Effects

EGCG is a prominent anti-oxidant and quenches reactive oxygen species (ROS), which facilitate oxidative DNA damage, mutagenesis, and tumor promotion, leading to anti-cancer effects [40]. EGCG can exhibit anti-oxidant activity through several mechanisms including catalytic metal chelation, hydrogen atom transfer, and electron transfer. Chemically, the anti-oxidant activity of EGCG can be interpreted by the existence of the polyhydroxyl structure and the gallate group which play key roles to scavenge free radicals and by the presence of phenolic groups with sensitivity to be oxidized, resulting in generation of a quinone [37,41]. Figure 2 illustrates a possible pathway through which EGCG exerts its anti-cancer actions via an anti-oxidant activity on the basis of present and previous findings and discussions [2,22,26,34,37,38,42–44]. Modulation of 5′-AMP activated protein kinase (AMPK) by tumor necrosis factor-α (TNF-α) is incorporated into Figure 2 based on the finding by Steinberg et al. [45] that TNF-α suppresses AMPK activity via transcriptional upregulation of protein phosphatase-2C, although this link remains to be explored in experiments using EGCG.

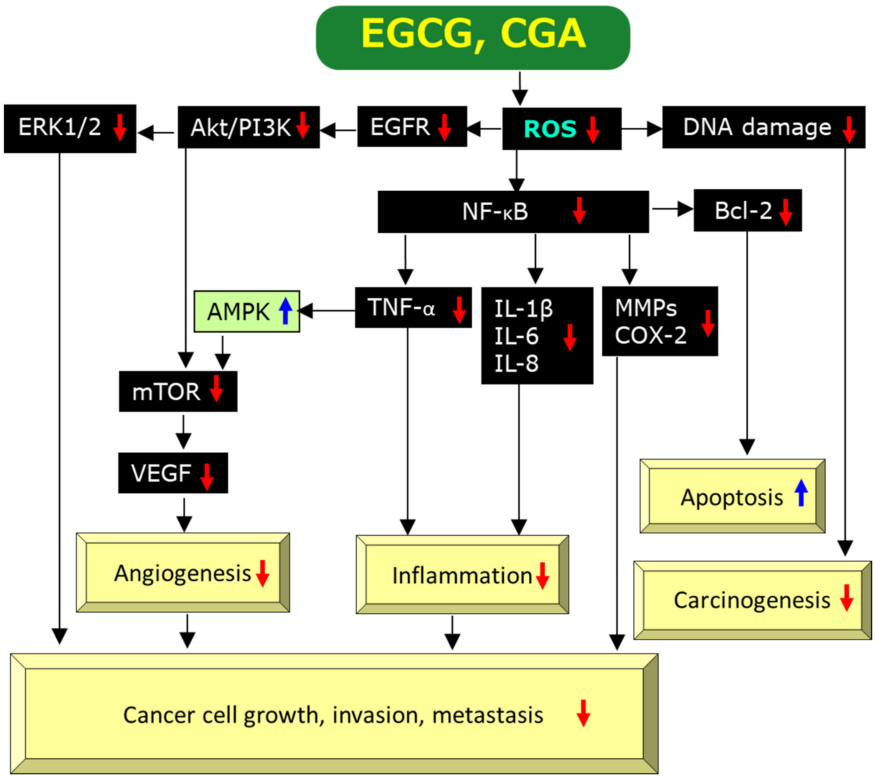

Figure 2. A possible mechanism by which EGCG and CGA exert anti-cancer effects via scavenging/downregulation of ROS. Red↓and blue↑marks represent downregulation/suppression and upregulation/stimulation, respectively.

Actually, Bulboaca, et al. showed that i.p. administration of EGCG or liposomal EGCG improved the oxidative stress parameters such as malondialdehyde levels and nitric oxide (NO) synthesis as well as those of anti-oxidant status as evaluated by total anti-oxidant capacity and levels of thiols and catalase in plasma of rats treated with streptozotocin [46].

Paradoxically, the pro-oxidant activity of EGCG has also been demonstrated by several studies and generation of ROS by EGCG is thought to be essential for the induction of apoptosis and inhibition of cell growth of cancer cells [37,40,42,47], as shown in Figure 3 which is compiled on the basis of previous data [2,34,42,48–52]. Since ROS generation induced by EGCG can upregulate AMPK, presumably through upregulation of Ca^{2+}/calmodulin-dependent protein kinase kinase (CaMKK) and/or liver kinase B1 (LKB1) [49,50], leading to downregulation of mechanistic target of rapamycin kinase (mTOR) which results in anti-cancer effects. There are some reports to show downregulation of nuclear factor-κB (NF-κB) by AMPK, if not directly [51,52]. Xiang et al. [52] demonstrated that AMPK inhibited NF-κB activity using mice treated with complete Freund's adjuvant. Therefore, ROS-mediated AMPK activation may also cause the downregulation of NF-κB, leading to anti-cancer effects through induction of apoptosis (Figure 3).

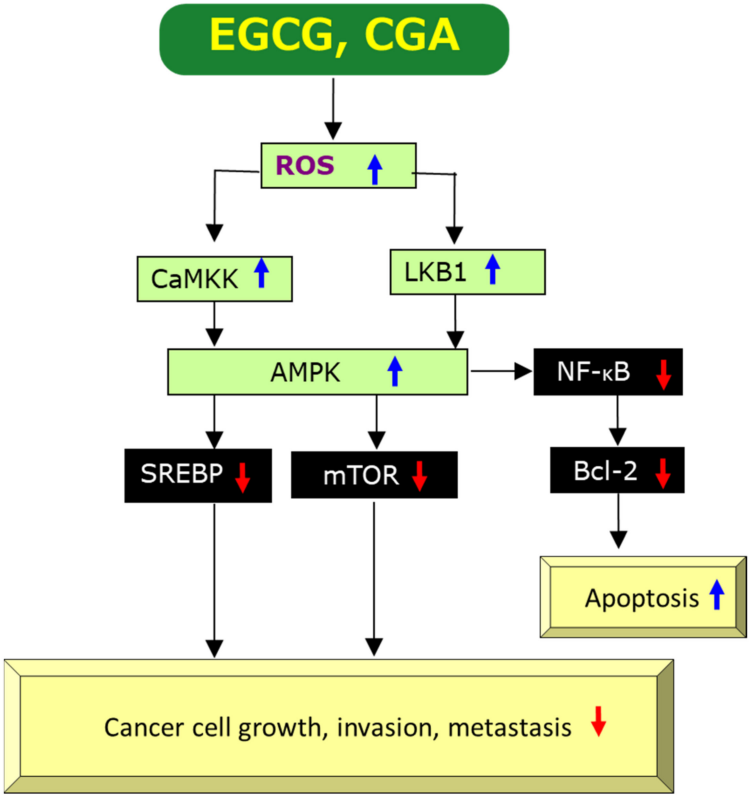

Figure 3. A possible mechanism by which EGCG and CGA exert anti-cancer effects via generation/upregulation of ROS. Red↓ and blue↑ marks represent downregulation/suppression and upregulation/stimulation, respectively.

However, it is not clear at present by what mechanism EGCG act's as an anti-oxidant or a pro-oxidant, although difference in cell types and different cellular concentrations including those of EGCG itself, metal ions, and the co-presence of other anti-oxidants may be important factors [42,53]. It can be assumed that either the anti-oxidant and pro-oxidant activities are involved in various mechanisms by which EGCG exerts anti-cancer effects (Figures 2 and 3).

2.3.2. Anti-Inflammatory Effects

Chronic inflammation is thought to have an important role on the onset and progression of human cancer by modulating the tumor microenvironment [54]. A number of studies have provided evidence EGCG's anti-inflammatory effects. These studies found that EGCG can inhibit activation of transcription factors such as NF-κB, activating protein-1 (AP-1), MyD88-dependent signaling pathway, Toll-interleukin-1 receptor domain-containing adaptor inducing interferon-β (IFN-β)-dependent signaling pathways of Toll-like receptors, and expressions of inflammatory genes including cyclooxygenase (COX), NO synthase, and TNF-α [42,43,55]. Many of these actions may be interpreted by EGCG's anti-oxidant activity (Figure 2). For example, ROS can induce NF-κB activation which in turn promotes biosynthesis of COX, NO, and TNF-α and, therefore, scavenging ROS by EGCG would lead to its anti-cancer effects [2,42] (Figure 2).

2.3.3. Anti-Angiogenic Effects

Angiogenesis is the process characterized by the development of new blood vessels from the pre-existing vessels, which supply a tumor with oxygen and nutrients to allow optimal growth. Anti-angiogenesis is thought to be one of the most promising methods of cancer treatment [56].

Cancer cells can adopt to the hypoxic microenvironment by expressing hypoxia-inducible factors-1 (HIF-1) and thereby increasing the levels of its downstream target vascular endothelial growth factor (VEGF), which promotes tumor growth, angiogenesis, and metastasis [57–59]. EGCG was shown to decrease the protein expression of HIF-1α and VEGF proteins in gastric cancer SGC7901 cells under hypoxia induced by cobalt chloride [59].

In a study in which C57BL/6J mice inoculated with 10^6 mouse breast cancer E0771 cells in the mammary gland fat pad, oral intake of EGCG at 50–100 mg/kg/day for 4 weeks reduced tumor weight, capillary density and tumor VEGF expression by 65, 30 and 23%, respectively, compared to control. EGCG at 50 μg/mL significantly inhibited the activation of HIF-1α and NF-κB as well as VEGF expression in cultured E0771 cells. These findings indicate that EGCG exerts anti-cancer effect by inhibiting angiogenesis mediated by the downregulation of VEGF, HIF-1α and NF-κB [60].

Wu et al. [61] found that EGCG inhibited the proliferation, viability, and cell cycle progression in three types of human thyroid carcinoma cells by decreasing the migration and invasion and increasing apoptosis. EGCG downregulated molecular signaling factors such as epidermal growth factor receptor (EGFR), extracellular signal-regulated kinase 1/2 (ERK1/2), and mitogen-activated protein kinase (MAPK) and inhibited tumor microvessel density dose-dependently in xenografts of these cells (Figure 2). Induction of angiogenesis by VEGF is caused by binding to its receptors on the surface of endothelial cells. Kondo et al. [62] reported that EGCG (1.56 to 100 μM) inhibited VEGF binding to its receptors in a dose-dependent manner.

Alternatively, EGCG's anti-angiogenic action may be related to its pro-oxidant activity. EGCG may induce generation of ROS to promote apoptosis which is known to be the primary action of many anti-cancer drugs. ROS can up-regulate, perhaps indirectly, AMPK which modulates expressions of a number of proteins [3]. ROS-mediated activation of AMPK can lead to downregulation of mTOR, resulting in downregulation of VEGF (Figure 3) [2,36]. Therefore, EGCG's pro-oxidant property can decrease the level of VEGF in cancer cells and tissues.

2.3.4. Induction of Apoptosis

Induction of apoptosis or programmed cell death is one of the most important mechanisms for EGCG to exert anti-cancer effects. Several studies have provided evidence for the induction of apoptosis by EGCG and its mechanism of action. ROS can stimulate gene expression of B-cell lymphoma-2 (Bcl-2) via activation of NF-κB and therefore, EGCG's scavenging activity of ROS is expected to downregulate the anti-apoptotic protein Bcl-2 (Figure 2), leading to apoptotic cell death of cancer cells (Figure 2).

Meanwhile, EGCG may induce apoptosis through enhancing ROS production (Figure 3). Das et al. [63] demonstrated that EGCG induced apoptosis via triggering ROS production with phosphorylation of p38 MAPK and activation of the redox-sensitive c-Jun N-terminal kinase-1 pathway. EGCG was also found to induce overexpression of apoptosis regulator Bcl-2 associated X (Bax) and activation of calpain, caspase-9, caspase-3, and caspase-8. It is noteworthy that EGCG did not induce apoptosis in human normal astrocytes [63].

Zan, et al. [64] reported that 5 and 20 μg/mL of EGCG induced apoptosis in breast cancer MCF-7 cells via the activation of caspase-9, caspase-3, and poly (ADP-ribose) polymerase-1 cleavage. Kwak et al. [65] also showed that 5 μg/mL of EGCG caused apoptosis in human cholangiocarcinoma HuCC-T1 cells through the increase of pro-apoptotic protein Bax and activation of caspase-9 and caspase-3, and cytochrome c release. Similarly, Jian et al. [66] found that EGCG induced apoptosis in human hepatocellular carcinoma (HCC) HepG2 cells and rat pheochromocytoma PC12 cells through downregulation of Bcl-2 and upregulation of Bax.

Sterol-response element binding protein-1 (SREBP-1), a nuclear transcription factor mainly involved in lipid metabolism, is also downregulated by AMPK (Figure 3). SREBP-1 is expressed at higher levels in patients with large tumor size, high histological grade and advanced tumor-node-metastasis stages. Downregulation of SREBP-1 inhibited cell proliferation and induced apoptosis in both HepG2 and MHCC97L cells and SREBP-1 knockdown inhibited cell migration and invasion in both cancer

cell types [67]. Since EGCG's suppression of the expression of SREBP-1 through the activation of the AMPK pathway in sebocytes was reported [68], this EGCG's inhibition may be expected to contribute to its anti-cancer effect (Figure 3).

2.3.5. Epigenetic Modifications

Epigenetic modifications represent post-translational changes in histones and DNA such as methylation and acetylation as well as dysregulation of microRNAs (miRNAs) expression [69]. Noncoding RNAs (ncRNAs) consist of miRNAs and long noncoding RNAs (lncRNAs) where miRNAs are defined as small single-stranded molecules (ca. 20 to 25 nucleotides) and lncRNAs as RNA molecules larger than 200 nucleotides. These ncRNAs are implicated in various cellular processes through regulating gene expression at the transcriptional and post-transcriptional level and thought to play roles in various diseases including cancer [70].

One mechanism involved in anti-cancer effects exerted by EGCG is such epigenetic modifications. The inhibitors of DNA methyltransferase (DNMT) and histone deacetylase (HDAC) are expected to be promising anti-cancer drugs. Fang et al. [71] demonstrated that EGCG inhibited DNMT activity with a Ki of 6.89 μM. Similarly, Pal et al. [72] showed that 10 μg/mL of EGCG decreased the proliferation of HeLa cells and expression of DNMT-1. Khan et al. [73] showed that EGCG inhibited the expression of DNMT-3B and HDAC-1 in a time-dependent manner in human cervical carcinoma HeLa cells.

In a review by Aggarwal et al. [32] the authors summarized the effects of EGCG on various cancers reported in 11 studies. In an experiment using cervical carcinoma cell lines, EGCG inhibited HeLa cells growth in a dose- and time-dependent manner [74]. EGCG caused downregulation of miR-125b and upregulation of miR-210 and miR-29 in HeLa cells and also upregulation of miR-210 and miR-29 expressions in CaSKi and SiHa cells. EGCG's upregulation of miR-210 was also found in experiments using lung cancer cells and a nude mouse model [75]. Overexpression of miR-210 led to reduction in cell proliferation and anchorage-independent cell growth [75].

In addition, Aggarwal et al. [32] described three studies in which EGCG upregulated the let-7 family miRNAs, which were implicated to function as a tumor suppressor and cause down-regulation of high mobility group-A2, a target gene related to tumor progression via 67-kDa laminin receptor (67LR)-binding in melanoma cells [76].

In another study, EGCG was demonstrated to decrease the expression of p53 gene-targeting miRNAs (miR-25, miR-92, miR-141, and miR-200a) in multiple myeloma cells [77]. The data suggest that EGCG can reverse the elevated expression of miRNAs which downregulate p53 in cancer cells and exert its anti-cancer effect via recovery of the activity of tumor suppressor p53. In harmony with this finding, EGCG was shown to stabilize p53 to upregulate its transcriptional activity leading to apoptosis in prostate cancer LNCaP cells [78]. It should be noted that EGCG downregulated miR-25 and miR-92 in multiple myeloma cells but upregulated them in HCC [77]. The difference may be due to cell-specific effect but further studies are required to understand the EGCG's effects on miRNAs.

Hu et al. [79] demonstrated that EGCG inhibited the growth of lung cancer A549 and NCI-H460 cells in a concentration-dependent manner. They identified an upregulation of RP1-74M1.3, AC087273.2, SNAI3-AS1, LINC02532, and AC007319.1 lncRNAs and downregulation of HMMR-AS1, AL392089.1, PSMC3IP, LINC02643, and H19 lncRNAs in EGCG-treated A549 cells. These lncRNAs are distributed across nearly all human chromosomes and EGCG affected lncRNAs expressions, suggesting that EGCG can regulate the expression of ncRNAs to exert anti-cancer activity in several types of cancer.

2.3.6. Molecular Docking Analysis of EGCG's Binding to Cancer-Related Proteins

A number of studies have demonstrated that the binding affinity of EGCG to proteins contributes to its anti-cancer mechanism. There are several physicochemical methods to examine molecular interaction between EGCG and proteins. In 1997, our research group conducted for the first time affinity chromatography using EGCG-agarose gel to demonstrate that EGCG binds to matrix metalloproteinase (MMP)-2 and MMP-9 which are intimately associated with cancer cell invasion and metastasis [80].

EGCG inhibited activities of these enzymes, leading to anti-cancer effects like batimastat (BB-94), a synthetic MMP inhibitor that inhibits tumor growth, local invasion, and lung metastasis of orthotopic metastatic human HCC in nude mice model [80,81]. Later, the binding interaction between EGCG and MMP-2 and MMP-9 was confirmed by computational molecular docking analysis (MDA) [82].

In our previous review article, we discussed binding interactions between EGCG and other cancer-related proteins revealed by affinity chromatography and pull-down methods using EGCG-agarose gel [82]. These include fibronectin, vimentin, heat shock protein 90, glucose-regulated protein 78 kDa (GRP78), insulin-like growth factor-1 receptor, Src-related proto-oncogene Fyn protein, ζ chain-associated 70-kDa protein, Ras-GTPase-activating protein Src homology (SH3) domain-binding protein-1, peptidyl-prolyl cis-transisomerase, and TNF receptor-associated factor-6. Most of these interactions were confirmed by MDA [82].

Similarly MDA revealed the binding interaction between EGCG and VEGF, VEGF receptors, tyrosine kinases, urokinase, chymotrypsin, DNMT, protein phosphatases, and signal transducer and activator of transcription-3 [83]. These protein-binding interactions are likely to be involved in EGCG's anti-cancer effects.

2.3.7. Roles of 67LR in EGCG's Anti-Cancer Effects

One of the EGCG's most important interactions may be that with 67LR, which was discovered by a surface plasmon resonance technique as discussed in several papers [84–86]. EGCG was shown to bind 67LR at physiologically available concentrations (0.1–1.0 μM) and to mediate many of its beneficial activities, including anti-cancer effect. EGCG binding to 67LR via eukaryotic elongation factor-1A causes the phosphorylation of myosin phosphatase targeting subunit-1 and activates myosin phosphatase which dephosphorylates its substrates such as myosin regulatory light chain, resulting in actin cytoskeleton rearrangement leading to cell growth inhibition [84,86].

3. Anti-Cancer Effects of Coffee

3.1. Human Studies on Anti-Cancer Effects of Coffee

Coffee is the second most consumed beverage worldwide after tea. Some early epidemiological studies suggested that coffee consumption was associated with an increased cancer risk [87]. For example, Yu et al. [88] described that daily coffee consumption is a risk factor in females for renal cell carcinoma. Based on the results of 32 epidemiological studies, Wierzejska found that several studies showed that coffee consumption had no or even unfavorable association with colorectal, breast, bladder, prostate, lung and pancreatic cancers, but emphasized that other studies showed promising results for these cancers and liver cancer [87].

Several early RCT suggested the coffee's favorable effects on cancers as exemplified by following findings: When 64 participants were randomly assigned into two groups and consumed 1000 mL of cafetière coffee daily or no coffee for intervention and washout periods, the result indicated that unfiltered coffee significantly increased the glutathione content by 8% in the colorectal mucosa and by 15% in plasma [89]. The increase in the detoxification capacity and anti-mutagenic properties in the colorectal mucosa through an increase in glutathione concentration suggests the possible lowering effect on the colon cancer risk [89].

A clinical trial with 10 participants found that consumption of 1L unfiltered coffee/day over 5 days resulted in a weak induction of glutathione-*S*-transferases (GSTs) and 3-fold increase in induction of placental type GST in blood, although other clinical markers for organ damage such as creatinine, aminotransferases, and alkaline phosphatase were not altered [90]. The finding suggests that coffee's induction of placental type GST may lead to protection from chemical carcinogenesis.

In a controlled intervention trial with a cross-over design with 38 participants, consumption of 800 mL coffee daily over 5 days demonstrated the decrease by 12.3% in the extent of DNA-migration attributable to formation of oxidized purines, although other biochemical parameters such as the total

anti-oxidant levels in plasma, glutathione concentrations in blood, and the activities of superoxide dismutase and glutathione peroxidase in lymphocytes were not markedly altered. The result indicates that coffee consumption prevents endogenous formation of oxidative DNA-damage in human [91].

Recent evidence has also suggested that coffee drinking may have health benefits on some types of cancer. A review by an International Agency for Research on Cancer working group conducted in 2016 on a large number of epidemiological and experimental studies on anti-cancer effects of coffee found an inverse association for liver and endometrial cancers [92].

Similarly, a comprehensive review of the beneficial effects of coffee and its components on gastrointestinal and liver carcinogenesis summarized observational epidemiological studies: four studies on oropharyngeal cancer, four on esophagus cancer, four on stomach cancer, four on CRC, and seven HCC [11]. Comparing the highest and lowest consumptions, all study results showed 31–37% risk reduction in oropharyngeal cancer, no risk reduction in esophagus cancer, no risk reduction in CRC and 34–43% risk reduction in HCC, although some subgroup analyses gave different results. In the case of stomach cancer, one study found reduced risk, two no effect and one increased risk. These results indicate that the coffee's benefit might be limited to liver cancer.

In addition, a recent meta-analysis of observational studies on associations between coffee intake and 26 different cancers including 364,749 cancer cases provided evidence to show that coffee intake is inversely associated with endometrial cancer, liver cancer, melanoma, oral cancer, and oral/pharyngeal cancer [93]. Additional evidence was also obtained to suggest the reduced risk of cancers of the mouth, pharynx and larynx, and skin cancer. Coffee consumption may also be inversely associated with breast, colon, colorectal, esophageal and nonmelanoma skin cancers.

Conversely, the same analysis showed the conflicting result whereby a higher coffee intake was associated with an increased risk of childhood acute lymphocytic leukemia, bladder cancer, and possibly lung cancer [93]. Similarly, a more recent pooled analysis of 12 cohort studies, comprising of 2601 cases out of 501,604 participants found a significantly increased risk for bladder cancer in male smokers: when compared the consumers of >4 cups/day with the non-consumers, hazard ratios were 1.75 (CI = 1.27–2.42) for current smokers and 1.44 (CI = 1.12–1.85) for former smokers [94].

In a review on the association of CRC risk with coffee, caffeinated coffee and decaffeinated consumptions, Buldak et al. [10] discussed eight, seven and three observational epidemiological studies showing no association, inverse association, and association with increased risk, respectively. These authors pointed out that caffeine is not an important component for coffee to exhibit the anti-cancer activity, since several studies found significant inverse correlation for both caffeinated and decaffeinated coffee consumptions.

A recent RCT on 160 healthy human subjects who consumed 3 or 5 cups of coffee per day for 8 weeks found that blood pressure, oxidation of DNA and lipids, blood levels of glucose, insulin, cholesterol, triglycerides, and inflammatory markers were unchanged, although a slight elevation of serum creatinine level and a significant elevation of serum γ-glutamyltransaminase levels in the 5 cups/day group [95]. The results indicated no detectable effects, either beneficial or harmful, on human health.

Thus, these findings from clinical studies are conflicting. The recall bias and confounding effects including tobacco smoking, a method for brewing coffee, differences in ingredients, and genetic background may contribute to these differences.

3.2. Comparison of Anti-Cancer Effects of Tea and Coffee in Simultaneous Human Studies

Several epidemiological studies have examined the anti-cancer effects of tea and coffee at the same time. For example, the European Prospective Investigation into Cancer and Nutrition on 486,799 subjects with a median follow-up of 11 years found that both coffee and tea intakes were inversely associated with HCC risk. Coffee and tea consumers in the highest compared to the lowest quintile had lower HCC risk by 72% and 59%, respectively [96]. In a study in which 10,399 of total 97,334 subjects developed cancers of 145 head and neck, 99 oesophageal, 136 stomach, 1137 lung, 1703 breast,

257 endometrial, 162 ovarian, 3037 prostate, 318 kidney, 398 bladder, 103 gliomas, and 106 thyroid, tea consumption of ≥1 cups/day was inversely associated with cancer overall combined (RR = 0.95, CI = 0.94–0.96) as compared to <1 cup consumption, but no association of coffee consumption with the risk of all cancers combined was found. However, coffee intake decreased a risk for endometrial cancer (RR = 0.69, 95% CI = 0.52–0.91 for ≥2 cups per day), while tea did not [97].

A meta-analysis of 12 case-control studies, comprising a total of 3649 cases and 5705 controls found that a high maternal coffee consumption increased a risk of acute lymphoblastic leukemia in childhood (OR = 1.43), whereas low to moderate tea consumption was inversely associated (odds ratio (OR) = 0.85, CI = 0.75–0.97), although the trend was not significant [98].

Table 2 shows a brief comparison of anti-cancer effects of tea and coffee in simultaneous studies reported since 2018 based on the Medline data base. Several investigations revealed that tea and coffee may have different effects in some cancer types. It is noticeable that coffee may increase a risk in certain types of cancer (bladder cancer, lung cancer, and childhood leukemia) in line with the finding from aforementioned studies which examined effects of either tea or coffee, individually [93].

Table 2. Comparison of anticancer effects in humans between tea and coffee.

Cancer Type	Tea/Green Tea/Black Tea *	Coffee/Caffeinated Coffee/Decaffeinated Coffee *	Type of Epidemiological Study [Reference]
Bladder	↓	+/−	Cohort study [100]
Bladder	+/−	↑	Meta-analysis of cohort study and case-control study [101]
Brain	↓	↓	Meta-analysis of cohort study and case-control study [102]
Breast	+/−	+/−	Cohort study [103]
Colorectal	+/−	+/−	Cohort study [104]
Colorectal	↓	+/−	Case-control study [105]
Endometrial	+/−	↓	Case-control study [103]
Glioma	↓	+/−	Cohort study [106]
Glioma	↓	↓	Case-control study [107]
Leukemia, acute myeloid	+/−	+/−	Cohort study [108]
Leukemia, childhood acute myeloid	+/−	↑	Meta-analysis of case-control study [109]
Leukemia, childhood acute lymphoblastic	+/−	↑	Meta-analysis of case-control study [99]
Liver	+/−	↓	Cohort study [110]
Liver	+/−	↓	Meta-analysis of cohort study and case-control study [24]
Lung	↓	↑	Cohort study [111]
Lymphoma, non-Hodgkin's	↓	+/−	Meta-analysis of cohort study and case-control study [112]
Melanoma, cutaneous	+/−	↓	Meta-analysis of cohort study [113]
Ovarian	+/−	+/−	Cohort study [103]
Prostate	+/−	+/−	Cohort study [114]
Renal cell carcinoma	+/−	+/−	Cohort study [100]
Skin cancer, non-melanoma	↓	↓	Cohort study [115]
Stomach	+/−	+/−	Meta-analysis of cohort study and case-control study [25]
Thyroid	+/−	+/−	Cohort [116]

* Risk decrease, risk increase and no effect are shown by ↓, ↑, and +/−, respectively.

The reason for the difference is not known at present. As pointed out by Milne et al. [99], the fact that both tea and coffee contain numerous different compounds, are prepared by various methods, and have differences in bioavailability makes it difficult to determine the factor(s) involved in the difference.

3.3. Basic Research on Anti-Cancer Action of Coffee and CGA

A number of cell-based and animal studies have provided evidence to support anti-cancer effects of coffee and CGA [117–120]. Salomone et al. [118] have elegantly discussed molecular basis of anti-cancer effects of coffee and some of its components including CGA. They summarized the results of 10 animal studies showing anti-cancer effects of coffee and CGA as examined in experimental models of liver cancer. For example, in an experiment of Miura et al. [119] coffee inhibited the proliferation and invasion of rat ascites hepatoma AH109A cells and the serum from rats given coffee orally also exhibited anti-proliferative and anti-invasive activities against these cells.

Similarly, Buldak et al. [10] reviewed the human and basic studies on anti-cancer effects of coffee and its components on CRC. These authors discussed the results of three cell-based studies on CGA. In an experiment by Hou et al. [120], CGA was shown to inhibit the proliferation of human colon cancer HCT116 and HT29 cells. CGA induced ROS generation and cell cycle arrest at the S phase, and suppressed the activation of ERK in both cell types, leading to the anti-cancer effect against CRC.

More recently, Romualdo et al. [11] discussed these issues on the basis of animal studies of the effects of coffee and CGA on oral and esophagus cancers (four studies), colon cancer (nine studies) and HCC (four studies). For example, the included data showed that two of four studies of coffee and four of five studies of CGA demonstrated beneficial effects on colon cancer. These authors summarized the mechanistic aspects of CGA's action which are associated with molecular pathways involving ROS and others such as Bax, interleukin (IL)-8, caspase-3, MMPs and miR-21. Although these articles also reviewed comprehensively other coffee components such as caffeine, caffeic acid, and kahweol, this review focuses CGA which is considered to be the major player in the coffee's anti-cancer mechanism as discuss below.

3.4. Mechanisms of CGA's Action against Cancer

3.4.1. Anti-Oxidant and Pro-Oxidant Properties, Anti-Inflammatory Effects, Anti-Angiogenic Effects and Apoptosis-Inducing Activity of CGA

CGA's involvement in anti- and pro-oxidant actions, anti-inflammatory effects, anti-angiogenic effects, and apoptosis-inducing activity of coffee has often been documented [7,11,48,118,120–122]. Examples are as follows:

Cha et al. [123] demonstrated that UVB gave damage to cellular DNA in human HaCaT cells, as demonstrated in a comet assay, but CGA pre-treatment prior to UVB irradiation prevented oxidative DNA damage and increased cell viability. Rakshit et al. [124] found that CGA induced an early accumulation of intracellular ROS and apoptosis in chronic myeloid leukemia cells.

Feng et al. [121] found that CGA inhibited the proliferation of A549 human cancer cells in vitro and that CGA suppressed 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced neoplastic transformation of JB6 P+ cells. CGA decreased UVB- or TPA-induced transactivation of AP-1 and NF-κB, the phosphorylation of c-Jun NH_2-terminal kinases, p38 kinase, and MAPK kinase-4 induced by UVB or TPA. The results also showed that CGA stimulated the nuclear translocation of NF-E2-related factor (Nrf2) as well as subsequent induction of GST-A1 anti-oxidant response element (ARE)-mediated GST activity. These results suggest that the chemopreventive effects of CGA may be through its up-regulation of cellular anti-oxidant enzymes via stimulation of Nrf2 and suppression of ROS-mediated NF-κB, AP-1, and MAPK activation [121].

Liang and Kitts reviewed anti-oxidative and anti-inflammatory effects of CGA [122]. They discussed five cell-based studies and two animal experiments, in which downregulation of ROS was demonstrated, and 10 experiments, most of which showed downregulation of inflammation-related cytokines such as IL-1β, TNF-α and IL-6. Four such studies revealed downregulation of NF-κB.

When anti-inflammatory effect of CGA was examined in lipopolysaccharide-stimulated murine RAW 264.7 macrophages and BV2 microglial cells, CGA inhibited NO production, the expression of

COX-2 and inducible NO synthase, and attenuated pro-inflammatory cytokines such as IL-1β and TNF-α via inhibition of the nuclear translocation of NF-κB [125].

In an attempt to evaluate the effects of CGA on retinal neovascularization in a mouse model of oxygen-induced retinopathy, Kim et al. [126] found that CGA inhibited VEGF-mediated tube formation in human vascular endothelial cells and that the neovascular area was significantly smaller in CGA-treated mice than in the vehicle-treated mice, demonstrating the CGA's anti-angiogenic property.

Most of these results are related to modulation of ROS and the aforementioned mechanisms by which EGCG exerts its anti-cancer effects might be applicable to CGA. Figures 2 and 3 show possible modulations by CGA, although individual pathways in which CGA is involved have not necessarily been reported. Figure 2 illustrates CGA's downregulation of ROS [2,11,48,120,127], DNA damage [123], EGFR [127], Akt/phosphatidyl 3-inositol kinase (PI3K) [127], ERK1/2 [11,127], NF-κB [2,121,125,127,128], TNF-α [2,122], IL-1β [122], IL-8 [2,11], IL-6 [122], MMPs [2,11], COX-2 [125,128], Bcl-2 [10,129], mTOR [10,127], VEGF [126], and upregulation of AMPK [7,49]. Although CGA's upregulation of CaMKK and LKB1 shown in Figure 3 has not been determined yet, Park et al. [130] reported upregulation of them by neochlorogenic acid, an isomer of CGA. Thus, EGCG and CGA would be expected to exert anti-cancer effects by modulating similar molecular pathways to each other in many cases.

3.4.2. Epigenetic Modification by CGA

Increased levels or alterations in the function of DNMT-1 are associated with the inactivation of tumor suppressor genes. Liu et al. [131] showed that CGA inhibited the proliferation, colony formation, invasion, and metastasis of HepG2 cells both in vitro and in vivo by down-regulating DNMT-1 protein expression, which enhanced p53 and p21 activity, and resulting in a significant reduction in cell proliferation and metastasis. Moreover, CGA inactivated ERK1/2 and reduced MMP-2 and MMP-9 expression in HepG2 cells. These findings suggest that CGA exhibits anti-cancer effects through several pathways. Using synthetic DNA substrates, Lee and Zhu found that CGA inhibited human DNMT-1 activity with an IC_{50} value of 0.9 μM. In MCF-7 and MAD-MB-231 human breast cancer cells, CGA inhibited the methylation of the promoter region of the retinoic acid receptor β gene [132].

Mira and Shimizu found the methanol extract of the medical herb *Angelica shikokiana* and some of its components including CGA showed cytotoxicity against various cultured cells and inhibited tubulin polymerization [133]. CGA was shown to inhibit activity of HDAC-8 (IC_{50} = 8.62 μM).

Hongtao et al. [134] found that CGA blocked the proliferation of non-small cell lung cancer cells. CGA inhibited HDAC-6 and MMP-2 activities through reduction in expression of acetylated NF-κB, the level of which is positively associated with the transcription of pro-inflammatory cytokines [134]. The results suggest CGA's anti-cancer effect through suppression of HDAC-6 activity. In line with these findings, an inhibition experiment with HeLa cell nuclear extracts and MDA conducted by Bora-Tatar et al. [135] demonstrated that CGA is the highly potent inhibitor compared to sodium butyrate, which is a well-known HDAC inhibitor.

Several studies have examined effects of CGA on miRNAs. The results of a study, in which hepatic stellate LX2 cells and CCl_4-induced liver fibrosis model rats are used, indicated that CGA inhibited the mRNA expression of miR-21, Smad7, connective tissue growth factor, α-smooth muscle actin, tissue inhibitor of metalloproteinase 1, MMP-9, and transforming growth factor-β1 (TGF-β1), suggesting that CGA relieves liver fibrosis through the miR-21-regulated TGF-β1/Smad7 signaling pathway [136]. Similar results were reported by Wang et al. [137] who showed that CGA could inhibit schistosomiasis-induced liver fibrosis through IL-13/miR-21/Smad7 signaling interactions in LX2 cells and schistosome-infected mice. Since liver fibrosis is a key factor for the risk of HCC [138], CGA might be useful for its prevention.

Induction of cancer differentiation may be a promising strategy to treat cancer. CGA reduced proliferation rate and migration/invasion ability in human hepatoma Huh7 and lung H446 cancer cells through elevation of small ubiquitin like modifier-1 expression by acting on its 3′-untranslated region and stabilizing the mRNA, leading to downregulation of miR-17 family member miR-20a, -93,

and -106b. The xenograft experiments using these cells gave similar results. NOD/SCID mice which received i.p. administration of 25–200 mg/kg/day of CGA demonstrated tumor growth inhibition and administration of 25 mg/kg caused downregulation of miR-17 family members [139].

3.4.3. MDA of CGA's Binding to Cancer-Related Proteins

The results of MDA showed that quercetin, rutin, and CGA can bind to MMP-1, MMP-3, and MMP-10 [140]. MDA of CGA and carbonic anhydrase IX showed the high affinity which is attributable to the strong interaction with enzyme active site through the formation of eight hydrogen bonds with the active site residue [141].

MDA for natural products which may interfere with SARS-CoV-2 attachment to the host cell found that CGA had the good average binding affinity to the cell-surface heat shock protein A5 (GRP78) of −7.10 ± 0.96 kcal/mol [142].

P-glycoprotein is associated with multidrug resistance as a drug efflux protein. CGA exhibited anti-proliferative effect on the mouse T-cell lymphoma L5178 cells with an IC_{50} = 0.06 ± 0.003 μg/mL and reversed multidrug resistance. MDA revealed that CGA can bind to three different sites which are known to be bound by verapamil with similar binding energies of around 7 kcal/mol [143].

CGA induced apoptosis in a dose-dependent manner with an IC_{50} of 75.88 ± 4.54 μg/mL and 52.5 ± 4.72 μg/mL in MDAMB-231 and MCF-7 cells, respectively. CGA binds to protein kinase C in a concentration-dependent manner with a Kd of 28.84 ± 3.95 μM and MDA suggested that CGA fits into the C1b domain of protein kinase C [144].

By UPLC-MS/MS analysis, Taha et al. [145] identified 22 compounds in the extracts of the fruits of *Nandina domestica* Thunb. which have served as a folk medicine in therapies of some types of cancer. MDA of CGA and some other compounds revealed strong interactions with the cancer-related proteins Akt1, caspase-3, MAPK-1 and tumor suppressor TP53.

4. Conclusions

The present review has discussed the anti-cancer effects of green tea and coffee based on epidemiological and intervention studies. These studies have provided evidence to show favorable effects on some types of cancer such as breast, colon, lung and blood cancers by green tea consumption (Tables 1 and 2) and those such as liver, endometrial, and skin cancers by coffee consumption (Table 2). Thus, green tea and coffee are likely to have some differences in site-specific anti-cancer effects.

Meanwhile, considerable studies have reported conflicting results, presumably due to confounding factors such as the method of quantifying consumption, beverage temperature, cigarette smoking, alcohol consumption, and differences in genetic and environmental factors such as race, sex, and age, lifestyle, intestinal microbiota and genetic polymorphisms [2,34,42]. Therefore, more rigorous human studies are necessary to establish the anti-cancer effects of consumption of these beverages.

This review has also provided evidence to show anti-cancer effects of EGCG and CGA based on cellular and animal experiments. These experiments have proposed several mechanisms through which EGCG and CGA exert their anti-cancer effects. Among them, the mechanism involving downregulation of ROS appears to explain commonly their anti-cancer actions (Figure 2). Another important mechanism may be related to ROS generation as shown in Figure 3.

Meanwhile, interpretations for the different anti-cancer effects between green tea and coffee need to be clarified. One possible explanation is the difference in interaction with target molecules. For example, binding interaction has not been reported between CGA and 67LR that is an important target of EGCG. The difference in co-existing molecules may also contribute to the different effects. For example, animal experiments showed that caffeic acid present in coffee exhibited carcinogenicity in the rat stomach [146,147] which may cancel the CGA's anti-cancer effect. Differences in by-products such as acrylamide generated during roasting and brewing and heavy metals and aflatoxin A which may have contaminated can be a reason [11,22,89].

In addition, some studies suggested a risk increase in certain types of cancers such as esophageal and gynecological cancers in green tea consumption [14] and bladder and lung cancers in coffee consumption (Table 2). The reason for these observations may be clarified in future studies.

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Theanine, the Main Amino Acid in Tea, Prevents Stress-Induced Brain Atrophy by Modifying Early Stress Responses

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Abstract: Chronic stress can impair the health of human brains. An important strategy that may prevent the accumulation of stress may be the consumption of functional foods. When senescence-accelerated mice prone 10 (SAMP10), a stress-sensitive strain, were loaded with stress using imposed male mouse territoriality, brain volume decreased. However, in mice that ingested theanine (6 mg/kg), the main amino acid in tea leaves, brain atrophy was suppressed, even under stress. On the other hand, brain atrophy was not clearly observed in a mouse strain that aged normally (Slc:ddY). The expression level of the transcription factor *Npas4* (neuronal PAS domain protein 4), which regulates the formation and maintenance of inhibitory synapses in response to excitatory synaptic activity, decreased in the hippocampus and prefrontal cortex of stressed SAMP10 mice, but increased in mice that ingested theanine. Lipocalin 2 (*Lcn2*), the expression of which increased in response to stress, was significantly high in the hippocampus and prefrontal cortex of stressed SAMP10 mice, but not in mice that ingested theanine. These data suggest that *Npas4* and *Lcn2* are involved in the brain atrophy and stress vulnerability of SAMP10 mice, which are prevented by the consumption of theanine, causing changes in the expression of these genes.

Keywords: brain atrophy; chronic stress; hippocampus; MRI analysis; prefrontal cortex; theanine; SAMP10

1. Introduction

It is well established that stress—especially chronic stress—seriously perturbs physiological and/or psychological homeostasis and affects cognitive function and brain structure, including that

of the hippocampus, prefrontal cortex and amygdala [1,2]. For example, in humans, the cumulative exposure to adverse life events is associated with a smaller gray matter volume in the prefrontal and limbic regions which are involved in stress [3]. Chronic restrained stress significantly decreased hippocampal volume and impaired hippocampal neurogenesis in mice [4]. In addition, animal models and human neuroimaging studies have suggested that stress-associated changes are mediated in part by glucocorticoids that are released from the adrenal gland in response to stressors, while circadian glucocorticoid oscillations are disrupted by chronic stress [5–7]. Neurogenesis in the hippocampus occurs throughout life in a wide range of animal species and could be associated with hippocampus-dependent learning and memory [8–10]. Hippocampal neurogenesis reportedly plays an important role in the regulation of the inhibitory circuitry of the hippocampus [11]. In addition, the maintenance of a balance between inhibitory and excitatory elements in the brain is believed to be important for synaptic plasticity and cognitive function [12,13], and the regulation of inhibitory neuronal activation may be especially important in the hippocampus during chronic stress [14–17].

We have demonstrated that long-term psychosocial stress using imposed male mouse territoriality, via confrontational housing, accelerates age-related alterations such as cerebral atrophy, oxidative damage, a shortened lifespan, cognitive dysfunction and behavioral depression in the senescence-accelerated mouse prone 10 (SAMP10) [18]. The average survival time of SAMP10 was about 18 months, but under confrontational housing, that was shortened to 14 months. Cognitive dysfunction of SAMP10 was significantly observed at 12 months, but this was already observed at 9 months in stressed mice. On the other hand, in a strain of mice that ages normally (Slc:ddY), a shortened lifespan and cognitive dysfunction were not observed under confrontational housing (unpublished data). These results indicate that aging is accelerated in SAMP10, and stress further accelerates SAMP10 aging. A significant increase in adrenal hypertrophy—a typical marker of the stress response—was similarly observed in ddY mice during confrontational housing [19]. In that case, adrenal hypertrophy developed within 24 h and persisted for at least one week under confrontational housing. However, stress response in SAMP10 mice is considered to continue longer than for ddY mice. Therefore, SAMP10 was used in this experiment as a stress-vulnerable mouse strain, and ddY was used as control.

On the other hand, in both SAMP10 and ddY mice, the intake of theanine—a non-protein amino acid that exists almost exclusively in tea (*Camellia sinensis* L.) leaves—was a potential candidate to suppress psychosocial stress. Although the circadian rhythm of corticosterone was blunted in ddY mice during confrontational housing, it was normalized in mice that ingested theanine (6 mg/kg) even if under the same stressful conditions [18,19]. To examine the reason for cerebral atrophy during confrontational housing, we measured the brain atrophy of SAMP10 and ddY mice using magnetic resonance imaging (MRI). In addition, we examined the mechanism of action of theanine in the brain.

2. Materials and Methods

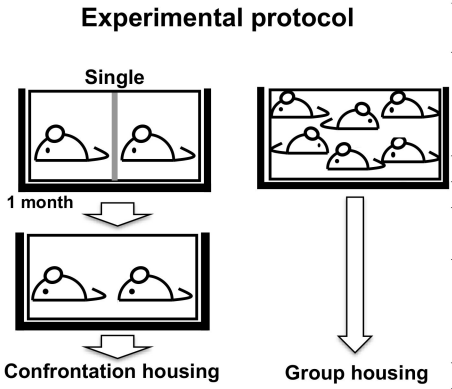
2.1. Animals and Preparation of Theanine

Four-week-old male SAMP10 (SAMP10/TaSlc) and ddY (Slc:ddY) mice were purchased from Japan SLC Co. Ltd. (Shizuoka, Japan) and kept in conventional conditions in a temperature- and humidity-controlled room with a 12 h–12 h light–dark cycle (light period, 08.00–20.00 h; temperature, 23 ± 1 °C; relative humidity, 55 ± 5%). Mice were fed a normal diet (CE-2; Clea Co. Ltd., Tokyo, Japan) and water ad libitum. All experimental protocols were approved by the University of Shizuoka Laboratory Animal Care Advisory Committee (approval No. 136068) and were in accordance with the guidelines of the US National Institutes of Health for the care and use of laboratory animals.

L-Theanine (suntheanine; Taiyo Kagaku Co. Ltd., Yokkaichi, Japan) was used at 20 μg/mL and dissolved in normal tap water according to previous methodology [18,19]. Mice consumed theanine solution, which was prepared freshly twice a week, ad libitum.

2.2. Housing Conditions for Stress Experiments

Four-week-old mice were housed in groups of six per cage for five days to habituate them to novel conditions. Mice were then divided into two groups, namely confrontational and group housing, according to a previously described method [18,19]. (Figure 1). In brief, for confrontational housing, a standard polycarbonate cage was divided into two identical subunits by a stainless steel partition. Two mice were housed in the partitioned cage for one month to establish territorial consciousness (single housing). These mice were further divided into two groups that ingested theanine or control water. The partition was then removed to expose the mice to confrontational stress, and the two mice subsequently cohabited in the same cage (confrontational housing). Mice were classified as follows: mice that ingested theanine under confrontational housing were termed CT; mice that ingested control water under confrontational housing, CC; mice that ingested theanine under group housing, GT; and mice that ingested control water under group housing were termed GC. Confrontation periods were 0, 1, 2, 4 and 6 months. The cages were placed in a styrofoam box (width 30 cm; length 40 cm; height 15 cm) in order to avoid visual social contact between cages.



SAMP10	Confrontation period (months)					Total
	0	1	2	4	6	
CC	8	6	9	7	6	36
CT	5	6	10	6	7	34
GC	4	6	12	1	3	26
GT	4	6	9	1	6	26
Total	21	24	40	15	22	122

ddY	Confrontation period (months)					Total
	0	1	2	4	6	
CC	5	6	6	6	6	29
CT	5	6	6	6	5	28
GC	4	6	6	6	6	28
GT	6	6	6	6	6	30
Total	20	24	24	24	23	115

Figure 1. Experimental protocol describing the housing condition. For confrontational housing, a standard polycarbonate cage was divided into two identical subunits by a stainless steel partition. Two mice were housed in the partitioned cage for one month (single housing). These mice were further divided into two groups that ingested theanine or control water. Then, the partition was removed, and subsequently the two mice cohabited the same cage (confrontational housing). Group-housing mice were housed in groups of six. Mice that ingested theanine under confrontational housing, CT; mice that ingested control water under confrontational housing, CC; mice that ingested theanine under group housing, GT; mice that ingested control water under group housing, GC. Confrontation periods were 0, 1, 2, 4 and 6 months. The number of mice for each group is shown.

2.3. Magnetic Resonance (MR) Sample Preparation

We performed ex vivo MR scanning in this study as it allows for longer scan times, higher resolution scans, and the use of a contrast agent such as a gadolinium complex [20–22]. Mouse brain samples were prepared according to the most widely used protocol in the literature [23]. Mice were anaesthetized with isoflurane (N01AB06, Pfizer Inc., Tokyo, Japan) and transcardially perfused with 30 mL of phosphate buffered saline (PBS) containing 2 mM of ProHance (V08CA04, Bracco-Eisai Co. Ltd., Tokyo, Japan), a gadolinium-based MR contrast agent. Subsequently, fixation was performed with 30 mL of 4% paraformaldehyde (PFA, 30525-89-4, Wako, Tokyo, Japan) that also contains 2 mM of ProHance. Bodies, along with the skin, lower jaw, ears, and the cartilaginous nose tip, were removed. The remaining skull structures containing the brain tissue were postfixed in 4% PFA + 2 mM ProHance at 4 °C overnight and then transferred to PBS + 0.01% sodium azide (26628-22-8, Wako, Tokyo, Japan) + 2 mM ProHance at 4 °C in a 15 mL plastic tube. The above procedures were performed at the University of Shizuoka (Shizuoka, Japan) and the ex vivo brain samples were transported to Tohoku University (Sendai, Japan) for MR scanning, which was performed within no longer than 5 months

after sampling [24]. Immediately prior to scanning, the ex vivo mouse brains were immersed in liquid fomblin (69991-67-9, Sigma-Aldrich, St. Louis, MO, USA), a perflorocarbon that minimizes susceptibility artifacts at the interface and limits sample dehydration during scanning.

2.4. MR Acquisition

All MRI data were acquired using a 7.0 T Bruker PharmaScan 70/16 system (Bruker Biospin, Ettlingen, Germany) with a 23 mm diameter birdcage coil that was designed to serve as both as a transmitter and receiver coil for the mouse brain [25]. The operational software of the MRI scanner was Paravision 5 or 6 (Bruker Biospin, Ettlingen, Germany). Prior to the acquisition of MRI data, global magnetic field shimming was performed inside the core and at the region of interest (ROI) using a point-resolved spectroscopic protocol [26]. The line width (full width at half maximum) at the end of the shimming procedure ranged from 15 to 20 Hz in the ROI. As the T1 tissue contrast between grey and white matter is less pronounced at a high magnetic field strength in rodents compared with humans [27], we used T2-weighted imaging in this study, as in our previous rodent studies [25,28,29]. The T2-weighted images were obtained using a spin-echo 3D-RARE (rapid acquisition with relaxation enhancement) pulse sequence with the following parameters: repetition time = 325 ms, effective echo time = 32 ms, RARE factor = 4, flip angle = 90 degrees, field of view = $12 \times 12 \times 17.4$ mm^3, matrix size = $200 \times 200 \times 290$, voxel size = $0.06 \times 0.06 \times 0.06$ mm^3, bandwidth = 60 kHz, fat suppression = on, and number of averages = 10. The total MRI scanning time for each mouse brain was approximately 13 h. The MRI acquisition parameters were set to achieve a reasonable signal-to-noise ratio (SNR) of the mouse brain image [30]. The SNR for each T2-weighted image was 43 ± 9 (mean ± standard deviation), which was measured as the mean image intensity in a single slice of the brain divided by the standard deviation of the intensity in the background outside the brain.

2.5. MR Image Preprocessing

Each T2-weighted image was reconstructed using Bruker's Paravision software, exported in Digital Imaging and COmmunications in Medicine (DICOM) format, and converted to The Neuroimaging Informatics Technology Initiative (NIfTI) format using the "dcm2niix" tool [31]. Each image was visually inspected for any possible artifacts, and a total of 237 mouse brain images (n = 122 for SAMP10 mice and n = 115 for ddY mice) were used for the analysis. Images were processed by a method used in Pagani et al. [32] in which semi-automated registration-based anatomical labeling for a high-resolution ex vivo mouse brain image can be achieved. Unless otherwise specified, computational steps were carried out using the Advanced Normalization Tools (ANTs) software package (version 2.2.0) [33]. ANTs software, which employs the symmetric diffeomorphic normalization (SyN) model, has been demonstrated to be the most accurate intensity-based normalization method among 14 nonlinear canonical registration algorithms [34]. Images were preprocessed as follows. First, each T2-weighted image was manually rotated and translated such that the origin of the coordinates occupied the midpoint of the anterior commissure to roughly match the standard reference space [35]. Second, each image was roughly skull stripped using Analysis of Functional NeuroImages (AFNI) "3dAutomask" tool [36], which estimates the clipping level of the image, and then the lower-intensity clusters were masked out (clip level fraction = 0.5). Third, the image non-uniformity inside the mask was corrected using the "N4BiasFieldCorrection" tool in ANTs [37] with default parameters. Fourth, to further remove the non-brain tissues, each image was segmented using the "Atropos" tool in ANTs, which employs Markov random field theory [38]. A standard k-means clustering algorithm was used to determine six classes, and the top one or two classes (i.e., higher-intensity clusters) were classified as non-brain tissues and finally removed. Fifth, the resulting skull-stripped image was further bias field corrected inside the mask and then used to create the study-specific template.

2.6. Minimum Deformation Template

While several mouse brain templates have been reported in the literature [39–41], the mouse strains, magnetic field, and imaging sequence, resolution, and contrast are different among studies and do not perfectly match the current study. The creation of a study-specific minimum deformation template (MDT) has been recommended to provide superior registration accuracy between subjects [42]. First, we computed the MDT at each age range for each mouse strain. The number of mice in each group is summarized in Figure 1. Second, the 10 computed MDTs from each group were used to create a single MDT that was consequently derived from all 237 mouse brains. Third, all subject MDTs were linearly (affine) and nonlinearly (SyN) registered to the mouse ex vivo brain template that was reported in the literature [43] and had 20 brain structure labels. All subject MDTs and structure labels are displayed in Figure 2. MDT computation was performed using the script in ANTs named "antsMultivariateTemplateConstruction2.sh" with the following SyN parameters: gradient step size = 0.1 voxels, update field variance = 3 voxels, and total field variance = 0.5 voxels. These SyN parameters were suggested by a recent mouse ex vivo brain study to account for a balance between registration quality and computation time [44]. Other parameters were as follows: iteration of template creation = 4, maximum iterations for each pairwise registration = 30 × 20 × 10, shrink factors = 4 × 2 × 1 voxels, smoothing factors = 2 × 1 × 0 voxels, similarity metric = cross-correlation, radius in brackets = 4 voxels, N4BiasFieldCorrection = on, and type of transformation model = Greedy SyN.

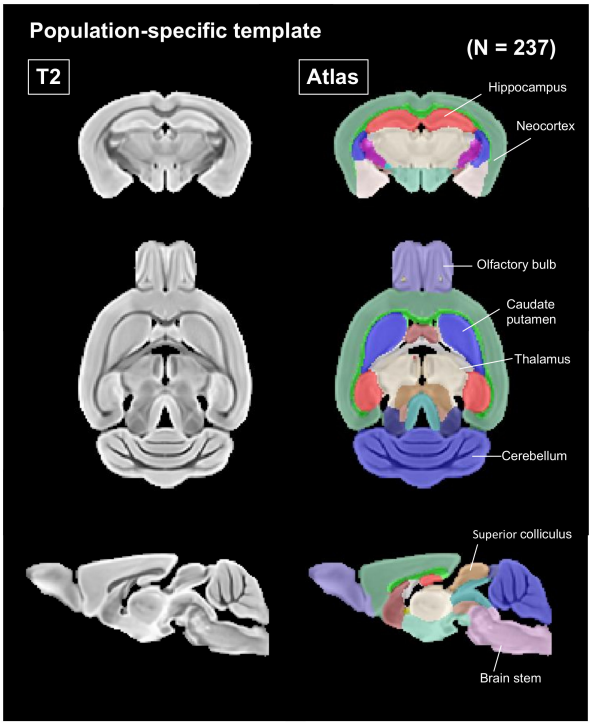


Figure 2. The population-specific minimum deformation template was constructed from the T2-weighted images of all SAMP10 and ddY mice (n = 237). The template was nonlinearly registered to the mouse atlas space [43]. Each anatomical structure was used for volume analysis.

2.7. Measurement of DNA Microarray and Principal Component Analyses

The mice were housed confrontationally for three days after single housing for one month. Group-housed mice were kept in a group of six for one month. Mice were anesthetized with isoflurane and blood was removed from the jugular vein. The hippocampus was removed and frozen immediately. Total RNA was extracted from the hippocampus using an RNeasy Mini Kit (74104, Qiagen, Valencia, CA, USA). Total RNA was processed to synthesize biotinylated cRNA using One-Cycle Target Labeling and Control

Reagents (Affymetrix, Santa Clara, CA, USA) and then hybridized to a Total RNA Mouse Gene 1.0 ST Array (Affymetrix), with three biological repeats per group. Raw data were parametrically normalized [45] by using the SuperNORM data service (Skylight Biotech Inc., Akita, Japan). The significance of theanine ingestion was statistically tested by two-way ANOVA [46] at $p < 0.001$.

To compare the effects of theanine ingestion in the control under group or confrontational housing, we performed principal component analysis (PCA) [47] on ANOVA-positive genes [48]. To reduce the effects of individual variability among samples, the axes of PCA were estimated on a matrix of each group's sample means and applied to all data, which were centered using the sample means of control mice under group housing.

2.8. Quantitative Real-Time Reverse Transcription PCR (qRT-PCR)

The mice were housed confrontationally from 0 to 7 months after one month of single housing. Group-housed mice were kept in a group of six for two months. Mice were anesthetized with isoflurane and blood was removed from the jugular vein. The brain was carefully dissected and the hippocampus and prefrontal cortex were immediately frozen. The brain sample was homogenized and total RNA was isolated using a purification kit (NucleoSpin® RNA, 740955, TaKaRa Bio Inc., Shiga, Japan) in accordance with the manufacturer's protocol. The obtained RNA was converted to cDNA using the PrimeScript® RT Master Mix kit (RR036A, Takara Bio Inc.). Real-time quantitative RT-PCR analysis was performed using the PowerUp™ SYBR™ Green Master Mix (A25742, Applied Biosystems Japan Ltd., Tokyo, Japan) and automated sequence detection systems (StepOne, Applied Biosystems Japan Ltd., Tokyo, Japan). Relative gene expression was measured by previously validated primers for *Npas4* [49] and *Lcn2* [50] genes: Npas4 (F; 5′-AGCATTCCAGGCTCATCTGAA-3′, R; 5′-GGGCGAAGTAAGTCTTGGTAGGATT-3′) and Lcn2 (F; 5′-TACAATGTCACCTCCATCCT GG-3′, R; 5′-TGCACATTGTAGCTCTGTACCT-3′). Since these expressions were significantly changed in the hippocampus of SAMP10 mice by theanine ingestion, the levels were compared among mice that were housed confrontationally from 0 days (only single housing) to 7 months and group-housed for 2 months. cDNA derived from transcripts encoding β-actin was used as the internal control.

2.9. Statistical Analyses

Each label volume was computed for each mouse using "ImageMath" and "LabelStats" tools in ANTs. Statistical analysis was performed using one-way ANOVA, and statistical significance was set at $p < 0.05$. Confidence intervals and significance of differences in means were estimated by using Tukey's honest significant difference method or Fisher's least significant difference test.

3. Results

3.1. Effect of Psychosocial Stress in SAMP10 and ddY Mice

The effects of confrontational housing and theanine ingestion on brain volume were examined in SAMP10 and ddY mice, respectively. The body weights of mice housed confrontationally were not different from the group-housed mice. The ingestion of theanine also did not affect body weight. The number of mice for each group was 26–36 (Figure 1). Each image was visually inspected for any possible artifacts, and a total of 237 mouse brain images ($n = 122$ for SAMP10 and $n = 115$ for ddY) were used for the analysis. The MDT and structure labels of all subjects are displayed in Figure 2. Brain volume was compared among the four groups (CC, CT, GC and GT) without distinguishing mouse age. Hippocampal volume was significantly lower in SAMP10 mice of CC than CT and GC (Figure 3a), indicating that atrophy was caused under confrontational housing and was significantly suppressed in mice that ingested theanine. The prevention of atrophy by the ingestion of theanine under confrontational housing was similarly observed in the neocortex of SAMP10 mice (Figure 3b). The time-course of brain atrophy was next examined in mice that were housed confrontationally. In the hippocampus, atrophy was significantly suppressed in aged mice that ingested theanine (6 months of

confrontation, Figure 3c). As brain atrophy with aging was not significant in mice housed in a group (Figure 3e), it was clarified that psychosocial stress due to confrontational housing promoted brain atrophy in the hippocampus of SAMP10 mice. The neocortex was significantly smaller one month after starting confrontational housing in SAMP10 mice (Figure 3d). While the lower volume recovered after 2 months of confrontation in CT, the volume gradually decreased with aging under confrontational housing (Figure 3d,f). A similar phenomenon was observed in other brain regions such as the caudate putamen, cerebellum, amygdala, olfactory bulb and brainstem (Table S1).

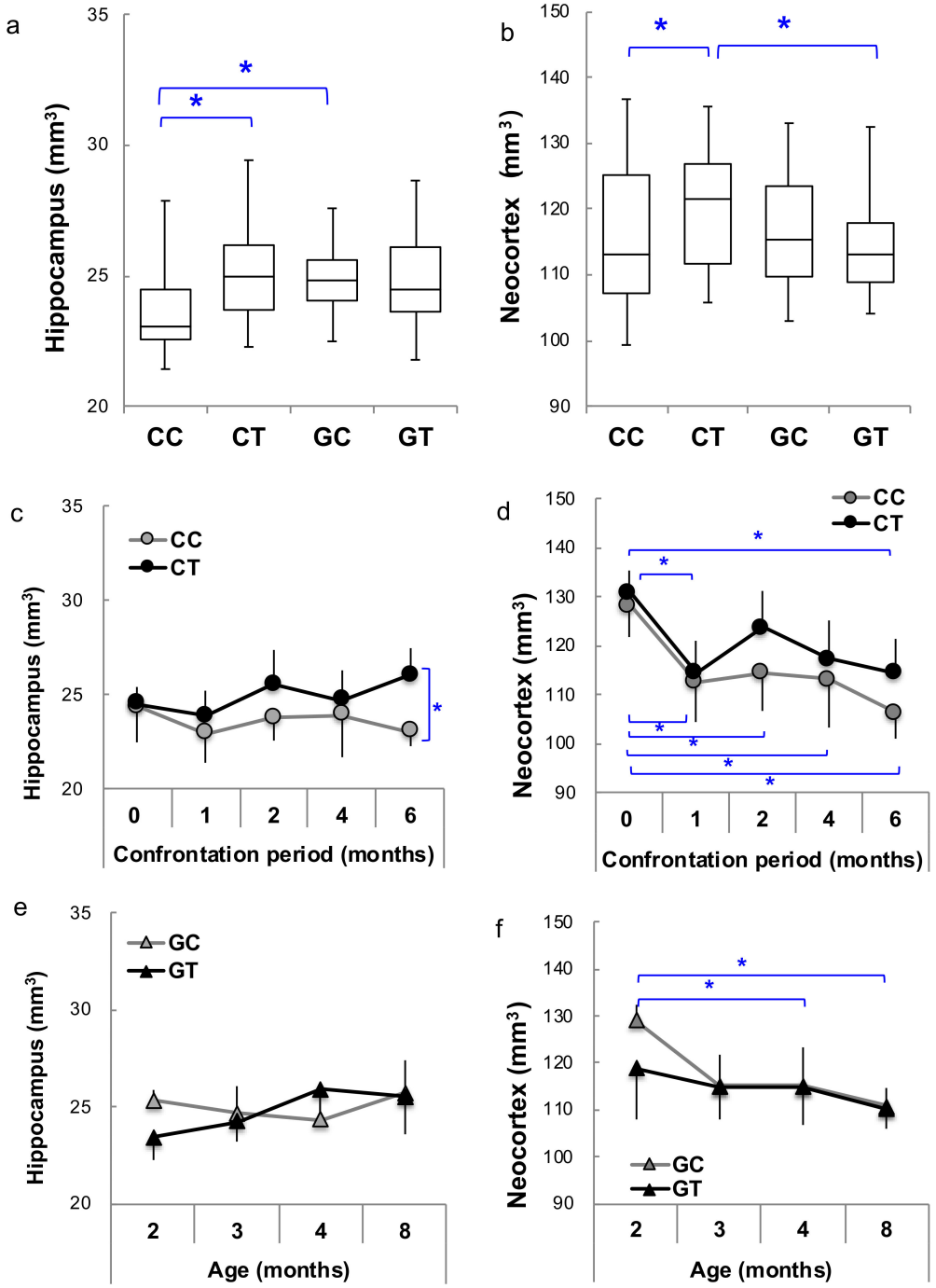


Figure 3. The brain volume of SAMP10 mice. Boxplot of the volumes of brain sections in the hippocampus (**a**) and neocortex (**b**) were compared among the four groups (CC, CT, GC and GT) without distinguishing age (n = 122, *; $p < 0.05$). The time-course of hippocampus and neocortex were compared between CC and CT (**c**,**d**) and between GC and GT (**e**,**f**) (n = 3–12; *, $p < 0.05$).

On the other hand, the effect of confrontational housing tended to be less in ddY mice than in SAMP10 mice, and the preventive effect of theanine was observed in ddY mice housed in a group (Figure 4a,b). The time-course of brain atrophy showed that brain atrophy was observed after one month of confrontational housing but that brain volume gradually increased and recovered within five months (Figure 4c,d). Almost no atrophy was observed in mice that ingested theanine. However, brain atrophy progressed in aged ddY mice in group housing, but was almost suppressed in mice that ingested theanine (Figure 4e,f).

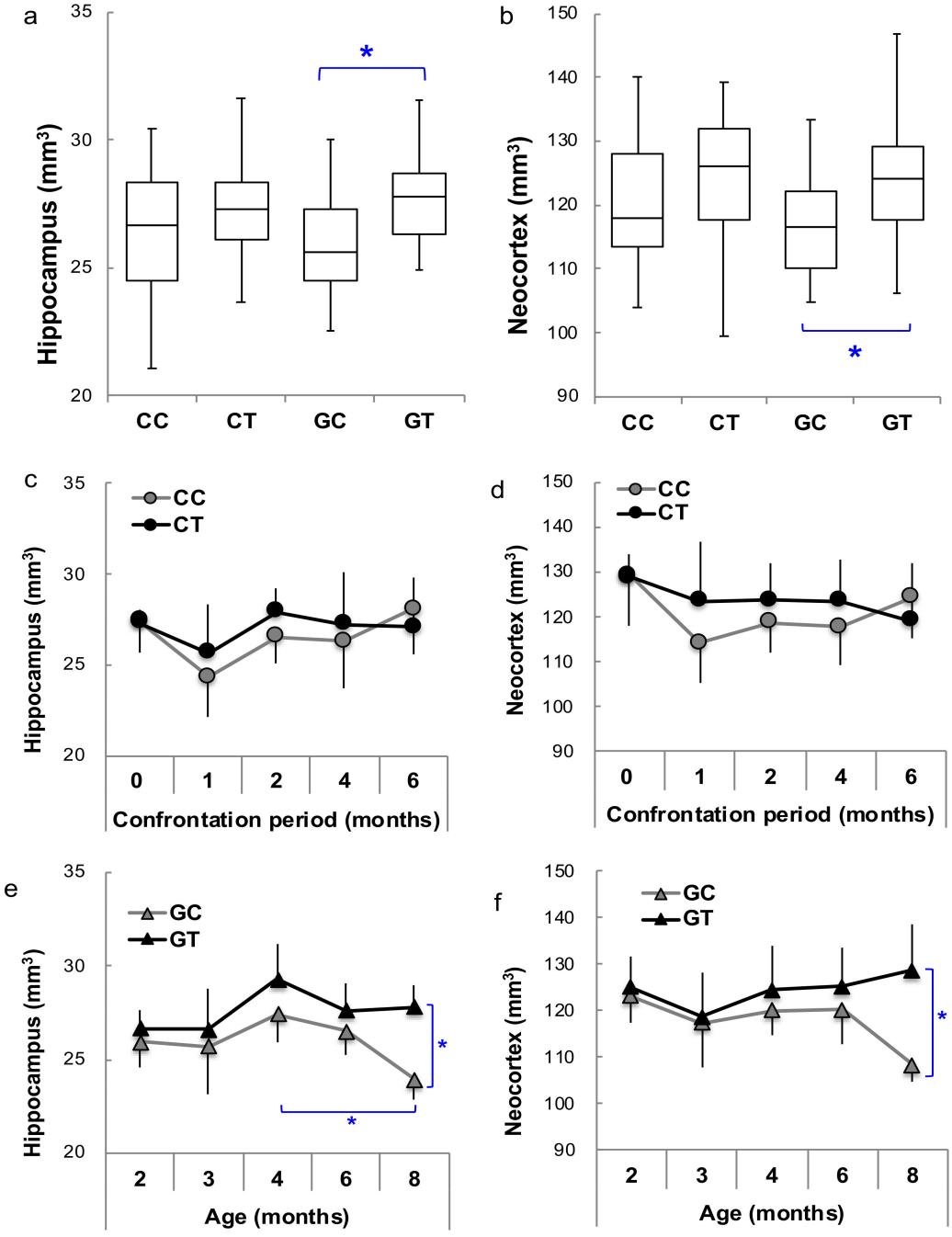


Figure 4. The brain volumes of ddY mice. Boxplot of the volumes of brain sections in the hippocampus (**a**) and neocortex (**b**) were compared among the four groups (CC, CT, GC and GT) without distinguishing age (n = 115, *; $p < 0.05$). The time-course of the hippocampus and neocortex were compared between CC and CT (**c**,**d**) and between GC and GT (**e**,**f**) (n = 4–6; *, $p < 0.05$).

These results indicate that brain atrophy occurred due to the psychosocial stress caused by confrontational housing not only in SAMP10 but also in ddY mice; however, atrophy progressed with aging in SAMP10 mice but was temporary in ddY mice. After temporary atrophy, an increase in brain volume was observed in ddY mice. Theanine ingestion suppressed brain atrophy with aging in both SAMP10 and ddY mice.

3.2. Effect of Psychosocial Stress and Theanine Intake on Gene Expression of the Hippocampus in SAMP10

To examine the effect of psychosocial stress on the brain, early gene expression was compared between mice in group and confrontational housing. Furthermore, the effect of theanine ingestion was examined in SAMP10 mice. The mice were housed confrontationally for three days. DNA microarray data of confrontational and group-housed mice that ingested theanine or water (control), obtained using high-density oligonucleotide microarrays, showed 2277 positively expressed genes based on two-way ANOVA ($p < 0.0034$). Principal component analysis (PCA) was applied to these genes. The PC scores for four groups and their gene expression are shown simultaneously in a biplot (Figure 5). To observe differences caused by stress, control mice housed in a group were used as the reference expression. The difference between group and confrontational housing appears on the PC1 axis, while the effect of theanine ingestion coincided with the PC2 axis. The effect of theanine ingestion on the magnitude of gene expression on the PC2 axis was larger in mice housed confrontationally than in group-housed mice. The top 10 biological processes that were significantly observed on the PC2 axis are shown in Table 1. Many processes, such as transcription and phosphorylation, were negatively regulated by theanine ingestion. On the other hand, oxidation-reduction, apoptosis and lipid metabolism were positively regulated. Several genes that were significantly up or down-regulated following theanine ingestion are summarized in Table 2. Neuronal Per-Arnt-Sim (PAS) domain protein 4, *Npas4*, was the most up-regulated gene. Npas4 is a transcription factor and plays a role in the development of inhibitory synapses [51]. Fatty acid binding protein 7, Fabp7, is a marker of neurogenesis [52]. B cell translation gene 2, *Btg2*, is related to the process of neurogenesis with memory function [53]. On the other hand, the expression level of lipocalin 2 (*Lcn2*), which is up-regulated following psychological stress [54], was down-regulated by theanine ingestion. The function of the most down-regulated gene, melanoma antigen, *Mela*, is unknown. In addition, the expression of *Neat1*, nuclear paraspeckle assembly transcript 1, which inhibits apoptosis [55], was down-regulated.

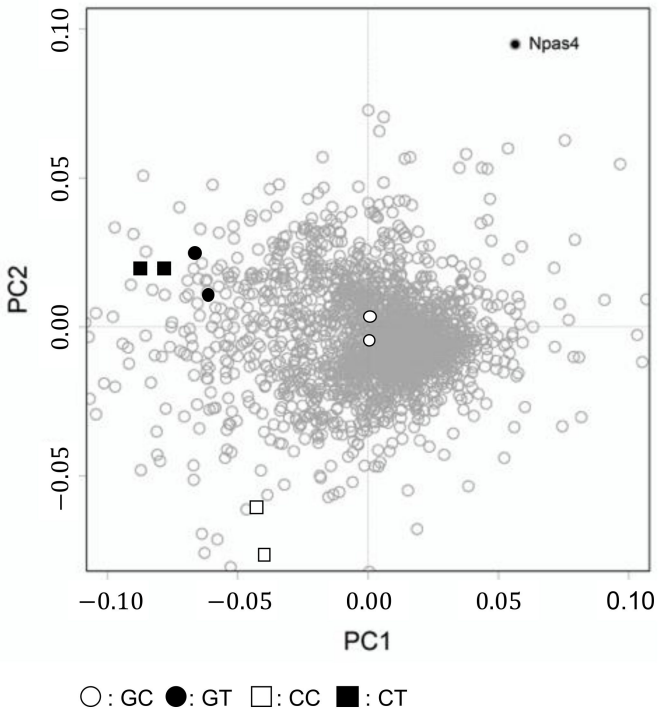


Figure 5. Principal component analysis of gene expression. Hippocampal samples were obtained from mice housed confrontationally for three days. Group-housed mice were kept in a cage for one month. The principal component (PC) ordination of ANOVA-positive genes us based on the transcriptome of hippocampal gene expression in mice of GC (open circle), GT (closed circle), CC (open square) and CT (closed square) groups (n = 2 for each group). Each small dot represents the expression of each gene. Neuronal Per-Arnt-Sim (PAS) domain protein 4 (Npas4) is shown as a small black circle.

Table 1. The effect of theanine ingestion on the magnitude of gene expression on the PC2 axis.

PC2	Biological Process	Selected	Total	p-Value
Positive	Oxidation-reduction process	40	854	0
	Transport	40	2011	4.22×10^{-7}
	Regulation of transcription, DNA-templated	31	2447	0.0140
	Multicellular organismal development	20	1074	0.0008
	Cell adhesion	16	530	1.32×10^{-5}
	Apoptotic process	16	607	6.54×10^{-5}
	Lipid metabolic process	14	472	5.48×10^{-5}
	Ion transport	13	633	0.0029
	Glucose metabolic process	13	79	3.03×10^{-13}
	Regulation of translation	13	140	3.28×10^{-10}
Negative	Regulation of transcription, DNA-templated	76	2447	0.0002
	Transcription, DNA-templated	70	1983	9.61×10^{-6}
	Signal transduction	69	2582	0.0152
	Transport	69	2011	2.70×10^{-5}
	Metabolic process	45	1595	0.0205
	Multicellular organismal development	42	1074	7.15×10^{-5}
	Cell adhesion	37	530	2.57×10^{-10}
	Phosphorylation	37	737	9.82×10^{-7}
	Protein phosphorylation	37	667	9.30×10^{-8}
	Positive regulation of transcription from RNA Polymerase II promoter	33	899	0.0012

Table 2. Top 10 genes that were significantly up or down-regulated following theanine ingestion.

	Symbol	Full Name	ΔZ	*p*
Up-regulated	Npas4	Neuronal PAS domain protein 4	0.3210	8.32×10^{-21}
	Fabp7	Fatty acid binding protein 7, brain	0.0926	1.25×10^{-31}
	n-R5s70	Nuclear encoded rRNA 5S 70	0.2585	0.0028
	Opalin	Oligodendrocytic myelin paranodal and inner loop protein	0.2031	2.67×10^{-24}
	Olfr1474	Olfactory receptor 1474	0.3071	0.0011
	Igkv14–130	Immunoglobulin kappa variable 14–130	0.2886	0.0027
	Vmn2r78	Vomeronasal 2, receptor 78	0.2541	0.0016
	Triml2	Tripartite motif family-like 2	0.2697	0.0032
	Btg2	B cell translocation gene 2, anti-proliferative	0.2027	1.86×10^{-11}
	Igkv18–36	Immunoglobulin kappa chain variable 18–36	0.2167	0.0034
Down-regulated	Mela	Melanoma antigen	−0.6668	6.16×10^{-64}
	Ly6a	Lymphocyte antigen 6 complex, locus A	−0.4346	1.01×10^{-8}
	Lcn2	Lipocalin 2	−0.3125	2.37×10^{-7}
	Prg4	Proteoglycan 4	−0.2707	4.82×10^{-7}
	Neat1	Nuclear paraspeckle assembly transcript 1	−0.3590	1.41×10^{-18}
	C1qb	Complement component 1, q subcomponent, beta polypeptide	−0.2347	1.54×10^{-17}
	Vwf	Von Willebrand factor homolog	−0.2057	7.46×10^{-21}
	C1qc	Complement component 1, q subcomponent, C chain	−0.2948	4.38×10^{-9}
	Hbb-b2	Hemoglobin, beta adult minor chain	−0.2340	1.30×10^{-8}
	Etnppl	Ethanolamine phosphate phospholyase	−0.2038	1.84×10^{-7}

ΔZ = expression level (confrontation theanine–confrontation control).

3.3. *Effect of Theanine Intake on Levels of Npas4 and Lcn2 in the Brain*

Since the expression of *Npas4* increased significantly and that of *Lcn2* decreased significantly in the hippocampus of SAMP10 mice that ingested theanine under confrontational housing on the third day (Table 2), the levels in the hippocampus and prefrontal cortex were compared among mice that were housed confrontationally from 0 days (only single housing) to 7 months and group-housed for 2 months. Although the level of *Npas4* expression tended to be high in mice on day 0 of confrontational housing relative to group housing, the levels decreased in mice housed confrontationally for three days and one month but recovered in mice housed confrontationally for 7 months (Figure 6). On the other hand, in the hippocampus of mice that ingested theanine, the level of *Npas4* expression was significantly higher in mice housed confrontationally for one month relative to day 0 of confrontational housing. The level of *Npas4* expression was not different between SAMP10 and ddY mice housed in a group. Although in ddY mice the level of *Npas4* expression was increased by confrontational housing for three days, the level was lowered after one month. The level of *Npas4* expression in the prefrontal cortex decreased in SAMP10 mice housed confrontationally for three days and recovered in mice housed confrontationally for 7 months. In the prefrontal cortex of SAMP10 mice that ingested theanine, the level of *Npas4* expression was significantly higher in mice housed confrontationally for 7 months than in other mice (Figure 6). The level of *Npas4* in ddY mice increased by confrontational housing for three days and lowered after one month.

The level of *Lcn2* expression was significantly higher in SAMP10 mice housed confrontationally for three days than in other mice (Figure 7). However, a significant increase was not observed in mice that ingested theanine. On the other hand, the level of Lcn2 expression in the hippocampus of ddY mice slightly increased by confrontational housing for three days, but the levels in ddY mice were one-tenth to one-fifth of those in SAMP10 mice. The level of *Lcn2* expression in the prefrontal cortex showed a similar changed to the expression level in the hippocampus (Figure 7).

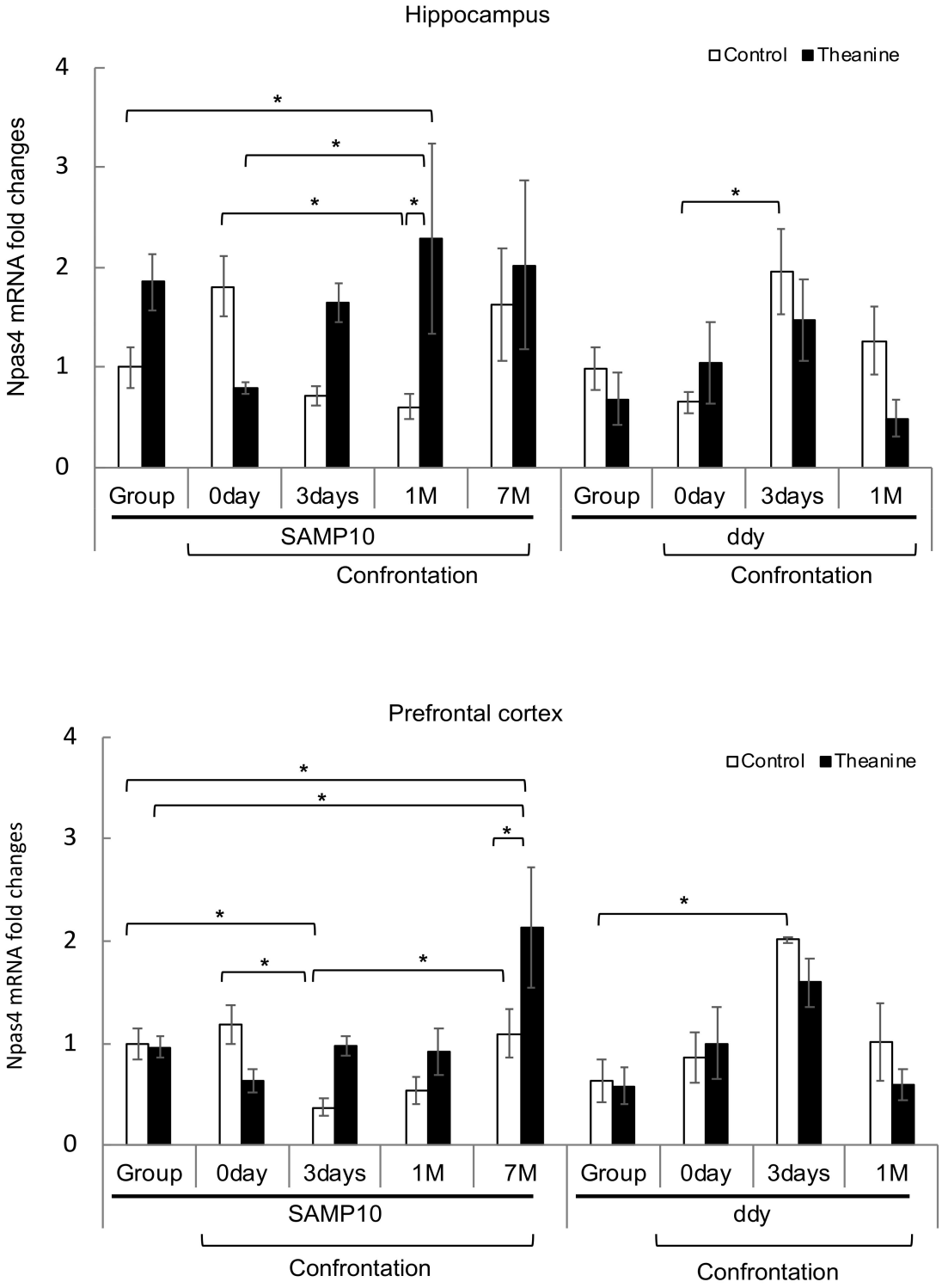


Figure 6. Expression levels of *Npas4* mRNA in the hippocampus and prefrontal cortex of SAMP10 and ddY mice. Mice consumed theanine (20 μg/mL water, closed bar) or normal tap water (control, open bar) ad libitum. After single housing for one month, hippocampal and prefrontal cortex samples were obtained from mice housed confrontationally for 0 days, 3 days, 1 month, and 7 months. Group-housed mice were kept in a cage for two months. Values are expressed as means ± SEM (n = 3–6, * $p < 0.05$).

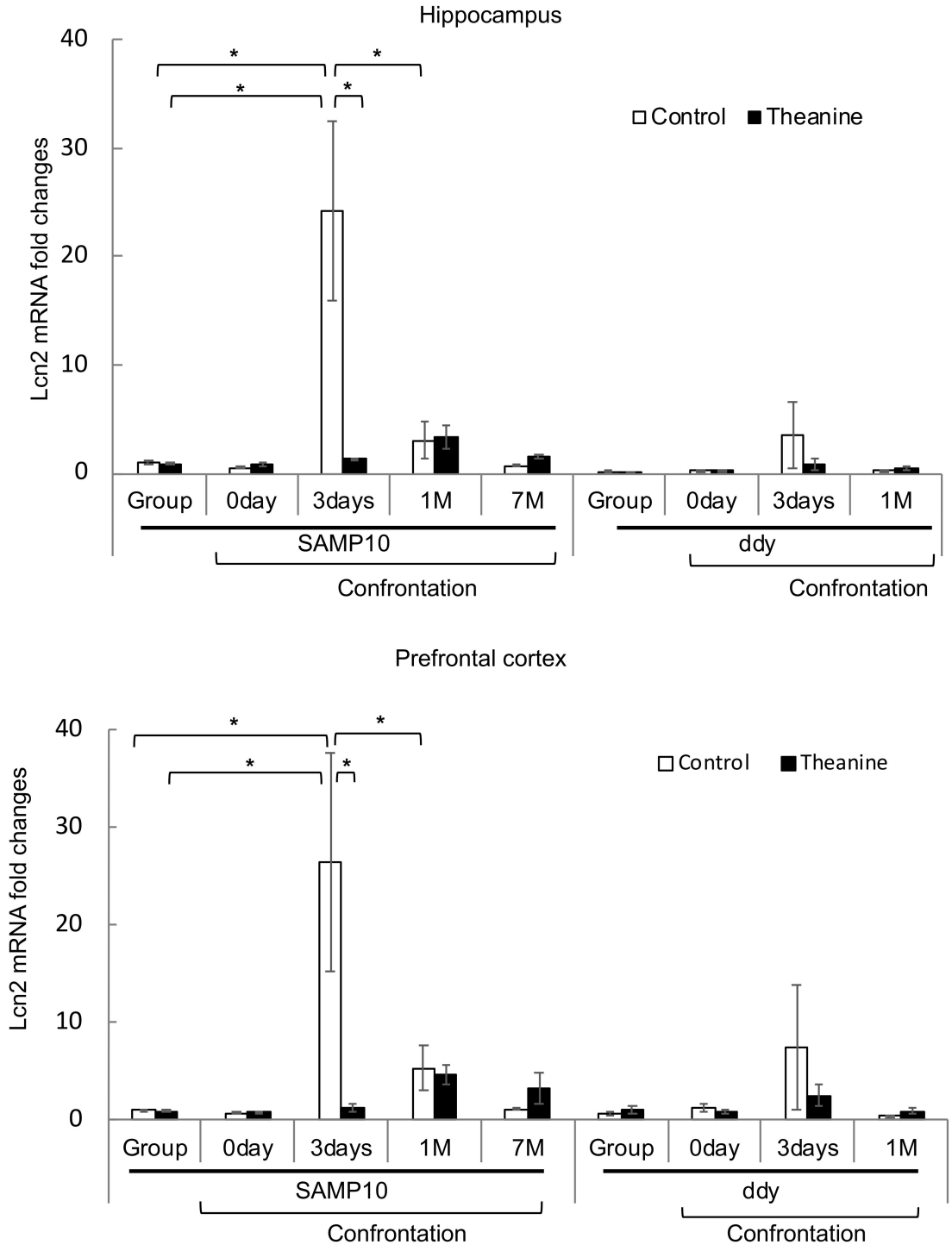


Figure 7. Expression levels of *Lcn2* mRNA in the hippocampus and prefrontal cortex of SAMP10 and ddY mice. Mice consumed theanine (20 μg/mL water, closed bar) or normal tap water (control, open bar) ad libitum. After single housing for one month, hippocampal and prefrontal cortex samples were obtained from mice housed confrontationally for 0 days, 3 days, 1 month, and 7 months. Group-housed mice were kept in a cage for two months. Values are expressed as means ± SEM (n = 3–6, * $p < 0.05$).

4. Discussion

SAMP10 mice are susceptible to aging and display characteristic age-related cerebral atrophy [56]. We previously found that cerebral atrophy was accelerated in aged SAMP10 mice that were psychosocially stressed by confrontational housing [18]. We examined whether cerebral atrophy

caused by psychosocial stress is a specific phenomenon in SAMP10 mice. In this study, volumetric changes induced by psychosocial stress were observed one month after confrontational housing in SAMP10 mice. Furthermore, subsequent brain atrophy with aging was observed after six months of age. On the other hand, in SAMP10 mice that ingested theanine, a reduction in and recovery from cerebral atrophy were observed. Cerebral atrophy was also observed in ddY mice—a strain that ages normally—but it was temporary within three months of age. Since significant adrenal hypertrophy was observed for at least one week after confrontational housing in ddY mice [19], it is considered that SAMP10 and ddY mice similarly feel psychosocial stress in confrontational housing. However, the stress due to confrontational housing may not last long in ddY mice.

Then, we examined the targets of theanine in the brain at the beginning of confrontational housing to determine the reason for the difference between SAMP10 and ddY mice. The expression of *Npas4* was significantly higher in mice that ingested theanine under confrontational housing than control SAMP10 mice. Npas4 is a neuronal transcription factor, the expression of which is enriched in the limbic system [51]. The detection of Npas4 protein in the soma, neurites and synapses suggests that Npas4 is involved in synaptic plasticity in the brain [57]. Using Npas4 knockout neurons, it has been suggested that Npas4 plays an important role in the structural plasticity of neurons [17]. Increased expression of Npas4 is considered to be important for preventing brain atrophy due to stress. Although the expression of Npas4 was reduced by stress loading in SAMP10, it was increased in mice which ingested theanine. On the other hand, in ddY mice, the expression increased during stress loading even without theanine ingestion. The difference between SAMP10 and ddY mice regarding the expression of Npas4 is considered to contribute to the difference in the degree of brain atrophy.

Npas4 expression is considered as a marker of hippocampus activation [58]. Data of *Npas4* knockout mice suggest that Npas4 plays a major role in the regulation of cognitive and social functions in the brain [59]. Although brain atrophy in Npas4 knockout mice is not mentioned, increased *Npas4* expression during stress appears to be needed to increase stress tolerance. That is, while exposure to acute unavoidable stress induced a long-lasting decrease in *Npas4* expression, resilient rats recovered the level of hippocampal Npas4 better than their vulnerable counterparts [60]. Npas4 regulates the formation and maintenance of inhibitory synapses in response to excitatory synaptic activity [59,61]. Npas4 in both excitatory and inhibitory neurons activates distinct programs of late-response genes that promote inhibition in excitatory neurons but induce excitation in inhibitory neurons [62]. Thus, γ-aminobutyric acid (GABA) release is increased, resulting in the overall lowering of the levels of circuit activity [62,63]. These lines of evidence strongly suggest that Npas4 plays an important role in the development of inhibitory synapses by increasing GABA release and lowering the overall levels of circuit activity. The increased expression of *Npas4* by theanine suggests increased GABA release in stressed mice. In addition, theanine inhibits glutamine uptake from the glutamine receptor, resulting in the inhibition of glutamate release [64]. Since chronic stress causes an imbalance of excitation–inhibition generated by a deficit of inhibitory neurotransmitters on principal glutamatergic neurons [65], theanine intake is thought to suppress excessive excitation by increasing the release of GABA through increased *Npas4* expression.

Npas4 has also been demonstrated to play a role in the neuroprotective response in various animal models of acute neurological injury and limits tissue damage through the modulation of the cell death pathway by directing damaged cells to undergo apoptosis instead of necrosis [51]. Furthermore, GABAergic neurons are particularly susceptible to aging-related alterations that are involved in many aging-induced cognitive impairments and brain disorders [66,67]. Increased *Npas4* expression in the prefrontal cortex by theanine may be involved in the suppression of brain atrophy and cognitive decline in aged SAMP10 mice that ingested theanine. Recent data indicates that the suppression of neural excitation by repressor element-1 silencing transcription factor (REST) regulates aging [68]. These studies suppose that increased expression of *Npas4* by theanine may play an important role in suppressing aging by reducing stress.

On the other hand, *Lcn2*, which is induced by acute stress [54,69], was significantly higher in SAMP10 mice three days after confrontational housing but not in mice that ingested theanine. *Lcn2* is up-regulated in the mouse hippocampus following psychological stress [54]. Since the ingestion of theanine suppresses adrenal hypertrophy under stress [19], the suppression of *Lcn2* could be caused by the suppression of excitation of the hypothalamus–pituitary–adrenal axis. The level of *Lcn2* was still high in SAMP10 mice after one month of confrontational housing, but the level in ddY mice was about 1/12 of that of SAMP10 mice. Lcn2, which is primarily secreted by reactive astrocytes, directly induces neuronal damage and amplifies neurotoxic inflammation under many brain conditions [70]. Since Lcn2 protein increases the sensitivity of neuronal cells to cell death [71], long-lasting *Lcn2* overexpression may be a reason for brain atrophy and stress vulnerability in SAMP10 mice. Lcn2 protein regulates cellular iron concentration [72]. Furthermore, Lcn2 modulates several behavioral responses such as cognitive function, depression, neuronal excitability and anxiety [69]. Mucha et al. [54] hypothesize that iron-free Lcn2 acts as an important regulator of neuronal morphological changes under physical conditions, whereas excess iron-free Lcn2 is harmful to neurons by sequestering intracellular iron and shutting down iron-responsive genes. The suppression of excess Lcn2 is thought to be a therapeutic target for chronic neuroinflammatory and neurodegenerative diseases including Alzheimer's and Parkinson's diseases, depression, schizophrenia and autism [70], suggesting that theanine may be important in protecting the brain not only from stress but also from many chronic neuroinflammatory and neurodegenerative diseases. Furthermore, the measurement of cerebrospinal fluid Lcn2 levels might be able to diagnose brain damage due to Alzheimer's disease, traumatic brain injury and chronic stress [73,74].

In addition, neurogenesis may be increased by the increased expression of *Npas4*, *Fabp7* and *Btg2*, because these genes are involved in neurogenesis [49,52,53,75]. The modulation of these genes involved in the early stress response may be important for the suppression of brain injury due to stress. In particular, *Npas4* and *Lcn2* may play key roles in this context. It is necessary to clarify how theanine modulates *Npas4* and *Lcn2* expression in the near future.

5. Conclusions

The brain volume of SAMP10—a stress-sensitive mouse—decreased by stress loading. However, theanine—the main amino acid in tea leaves—suppressed brain atrophy. Theanine was suggested to prevent stress-induced brain atrophy by modifying early stress responses such as Npas4 and Lcn2.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/12/1/174/s1, Table S1: Brain volume of each part.

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L-Arginine Exerts Excellent Anti-Stress Effects on Stress-Induced Shortened Lifespan, Cognitive Decline and Depression

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Abstract: The anti-stress potential of dietary L-arginine (Arg) was assessed in psychosocially stress-loaded senescence-accelerated (SAMP10) mice. Although this strain of mouse is sensitive to stress, daily administration of Arg at 3 mg/kg significantly suppressed aging-related cognitive decline and behavioral depression at nine months of age and counteracted stress-induced shortened lifespan. To investigate the mechanism of the anti-stress effect of Arg in the brain, early changes in oxidative damage and gene expression levels were measured using SAMP10 mice that were stress-loaded for three days. Increased lipid peroxidation in the brains of stressed mice was significantly lowered by Arg intake. Several genes associated with oxidative stress response and neuronal excitotoxic cell death, including *Nr4a1*, *Arc*, and *Cyr61*, remarkably increased in response to psychosocial stress; however, their expression was significantly suppressed in mice that ingested Arg even under stress conditions. In contrast, the genes that maintain mitochondrial functions and neuronal survival, including *Hba-a2* and *Hbb-b2*, were significantly increased in mice that ingested Arg. These results indicate that Arg reduces oxidative damage and enhances mitochondrial functions in the brain. We suggest that the daily intake of Arg plays important roles in reducing stress-induced brain damage and slowing aging.

Keywords: aging; arginine; brain; chronic psychosocial stress; depression; oxidative damage; shortened lifespan

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1. Introduction

We have conducted research on the anti-stress effect of theanine, an amino acid that is mainly found in the leaves of *Camellia sinensis* L. (green tea) [1–8]. In our previous studies wherein the anti-stress effects of other amino acids in green teas were examined, L-arginine (Arg) was found to exert an excellent anti-stress effect that is similar to or better than that exerted by theanine [4]. Almost the same anti-stress effect of Arg was observed at 0.032–3.2 mg/kg/day. Arg is the second most abundant amino acid, after theanine, in high-grade green teas [4]. Chronic psychosocial stress has been demonstrated to shorten lifespan and accelerate age-related alterations such as cerebral atrophy, oxidative damage, cognitive dysfunction, and behavioral depression in stress-loaded senescence-accelerated mouse prone 10 (SAMP10) [1,2]. Mice of this strain have been reported to exhibit a short lifespan, aging-related brain atrophy, and cognitive decline even under normal conditions [8–11] and are sensitive to stress [8]. Therefore, we aimed to further elucidate whether Arg has anti-stress potential against chronically-stressed SAMP10 mice in the present study.

Fundamentally, Arg, one of the 20 basic natural amino acids, is functionally classified as an essential amino acid in birds, carnivores, and young mammals and semi-essential

for adults, and has been identified to play critical roles in health, including immune response [12], wound healing [13], growth hormone release [14], and cell proliferation [15]. Dietary sources of Arg include meat, wheat, sea foods, milk, cheese, corn, soy, nuts, and others [16]. In Japanese green tea, Arg is contained in the range from 0.85 to 3.14 mg/g as a free amino acid [17]. In previous studies, dietary Arg has been reported to suppress oxidative stress [18] and inflammatory responses [19]. Ingested Arg via the gastrointestinal tract is absorbed in the small intestine. Thereafter, approximately 40% of the ingested Arg is circulated systemically [20]. Dietary Arg is degraded by arginase, which converts Arg into urea, ornithine, proline, polyamines, glutamate, and glutamine [21]. In addition, nitric oxide, which can be converted from Arg by nitric oxide synthase [21], acts as a precursor of signaling molecules [22] and is involved in several functions, including the vasodilation of blood vessels [21], synaptic plasticity [23], learning and memory processing [24], and modulation of neuronal function during stress and anxiety [25]. Therefore, Arg and its metabolites play many important roles in health.

Chronic psychosocial stress has been associated with various mental disorders such as depression and anxiety [26]; it elevates the risk of neurodegenerative diseases, including Alzheimer's disease, dementia [27], and cardiovascular diseases, accelerates aging, and shortens lifespan [28]. Numerous animal and human studies have shown the deleterious effects of stress on the brain, behavior, and cognitive function [1,8,29–31]. The brain is highly susceptible to stress during both early childhood and old age [32]. Stress activates the hypothalamic–pituitary–adrenal axis which leads to the secretion of glucocorticoids from the adrenal glands [32,33]. Increased levels of glucocorticoids have been associated with neuronal loss [34,35], cognitive impairment, and Alzheimer's disease development [36].

In the present study, to elucidate the anti-stress potential of Arg on stress-loaded SAMP10 mice, the long-term effect of stress was observed by measuring the cognitive function and depressive-like behavior of mice at nine months of age. Furthermore, the lifespan of these mice was measured. Next, to elucidate the mechanism of Arg in the brain, the initial responses of SAMP10 mice loaded with stress for three days were used to observe the changes in lipid peroxidation (LPO) and gene expression levels in the hippocampus and prefrontal cortex.

2. Results

2.1. Long-Term Effect of Stress

2.1.1. Improving Effect of Arg on Learning Ability and Behavioral Depression

The learning ability of mice was measured at nine months of age using a step-through passive avoidance test. A longer learning time implies lower learning ability. The mice that ingested Arg under both confrontation and group housings showed a significantly shortened learning time than the control mice that consumed only water under both housing conditions (Figure 1A). The group-housed mice were defined as mice under a low-stress condition; however, the group-housed mice used in this experiment sometimes fought and might have been stressed similarly as the mice that were confrontationally housed. Therefore, no difference in terms of learning ability between the mice that were group-housed and confrontationally housed was observed in this experiment.

The effect of Arg intake on behavioral depression was investigated using a tail suspension test at nine months of age. The immobility duration was significantly shorter in the confrontationally housed and group-housed mice that ingested Arg than the control mice that were also confrontationally and group-housed (Figure 1B).

2.1.2. Improving Effect of Arg Intake on Lifespan

Many among the confrontationally housed mice died earlier than the group-housed mice (Figure 2A). The median survival time (MST) of the confrontationally housed mice that consumed only water was 10.5 months, whereas that of the mice administered Arg at 3 mg/kg under confrontational housing was 16.6 months (Figure 2B,D); this MST was 1.58 times longer ($p = 0.032$) than the MST of confrontationally housed mice that consumed

only water. In contrast, no difference in MST was observed in the group-housed mice by Arg intake (Figure 2C) ($p = 0.75$) and between confrontational and group housing control mice (Figure 2A) ($p = 0.17$).

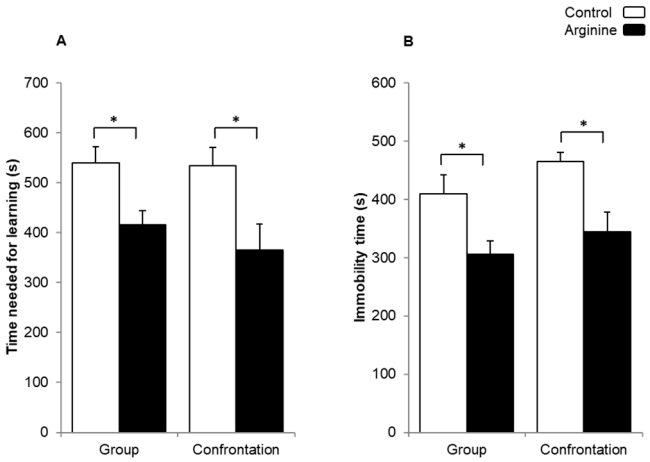

Figure 1. Effect of Arg intake on learning ability and behavioral depression in SAMP10 mice. The step-through passive avoidance test (**A**) and the tail suspension test (**B**) were performed using 9-month-old mice. Mice ingested Arg (3 mg/kg, closed column) or only water (control, open column) (the number of mice in each group = 12; * $p < 0.05$).

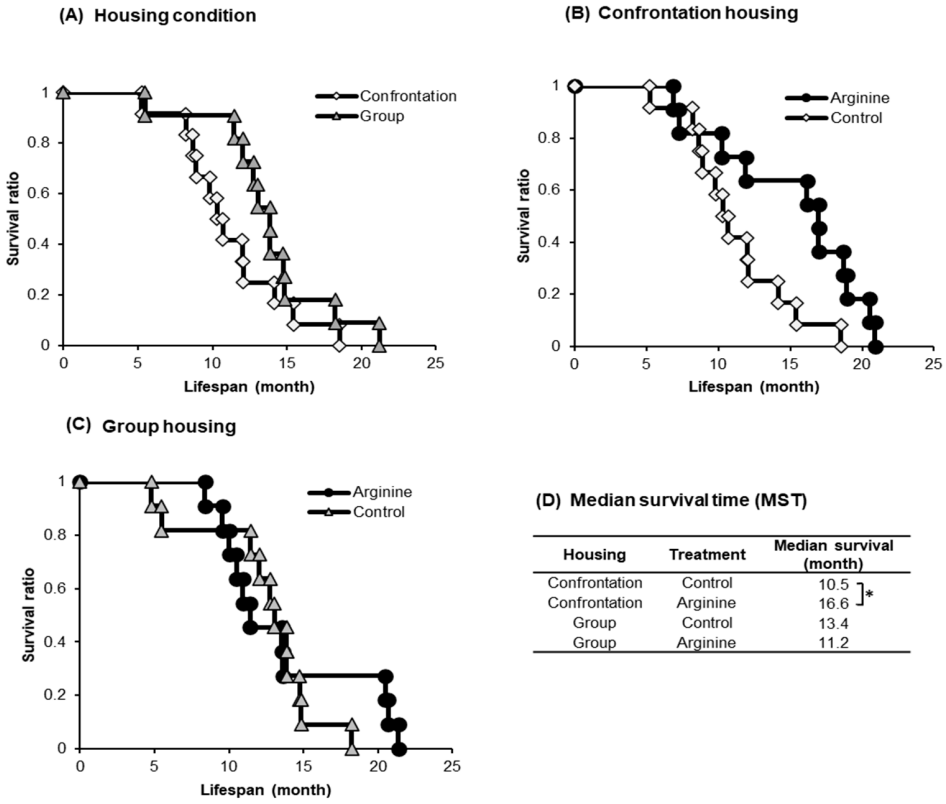

Figure 2. Effect of Arg intake on longevity in SAMP10 mice. Effect of housing condition (**A**), effect of Arg intake on confrontation housing (**B**), effect of Arg intake on group housing (**C**), and median survival time of each group (**D**). Four groups of mice, the number of mice in each group, $n = 12$ (6 mice were housed per cage for group housing and 2 mice per cage for confrontation housing). Mice ingested Arg at 3 mg/kg or only water (control) freely from 1 month of age. Arg-water (10 μg/mL) was freshly prepared twice a week. * $p < 0.05$.

2.2. Initial Response to Stress

2.2.1. Oxidative Damage in the Brain

The LPO levels in the cerebral cortex, a marker for oxidative damage, were measured using the mice that were confrontationally housed for three days. Same-aged group-housed mice were used as a reference. LPO levels were significantly higher in the confrontationally housed control mice than those of the group-housed control mice and the mice that ingested Arg under confrontational housing (Figure 3). No difference in LPO levels due to Arg intake was observed in group-housed mice (Figure 3).

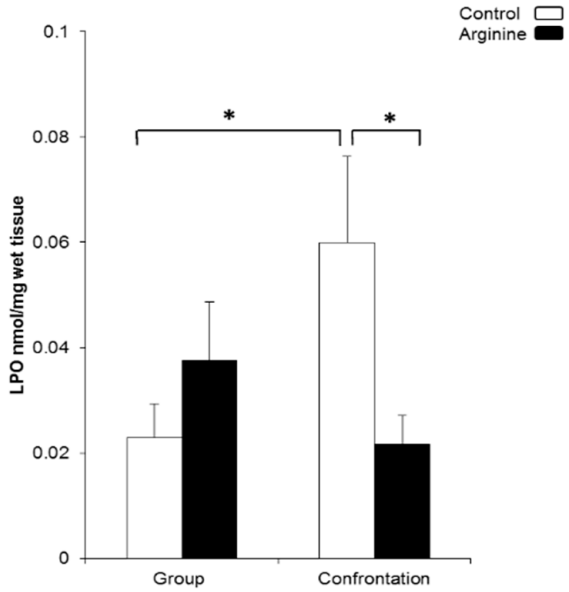

Figure 3. Levels of oxidative damage in the brain. Lipid peroxidation (LPO) in the brain was measured using the cerebral cortex. Mice were housed confrontationally for 3 days after single housing for 1 month. Mice ingested Arg (3 mg/kg, closed column) or only water (control, open column) (n = 5~6, * $p < 0.05$, Fisher's least significant differences).

2.2.2. Effect of Arg Intake on Gene Expression in the Hippocampus of Stressed SAMP10 Mice

To investigate the mechanism of action of Arg in the brain, we performed microarray analysis to assess the comprehensive changes in gene expression using the hippocampus of mice. The early changes in gene expression on the third day of stress loading were examined. In the microarray analysis, all four groups of mice's hippocampus tissues were used including group-control, group-Arg, confrontation-control, and confrontation-Arg. As a result of principal component analysis (PCA), changes due to Arg intake appeared along with the PC1 axis. Meanwhile, changes due to Arg intake under the stress condition coincided with the PC3 axis. The data are shown simultaneously in a biplot (Figure 4). The top 10 genes that were significantly downregulated and upregulated in the mice that ingested Arg are listed in Table 1. Among these genes, nuclear receptor subfamily 4, group A, member 1 (Nr4a1), also known as Nur77, was the most downregulated gene after Arg intake. Nr4a1 is a potent pro-apoptotic member of the nuclear receptor superfamily and is associated with neuronal excitotoxicity and neuronal cell death [37–39]. Activity-regulated cytoskeleton-associated protein (Arc) has been shown to induce neuronal cytotoxicity and cell death [40]. Membrane-spanning 4-domains, subfamily A, member 6 D (Ms4a6d) is a transmembrane protein involved in inflammatory signaling [41] and Alzheimer's disease [42,43]. Moreover, midline 1 (Mid1) has been reportedly expressed in the brains of patients with Alzheimer's disease [44]. Cysteine rich protein 6 (Cyr61) has been associated with neuronal cell death [45].

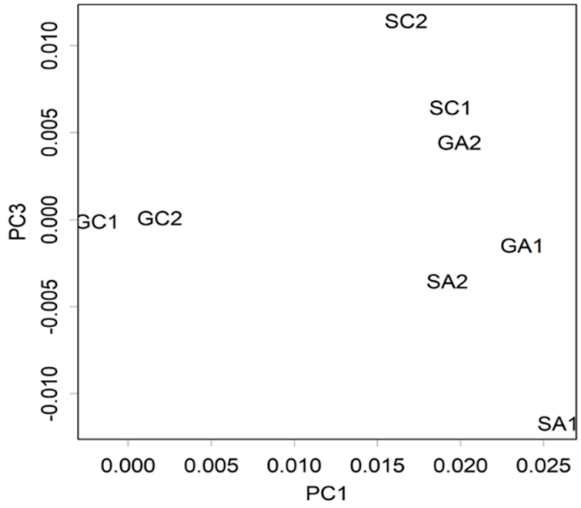

Figure 4. Principal component analysis of gene expression. Hippocampal samples were obtained from mice housed confrontationally for three days. Group-housed mice were kept in a cage for one month. The principal component (PC) ordination of ANOVA-positive genes is based on the transcriptome of hippocampal gene expression in mice of GC: group-control; GA: group-Arg; SC: confrontation stress-control, SA: confrontation stress-Arg (n = 2 for each group).

Table 1. Top 10 significantly downregulated and upregulated genes in the hippocampus of mice that ingested Arg under confrontational housing.

	Symbol	Full Name	ΔZ	p
Downregulated	Nr4a1	Nuclear receptor subfamily 4, group A, member 1	−0.2726	3.51×10^{-10}
	Arc	Activity regulated cytoskeleton-associated protein	−0.2345	2.51×10^{-7}
	Olfr1384	Olfactory receptor 1384	−0.2446	0.00702
	Cryba1	Crystallin, beta A1	−0.2270	0.00280
	Ms4a6d	Membrane-spanning 4-domains, subfamily A, member 6D	−0.2371	0.00674
	Mid1	Midline 1	−0.2399	2.93×10^{-9}
	Cyr61	Cysteine rich protein 61	−0.2591	8.73×10^{-10}
	Prss2	Protease, serine 2	−0.2197	0.00620
	H2-Q6	Histocompatibility 2, Q region locus 6	−0.2550	0.00548
	Mir1983	MicroRNA 1983	−0.1860	0.00375
Upregulated	Mela	Melanoma antigen	0.3509	1.42×10^{-5}
	Olfr2	Olfactory receptor 2	0.3302	0.00402
	Slitrk6	SLIT and NTRK-like family, member 6	0.1712	0.00023
	Hbb-b2	Hemoglobin, beta adult minor chain	0.3780	3.55×10^{-8}
	Itih2	Inter-alpha trypsin inhibitor, heavy chain 2	0.2867	7.42×10^{-6}
	Olfr535	Olfactory receptor 535	0.3544	0.00262
	Zic1	Zinc finger protein of the cerebellum 1	0.0703	4.38×10^{-9}
	LOC666331	Uncharacterized LOC666331	0.2908	0.00262
	Hba-a2	Hemoglobin alpha, adult chain 2	0.3284	6.38×10^{-17}
	Tcf712	Transcription factor 7 like 2, T cell specific, HMG box	0.1181	1.27×10^{-11}

ΔZ = expression level (confrontation Arg-confrontation control).

In contrast, melanoma antigen (Mela), whose function is unknown, is the most upregulated gene in the hippocampus of mice that ingested Arg. Hemoglobin is the iron-containing protein that carries oxygen in vertebrate erythrocytes. Hemoglobin α- and β-chains (Hba and Hbb) were found to be expressed in several brain regions, including the cortex and hippocampus of rats [46]. Furthermore, these proteins have been identified within the mitochondrion of neurons [47] and are important in maintaining mitochondrial functions and neuronal survival.

2.2.3. Effect of Arg Intake on Nr4a1, Arc, and Cyr61 Levels in the Brain

As the *Nr4a1*, *Arc*, and *Cyr61* genes are the most significantly downregulated genes after Arg intake (detected using microarray analysis), we focused on these genes and compared their expression levels in both the hippocampus and prefrontal cortex tissues of group-housed mice using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) (Figure 5). Arg intake exerted no effect on these genes in group-housed mice; however, the gene expression levels of *Arc* in confrontationally housed mice were significantly higher than those of group-housed mice. Furthermore, the expression levels of *Nr4a1* and *Cyr61* in confrontationally housed mice tended to be higher than those in group-housed mice (Figure 5A). In the mice that ingested Arg at 3 mg/kg under the stressed condition, the expression of these genes was significantly suppressed (Figure 5A). In the prefrontal cortex, the expression levels of *Nr4a1*, *Arc*, and *Cyr61* between the control mice that were group-housed and confrontationally housed did not differ (Figure 5B). The expression levels of these genes were significantly suppressed in mice that ingested Arg under confrontational housing (Figure 5B).

2.2.4. Effect of Arg Intake on Hba-a2 and Hbb-b2 Levels in the Brain

As *Hba-a2* and *Hbb-b2* are the most significantly upregulated genes in the hippocampus after Arg intake and are important factors in maintaining the function of neuronal mitochondria, we focused on these genes. qRT-PCR results indicate that *Hba-a2* levels in the hippocampus of confrontationally housed mice were similar to those of group-housed mice (Figure 5A); however, in the mice that ingested Arg, *Hba-a2* levels were significantly increased in both group-housed and confrontationally housed mice (Figure 5A). Similarly, in the prefrontal cortex, *Hba-a2* expression levels were significantly increased in mice that ingested Arg under group housing (Figure 5B). The levels of *Hbb-b2* expression in the hippocampus (Figure 5A) and prefrontal cortex (Figure 5B) were significantly increased in mice that ingested Arg under group housing and tended to increase in mice that ingested Arg under confrontational housing.

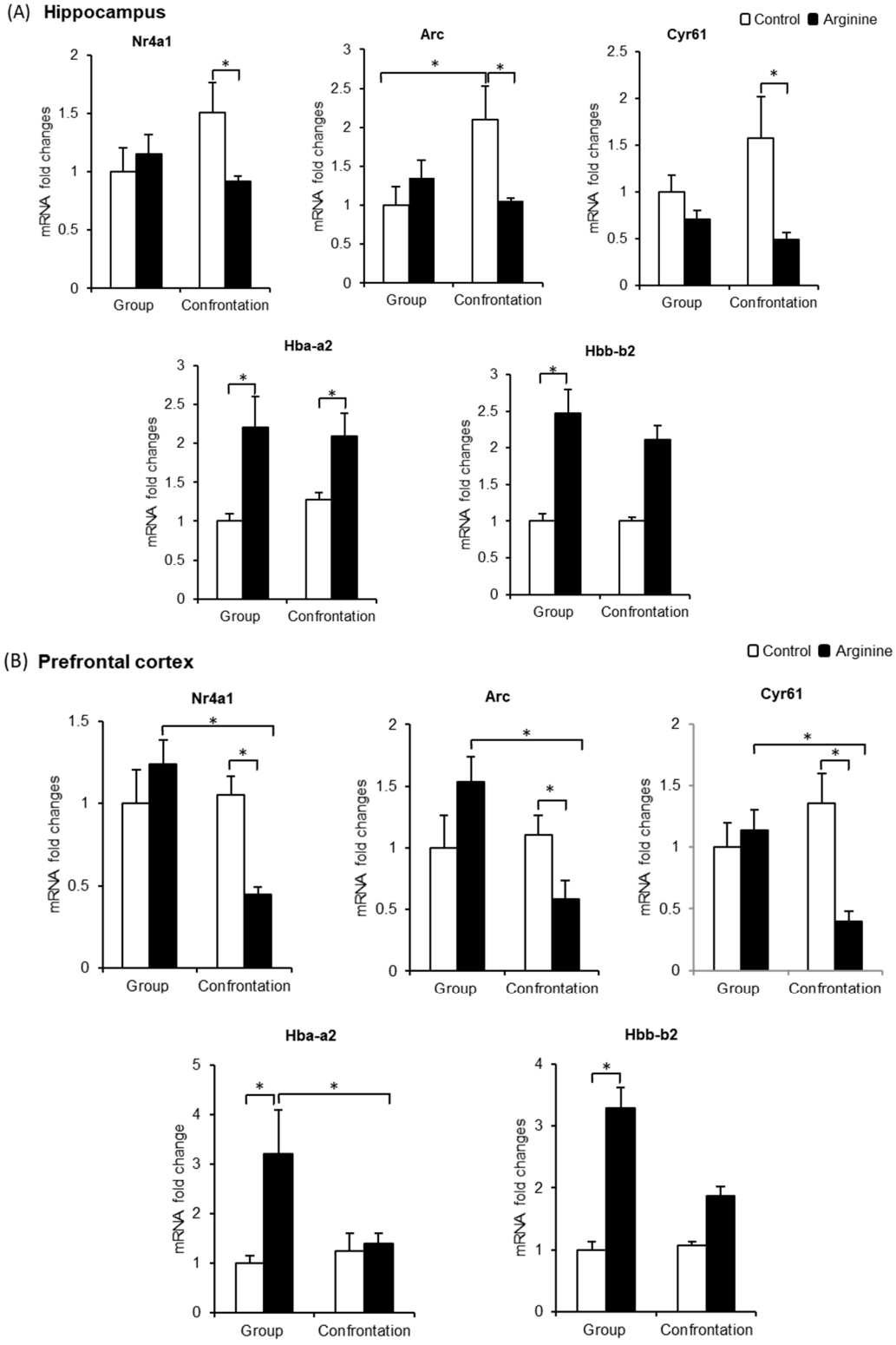

Figure 5. (**A**) Expression of *Nr4a1*, *Arc*, *Cyr61*, *Hba-a2*, and *Hbb-b2* in the hippocampus of mice under group and confrontation housing. Mice ingested Arg (3 mg/kg, closed column) or only water (control, open column) (n = 5~6, * $p < 0.05$, Fisher's least significant differences). (**B**) Expression of *Nr4a1*, *Arc*, *Cyr61*, *Hba-a2*, and *Hbb-b2* in the prefrontal cortex of mice under group and confrontation housing. Mice ingested Arg (3 mg/kg, closed column) or only water (control, open column) (n = 5~6, * $p < 0.05$, Fisher's least significant differences).

3. Discussion

In this study, we found that Arg exerts a remarkable anti-stress effect on the brain as a new function. A significant increase in oxidative damage was observed in the brain of stress-loaded SAMP10 mice, and these mice exhibited cognitive decline as well as a shortened lifespan as they aged. No lifespan-shortening effect due to confrontational housing was observed in WT mice such as ddY and C57BL/6 (data unpublished). Oxidative stress plays a crucial role in the aging process, particularly in cognitive dysfunctions [48]. Previous data suggested that dietary Arg supplementation ameliorated oxidative stress and that oral administration of Arg at a dose of 1.6 g/day for three months substantially reduced LPO levels in patients with senile dementia [49]. Our Arg dose administered to SAMP10 mice (3 mg/kg) was lower than the dose in the above report, but it supports that Arg has an inhibitory effect on LPO.

Next, we looked at the molecular target of Arg in the brain to elucidate its mechanism in suppressing oxidative damage. The expression of several immediate-early genes (IEGs) such as *Nr4a1*, *Arc*, and *Cyr61* significantly increased in the hippocampus of stressed mice and was suppressed in mice that ingested Arg (Figure 5A). *Nr4a1* is associated with adrenal stress response and excessive neuronal excitotoxicity [37] and is known to be a potent pro-apoptotic molecule that induces nerve cell death [38,39]. Most importantly, Nr4a1 activated in response to oxidative stress has been reported to translocate from the nuclei to the mitochondria and induce mitochondrial damage and cell death [50]. Arc reportedly plays a critical role in the neuronal excitotoxicity mediated by glutamate receptor signaling. Moreover, an elevated mRNA and protein expression of Arc has been detected in rat cortical neurons via neurotoxic stimulation [40]. Cyr61 induction has been associated with neuronal cell death [45], and Cyr61 elevation has been reported to be associated with oxidative stress as it was markedly upregulated at both the gene and protein levels against reactive oxygen species induction in human dermal fibroblasts cells [51].

Arg incorporated into the brain has been shown to completely block glutamate-induced neuronal excitation in the ventromedial hypothalamus of rats [52]. Our data suggest that dietary Arg incorporated into the brain suppresses the stress-induced elevation of *Nr4a1*, *Arc*, and *Cyr61* in the hippocampus and that the suppression of these genes may be involved in preventing neuronal cell death through the regulation of excessive neuronal excitotoxicity and mitochondrial damage via the suppression of oxidative damage in the brain.

Contrarily, *Hba-a2* and *Hbb-b2* expression levels were increased in the hippocampus and prefrontal cortex of mice that ingested Arg under both group and confrontational housing. As both Hba and Hbb are co-localized within the mitochondrion of neurons and are closely associated to maintain the neuronal mitochondrial function as well as survival of neurons [47], we speculate that Arg protects neurons by maintaining the neuronal mitochondrial function. An increase in the expression of Hba and Hbb reportedly has therapeutic effects against neurodegenerative disease, and increasing evidence suggests that a deficiency in these chains in the brain is associated with neurodegenerative disease [53,54]. Our results suggest that Arg may also be important in protecting the brain from neurodegenerative diseases such as Alzheimer's disease.

Arg revealed a similar protective effect to that of theanine on stress-induced shortened lifespan, cognitive decline, and depression in SAMP10 mice. We have shown that theanine significantly altered the gene expression of neuronal PAS domain protein 4 *(Npas4*) and lipocalin 2 (*Lcn2*) in the hippocampus of stressed SAMP10 mice [8]. Therefore, theanine and Arg suppress stress in different ways.

In the present study, we demonstrated that orally administered Arg modulates psychosocial stress-induced gene expression in the hippocampus and prefrontal cortex of SAMP10 mice. Further study is necessary to elucidate how dietary Arg modulates the gene expression of *Hba-a2*, *Hbb-b2*, and IEGs (*Nr4a1*, *Arc*, and *Cyr61*) in the brain. It may be necessary to determine by immunofluorescence study that which cell types in the hippocampus and frontal cortex, namely, glial or neuron, is primarily reduce lipid peroxidation

by Arg. In addition, the effect of Arg on mitochondrial morphology and function needs to be elucidated.

4. Materials and Methods

4.1. Animals, Arg Preparation, and Housing Condition

Four-week-old male SAMP10/TaSlc (SAMP10) mice were purchased from Japan SLC Co., Ltd. (Shizuoka, Japan), and bred under conventional conditions in a temperature- and humidity-controlled room with 12/12 h light–dark cycle (light period, 08:00–20:00; temperature, 23 ± 1 °C; relative humidity, 55 ± 5%). Female mice were not used in this experiment because females are not as territorial as males. The mice were fed a normal diet (CE-2; Clea Co., Ltd., Tokyo, Japan). Arg (Wako Pure Chemical Co., Ltd., Osaka, Japan) was dissolved in water at 10 μg/mL. The volume of water containing Arg consumed by the mice was measured. Forty-eight mice were divided into four groups and observed to determine the long-term effects of Arg on their cognitive function and depression at nine months of age. Thereafter, the mice were fed continually to measure their lifespan. To study the effect of Arg on the brain, another group of 24 mice was used: 12 mice ingested Arg by drinking water ad libitum at 10 μg/mL (3 mg/kg) from one month of age. The remaining 12 mice ingested water as a control. Arg solution was freshly prepared twice a week. All experimental protocols were approved by the University of Shizuoka Laboratory Animal Care Advisory Committee (approval No. 166197, 5 April 2016) and were in accordance with the guidelines of the US National Institutes of Health for the care and use of laboratory animals.

4.2. Housing Condition for Confrontation

To induce psychosocial stress in mice, confrontational housing was used, as previously described [1,2,4,8]. To build territoriality, two mice were housed for one month in a standard polycarbonate cage that is equally divided into two units by a stainless-steel partition. Next, the partition was removed, and the mice were co-housed confrontationally to induce psychosocial stress (Figure 6). Group housing was used as a model of the low-stressed condition. Two experiments in a linear diagram are shown including each experimental step for long term and short term (Figure 6).

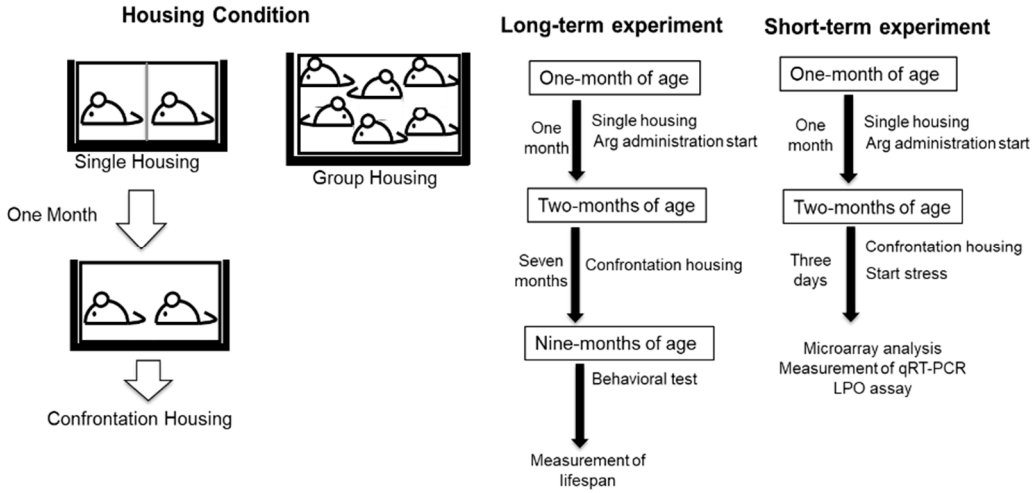

Figure 6. Two mice were housed in a cage with a stainless-steel partition (single housing) for one month. The partition was withdrawn, and mice were co-housed confrontationally in the same cage (confrontation housing) to load psychosocial stress. Group housing mice were kept with six in a cage. For the long-term experiment, after confrontationally housing mice for seven months, a behavioral test was performed at nine months of age; thereafter, their lifespan was measured. For the short-term experiment, after confrontationally housing mice for three days, microarray analysis, qRT-PCR, and LPO assay were performed.

4.3. Memory Acquisition Test

To measure the learning ability of mice, a step-through passive avoidance task was tested on 9-month-old mice, as previously described [2]. Briefly, when a mouse enters a dark chamber from a light chamber, the door in the chamber is closed and an electric foot-shock is delivered at 50 μA for 1 s (SGS-003, Muromachi Kikai. Co., Ltd., Tokyo, Japan). The acquisition of the avoidance response was considered successful if the mouse remained in the light chamber for 300 s. The trial was repeated until the mouse satisfied the acquisition criterion within five trials. For each trial, the time spent by the mice in the light chamber was subtracted from 300 s; the results from the successive trials were summed up for each mouse to determine the time required for learning ("learning time").

4.4. Measurement of Immobility in the Tail Suspension Test

To examine behavioral depression, mice were individually suspended by their tails at a height of 30 cm using a clip for tail suspension (MSC2007, YTS Yamashita Giken, Tokushima, Japan). The duration of immobility was recorded for 15 min, as previously described [1]. The mice were considered immobile only when they were both passively hanging and completely motionless. The duration during which the mice were immobile was measured.

4.5. Measurement of Oxidative Damage in the Brain

Mice that were confrontationally housed for three days after being singly housed for one month were used for the quantification of LPO. LPO in the brain of SAMP10 mice was measured using a lipid hydroperoxide assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer's instructions. Briefly, approximately 50 mg of the cerebral cortex was homogenized in 500 μL of HPLC-grade water. An equal volume of methanol solution saturated with Extract R® was added following the addition of 1 mL chloroform–methanol (2:1, v/v) solvent. After centrifugation at 1500× g for 5 min, the bottom layer of the chloroform was collected, and the lipid hydroperoxide content was measured via redox reactions with ferrous ions. The resulting ferric ions produced from the reaction of hydroperoxide with the ferrous ions were detected using thiocyanate ion as the chromogen. The absorbance of each sample at 500 nm was obtained (n = 6/group).

4.6. Measurement of DNA Microarray and qRT-PCR

The mice that were confrontationally housed for three days after being singly housed for one month were used for DNA microarray analysis. The mice were provided 10 μg/mL (3 mg/kg) Arg-water ad libitum. RNeasy Mini Kit (NucleoSpin® RNA, 740955, Takara Bio Inc., Shiga, Japan) was used to extract total RNA from the hippocampus. To synthesize biotinylated cRNA, total RNA was processed using One-Cycle Target Labeling and Control Reagents (Affymetrix, Santa Clara, CA, USA) and hybridized to a Total RNA Mouse Gene 2.0 ST Array (Affymetrix) using three biological repeats per group. The significance of Arg intake was statistically tested using two-way ANOVA at $p < 0.001$ [55,56].

For the measurement of qRT-PCR, group-housed same-aged mice were used as the reference. Total RNA was isolated from the homogenized hippocampus and prefrontal cortex as described above. cDNA was prepared from the obtained RNA using PrimeScript® RT Master Mix (RR036A, Takara Bio Inc.). qRT-PCR analysis was performed using PowerUp™ SYBR™ Green Master Mix (A25742, Applied Biosystems Japan Ltd., Tokyo, Japan) and automated sequence detection systems (StepOne, Applied Biosystems Japan Ltd., Tokyo, Japan). Previously validated primers for *Nr4a1*, *Arc*, *Cyr61*, *Hba-a2*, and *Hbb-b2* [37,57–60] (Table 2) were used to quantify their relative gene expression. β-actin was used as the internal control.

Table 2. Primer sequences for qRT-PCR.

Gene	Forward Sequence (5′–3′)	Reverse Sequence (5′–3′)	Ref.
Nr4a1	CTGCCTTCCTGGAACTCTTCA	CGGGTTTAGATCGGTATGCC	[37]
Arc	ACGATCTGGCTTCCTCATTCTGCT	AGGTTCCCTCAGCATCTCTGCTTT	[57]
Cyr61	CCCCCGGCTGGTGAAAGTC	ATGGGCGTGCAGAGGGTTGAAAAG	[58]
Hba-a2	GAAGCCCTGGAAAGGATGTT	GCCGTGGCTTACATCAAAGT	[59]
Hbb-b2	CACCTGACTGATGCTGAGAAGT	CCCTTGAGGTTGTCCAGGTTT	[60]

4.7. Statistical Analysis

Statistical data are presented as the mean ± standard error of the mean. Statistical analysis was performed using one-way ANOVA followed by Tukey–Kramer's honest significant difference method for cognition activity and a tail suspension test. Fisher's least significant differences were used for qRT-PCR and the LPO assay. After calculating survival rates using the Kaplan–Meier method, the difference in survival rate was tested using the log-rank test. $p < 0.05$ was considered statistically significant.

5. Conclusions

The present study revealed that the daily intake of Arg at a dose of 3 mg/kg suppressed cognitive decline and depression-like behavior and counteracted shortened lifespan in chronic stress-loaded SAMP10 mice. We speculate that Arg reduces stress by suppressing oxidative damage in the brain, resulting in slowed aging. The suppression of *Nr4a1*, *Arc*, and *Cyr61*, which are associated with oxidative damage and nerve cell death, and an increase in *Hba-a2* and *Hbb-b2*, which protect neuronal mitochondrial dysfunction, were observed in mice that ingested Arg. Arg may be a potential candidate for the suppression of the deleterious effects of chronic psychosocial stress.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

Arg	Arginine
Arc	Activity regulated cytoskeletal-associated protein
Cyr61	Cysteine rich protein 61
Hba-a2	Hemoglobin alpha, adult chain 2
Hbb-b2	Hemoglobin, beta adult minor chain
IEGs	Immediate-early genes
LPO	Lipid peroxidation
MST	Median survival time
Nr4a1	Nuclear receptor subfamily 4, group A, member 1
qRT-PCR	Quantitative real-time reverse transcription polymerase chain reaction
SAMP10	Senescence-accelerated mouse prone 10

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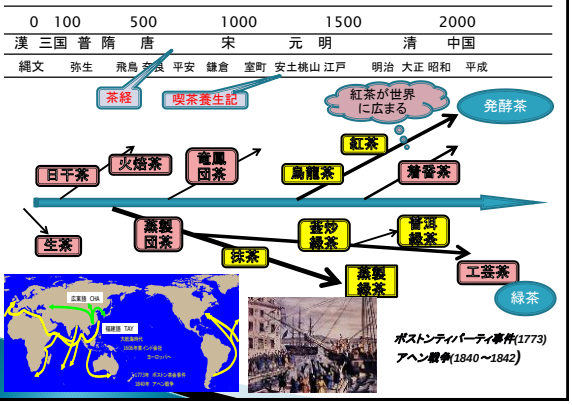
３．セミナー等関連 PPT 資料

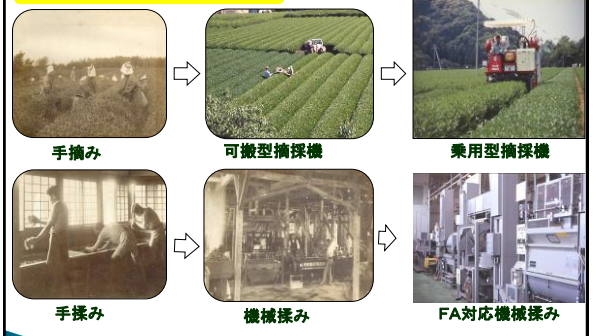
次世代に展開する茶の魅力

茶学総合研究センター
中村順行

2000年の歴史。時代とともに多様に進化してきた



緑茶生産方法の推移



摘採は手摘みから機械摘みに変わり、著しく摘採能率を向上してきた。製造は手揉みから機械化され、徐々に投入量を増加させるとともに最近ではコンピュータ制御による自動化に技術革新したことで、日本独自の生産加工技術を確立し、品質の高位平準化に貢献してきた

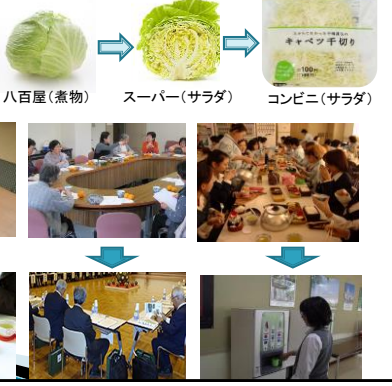
茶種や新製品などへの変化

昭和初期までの輸出用各種茶の製造から昭和40年代には高度経済成長下で茶価は上昇し、消費の拡大した国内需要向けの高品質煎茶に急速に移行。その後、嗜好の多様化と健康志向による多用途利用に対応した新製品開発など時代に対応した新商品の開発に貢献した

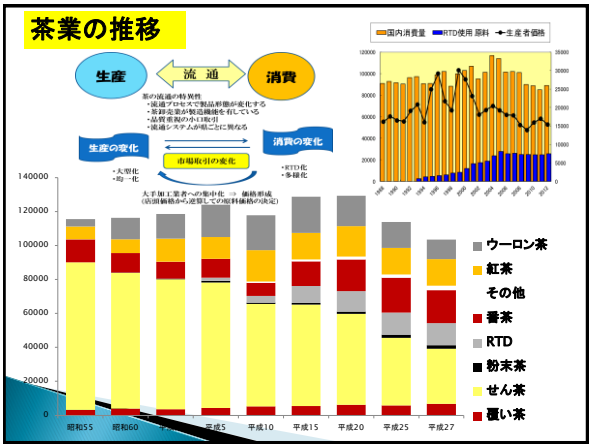
茶の飲み方は変わる

ライフスタイルに対応した日本茶とは？

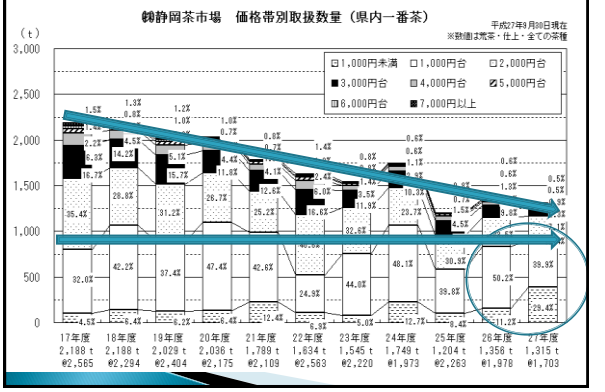
ライフスタイルが変われば、必要な物も食べ方や飲み方も変わる。
⇒対応したものと技術が必要(販売方法も変わる)



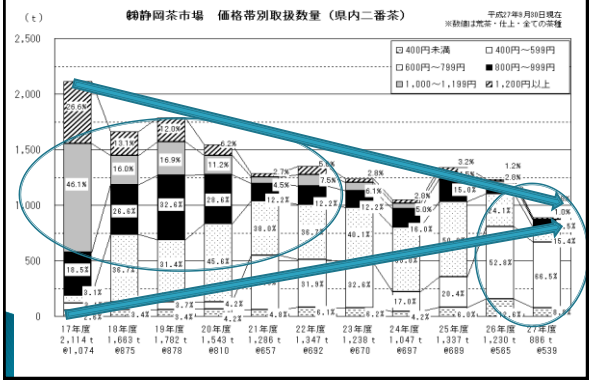
茶業の推移



静岡茶市場の取扱量の推移 (静岡茶市場HPより引用)



静岡茶市場の取扱量の推移 (静岡茶市場HPより引用)



流通の変化

低価格化の波

生産の変化
- 大型化
- 均一化
- 効率化

市場取引の変化

大手加工業者への集中化 ⇒ 価格形成（店頭価格から逆算しての原料価格の決定）

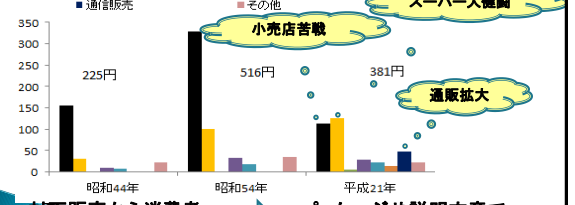
消費の変化
- 簡便化
- ティーバッグ
- 粉末茶
- ペットボトル

☆新ジャンル商品への取り組み
- 取引先との連携強化
- 原料供給と商品との連動
  ⇒直結による低コスト化
  ⇒商品に応じた生産

購入先にも大きな変化が

お茶は多くの茶商品の中からパッケージや価格で購入される

多極化
- 一般小売店
- コンビニ
- 生協・購買
- 通信販売
- スーパー
- 百貨店
- ディスカウントストア・量販店
- その他



対面販売から消費者の選択購入に移行

パッケージや説明文章で勝負、ブランディング

まだ日常茶飯事のお茶もある

625gの世界で
日常茶としての販売
番茶が200g～500gで販売される

しかし、基本的には

美味しくて簡便に飲める茶

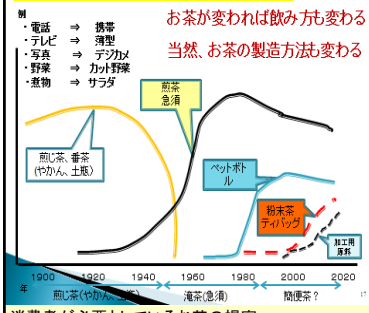
ポスト急須も必要では

急須いらずの新規本格的緑茶

粉末茶　ティバッグ　煎茶ラテ　高級ボトリング茶

ティーバッグも増加 （遊び心、高級化路線が重要）

緑茶消費の推移の概観



お茶が変われば飲み方も変わる

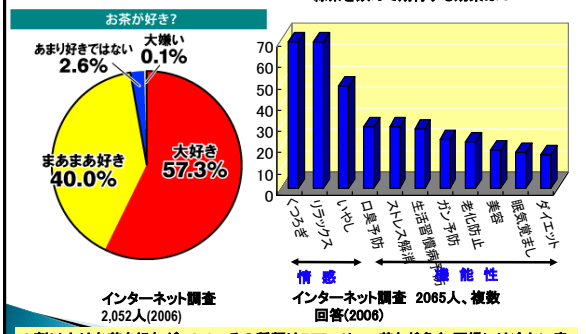
当然、お茶の製造方法も変わる

日常茶飯事の茶
- ☆ コスト低下
- ☆ 簡便化
- ☆ 飲用水化

愉しみのお茶
- ☆ かわいさ
- ☆ 話題性
- ☆ 面白さ
- ☆ インスタ映え
- ☆ 高級品
- ☆ ブランド

消費者が必要としているお茶の提案
- ・妊婦さん、子供、高齢者 ⇒ 低カフェイン茶
- ・体脂肪の気になる方 ⇒ カテキン強化茶
- ・アレルギー ⇒ べにふうき緑茶 ・ストレスの気になる方 ⇒ テアニン強化茶
- ・肉食に合うお茶 ⇒ ・パンに合うお茶 ⇒ その他

消費者はお茶が好き ？

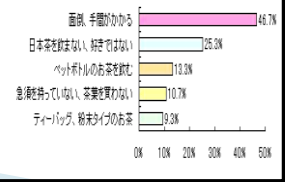


9割以上はお茶を好むが ？？、その種類はRTD、リーフ茶など多く、夏場には冷たい麦茶やウーロン茶に人気が集中する。

消費者のお茶は急須のお茶ばかりではない

急須で淹れるお茶への期待は
- ・ゆったりとした時に
- ・お茶会
- ・団らんの時に
- ・こだわりのお茶を

急須で淹れるお茶を「ほとんどいれない」「全くいれない人」の理由は



フレーバードティも人気

店舗前には三越の高級茶葉が売られているが、若い女性がひっきりなしに訪れ、可愛いとのことで数種類のお茶を購入する

- ・香り
- ・かわいさ
- ・面白さ
- ・話題性
- ・多種類

高級茶の飲用の場を広げよう
～ボトルティは面白い～






☆最高の旨味抽出が可能
☆誰でもが同じ味で出せる
☆演出が可能
☆付加価値向上

高～～い お茶もある

324,000円

21,600円/100g

高価格茶の要因

・物語性があること
・こだわりのお茶であること
・数量が少ないこと
・品質的にも上級であること
・販売店の格

高価格茶の戦略

・販売店の格づくり
・他の商品価格の上昇

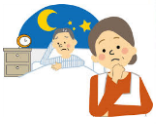
10,800円/100g

10,800円/100g

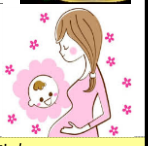
特定需要者向け茶も増加

H.Pより引用

若い女性や高齢者は睡眠阻害、妊娠時には乳児への影響を避けるため、茶の飲用を遠慮する人が多い





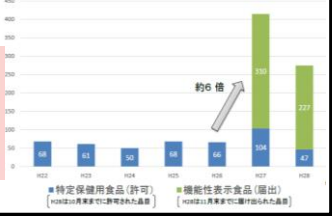


最近では、様々な低カフェイン茶が販売されるようになってきました

茶関係の機能性表示食品も増加

（各メーカーHPより引用）

新たに機能性の表示が可能とされた食品数



微生物発酵茶も増加

H.Pより引用

香味豊かなで新たな機能性をもつ後発酵茶も増加

11年振りに復活
浜松市胡桃平

日本の後発酵茶
（宮川編）さんえい出版より

今年大流行、生産追い付ず

食べるお茶も関心事

食茶のメリット

①溶出液では35％しか利用できないお茶の機能成分が100％利用できます。

②茶葉には、つぎのような身体の機能を高める成分が沢山含まれています。（カテキン類、テアニン、カフェイン、ビタミン類（β－カロテン、B2、C、E）、フラボノール、サポニン、葉緑素、カリウム、カルシウム、鉄、
リン、植物繊維など）

③常日頃、茶をまるごと食べることによって健康・美容パワーや生活習慣病の予防などが期待されます。

◆創作料理の例
[和風料理]
お茶葉懐石
[洋風料理]
お茶葉イタリアン、お茶葉カルボナーラ、緑茶ニョッキ、アクアパッツア緑茶
[中華料理]
海老のお茶蒸し、春巻き
[和菓子]
茶ゼリー、茶葛餅
[洋菓子]
茶チーズケーキ、茶シフォンケーキ、茶ロールケーキ
[調味料]
ソース、お茶シロップ、お茶醤油、茶湯
[加工食品]
茶葉入りやりいかの塩辛、茶葉入りやりいかの糀漬け

茶の新需要の事例

表　茶の新需要の事例

区　分	需　要　分　野　と　応　用　例
茶として利用	水出し茶、各種発酵茶、新香味茶、ギャバロン茶、低カフェイン茶、濃縮茶、混合茶　など
飲用・形態を変えて利用	ドリンク茶、ティバッグ、インスタントティ、粉末茶、微粉末茶(食用、即席飲用、酒割用)、カード茶、錠剤茶、カプセル茶、茶ワイン、緑茶酒、スポーツ飲料、カテキン粉末など
食品・食用として利用	☆　形態を変えてそのまま食用として利用 ☆　食品素材として利用 「素材」「食品」「菓子類」「その他」健康補助食品
飲食料以外に利用	☆　衣料用など ☆　医療用 ☆　化粧品、石鹸用など ☆　消臭剤、脱臭剤など ☆　日用品など ☆　建材、家具、家電用品など ☆　家畜、ペット用品 ☆　植物活性用 ☆　その他

茶は飲用だけでなく、食品素材として、さらには機能性成分を活かした様々な飲食料以外にも利用され、新しいビジネスを創造している

茶として利用

水出し茶、各種発酵茶、新香味茶、ギャバロン茶、低カフェイン茶、濃縮茶、混合茶　など

飲用形態を変えて利用

ドリンク茶、ティバッグ、インスタントティ、粉末茶(食用、即席飲用、酒割用)、カード茶、錠剤茶、カプセル茶、茶ワイン、緑茶酒、スポーツ飲料、カテキン粉末　など

食品・食用として利用 ～形態を変えて食用～

いしびき茶、食べる茶、茶のふりかけ、ペースト茶、佃煮　など

食品・食用として利用 ～食品素材・食品～

「素材」
フレーバー、エキス、多用途茶
「食品」
茶そば、茶団子、茶かゆ、茶かまぼこ、ハム、茶料理、ジャム、食用油、ドレッシング、マヨネーズ

食品・食用として利用
～菓子類など～

茶飴、茶羊かん、茶入り菓子、クッキー、パイ、サブレ、カステラ、プリン、ガム、キャンディー、チョコレート、アイスクリーム　など





飲食料以外に利用
～衣料、医療、化粧など～

衣料用；シーツ、タオル、シャツ、靴下、寝具、のれん　など
医療用；消臭シーツ、消臭カバー、紙おむつ、マスク　など
化粧品、石鹸用；化粧品、化粧水、スキンクリーム、洗顔パック、石鹸、シャンプー、リンス、入浴剤、歯磨き粉、虫歯予防剤　など





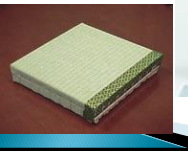

飲食料以外に利用
～消臭剤、脱臭剤、日用品など～

消臭、脱臭剤：トイレ用、冷蔵庫用、消臭スプレー、除菌シート等
日用品など：ノート、ティッシュ、トイレットペーパー、うちわ、スリッパ等

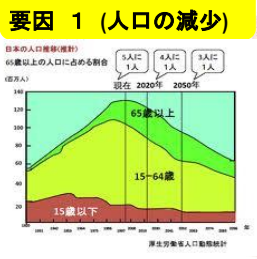


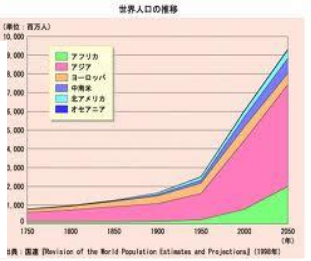

飲食料以外に利用
～建材、家具、ペット用品、その他

建材、家具、家電用品；塗料、ワックス、抗菌量、空気清浄機、布団乾燥機
家畜、ペット用品；ペット用飼料、卵、豚、さなかの肉質改善、脱臭剤
その他；植物活性用、植物活力剤、土壌改良剤、酸化防止剤など

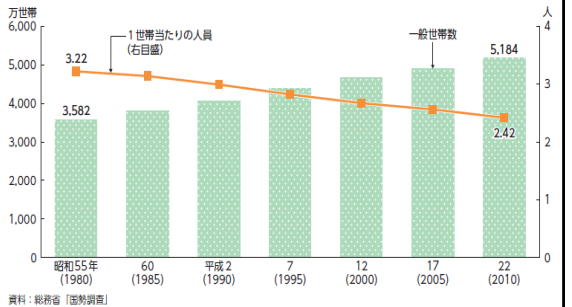

これからのお茶は？
要因　1　(人口の減少)





・人口の減少　⇒　消費者の自然減
・高齢者層の増加　⇒　淡白化、健康意識化、低カフェイン
・アフリカ、アジア人口の急増　⇒　消費量の増大

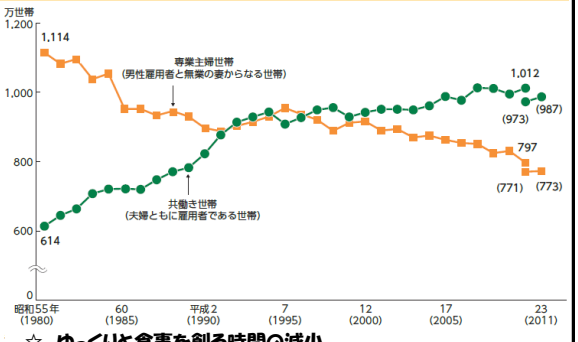
要因　2　(世帯も少人数化)



資料：総務省「国勢調査」

☆大型(大量)から小型化(少量)に
☆単身世帯も増加　個食化

要因 3 (共働きの増加)



☆ ゆっくりと食事を創る時間の減少
☆ 惣菜、外食文化　　通販、ネットショップの増加

茶業の今後の展開を考える上での重要要因

来るべき社会は？

社会構造の変化
Keyword;グローバル化、高度情報通信社会、少子高齢化、サービス産業社会化、人口減少
→ 消費者の減少、ストレス社会、情報化社会

需要・供給構造の変化
Keyword;自然健康志向、価値観・生活様式の多様化、女性の社会進出化、
→ 多様化社会、小世帯化社会、惣菜社会、健康願望社会

環境保全の強化
keyword;温暖化、低炭素社会化、自然循環機能重視型
→ 省エネルギー、自然保護社会

情報発信

オスカルの旅する急須(キューチャン)

抹茶アート

グリーンティーリズムは伸びる

憩いの場を 縁側カフェから

日本茶をテーマにした漫画11作品

静岡の老舗茶屋の娘・鈴(すず)は人助けがきっかけで、かつてないほど素晴らしいお茶に出会う。それを追い求めて脱毒茶店「茶柱倶楽部」を営みながら、お茶をくれた人を捜すことに

日本茶の輸入を突然禁止したフランスと交渉する農水省新米女性職員。日本茶を求めパリを彷徨う青年。この二人が、フランス農労相に立ち向かう本格日本茶コミック！

中学時代、喧嘩ばかりの日々を送っていた主人公・船橋雅矢は、高校入学を機に暴力の道から抜け出す為、勧誘された茶道部に入部。雅矢の脱不良計画が始まる…。

乱暴グセを直して！ と妹によってムリヤリ茶道部に入部させられた僚矢。教室ではボーっとしてばかりの薄幸の、別人のように厳しい部長ぶりにビックリするが、そのうちに彼の様々な面が見えてきて…!?

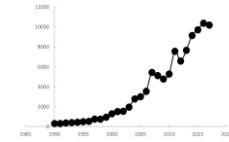
アメリカ人留学生と茶道家元の息子を中心に初心者だらけの高等学校の小さな茶道部での活動を描いた物語。

Enhancement of Ceylon tea brand power and marketing

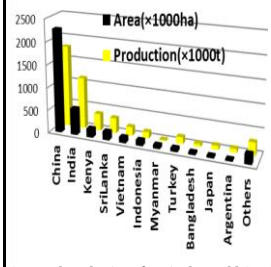
University of Shizuoka
Tea science center
Project Professor
Yoriyuki NAKAMURA

Production of tea in the world

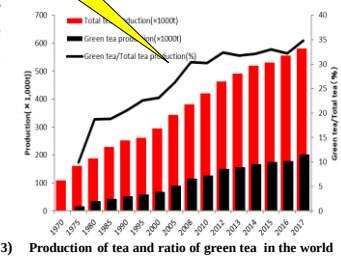
Green tea production increase steadily for its function



Changes in the number of papers on catechins in Google Scalar

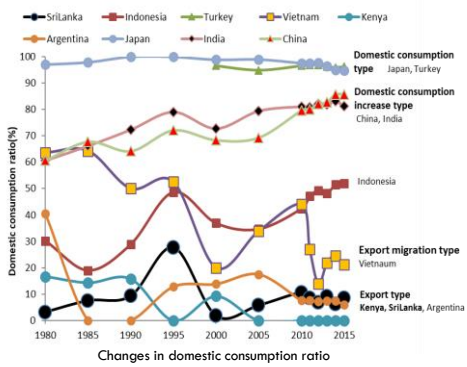


Area and production of tea in the world (2013)



Production of tea and ratio of green tea in the world

Changes in the ratio of domestic consumption to production in major tea producing regions



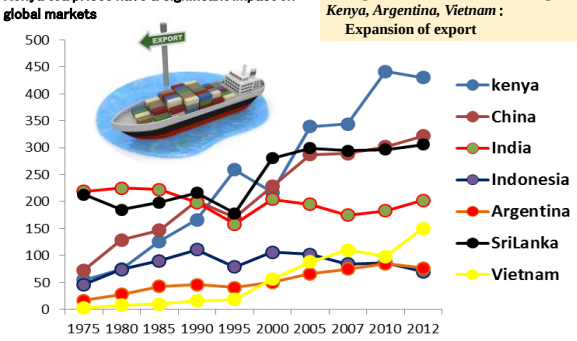
Changes in domestic consumption ratio

Increasing domestic consumption in China and India, the world's leading tea producers, is changing exports in other countries

Changes in export countries

Kenya tea prices have a significant impact on global markets

India, China, Indonesia:
Expansion of domestic consumption
Kenya, Argentina, Vietnam:
Expansion of export

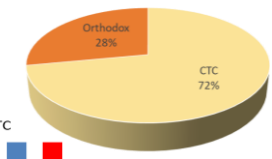


Change in the tea export volume in the major country

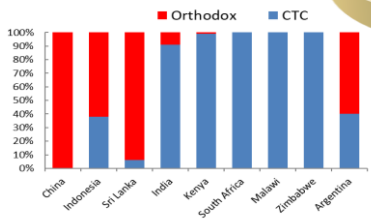
Ratio of orthodox and CTC black tea in major tea producing countries

Orthodox

CTC



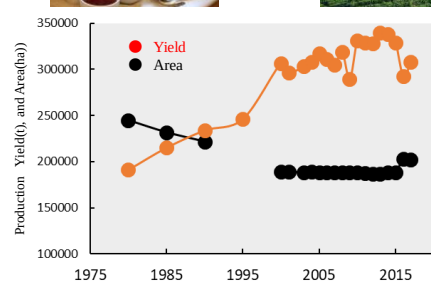
Ratio of tea types



Orthodox and CTC ratio in major tea producing countries

The majority of black tea exported to the world is CTC

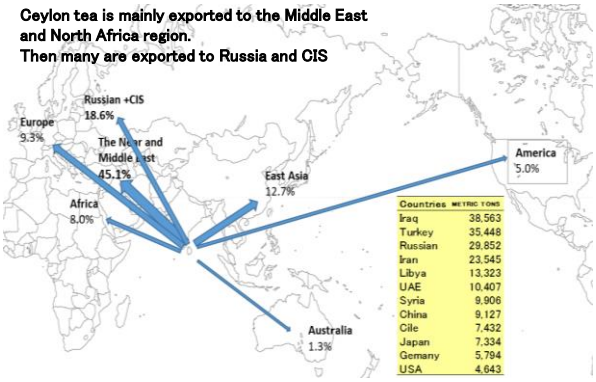
Current status of Ceylon tea



Changes in tea production area and yield in Sri Lanka

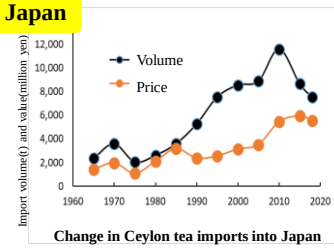
Major export destinations and ratios of Ceylon tea

Ceylon tea is mainly exported to the Middle East and North Africa region.
Then many are exported to Russia and CIS

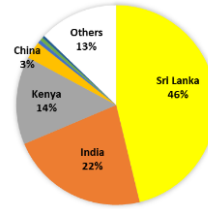


Countries	METRIC TONS
Iraq	38,563
Turkey	35,448
Russian	29,852
Iran	23,545
Libya	13,323
UAE	10,407
Syria	9,906
China	9,127
Cile	7,432
Japan	7,334
Gemany	5,794
USA	4,643

Export of Ceylon tea to Japan



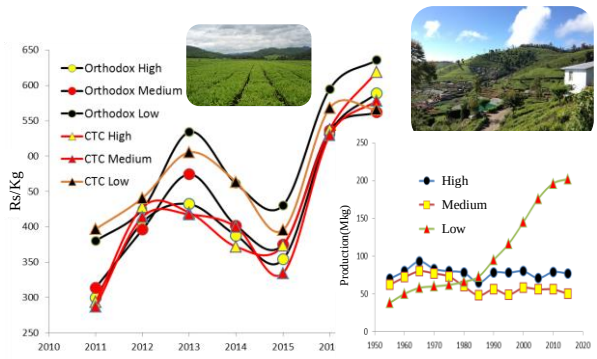
Change in Ceylon tea imports into Japan



Ratio of black tea imported to Japan

There are many Ceylon tea specialty stores in Japan

Trends in orthodox and CTC tea prices in Ceylon tea



Price changes by elevation in Sri Lanka

Production changes by elevation

Ceylon tea production by altitude

Tea production is overwhelmingly in the low districts, and the high districts are mostly in the area around Nuwara Eliya

Table District wise Tea Production (in ton) 2015

District	High	Medium	Low	Total	Ratio(%)
Badulla	14,942	14,164		**29,107**	**8.9**
Colombo			825	**825**	**0.3**
Galle			48,101	**48,101**	**14.6**
Hambantota			246	**246**	**0.1**
Kalutara			18,325	**18,325**	**5.6**
Kandy		20,924	11,790	**32,714**	**10.0**
Kgalle		666	9,575	**10,241**	**3.1**
Matale		2,168	861	**3,029**	**0.9**
Matara		190	41,378	**41,567**	**12.6**
Nuwara Eliya	60,323	10,766	762	**71,851**	**21.9**
Ranapura	160	2,088	70,517	**72,765**	**22.1**
Total	75,426	50,966	201,379	**328,771**	**100.0**

Supply ratio of raw leaves of Ceylon tea

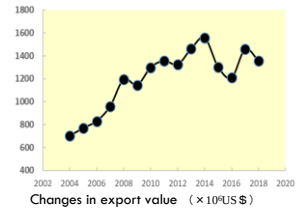
In general, low districts tends to have a high bought leaf ratio, and high districts tends to have more own leaf.

Table. District wise Tea Production (Ratio) 2015

District	Own Leaf	Estate Leaf	Bought Leaf	Total
Badulla	43.2	23.9	32.9	100.0
Colombo	0.0	0.0	100.0	100.0
Galle	1.8	1.0	97.2	100.0
Hambantota	0.0	0.0	100.0	100.0
Kalutara	3.0	0.5	96.6	100.0
Kandy	16.0	5.2	78.8	100.0
Kgalle	11.7	2.8	85.5	100.0
Matale	7.7	3.3	89.0	100.0
Matara	5.0	1.2	93.8	100.0
Nuwara Eliya	64.3	14.6	21.1	100.0
Ranapura	6.4	1.3	92.3	100.0
Total	22.4	6.5	71.1	100.0

Major issues to be solved in Ceylon tea

The cost of production of made tea has rapidly increased.
However, there has been no increase in export prices



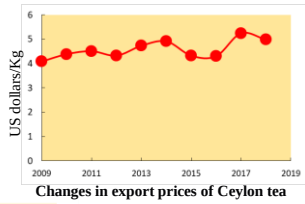
Changes in export value (×10⁶US$)

Task

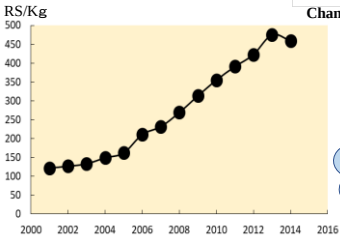
- ★Decline in tea price
- ★Rising production costs
- ★Production instability due weather fluctuation
- ★Small holder vulnerability
- ★Diversification etc.

Key issues in Ceylon tea

☆ *Decline in tea prices*
☆ *Rising wages*



Changes in export prices of Ceylon tea



Total cost per kilogram of made tea

Ceylon tea brand strengthening and marketing

Enhancement of Ceylon tea brand power

CEYLON TEA
SYMBOL OF QUALITY

Personal idea

PREMIUM CEYLON TEA
SYMBOL OF SUPER QUALITY

・100% pure Ceylon tea
・100% packaged in Sri Lanka

With a premium lion logo
Need to add value

What can be considered to further enhance the brand power of the Ceylon tea lion logo

For example,
☆Development of premium lion logo products
☆Development of functional tea
☆Development of high quality tea

Ceylon tea flag product creation

PREMIUM CEYLON TEA
SYMBOL OF SUPER QUALITY

Silver Lion Tea

Silver chips made only from buds
→ I make it with a bud and one leaf and make silver lion tea

Pearl Ceylon Silver Tea

Shining island, Creation of Ceylon tea inspired by Indian Ocean pearls

Development of tea with added functionality

In the case of Japan

Low caffeine tea

・For children and pregnant women
・For those who can't sleep after drinking tea at night

GABA(γamino butyric acid) tea

・For improvement of hypertension
・For get a good night's sleep

High concentration catechin tea

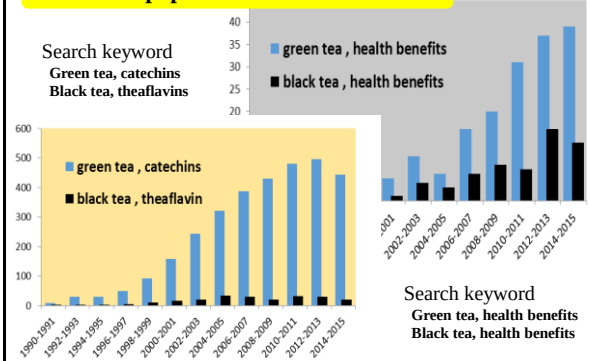
・For obesity prevention
・For burning fat

Physiological functions of black tea components

Black Tea Components	Contents	Functions
Theaflavins (+Thearubigins)	1～2%	***Blood flow improvement effect.*** Anti-oxidative, Anti-mutagenic, Anti-hypercholesterolemic, ***Anti-virus***, Anti-hyperglycemic, Fat reducing, Anti-hypertensive, Anti-ulcer, ***Anti-bacterial*** etc.
Caffeine	3～4%	Removal of fatigue, Sleepy feeling, Diuretic etc.
Vitamin C	0%	
Vitamin B	0. 1mg%	Excitometabolic action of carbohydrates and amino acids
Vitamin E	11.4mg%	Anti oxidative, Aging prevention
γ amino butyric acid		Anti hypertensive
Flavonoids	1～3%	Halitosis prevention
Theanine	1.40%	Anti hypertensive

Number of papers from 1990 in PubMed

Search keyword
Green tea, catechins
Black tea, theaflavins



Search keyword
Green tea, health benefits
Black tea, health benefits

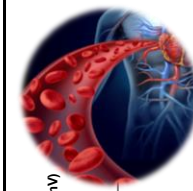
Number of papers containing green tea, black tea, catechins, theaflavins and health benefits from 1990

Characteristic of health benefits of black tea

☆ Blood flow improvement effect
(Nothing in Catechins)
☆ Anti-viral, bactrial action
(Stronger than that of Catechins)

☆ Anti-hypercholesterolemic action
☆ Anti-hyperglycemic action
☆ Anti-hypertensive action
☆ Fat reduce action

Blood flow improvement effect of theaflavins



Relationship between theaflavin type and blood flow

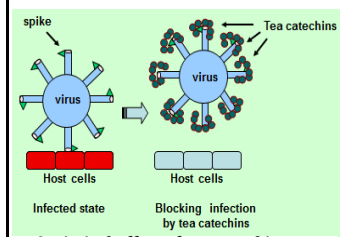
Anti-bacterial action in polyphenol

Theaflavins > Catechins

Comparison of minimum growth inhibiting concentration of tea polyphenol against bacteria (Hara. Y & Watanabe. M: 1989)

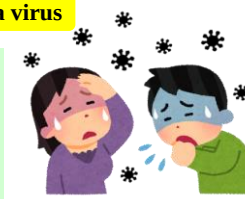
	B.subtilis	*B.slearothermophilus*	*D.nigrificans*
	minimum growth inhibiting concentration(ppm)		
EGC	>800	300	>1000
EC	>800	800	>1000
EGCG	>800	200	>1000
ECG	>800	<100	>1000
TF1	>1000	200	>1000
TF2A	500	300	>1000
TF2B	450	300	>1000
TF3	400	200	>1000

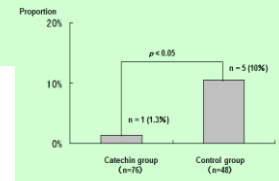
Effects of catechins on influenza virus



Anti-viral effect of tea catechins against influenza

Tea catechins bind to the spikes on the surface of influenza virus and inhibit viral adsorption onto the host cell surface, thus preventing infection

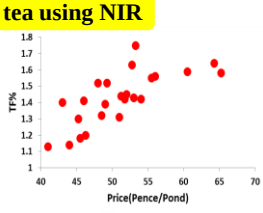




Comparison of the incidence of influenza infection

Development of high quality tea using NIR

The higher the theaflavin content, the better the taste and color, and the higher the quality and price.



Faster analysis
Evaluate numerically

Use NIR to measure chemical components

Markething (Demand varies by export destination)

Since the needs differ depending on the export destination, it is important to supply tea that matches the needs

Tea bags are interesting (Tea bags need to be fun and fashionable etc.)

Expand the place to drink fine tea

Bottle tea; Wine without alcohol

- ☆*Highest umami extraction possible*
- ☆*Anyone can serve with the same taste*
- ☆*Direction is possible*
- ☆*Improve added value*

Tea Foods for specified function uses

Tea Foods with function claims

For low cost production of Cylon tea

Mechhanization is important

With the boom in the tea industry, From hand-picking to machine-picking are rapidly progressing worldwide to reduce costs

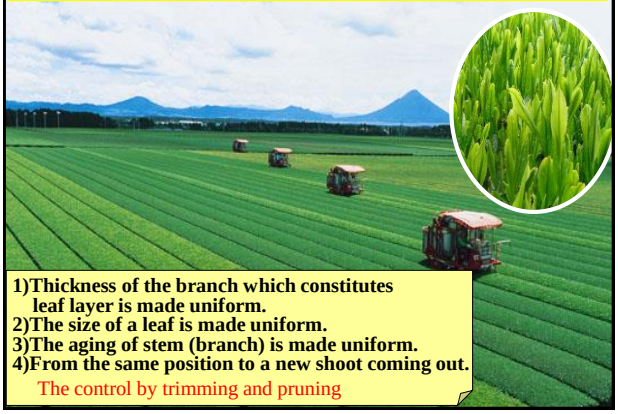
Mechanization has become necessary due to rise in labor costs and declining labor force

Difference between the hand and the mechanical plucking

- ★ **A new shoot is chosen and it plucking**
- ★ **The amount of plucked shoot is 10 -15kg/day**
- ★ **Plucking method for high grade tea**

- ★ **All the new shoots are plucked in fixed height**
- ★ **The amount of plucked shoot is 700 – 1000kg/day using portable machine for two person**
- ★ **Plucking method for midle grade tea**

The point for making a plucking surface of tea uniform



1)Thickness of the branch which constitutes leaf layer is made uniform.
2)The size of a leaf is made uniform.
3)The aging of stem (branch) is made uniform.
4)From the same position to a new shoot coming out.
The control by trimming and pruning

Organic tea is also a boom

Organic farming is increasing in high altitude tea fields

Good tea tourism

It is also necessary to make tea a tourist destination in the future

For the success of Ceylon tea

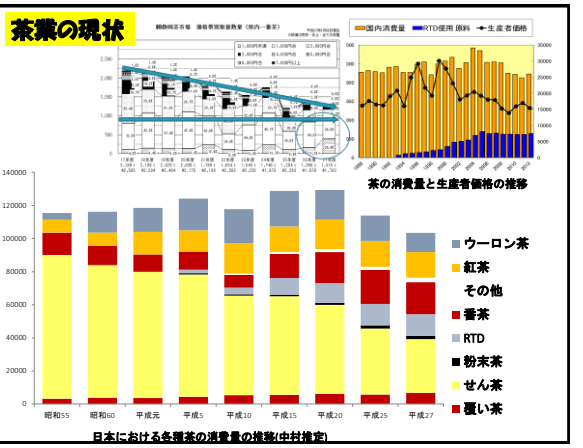
Five key issue

1. Enhancement of brand power
   ⇒ *Promotion the premium quality of Ceylon tea*
2. Increase in labor cost, decrease in labor force
   ⇒ *From hand plucking to mechanization*
3. Promoting the functionality of Ceylon tea
   ⇒ *Deepening of functional research and PR*
4. Demand varies by export destination
   ⇒ *Build marketing strategies by export destination*
   other uses
5. Raising the cultural values of tea
   ⇒ *Preservation of tea tradition, habitalization and utilization as tourism resources*

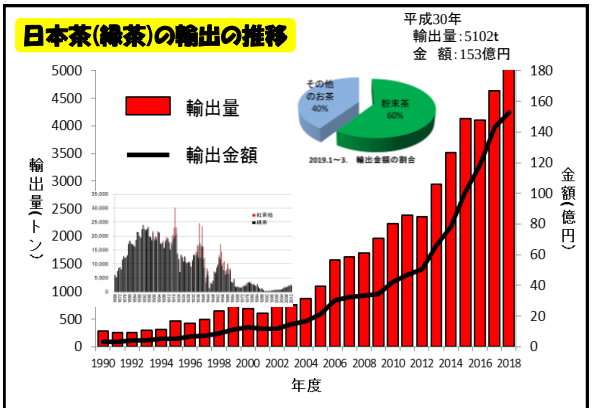
静岡抹茶の特性と品質向上

代表研究者；静岡県立大学
茶学総合研究センター　中村順行

茶業の現状



日本茶(緑茶)の輸出の推移



静岡抹茶も急増しているが、その位置づけや高品質化が重要

目　的

国内外で市販されている抹茶のなかでの静岡抹茶の特質を明らかとし、品質向上を図ることにより海外輸出振興の強化を目的とする

抹茶とは?!　（国内における抹茶の定義）

日本茶業中央会基準
覆下栽培した茶葉を揉まずに乾燥し、茶臼等で微粉末状に製造したもの

日本茶業技術協会(茶の科学用語辞典)
てん茶を臼で挽き微粉にしたもの。茶の湯に用いるが、最近は菓子や飲物の原料への使用が増加している。

三重県茶業会議所
茶葉を揉まずに乾燥した茶葉(てん茶など)を微粉状に製造したもの

京都府茶業会議所
業者間取引における「てん茶の表示区分」

表示区分	原料生産茶園など	茶期など	被覆	乾燥機	揉捻	主用途	粉砕機
宇治てん茶	棚施設による覆い下茶園。手摘み	一番茶、被覆期間20日以上	蒸し	てん茶機	揉まない	多用途（茶道等）	茶臼
初茶てん茶	覆い下茶園	一番茶、被覆期間20日程度	蒸し	てん茶機	揉まない	多用途	茶臼等（粉砕機含）
2茶てん茶	覆い下茶園	二番茶、被覆期間14日程度	蒸し	てん茶機	揉まない	多用途	茶臼等（粉砕機含）
秋てん茶	露天栽培	秋番茶	蒸し	てん茶機	揉まない	多用途	粉砕機

供試材料：
本試験では、市販品及び静岡抹茶は一番茶期、二番茶期、秋冬番茶期に生産された抹茶を供試した

供試材料及び方法

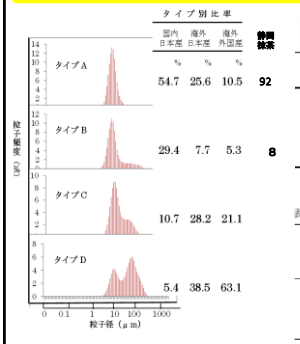
供試材料
国内で購入した抹茶は京都府、愛知県、三重県、静岡県、鹿児島県などで製造された**日本産抹茶76点**、および海外の米国、オランダ、イギリス、フランス、台湾など海外**11カ国**から購入した**日本産抹茶40点**、**外国産抹茶17点の計57点**の合計133点を供試。また、**静岡抹茶**は、一番茶期9点、二番茶期8点、秋冬番茶期8点の**計25点**を供試した。

①抹茶の粒度特性
抹茶の粒度分布特性をHORIBA製のLaser Scattering Particle Size Distribution Analyzer LA-950を用い、湿式法で0.01～3,000μm間の粒度の大きさを計測した。

②測色値特性
測色値はKONICA MINORUTA製の分光測色計 CM-5 Spectrophotometerを用いてL、a＊、b＊表色系、L、c＊、h表色系値を計測した。

③化学成分特性
高速液体クロマトグラフィー(HPLC)によりアミノ酸類、カテキン類、カフェインを分析した。

国内外で市販される抹茶の粒度特性



図B-1-(5)-3　国内外から購入した抹茶のタイプ別比率

表　海外購入抹茶の粒度特性

タイプ	購入抹茶の種類 日本産	外国産	平均径（μm）	標準偏差	モード径（μm）	メジアン径（μm）
A	10	2	16.5	12.9	11.7	12.7
B	3	1	21.4	22.3	12.0	14.2
C	11	4	30.2	33.3	12.1	16.6
D	15	12	44.5	41.8	31.9	29.8

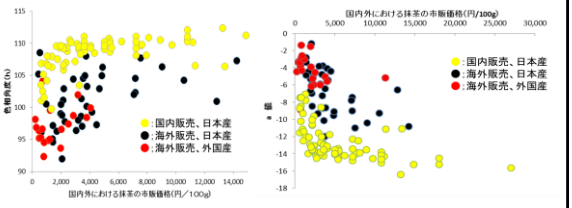
表B-1-(5)-2　国内外から購入した抹茶のタイプ別価格と粒径の大きさ

タイプ	国内市販品 日本産 100g当たり価格(円)	平均径（μm）	海外市販品 日本産 100g当たり価格(円)	平均径（μm）	外国産 100g当たり価格(円)	平均径（μm）
A	6,902	15.5	5,801	16.5	1,569	16.7
B	4,010	23.8	2,515	23.7	3,780	19.2
C	2,698	26.2	4,178	30.1	1,241	30.8
D	1,572	52.6	3,461	47.9	1,599	38.8

国内外で市販される抹茶の測色特性

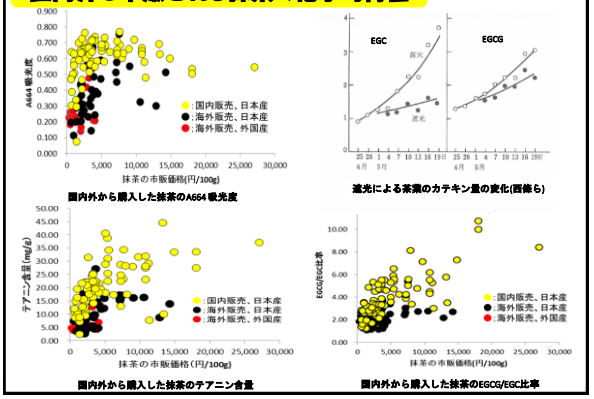
国内外から購入した抹茶の価格と測色値

100g当たり単価（円） 価格帯	平均単価	L*	a*	b*	C*	h
～1,000	654	60.31	−3.63	34.08	34.30	96.03
1,001～2,000	1,575	56.25	−3.89	34.75	35.02	96.28
2,001～3,000	2,247	57.94	−5.73	35.88	36.39	99.00
3,001～4,000	3,492	59.13	−6.87	34.64	35.38	101.09
4,001～5,000	4,404	57.24	−6.35	34.11	34.78	100.65
5,001～10,000	7,582	55.16	−9.13	35.01	36.21	104.61
10,001～	12,225	57.64	−7.95	34.64	35.60	102.83



国内外から購入した抹茶の色相角度(h)　　国内外から購入した抹茶のa＊値

国内外で市販される抹茶の化学的特性



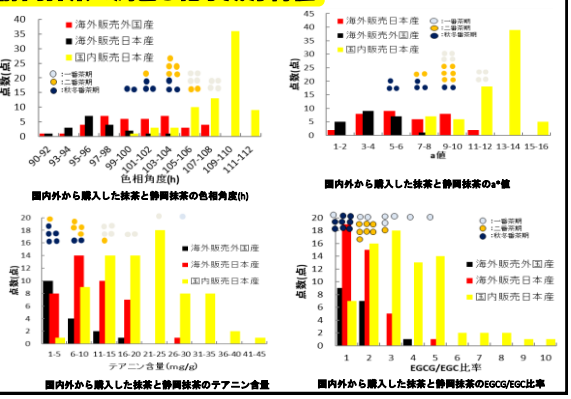
国内外から購入した抹茶のA664吸光度

遮光による茶葉のカテキン量の変化(西條ら)

国内外から購入した抹茶のテアニン含量

国内外から購入した抹茶のEGCG/EGC比率

静岡抹茶の測色と化学成分特性



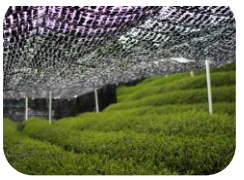
国内外から購入した抹茶と静岡抹茶の色相角度(h)

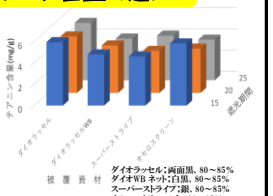
国内外から購入した抹茶と静岡抹茶のa*値

国内外から購入した抹茶と静岡抹茶のテアニン含量

国内外から購入した抹茶と静岡抹茶のEGCG/EGC比率

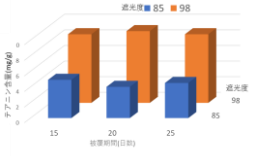
被覆の遮光度や資材によるテアニン含量の違い





ダイオラッセル：両面黒、80～85%
ダイオNB ネット：白黒、80～85%
スーパーストライプ：銀、80～85%
オセロスクリーン：白、80～85%

被覆資材と遮光度がテアニン含量に及ぼす影響



被覆日数と遮光度がテアニン含量に及ぼす影響

静岡抹茶の品質向上には

1. 品種の利用、選定
   - やぶきた主体から、被覆特性に優れた品種の採用
2. 栽培技術の確立(施肥、整せん枝)
   - 効率的な施肥法
3. 被覆技術(資材の選定含
   - 遮光度や被覆期間
4. てん茶製造法

各種茶品評会の審査法の概要と審査法の変化

静岡県立大学　茶学総合研究センター　中村順行

審査とは　何のために審査し評価するのか！が　重要

・物事・性質などの良し悪しなどを調べて価値を定めること。
・品物の値段を定めること、またはその値段。
⇒お茶では、茶園、茶樹、生葉、荒茶、仕上げ茶、商品などを多少に特徴や優劣などを表現する

官能評価

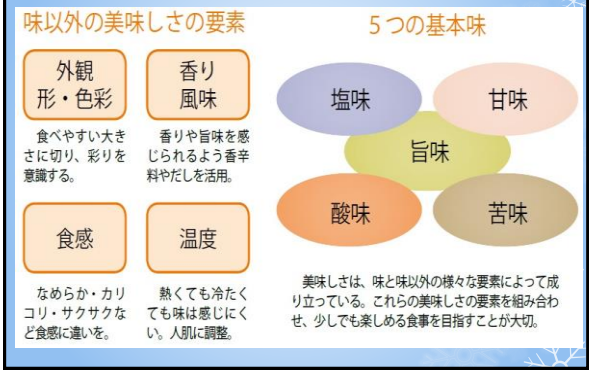
人間の五感を駆使して評価する方法
方法
カテゴリー尺度法
採点法
順位法
一対比較法　　などなど

理化学的評価

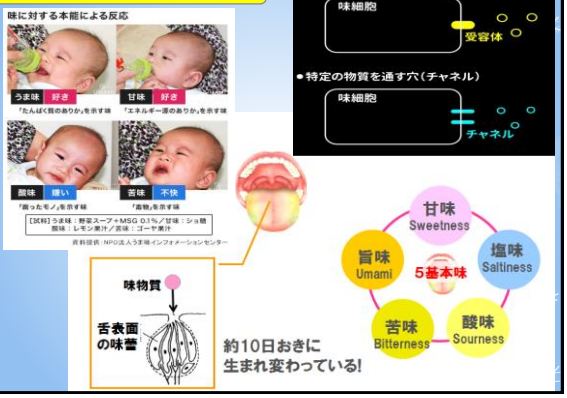
物理化学的機械を用いて評価する方法
形状、色、化学成分　などなど
味覚センサー、においセンサーなど
非破壊計測法として近赤外分光分析計

おいしさ　とは

味の評価　美味しさは様々な要素によって成立している



基本味は受容体で感じる



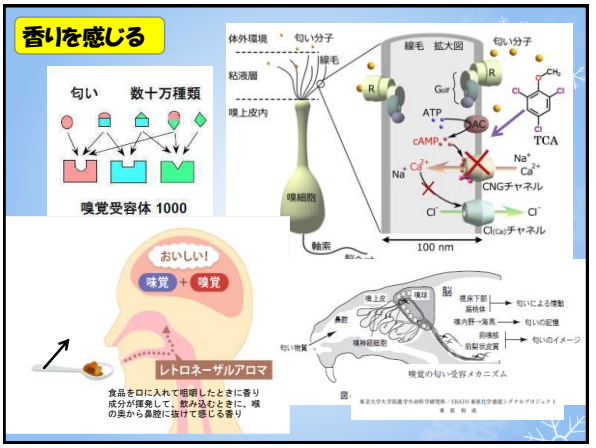
渋味や辛味は感覚で感じる

渋味と苦味は異なるものであり、例えば、柿渋の渋味はタンニン、茶葉の渋味はカテキン、苦味はカフェインによる。

渋味は、生理学的定義に基づく味覚のいわゆる五原味（甘・酸・塩・苦・旨味）には含まれず、辛味と同様、渋味は触覚に近い感覚だと考えられている。

渋味は、カテキンが舌や口腔粘膜のタンパク質と結合して変性させることによると言われている。このような粘膜の変性作用のことを「収斂作用」と呼ぶ。渋味は厳密には味覚の一種というよりも、このタンパク変性によって生じる痛みや触覚に近い感覚だと言われている。

香りを感じる



お茶の香気成分

表 4-14　現在までに発表された茶香気成分表(官能基別表)(山西)

Ⅰ. 炭化水素類	(72)	Ⅵ. エステル類	(82)
1. 脂肪族	14	1. 脂肪族	52
2. 芳香族	25	2. 脂環式	3
3. テルペノイド	33	3. 芳香族	19
Ⅱ. アルコール類	(89)	4. テルペノイド	8
1. 脂肪族	51	Ⅶ. ラクトン類	(25)
2. 芳香族	5	Ⅷ. フェノール類	(22)
3. テルペノイド	33	Ⅸ. その他の含酸素化合物	(36)
Ⅲ. アルデヒド類	(68)	1. フラン化合物	17
1. 脂肪族	45	2. 芳香族	10
2. 芳香族	18	3. ヨノン誘導体	5
3. テルペノイド	5	4. その他	4
Ⅳ. ケトン類	(75)	Ⅹ. 含窒素化合物	(86)
1. 脂肪族	30	1. ピロール類	12
2. 脂環式	10	2. ピリジン類	17
3. 芳香族	16	3. ピラジン類	24
4. テルペノイド	3	4. その他	33
5. ヨノン系	16	Ⅺ. 含イオウ化合物	(14)
Ⅴ. 酸類	(69)	計	638種
1. 脂肪族	63		
2. 芳香族	3		
3. テルペノイド	3		

あなたにとってのお茶の香り

どのような香りを思い浮かべますか??

代表的な茶の香りの種類とその主要香気成分 (山西より)

香りの種類	関係する主要な香り成分	
若葉の爽やかな青香	シス-3-ヘキセノール	cis-2-Hexenol
	トランス-2-ヘキセナール	trans-2-Hexenal
スズラン系の軽く爽やかな花香	リナロール	Linalool
	リナロールオキサイド	Linalool oxide
バラ系の温かい花香	ゲラニオール	Geraniol
	2-フェニルエタノール	2-Phenylethanol
ジャスミン、クチナシ系の甘く重厚な花香	β-イヨノン	β-Ionone
	シスジャスモン	cis-Jasmone
	ジャスモン酸メチル	Methyljasmonate
果実、乾果系の香り	ジャスミンラクトン	Jasmin lactone
	テアスピロン	Theaspirone
木質系の甘い香り	4-ビニルフェノール	Benzylalcohol
	ネロリドール	Nerolidol
青苦く重い香り	インドール	Indole
加熱香気系の香ばしい香り	ピラジン類	Pyrazine
	ピロール類	Pyrrole
保存中に増加する古い香	2,4-ヘプタジェナール	2,4-Heptadienal

官能評価について

官能審査とは

味・香りの強さや性質の評価、それらの嗜好性、市場性などの評価を**人間の五感によって行う方法**。

官能検査には計測器である人間の生理・心理が絡んでくるため、理化学的検査法とは異なり下記のような誤差、特殊な効果が入り込む。

①個人差：感覚の質的な個人差。判定基準の個人差
②特殊な効果：練習の効果。疲労の効果。順序の効果。
③周辺の影響：試料のサイズ、温度、容器の形状、デザインなどによる影響。検査場の環境の影響
④判定の尺度：同じものでも人により表現する言葉、方法が異なる

茶における品評会などの種類

茶園	収穫	荒茶	仕上茶	商品
茶園共進会	摘採競技会	**荒茶品評会**	仕上茶品評会	**世界緑茶コンテスト**
		荒茶審査会	**日本茶アワード**100銘茶コンテスト	
		手揉茶品評会	**国際銘茶品評会**	

その他、研修会、講習会、闘茶会、互評会など

品評会：産物・製品を一堂に集めて、その優劣を定める会
審査会：ある特定の事項について審査する会
競技会：一定のルールの下で競い合い優劣を決める会
アワード：ヒトやモノに賞与などを与えること
コンテスト：出来ばえなどの優劣を競うこと
互評会：互いに評価しあう会

品評会などの役割

① **技術の向上**
② **技術の改良**
③ **新しい茶種の普及**
④ **競技性**
⑤ **評価による付加価値化**
⑥ **その他**

全国茶品評会

100銘茶コンテスト

手もみ茶競技会

品評会などの果たしてきた役割

輸出促進のために

品評会や共進会は輸出用茶の品質向上に大きな貢献を果たしてきた

標準茶を目指して
目指すべき尺度の一つとして
各種輸出茶の標準茶の提示

技術の向上
番茶から煎茶に
機械化が進むとともに、新しい各種
各様の機械の開発に伴い、より品質
向上が図られてきた

品評会などの果たしてきた役割

深蒸し煎茶の創造

新しい茶種の創造に関し、全品では協賛としてフリースタイル茶の審査を経て深蒸し煎茶の採用

審査の全国統一的な「ものさし」の決定　⇒技術の向上を目指して

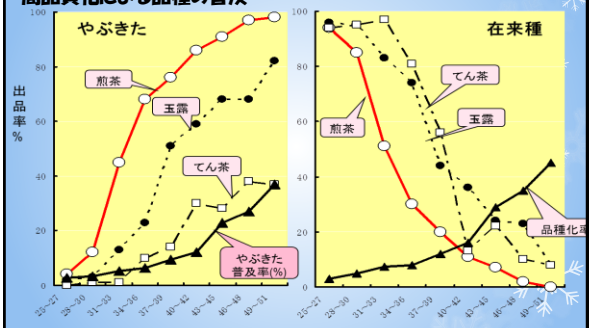
当初の審査基準
- 形状：細よれで締りがよく、葉切れの少ないもの
- 色沢：煎茶より黄色みの強い黄緑色でさえのあるもの
- 香気：青臭が完全に抜けた深みのある芳香を持つもの
- 水色：煎茶よりも黄色が濃く、濃度を感じさせるもの

全品における深蒸し煎茶の正種目化への流れ

年次	種目	茶種名	開催権
昭44年	協賛	フリースタイル	静岡
昭46年	協賛	フリースタイル	静岡
昭47年	協賛	フリースタイル	中央会
昭48年	協賛	フリースタイル	埼玉
昭52年	正種目	せん茶深蒸し	静岡
昭53年	不採用		京都
昭54年	正種目	深蒸し煎茶	佐賀

品評会などの果たしてきた役割

高品質化による品種の普及



全国茶品評会(第4回～30回)における茶種別出品茶の推移

目的に合った評価法の導入

① レベルの向上を目指した評価法

標準茶あるいは想定目標品質を満点とし、減点により評価(大部分の品評会)

⇒減点法

② 新茶種や新香味の導入を目指した評価法

社会的ニーズ、目的、志向に適合したものを選定(商品開発、育種分野など)

⇒適合法、選定法

③ 特性の把握を目指した評価法

各々の持つ特性を明らかにする。主に品質表示や説明に応用される(商品特性評価など)

⇒加点法、説明法

④ 競技性を重んじた場合の評価法

優劣を決める場合には絶対評価法が多い

⇒減点法(減点法＋加点法も有り)

代表的な評価法例

品評会の種類	茶の種類	評価法	備考
全国茶品評会	荒茶	減点法	相対評価
日本茶Award 国際銘茶品評会	仕上げ茶	基本点＋加減点法	絶対評価
世界緑茶コンテスト	商品	減点法	絶対評価
その他、商品特性把握	商品	加点法	個別評価

品評会の種類による利点・欠点など

減点法：審査が容易。目標値の設定が困難。新茶種や新香味には応用しにくい

加点法：審査員によるばらつきが出る。加点項目が不明確。

基本点＋加減点法：最初にクラス分類配点、その後に加減点数。減点法と加減点法の折衷法

外観順位並替方式：時間の短縮が可能。外観と香味に相関の高いことが前提

絶対評価：審査員の資質が重要。審査順位はつけにくい。

商品特性把握：コメントが重要。プラスイメージの表現が不足。

全国茶品評会

趣旨；日本茶業の将来を展望し、茶生産の近代化と我が国茶業経営の一層の発展を図ることを目的とする

審査；各茶種の特質の良否及び内容等に重点をおき実施する。

審査方法など；
- **審査は外観から始める**
- **審査器具はネットカップと茶碗のセット方式**
- **審査器具の並べ替えあり**

	内質				外観	合計
	香気	水色	滋味	から色		
普通煎茶(10kg)	75	30	75		20	200
普通煎茶(4kg)	75	30	75		20	200
深蒸し煎茶	70	30	80		20	200
かぶせ茶	70	30	70		30	200
玉露	65	30	65		40	200
てん茶	65	20	65	10	40	200
蒸し製玉緑茶	75	30	75		20	200
釜炒り茶	75	30	75		20	200

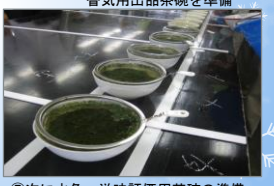
全国茶品評会

①まず外観から評価

②外観のコメント

③外観の順位に並べ替えた香気用出品茶碗を準備

④香気の審査



⑤次に水色・滋味評価用茶碗の準備する。全品ではネットカップ方式

全国茶品評会

⑥ネットカップ方式により淹れられた水色を評価

⑦水色の評価時にも高得点順に並べ変えられる

⑧水色の高得点順で滋味の評価を行う。いずれの評価項目とも最高得点からの減点法が用いられる。

日本茶Award

趣旨；新たな時代に適応した価値あるお茶の発掘と創造を通じ、日本茶の持つ幅広い魅力を世界に伝えることを目的

審査；消費者の求める「うまいお茶」「香りのお茶」に重点を置き審査する。専門審査員による一次・二次審査を行いその優秀品について消費者による三次審査を行う。

審査方法など；
現実のお茶の淹れ方に則し、急須を用い、二煎目まで審査。
採点は合議制によらず個人制。
審査は、内質から始め、並べ替えはしない

	香気	滋味	水色	外観	合計
うまいお茶	20	60	10	10	100
香りのお茶	60	20	10	10	100

日本茶Award

①まず全体の概要を把握

②二煎目まで出された茶の香気・滋味・水色を1分間で絶対評価する。茶ごとにコメントを入れる。

②香気は英国式試験急須内の茶殻から評価

②滋味は二煎目までの浸出液の両者で判断

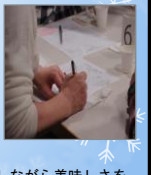
③外観は最後に拝見盆を動かさずに評価

日本茶Award

深蒸し煎茶の場合には、急須を使用

抹茶・粉末茶の場合には電動式茶筅を使用

消費者審査



一碗づつ配布されるお茶に対して、それぞれのコメントを記入しながら美味しさを評価し、最後にどれが総合的に一番良かったかを記入する

国際銘茶品評会

趣旨；各種銘茶の品質向上や茶文化の普及、国際的な茶産業の発展と進歩を目的とする

審査；各茶種の特質に基づき評価し、甲乙丙の等級を決めたうえで採点をする。

審査方法など；
審査は外観から始めるが、並べ替えはしない
審査器具は蓋付き審査茶杯と審査茶碗のセット方式
いずれの茶種も茶殻の審査あり

茶　種	外観	水色	香気	滋味	茶殻	合計
名優緑茶	25	10	25	30	10	100
普通緑茶	20	10	30	30	10	100
工夫紅茶	25	10	25	30	10	100
紅砕茶	20	10	30	30	10	100
烏龍茶	20	5	30	35	10	100
黄　茶	25	10	25	30	10	100
白　茶	25	10	25	30	10	100
黒　茶	20	15	25	30	10	100
花　茶	20	5	35	30	10	100

国際銘茶品評会

審査用茶杯

審査にあたり、個々の出品茶ごとに評価用紙、茶杯、茶碗、拝見盆が準備され、浸出液が入れられる

①外観の審査から開始

拝見盆は木製の白色盆

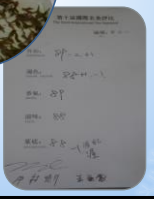
②茶種担当主任による基本点の提示

国際銘茶品評会

③香気の評価

④滋味の評価

⑤茶殻の評価。茶杯の蓋を使用する場合が多いが、トレイに水を張りその中に茶殻を入れて評価する方法もある



評価用紙：審査茶ごとに1枚の用紙が準備され、審査主任の基本点に対し、個々の審査員はプラス、マイナス(±)評価点を記入し、全員の点数を加減して最終評価点とする

世界緑茶コンテスト

趣旨；市場性の高い“斬新でお茶の未来を感じさせる商品”を提案し、消費者の選択の幅を広げることで、茶の新たな需要を創造し、消費の拡大に繋げることを目的とする

審査；商品として販売可能なものとする。商品形態と内質について審査する。

審査方法など；
商品のコンセプト、パッケージ、ネーミング、コストパフォーマンスなどの商品形態と香気・滋味の内質により評価する。
採点は合議制によらず個人制。審査は、商品形態から始め、並べ替えしない

商品形態			内質		合計
コンセプト・名称	パッケージデザイン	コストパフォーマンス	香気	滋味	
20	20	20	20	20	100

世界緑茶コンテスト

①商品形態の評価
コンセプト、名称については事前に書類で把握した後、出品商品で確認。パッケージデザイン、コストパフォーマンスなどは商品の包装と茶の外観から判断する

②香気の評価

③滋味の評価

1碗づつコメントと評価点を筆記者が記入

各種品評会の課題など

品評会の種類	課題など
全国茶品評会	・時代のニーズに応じた配点方法 ・審査基準の改善 ・需要拡大目線の低下
日本茶Award	・審査法のグレードアップ ・目標設定の明確化 ・出品茶の意向と淹れ方の不整合性 ・審査の安定性
国際銘茶品評会	・情報不足 ・日本茶審査員の養成
世界緑茶コンテスト	・商品価値と評価の不整合性 ・商品と淹れ方の不整合性 ・経済背景の異なる商品に対する評価基準

今後、望まれる評価法(品評会)等について

☆ **目的に応じた評価法の採用**

- ★ **茶の魅力を高めるための評価法**
- ★ **茶の幅を広げるための評価法**
- ★ **新技術・品種導入を誘発するための評価法**
- ★ **消費拡大を目的とした評価法**

☆ **評価に係る用語、技術、道具等の検討**

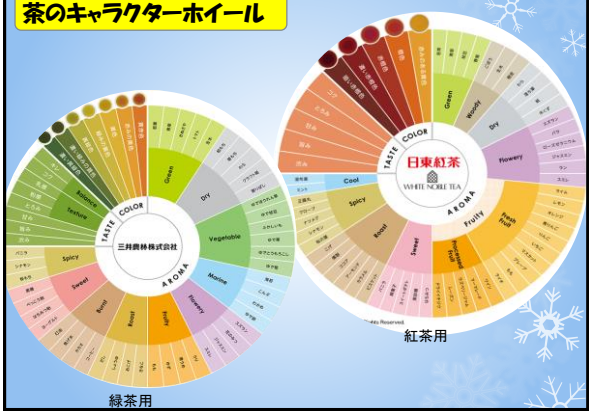
お茶の魅力を消費者にわかりやすく伝えるための評価

各々の茶が持つ特性を誰にでも理解できる用語で説明することが必要

⇒説明法、コメント法、キャラクターホイールの活用

心向樹さんの
品種茶シリーズ

茶のキャラクターホイール



緑茶用　　紅茶用

消費者には茶のわかりやすい表現方法が必要

味わい
すっきり ― こく

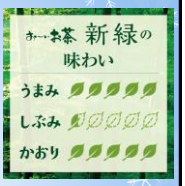
水色（すいしょく）
薄い ― 濃い

旨み
あまみ ― しぶみ

蒸度（むしど）
浅い ― 深い

香り
みるめ香 ― 熟成香

「みるめ香」･･･新芽の新鮮で若々しい爽やかな香り
「熟成香」･･･日光を充分に浴び、養分を蓄えた濃厚な香り



	普通煎茶	深蒸し煎茶	第三の煎茶
花の香り	★★	★	★★★★★
香りの強さ	★★★	★	★★★★★
新鮮な緑の香り	★★★★★	★★★	★
コク	★★	★★★★★	★★
渋み	★★	★	★★★

闘茶会

茶鑑定能力の研磨

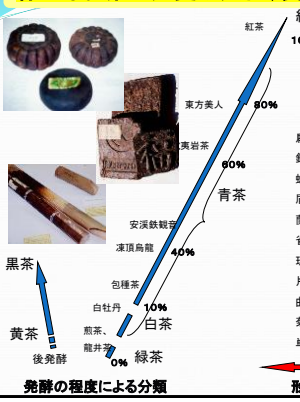
闘茶（とうちゃ）とは、中世に流行した茶の味を飲み分けて勝敗を競う遊び。日本では**回茶**・**飲茶勝負**・**茶寄合**・**茶湯勝負**・**貢茶**、中国では**茗茶**・**銘闘**などの異名もある。

令和2年度　ふじのくに学（お茶）

お茶の淹れ方

茶学総合研究センター　中村順行

様々なお茶が歴史や文化、食生活の中で生み出された



品種――――
製造法――――
素材の部位――
摘採時期――
二次加工――
着香――――
その他の分類

扁平形――龍井、旗槍
針型――玉露
螺型――碧螺春、
盾型――屯緑、茗眉
蘭花型――蘭花茶
雀舌型――雀舌茶
珠型――珠茶
片型――六安瓜片
曲型――天柱弦月
菊花型――緑牡丹
単芽型――白豪銀針
形状による分類

蒸熱――日本茶
湯熱――低カフェイン茶
炒青型――龍井茶
半焙炒型―碧螺春、
焙青型――毛峰
殺青による分類

日本茶の成分と味との関係

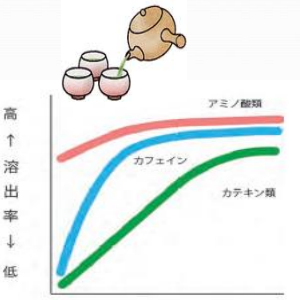
主な味成分とその味

分　類	成　分	味
アミノ酸類	テアニン	甘味、うま味
	グルタミン酸	うま味、酸味
カテキン類	エピカテキン	苦味
	エピガロカテキン	苦味
	エピカテキンガレート	渋味、苦味
	エピガロカテキンガレート	渋味、苦味
カフェイン		軽い苦味

●アミノ酸類 ……お湯の温度の高低にあまり関係なく溶け出す
●カフェイン ……低温では溶け出しにくいが、高温になると一気に溶け出す
●カテキン類 ……お湯の温度が高くなるにつれて溶け出す

3

茶の成分は温度の違いで浸出量が異なる



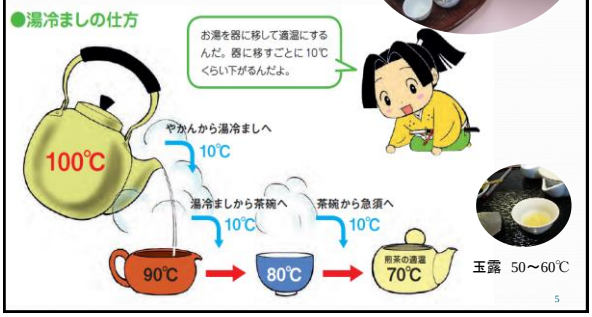
【湯温と成分の溶け出し方のイメージ】

【湯温の違いによる味のイメージ】

浸出温度を制御する

湯温で味をお好みのお茶を創る

●湯冷ましの仕方



玉露　50～60℃

5

お茶の種類により淹れ方が変わる

茶種	いれる人数(人)	急須の大きさ(ml)	茶碗の大きさ(ml)	茶量(g)	湯温(℃)	湯量(ml)	浸出時間(秒)	浸出液温(℃)	1人あたり浸出量(ml)
玉露（特）	3	90	40	10	50	60	150	33	12
玉露（並）	3	90	40	10	60	60	120	40	13
煎茶（上）	3	250	100	6	70	170	120	51	50
煎茶（並）	5	600	150	10	90	430	60	65	80
焙じ茶	5	800	240	15	熱湯	650	30	75	120
番茶	5	800	240	15	熱湯	650	30	75	120

茶のいれ方研究会；茶研報

6

実際の淹れ方

①水は必ず沸とうさせる

水道水は十分に沸とうさせたものを使いましょう。

②お湯の量を量りながら適温に冷ます

お湯を茶碗に８分目まで注いで、湯量を量りながら湯冷ましをします。

③お茶の葉を量る

さじでお茶の分量を量り、急須に入れます。

実際の淹れ方

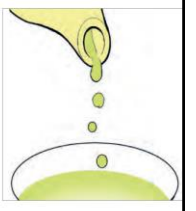
④茶碗で湯冷ましした お湯を急須に入れる

急須にお湯を入れ、蓋をしてじっと待ちます。

⑤茶碗にお茶を注ぐ

まわし注ぎをして、それぞれの茶碗を同じ量、濃度にします。

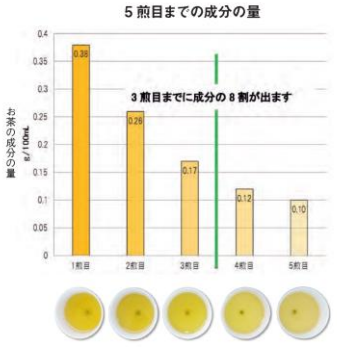
⑥最後の一滴まで注ぐ



急須の中の最後の一滴まで注ぎ切りましょう。

同じ茶葉で何煎目まで淹れれるか？

5煎目までの成分の量



各煎目のいれ方条件

煎茶　6g
◆１煎目
湯温 70℃、湯量 170mL、浸出時間 90 秒
◆２煎目以降（１煎目をいれた急須を使用）
湯温 90℃、湯量 170mL、浸出時間 10 秒

	浸出液中の成分量
１煎目	0.38
２煎目	0.26
３煎目	0.17
４煎目	0.12
５煎目	0.10
合　計	1.03

単位：g/100mL
※100mL 中の成分量（g）=%
データ提供　日本茶インストラクター協会

茶の香りで癒し効果

★茶の香りの主要成分（日本茶インストラクターH.Pより）

煎茶の香り成分
- ・新茶の青々しい香り → 青葉アルコール
- ・新茶の花のような香り → リナロール、ゲラニオール、シスジャスモン
- ・古茶の香り → 1-ペンテン-3 オール、2,4 ヘプタジノール
- ・火入れの香り → カルボニル化合物

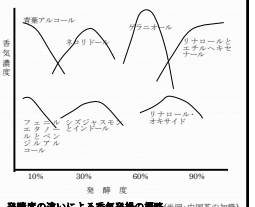
玉露の香り成分
- ・青のりのような深い香り → ジメチルスルフィド

焙じ茶の香り成分
- ・焙煎香 → ピラジン類、フラン類、ピロール類

包種茶と烏龍茶の香り成分
- ・烏龍茶の花のような香り → ネロリドール、ジャスモン酸メチル、インドールなど

紅茶の香り成分
- ・花のような香り → リナロール、ゲラニオール
- ・爽やかな鈴蘭の香り（セイロン紅茶の特徴） → リナロールオキサイド
- ・バラのような香り（ダージリン紅茶、中国紅茶の特徴） →ゲラニオール

微生物発酵茶の香り成分
- ・碁石茶や阿波番茶の香り → 乳酸メチル、アセトン



発酵度の違いによる香気発揚の概略（光岡・中国茶の知識）

こんな時に合うお茶とは？

（1）スポーツの前後

上級煎茶に多く含まれるカフェインは、筋肉を刺激する効果や、心臓の動きを活発にする作用が期待できるので、カフェインが溶け出しやすいように、やや高めの湯温でいれたお茶をスポーツ前に飲んでおくのが良いでしょう。スポーツのあとは、汗をかいて水分が不足しているので、たくさん飲める番茶や焙じ茶などの軽いお茶を冷やしたものが適しています。

（3）いつもの食事のあと

中級煎茶や番茶、焙じ茶、釜炒り茶などは、どのような食事にも合い、たっぷり飲むことができます。お茶に含まれるカテキンには、殺菌効果や虫歯予防効果がありますから、カテキンを多く含む上・中級煎茶をやや高めの湯温でいれると、食中毒や虫歯の予防にもなります。

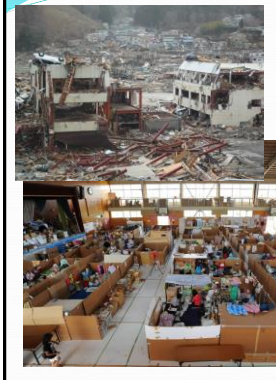
（2）脂っこい食事のあと

天ぷらやフライ、洋食など脂っこい食事のあとは、口の中をさっぱりさせる焙じ茶や烏龍茶が合います。濃厚な味の玉露や上級煎茶はやや不向きです。

（4）試験勉強のとき

夜遅くまで勉強しなければならないときは、カフェインとテアニンを多く含む玉露や上級煎茶を、やや高めの湯温でいれたお茶がおすすめです。カフェインには眠気を覚ます作用、テアニンには落ち着いて集中できる効果があるからです。お父さんの二日酔いにも効果があるよ。

震災時にもお茶の癒し効果発揮



静岡からお茶が届けられ非常に喜ばれた

最近では中国式の6茶種に分類されることが多い

茶の分類

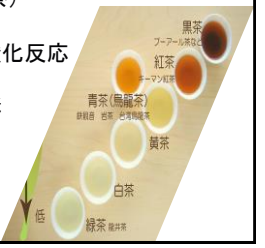
緑茶（不発酵茶）
- 蒸し製緑茶(日本式)
- 釜炒り製緑茶(中国式)

青茶「ウーロン茶」（半発酵茶）

紅茶（発酵茶）
- ※発酵：葉の酵素による酸化反応

黒茶「後発酵茶」（堆積茶）
- ※発酵：微生物発酵

その他
- 白茶
- 黄茶
- 二次加工茶

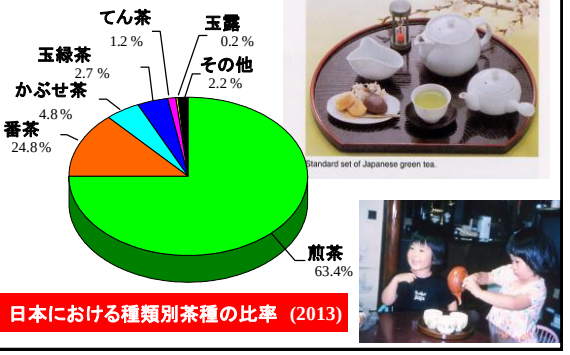


茶の製造法の概略(六大茶種)

不発酵茶	不発酵茶	緑茶	殺青	（蒸し）	揉捻		乾燥
				（釜炒り）	揉捻		乾燥
	軽後発酵茶	黄茶	殺青		揉捻	悶黄	乾燥
	後発酵茶	黒茶	殺青		揉捻	渥堆	乾燥
発酵茶	軽発酵茶	白茶	萎凋				乾燥
	半発酵茶	青茶	萎凋	攪拌	殺青	揉捻	乾燥
	完全発酵茶	紅茶	萎凋		揉捻	発酵	乾燥

3

日本茶の種類別生産量比率



日本における種類別茶種の比率（2013）

日本茶の種類

日本茶の種類

同じ茶葉から様々なお茶が作れ、成分も変わる

生 葉	緑 茶	紅 茶
カテキン類	カテキン類 ⇒	テアフラビン、テアルビジン
クロロフィル	クロロフィル ⇒	フェオフイチン
ビタミンC	ビタミンC ⇒	消失(酸化物、分解物)
香り	青葉様香気 ⇒	花様香気

緑茶の製造工程

生葉 → 殺青 → 蒸気 / 釜炒り → 揉捻 → 乾燥

日本式 蒸熱殺青

中国式 釜炒り殺青

緑茶は殺青が重要

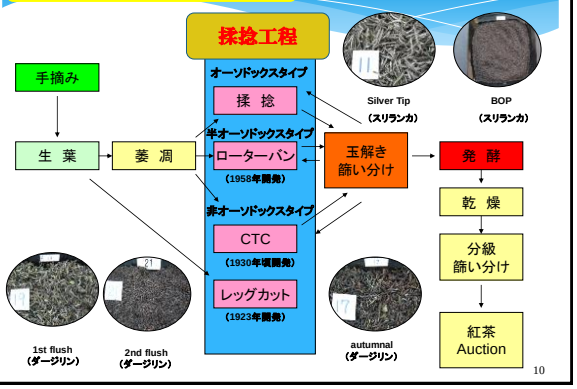
中国式釜炒り殺青の簡便法

新鮮な生葉をホットプレート上で炒ることにより、釜炒りと同様な殺青状態となる。
⇒釜炒り式

日本式蒸熱殺青の簡便法

新鮮な生葉をラップで包み、電子レンジを用いて葉の持つ水分を蒸気化することにより、蒸した状態となる。 ⇒蒸気で蒸す

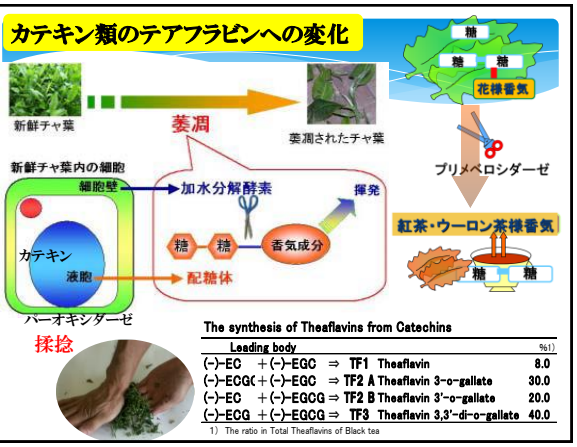
発酵茶(紅茶)製造工程



紅茶は発酵が重要

紅茶には発酵が重要
細胞壁にある酵素と細胞質内にあるカテキンが酸化することにより発行が始まる

カテキン類のテアフラビンへの変化



The synthesis of Theaflavins from Catechins

Leading body				%1)
(-)-EC	+(-)-EGC	⇒ TF1	Theaflavin	8.0
(-)-ECG	+(-)-EGC	⇒ TF2 A	Theaflavin 3-o-gallate	30.0
(-)-EC	+(-)-EGCG	⇒ TF2 B	Theaflavin 3'-o-gallate	20.0
(-)-ECG	+(-)-EGCG	⇒ TF3	Theaflavin 3,3'-di-o-gallate	40.0

1) The ratio in Total Theaflavins of Black tea

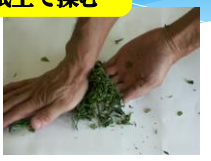
ホットプレートを用いたお茶づくり
①準備するもの

新鮮な生葉

ホットプレート、割りばし、軍手、ラップ、紙、電子レンジ　など

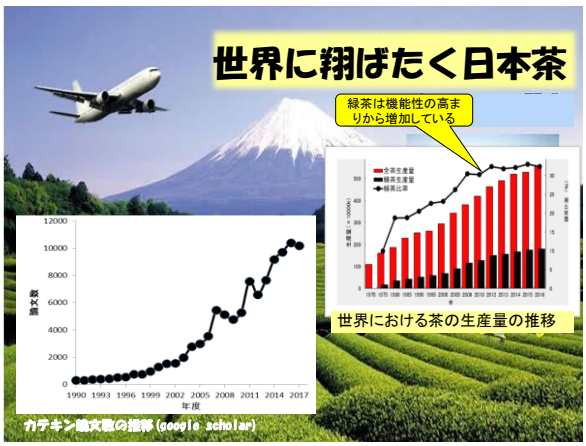
ホットプレートを用いたお茶づくり
②ホットプレート上で乾燥させながら揉む

ホットプレートを用いたお茶づくり
③ホットプレート上あるいは紙上で揉む



ホットプレートを用いたお茶づくり
④紅茶を作る場合に等には洗濯板上で揉んでもよい

ホットプレートを用いたお茶づくり
⑤ホットプレート上で乾燥



海外では緑茶が好況

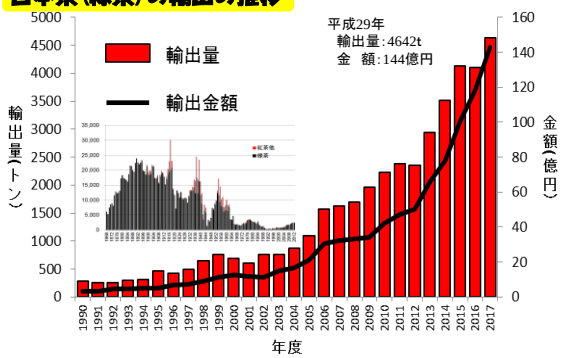
各種H.Pより引用

ハワイの日本茶販売店舗

伊藤園「お～いお茶HPより引用」

オーストラリアの茶店

日本茶(緑茶)の輸出の推移



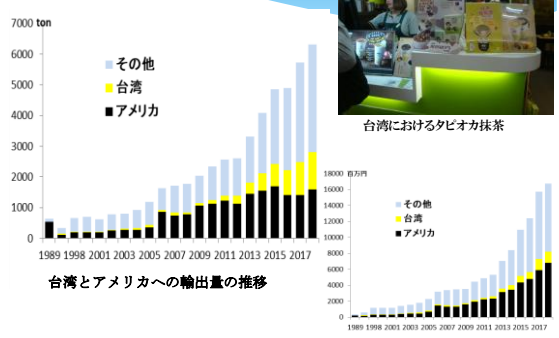
日本茶の輸出量と金額の推移

日本茶の主な輸出先(平成29年度)

近年の緑茶の輸出状況　(単位:トン)

	全体	輸出量		輸出量		輸出量
2005	1,098	アメリカ	353	ドイツ	96	台湾	84
2006	1,570	アメリカ	865	シンガポール	103	香港	93
2007	1,825	アメリカ	750	カナダ	137	シンガポール	94
2008	1,701	アメリカ	776	カナダ	162	ドイツ	124
2009	1,858	アメリカ	1,063	カナダ	149	シンガポール	131
2010	2,232	アメリカ	1,136	カナダ	172	シンガポール	169
2011	2,387	アメリカ	1,228	カナダ	198	台湾	171
2012	2,351	アメリカ	1,127	台湾	262	シンガポール	257
2013	2,848	アメリカ	1,406	台湾	365	シンガポール	205
2014	3,516	アメリカ	1,550	台湾	570	シンガポール	256
2015	4,127	アメリカ	1,698	台湾	735	ドイツ	305
2016	4,108	アメリカ	1,420	台湾	793	シンガポール	342
2017	4,642	アメリカ	1,407	台湾	1,080	シンガポール	343

主要な輸出先「米国と台湾」

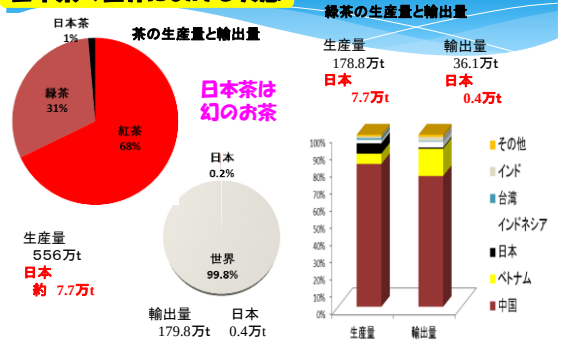


台湾とアメリカへの輸出量の推移

台湾におけるタピオカ抹茶

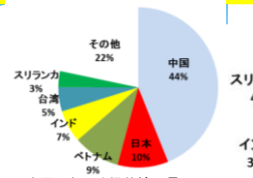
台湾とアメリカへの輸出金額の推移

日本茶の世界における実態

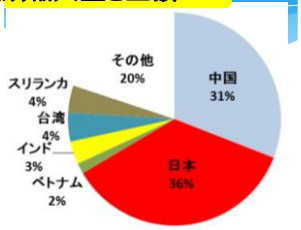


世界の**茶**の生産量と輸出量(2016)　　世界における**緑茶**の生産量と輸出量

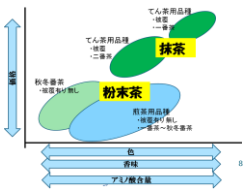
米国における緑茶の国別輸入量と金額



米国における緑茶輸入量



米国における緑茶輸入金額



茶の輸入価格(2017)

中国	680円/kg
日本	3,343円/kg

海外で市販されている抹茶

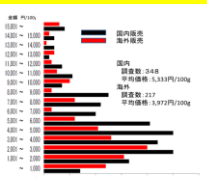
- 全ての成分が摂取可能なこと
- 健康に良さそう
- 緑色がきれいで美味しい
- 文化性が豊かである
- 他用途利用しやすい

海外で市販されている抹茶

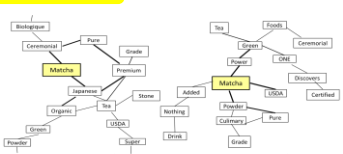
抹茶道具販売(イギリス)

有機日本茶専門店
(オランダ)

国内外における市販抹茶の現状



図B-1-(5)-1　国内外で市販される抹茶の金額別比率



5,000円/100g以上の抹茶　　1,000円/100g以下の抹茶

図B-1-(5)-2　米国で市販される抹茶のパッケージにみられるキーワード
(塗　パッケージに記載されている文章のトレンドサーチ解析。太線ほど関連性が高い)

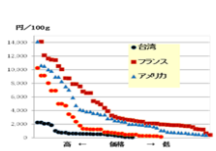


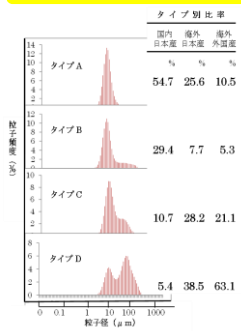
図 B-1-(5)-3　海外で市販される抹茶価格の国別推移

表B-1-(5)-1 各国のネットで市販されている抹cha あるいは抹茶の価格

国名	抹茶のみ 点数	抹茶のみ 比率(%)	混合抹茶 点数	混合抹茶 比率(%)	平均価格(円/100g) 全体	上位10点	下位10点
イギリス	44	88.0	6	12.0	4,266	9,812	1,185
アメリカ	44	88.0	6	12.0	3,636	9,586	609
フランス	41	82.0	9	18.0	4,795	11,412	1,346
ドイツ	45	90.0	5	10.0	4,459	10,808	1,050
台湾	8	26.7	22	73.3	787	1,445	295
シンガポール	32	84.2	6	15.8	2,532	6,850	362
日本	34	77.3	10	22.7	2,042	5,330	304

※ 各々の国のアマゾンネットで市販されている抹cha あるいは抹茶の販出順位別50点(台湾、シンガポールは掲出されたもの全て)程度を調査対象とした。

国内外で市販される抹茶の粒度特性



図B-1-(5)-3　国内外から購入した抹茶のタイプ別比率

表　海外購入抹茶の粒度特性

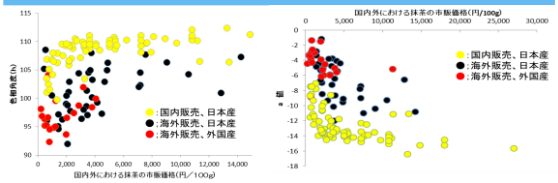
タイプ	購入抹茶の種類 日本産	購入抹茶の種類 外国産	平均径(μm)	標準偏差	モード径(μm)	メジアン径(μm)
A	10	2	16.5	12.9	11.7	12.7
B	3	1	21.4	22.3	12.0	14.2
C	11	4	30.2	33.3	12.1	16.6
D	15	12	44.5	41.8	31.9	29.8

表B-1-(5)-2　国内外から購入した抹茶のタイプ別価格と粒径の大きさ

タイプ	国内市販品 日本産 100g当たり価格(円)	国内市販品 日本産 平均径(μm)	海外市販品 日本産 100g当たり価格(円)	海外市販品 日本産 平均径(μm)	海外市販品 外国産 100g当たり価格(円)	海外市販品 外国産 平均径(μm)
A	6,902	15.5	5,801	16.5	1,569	16.7
B	4,010	23.8	2,515	23.7	3,780	19.2
C	2,698	26.2	4,178	30.1	1,241	30.8
D	1,572	52.6	3,461	47.9	1,599	38.8

国内外で市販される抹茶の測色特性

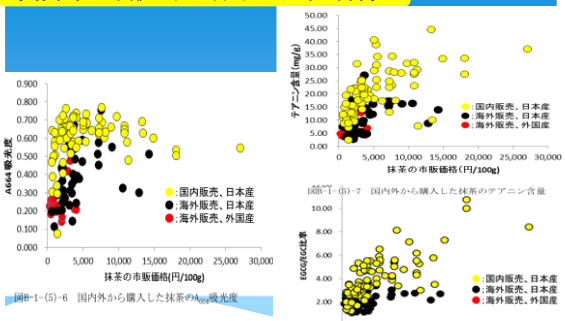
100g当たり単価(円)		測色値				
価格帯	平均単価	L*	a*	b*	C*	h
～1,000	654	60.31	−3.63	34.08	34.30	96.03
1,001～ 2,000	1,575	56.25	−3.89	34.75	35.02	96.28
2,001～ 3,000	2,247	57.94	−5.73	35.88	36.39	99.00
3,001～ 4,000	3,492	59.13	−6.87	34.64	35.38	101.09
4,001～ 5,000	4,404	57.24	−6.35	34.11	34.78	100.65
5,001～10,000	7,582	55.16	−9.13	35.01	36.21	104.61
10,001～	12,225	57.64	−7.95	34.64	35.60	102.83



図B-1-(5)-4 国内外から購入した抹茶の色相角度(h 値)

図B-1-(5)-5 国内外から購入した抹茶のａ*値
※ａ*値が低いほど緑色、高いほど赤色を表す

国内外で市販される抹茶の化学的特性

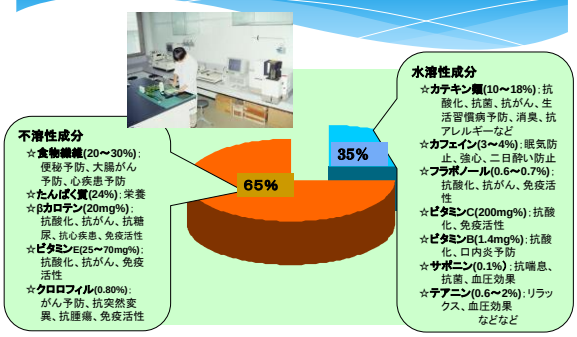


図B-1-(5)-6 国内外から購入した抹茶のA_{646}吸光度

図B-1-(5)-7 国内外から購入した抹茶のテアニン含量

図B-1-(5)-8 国内外から購入した抹茶のEGCG/EGC比率

機能性を主体とした茶成分とその特性



茶はカテキンを始め多くの特異的な成分を含有し、それぞれ機能性をもつため、その機能性を活かした商品も数多く開発されている

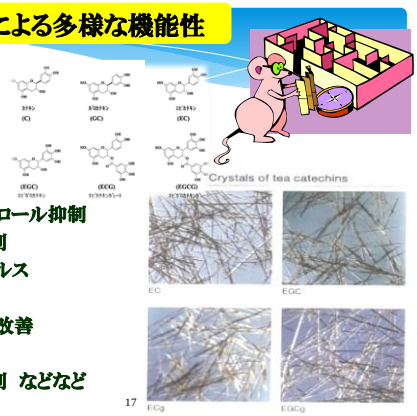
主要茶成分の機能性

表　茶の主要三次機能(機能性)成分の特性と用途

成　分	機　能　性	用　途
カテキン類	抗酸化、抗突然変異、抗がん、コレステロール低下、血圧上昇抑制、血糖上昇抑制、血小板凝集抑制、抗菌、抗ウイルス、虫歯予防、抗アレルギー、消臭	食品酸化防止、抗菌剤、脱臭剤、抗虫歯剤など
フラボノール	毛細血管抵抗性増加、抗酸化、血圧降下、消臭	脱臭剤
カフェイン	中枢神経興奮、睡眠防止、強心、利尿、抗喘息、代謝亢進	眠気防止剤、感冒剤、強心剤、アレルギー軽減剤
ビタミンC	抗壊血病、抗酸化、がん予防	酸化予防剤
ビタミンE	抗酸化、がん予防、抗不妊	酸化防止剤
γアミノ酪酸	血圧上昇抑制、抑圧性神経伝達	ギャバロン茶
テアニン	興奮抑制、リラックス効果、血圧低下、脳・神経機能調節	神経機能調節剤

カテキン類による多様な機能性

- 抗酸化
- 抗突然変異
- 抗がん
- 酸化防止
- 抗動脈硬化
- 血中コレステロール抑制
- 脂肪吸収抑制
- 抗菌、抗ウイルス
- 虫歯予防
- 腸内フローラ改善
- 消臭
- 血圧上昇抑制　などなど



カテキン類による抗がん作用

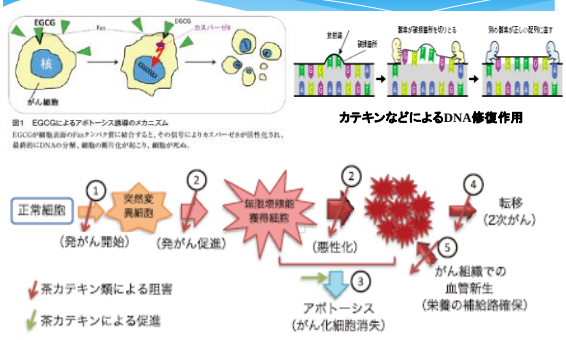


図1　EGCGによるアポトーシス誘導のメカニズム
EGCGが細胞表面のリセプタパク質に結合すると、その信号によりカスパーゼ8が活性化され、最終的にDNAの分解、細胞の断片化が起こり、細胞が死ぬ。

図1　茶カテキン類による発がん過程の抑制効果

がんと緑茶に関する疫学的調査研究のまとめ

表１　がんと緑茶に関する疫学調査研究（伊勢村護）

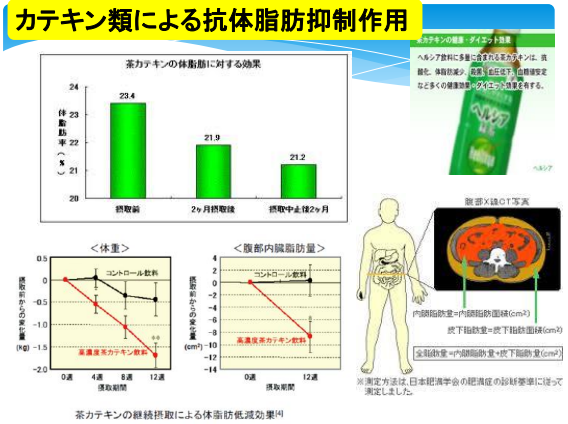
	前向きコホート研究		症例対照研究	
がんの部位	リスク軽減あり	リスク軽減なし	リスク軽減あり	リスク軽減なし
大腸	3	6	4	3
肺	0	4	2	3
胃	2	6	8	8
食道	0	2	4	5
乳房	3	5	3	0
前立腺	2	1	2	0
卵巣	1	0	2	0
すい臓	0	2	2	1
腎臓、膀胱	0	1	1	4
肝臓	1			
子宮内膜			2	1
甲状腺	1	1		
血液	1			

データは、～緑茶と健康のメカニズム～　機能効用ナビゲーション 2013
（静岡県経済産業部農林業局茶業農産課）

前向きコホート研究：
まだ病気になっていない人達を対象に調査し、数年後の追跡で発病を調査する方法

症例対照研究：
特定の病気が発症した人を対象に、健常人との比較調査する方法

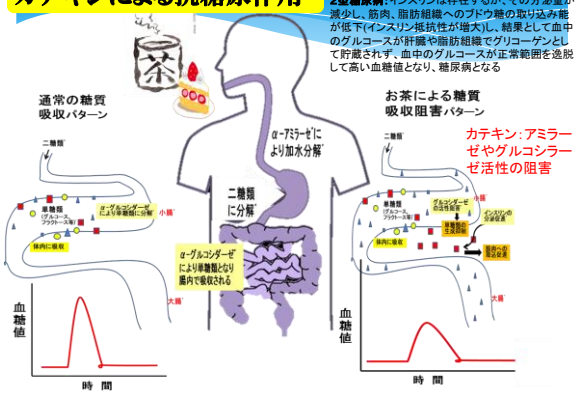
カテキン類による抗体脂肪抑制作用



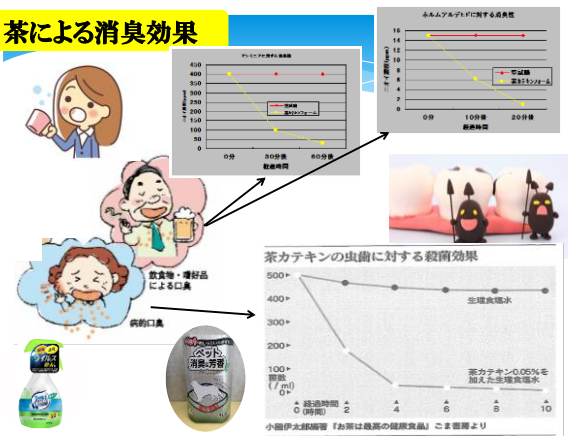
茶カテキンの継続摂取による体脂肪低減効果[4]

カテキンによる抗糖尿作用

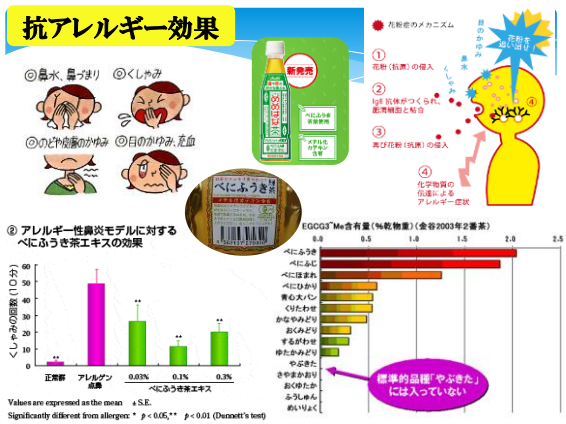
1型糖尿病：膵臓のβ細胞の破壊によるインスリン欠乏により、高血糖、糖尿病へと至る。
2型糖尿病：インスリンは存在するが、その分泌量が減少し、筋肉、脂肪組織へのブドウ糖の取り込み能が低下（インスリン抵抗性が増大）し、結果として血中のグルコースが肝臓や脂肪組織でグリコーゲンとして貯蔵されず、血中のグルコースが正常範囲を逸脱して高い血糖値となり、糖尿病となる



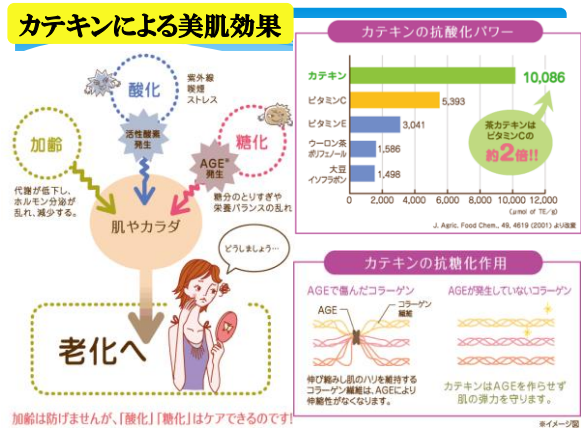
茶による消臭効果



抗アレルギー効果



カテキンによる美肌効果



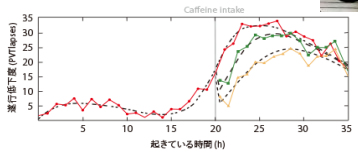
カフェインの機能性

- 覚醒作用
- 大脳刺激作用
- 疲労回復
- 強心作用
- 利尿作用

カフェインの別名は「目覚まし草」

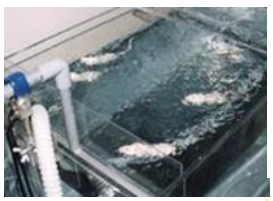
カフェインの覚醒効果（遂行の改善）

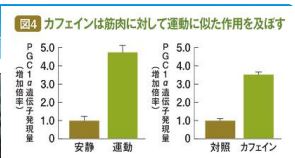
20時間断眠後の睡魔による遂行低下を改善



出典元：「Journal of Theoretical Biology」Volume 358: I（2014 年 Ramakrishman 他）

カフェインによる運動機能の向上





運動によるマウス骨格筋およびカフェイン処理による骨格筋培養細胞における PGC1α遺伝子の発現量増加

マウスを強制的に運動させると骨格筋ではミトコンドリア機能の活性化や脂肪酸燃焼に重要な役割を担う遺伝子である PGC1α遺伝子発現量が増加する。PGC1α遺伝子発現量が増加すると代謝が活性化して脂肪が燃焼し、インスリン感受性が強まる。培養骨格筋細胞をカフェインで刺激しても、PGC1α遺伝子発現量が増加する。

テアニンの機能性

- 血圧降下
- 脳神経機能調整
- 血管性痴呆症予防作用
- 抗ストレス作用
- 記憶学習行動促進作用

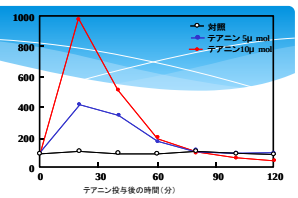
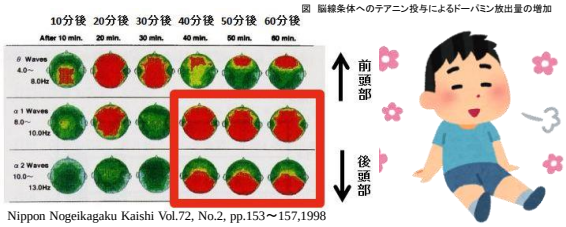


図 脳線条体へのテアニン投与によるドーパミン放出量の増加



Nippon Nogeikagaku Kaishi Vol.72, No.2, pp.153～157,1998

ストレスの解消から寿命の延伸を

高齢者・次世代向けお茶の開発

☆ 睡眠の質の改善
☆ ストレスの緩和

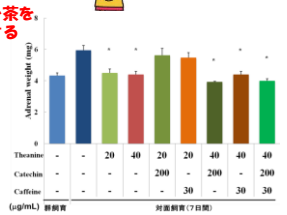
低カフェン茶を、水出しにする

水出し煎茶の特質

・苦渋みが少ない
　ガレート型カテキンの溶出が少
　カフェインが少ない
・甘味が強い
　アミノ酸の溶出比率が高い

↓

免疫機能の強化



カテキン、カフェイン、テアニンの抗ストレス効果の相互作用

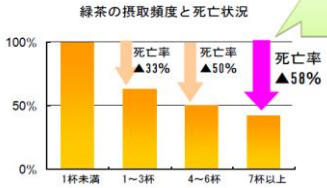
お茶は長寿の秘訣

多く緑茶を飲む高齢者ほど、長生き

Q（この1ヶ月で）緑茶を1日に何杯くらい飲みましたか？

緑茶を「1日に7杯以上」飲む人は 長生き 死亡が半分に!!

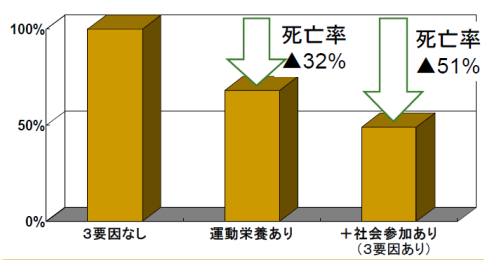
緑茶の摂取頻度と死亡状況



出典：静岡県高齢者コホート調査
Suzuki(2009) Annual Epidemiology

長寿の秘訣　3分野

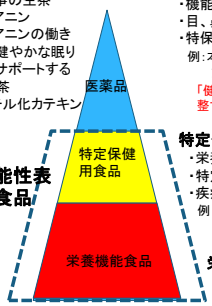
- 高齢者14,001人の追跡結果
  - 運動・栄養・社会参加について良い習慣がある人は長生き



・性別、年齢、体格指数、喫煙状況で調整したハザード比
・H24．7．21東海公衆衛生学会にて発表

各種表示食品の分類

・食事の生茶
・テアニン
・テアニンの働き
で健やかな眠り
をサポートする
麦茶
・メチル化カテキン



機能性表示食品

機能性表示食品
・栄養成分の含有表示が可能
・機能性関与成分の機能性が表示可能
・目、鼻などの部位を表示可能
・特保にない疲労、ストレス、睡眠などの用語が使用可能
例：本品はメチル化カテキンを含んでいるため花粉が気になる方の目や鼻の調子を整えます
「健康を維持する」「働きをサポートする」「健康を増進する」「調整する」など高低、上昇、低下などの程度を示す用語は不可

特定保健用食品(特保)
・栄養成分の含有表示が可能
・特定の保健用途の表示可能
・疾病リスクの低減表示が可能
例：糖の吸収を穏やかにし、血糖値の気になる方に適します

栄養機能性食品
・栄養成分の含有表示が可能
・栄養機能について表示可能
例：カルシウムは骨や歯の形成に必要な栄養素です

茶を中心とした特定保健用食品例
最近では　W効果の商品が出現

茶関係の機能性表示食品も増加　（各メーカーHPより引用）

茶の新需要の事例

表　茶の新需要の事例

区　分	需　要　分　野　と　応　用　例
茶として利用	水出し茶、各種発酵茶、新香味茶、ギャバロン茶、低カフェイン茶、濃縮茶、混合茶　など
飲用・形態を変えて利用	ドリンク茶、ティバッグ、インスタントティ、粉末茶、微粉末茶(食用、即席飲用、酒割用)、カード茶、錠剤茶、カプセル茶、茶ワイン、緑茶酒、スポーツ飲料、カテキン粉末など
食品・食用として利用	☆　形態を変えてそのまま食用として利用 ☆　食品素材として利用 「素材」「食品」「菓子類」「その他」健康補助食品
飲食料以外に利用	☆　衣料用など ☆　医療用 ☆　化粧品、石鹸用など ☆　消臭剤、脱臭剤など ☆　日用品など ☆　建材、家具、家電用品など ☆　家畜、ペット用品 ☆　植物活性用 ☆　その他

茶は飲用だけでなく、食品素材として、さらには機能性成分を活かした様々な飲食料以外にも利用され、新しいビジネスを創造している

茶として利用

水出し茶、各種発酵茶、新香味茶、ギャバロン茶、低カフェイン茶、濃縮茶、混合茶　など

飲用形態を変えて利用

ドリンク茶、ティバッグ、インスタントティ、粉末茶(食用、即席飲用、酒割用)、カード茶、錠剤茶、カプセル茶、茶ワイン、緑茶酒、スポーツ飲料、カテキン粉末　など

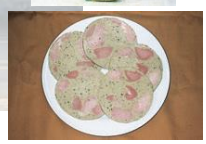
食品・食用として利用
～形態を変えて食用～

いしびき茶、食べる茶、茶のふりかけ、ペースト茶、佃煮　など

食品・食用として利用
～食品素材・食品～

「素材」
フレーバー、エキス、多用途茶
「食品」
茶そば、茶団子、茶かゆ、茶かまぼこ、ハム、茶料理、ジャム、食用油、ドレッシング、マヨネーズ





食品・食用として利用
～菓子類など～

茶飴、茶羊かん、茶入り菓子、クッキー、パイ、サブレ、カステラ、プリン、ガム、キャンディー、チョコレート、アイスクリーム　など




飲食料以外に利用
～衣料、医療、化粧など～

衣料用；シーツ、タオル、シャツ、靴下、寝具、のれん　など
医療用；消臭シーツ、消臭カバー、紙おむつ、マスク　など
化粧品、石鹸用；化粧品、化粧水、スキンクリーム、洗顔パック、石鹸、シャンプー、リンス、入浴剤、歯磨き粉、虫歯予防剤　など




飲食料以外に利用
～消臭剤、脱臭剤、日用品など～

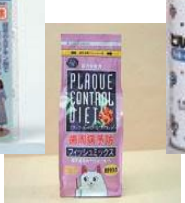
消臭、脱臭剤：トイレ用、冷蔵庫用、消臭スプレー、除菌シート等
日用品など：ノート、ティッシュ、トイレットペーパー、うちわ、スリッパ等




飲食料以外に利用
～建材、家具、ペット用品、その他

建材、家具、家電用品；塗料、ワックス、抗菌量、空気清浄機、布団乾燥機
家畜、ペット用品；ペット用飼料、卵、豚、さなかの肉質改善、脱臭剤
その他；植物活性用、植物活力剤、土壌改良剤、酸化防止剤など





令和2年度
ふじのくに学(お茶)

茶学概論

静岡県立大学
茶学総合研究センター 中村順行

チャの分類

Genus Camellia
- Subgen. Protocamellia
- Subgen. Camellia
  - Sect. Oleifera
    - C.oleifera
    - C.sasanqua
  - Sect. Camellia
    - C.japonica
  - ***Subgen. Thea***
    - ***Sect. Thea***
      - ***C.sinensis***
        - ***var.sinensis***
        - ***var.assamica***
      - C.taliensis
      - C.irawadiensis
  - Sect. Chrysantha
    - C.chrysantha
- Subgen. Metacamellia

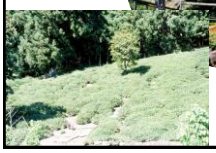
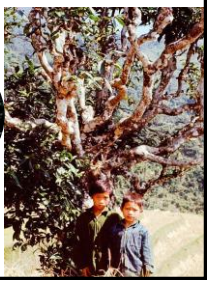




チャの特質

チャが他の植物と異なる点
- ☆カフェイン
- ☆ガレート型のカテキン
- ☆テアニン
- ☆その他(フッ素、アルミ等)



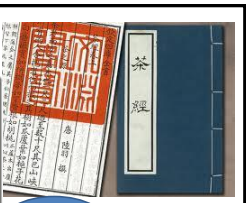



茶の始まり

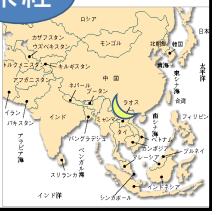
神　農

西暦500年前後に陶弘景(452-536)がまとめた『神農本草経』に**「神農嘗百草、日遇七十二毒、得茶而解之**

陸　羽

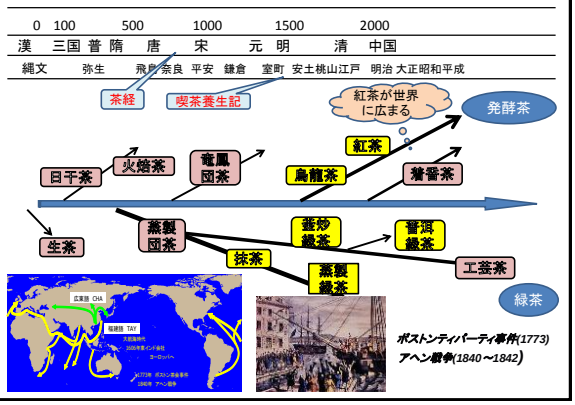


茶経

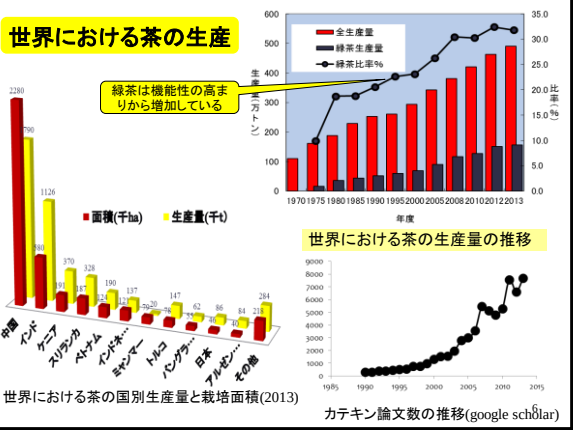


Camellia sinensis から作られる多様なお茶は、カフェイン、カテキン、テアニンなどの特異成分を含むが故に世界中の人々を虜にした

茶には2000年の歴史。時代とともに多様に進化してきた



世界における茶の生産



世界における茶の国別生産量と栽培面積(2013)

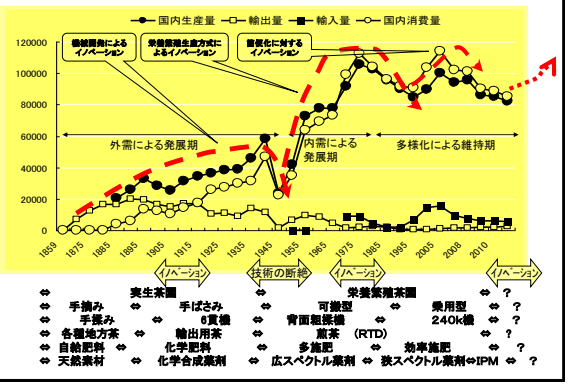
カテキン論文数の推移(google scholar)

喫茶養生記(栄西:1211)

茶者養生之仙藥也　延齢之妙術也

①茶は身体衰弱、意志消沈のときは、気力を強くする。
②茶は人を愉快な気持ちにさせ、酒の酔いを醒まし、睡気を起こさない。
③茶は小便の通じが良く、喉の渇きをとりさり、消化不良をなくす。
④茶は身を軽くし、脚気によい。
⑤茶は精神を整え、内臓を和らげ、身体の疲労をやすらかに除く。

産業化以降の需給とイノベーションの推移



緑茶生産方法の推移


手摘み


可搬型摘採機


乗用型摘採機


手揉み


機械揉み


FA対応機械揉み

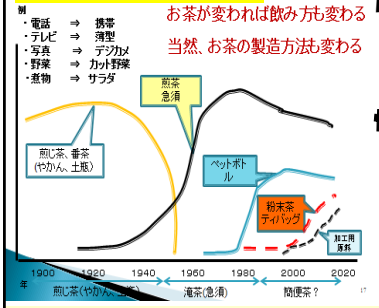
摘採は手摘みから機械摘みに変わり、著しく摘採能率を向上してきた。製造は手揉みから機械化され、徐々に投入量を増加させるとともに最近ではコンピュータ制御による自動化に技術革新したことで、日本独自の生産加工技術を確立し、品質の高位平準化に貢献してきた

緑茶消費方法の推移

現在の生活の中では
お茶を飲む風景も激変しています
⇒ 生産されるお茶も変わります

番茶 ⇒ せん茶 ⇒ 茶素材

緑茶消費の推移の概観

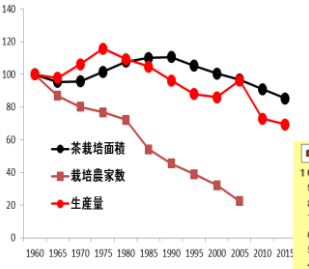


日常茶飯事の茶
- ☆ コスト低下
- ☆ 簡便化
- ☆ 飲用水化

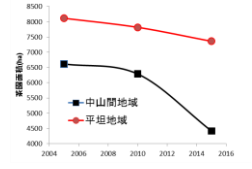
愉しみのお茶
- ☆ かわいさ
- ☆ 話題性
- ☆ 面白さ
- ☆ インスタ映え
- ☆ 高級品
- ☆ ブランド

消費者が必要としているお茶の提案
- 妊婦さん、子供、高齢者 ⇒ 低カフェイン茶
- 体脂肪の気になる方 ⇒ カテキン強化茶
- アレルギー ⇒ べにふうき緑茶 ・ストレスの気になる方 ⇒ テアニン強化茶
- 肉食に合うお茶 ⇒ ・パンに合うお茶 ⇒ その他

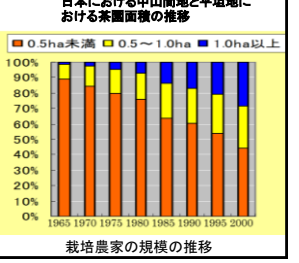
緑茶の生産実態の推移



県内の茶栽培面積・農家数・生産量の推移

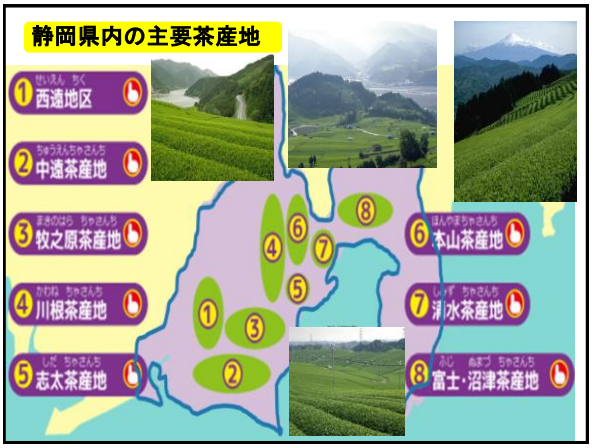


日本における中山間地と平坦地における茶園面積の推移

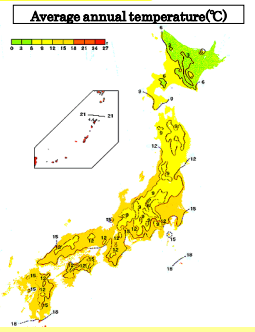


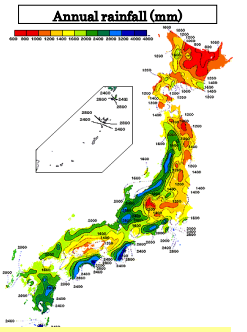
栽培農家の規模の推移

静岡県内の主要茶産地



茶の栽培適地





国内茶生産地の年平均気温11.5～18℃。冬季の最低気温−4.1～4.5℃程度。降水量1300mm以上。茶芽の生育する4～10月に900mm以上。

日本における茶の生産

平成25年度

県 名	面積 (ha)	生産量 (1000t)
静岡県	18,300	32,200
鹿児島県	8,660	25,600
三重県	3,150	7,130
熊本県	1,640	1,300
京都府	1,580	3,020
福岡県	1,570	2,290
宮崎県	1,540	4,100
埼玉県	925	537
佐賀県	953	1,510
その他	7,082	5,113
合 計	45,400	82,800

茶栽培の年間スケジュール

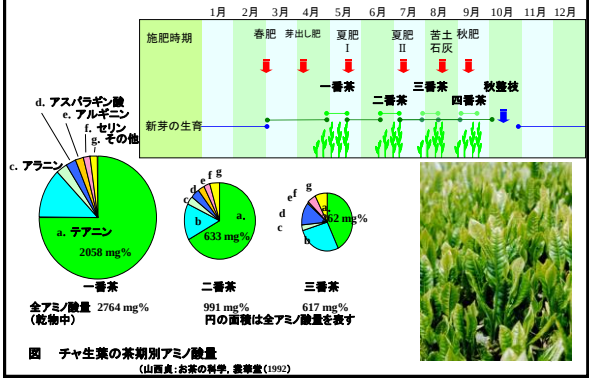
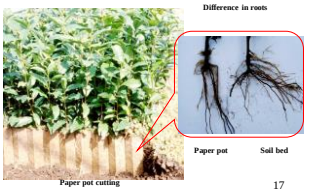


図 チャ生葉の茶期別アミノ酸量
（山西貞：お茶の科学，養賢堂(1992)）

チャの育苗

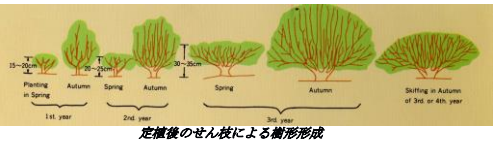


チャの定植と仕立

苗の植え付け

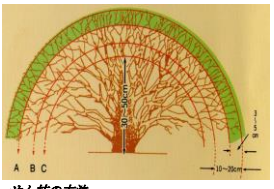
植え付け後のせん枝

定植1年後の茶園



定植後のせん枝による樹形形成

チャせん枝(更新)



せん枝後の茶園

せん枝の方法
A: 浅刈り
B: 深刈り
C: 中切り

Portable pruning machine

Riding pruning machine

代表的な摘採方法

Hand plucking

摘採方法と一人当たり摘採量

摘採方法	一人、一日当り摘採量
手摘み	10 ～ 15 kg
手はさみ	100 ～ 200
機械摘採	
二人用可搬型摘採機	700 ～ 1,000
乗用型摘採機	4,000 ～ 5,000
レール走行式摘採機	2,000 ～ 3,000

Hand-shear plucking

Portable machine for two persons

Riding-type plucking machine

防霜対策

Anti-frost fan

First crop injured by frost

Sprinkler

持続型茶業を目指した施肥と防除

White Roots

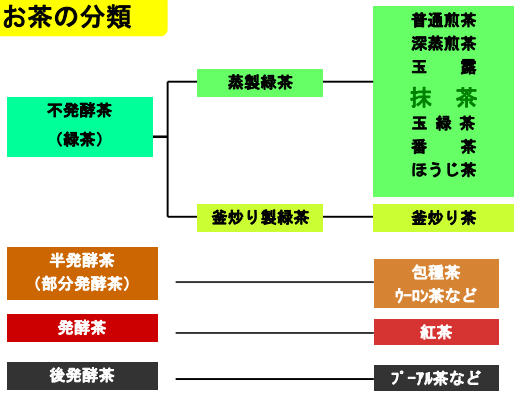
Deep plow subsailer

Natural enemies

Dispenser releasing sex pheromone

Sex pheromone dispensers disruption of communication

お茶の分類



世界のお茶

茶の分類

緑茶（不発酵茶）
- 蒸し製緑茶(日本式)
- 釜炒り製緑茶(中国式)

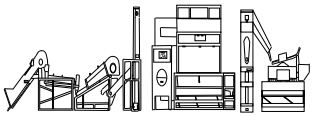
青茶「ウーロン茶」（半発酵茶）

紅茶（発酵茶）
- ※発酵：葉の酵素による酸化反応

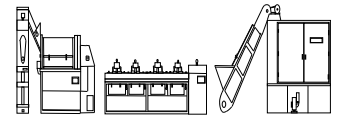
黒茶「後発酵茶」（堆積茶）
- ※発酵：微生物発酵

その他
- 白茶
- 黄茶
- 二次加工茶

煎茶(荒茶)製造工程




Tea steaming machine

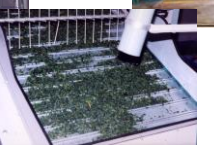


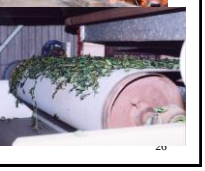

Primary drying tea roller

てん茶(抹茶原料)製造工程

レンガ造り、てん茶機(乾燥機)


冷却散茶機





釜炒り茶製造工程

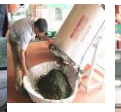

手作り用釜


炒葉機


締炒機

半発酵茶(ウーロン茶)製造工程

生葉 → 日光萎凋 → 室内萎凋 → 殺青 → 揉捻 → 乾燥



発酵茶(紅茶)製造工程

生葉 → 萎凋 → 揉捻 → 発酵(酸化) → 乾燥

日本茶の特質は

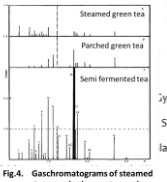
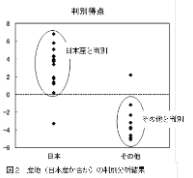
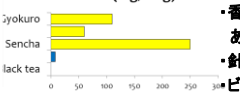


Fig.4. Gaschromatograms of steamed green tea, parched green tea and semifermented tea made from fresh leaves plucked on the same day. (Tadakazu TAKEO etc. 1985)





Vitamin C content of various tea

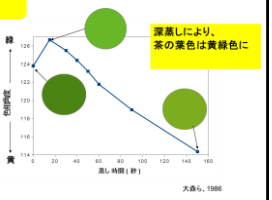
世界の茶のなかでの日本茶の特質は!!

- 浸出液が緑色であること
- 蒸熱処理のため、浸出液中に成分が溶出しやすいこと
- 旨味のアミノ酸含量が高く、カテキン類の濃度が低いこと
- 香りに若葉の新鮮香があること
- 針状のお茶であること
- ビタミンCを多く含むこと
- 歴史、文化性が高いこと

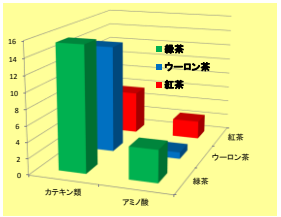
針状のお茶であること

蒸して細く撚れるお茶
は日本茶しかない

浸出液が緑色であること



アミノ酸含量が高く、カテキン類の濃度が低い

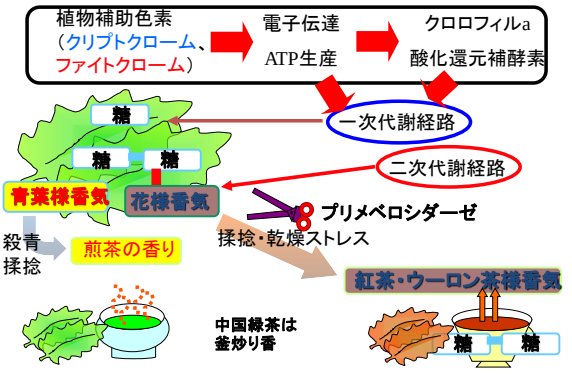


生葉のカテキン類含量

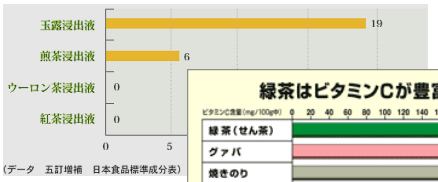
中　国　種：13～17%
中　葉　種：16～23%
アッサム種：25～30%

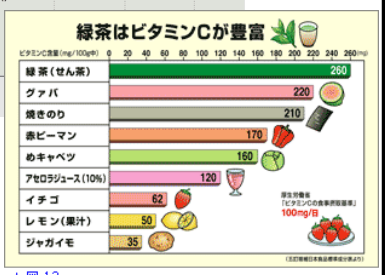
※紅茶茶はカテキン類が酸化され、重合しテアフラビン類、テアルビジン類に変化し、赤色となる。

青葉の香りがすること



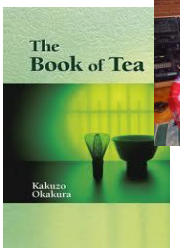
ビタミンCを多く含有すること





▲図 12

日本茶文化の海外波及



岡倉天心

*「一碗からピースフルネスを」をテーマに15世千宗室（鵬雲斎玄室大宗匠）が平和の尊さを茶道の精神をもって訴える活動をハワイを皮切りに世界中で行い、茶道は日本文化を代表するものになった。

高級抹茶の特性を活かした輸出戦略マニュアル

目　　次

２　抹茶生産と輸出の推移
２.１ 抹茶の生産量の推移
２.２ 輸出の推移

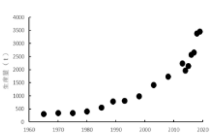


図１　てん茶生産量の推移

図３　世界各国への日本茶の輸出状況(2019)

表１　各国への日本茶の輸出概要

項　目	茶消費量	一人当たり消費量	日本茶輸出量	日本茶輸出金額	輸出kg当り価格	粉末茶輸出量比率
	千トン	kg	トン	百万円	円	%
アメリカ	129	0.40	1,595	6,811	4,271	62
カナダ	17	0.47	205	688	3,347	42
ロシア	253	0.89	15	39	2,555	75
イギリス	110	1.67	35	195	5,570	36
ドイツ	31	0.80	374	1,397	3,740	45
フランス	14	0.21	104	310	2,971	26
イタリア	7	0.12	36	139	3,862	74
オーストラリア	11	0.44	87	280	3,221	43
シンガポール	—	—	307	925	3,008	55
台湾	37	1.37	1,216	1,407	1,157	7
香港	11	1.51	172	862	4,992	61
タイ	—	—	291	541	1,862	77
ベトナム	—	—	72	121	1,688	58
マレーシア	25	0.79	178	323	1,822	42
中国	1,956	1.42	53	124	2,362	—
資料統計年度	2015～2017平均	2015～2017平均	2018	2018	2018	2019.1～8月

表２　各国のネットで市販されている抹茶価格

国名	抹茶のみ		混合抹茶		平均価格(円/100g)		
	点数	比率(%)	点数	比率(%)	全体	上位10点	下位10点
イギリス	44	88.0	6	12.0	4,266	9,812	1,185
アメリカ	44	88.0	6	12.0	3,636	9,586	609
フランス	41	82.0	9	18.0	4,795	11,412	1,346
ドイツ	45	90.0	5	10.0	4,459	10,808	1,050
台湾	8	26.7	22	73.3	787	1,445	295
シンガポール	32	84.2	6	15.8	2,532	6,850	362
日本	34	77.3	10	22.7	2,042	5,330	304

※ 各々の国のアマゾンネットで市販されているMatchaあるいは抹茶の掲出順位別50点(台湾、シンガポールは掲出されたもの全て)程度を調査対象とした。

３　海外市場の特性
3.1 マーケットの特性
3.2 嗜好、消費、購買特性の違い

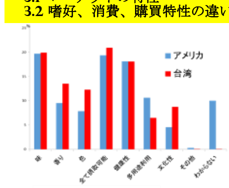


図７　抹茶の優位性

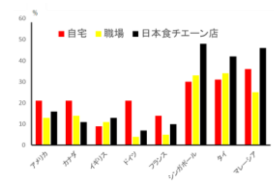


図１０　日本茶の飲用場所

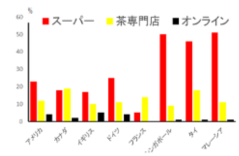


図１１　日本茶の購入場所

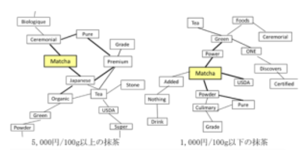


図１３　価格の異なる抹茶のトレンドサーチ解析

３　海外市場の特性
3.3 輸出障壁
3.4 プロモーション方法、特にEC市場の特性

輸出障壁

残留農薬規制
動植物検疫
関税
認証制度　など

プロモーション方法、特にEC市場の特性

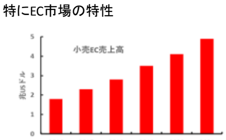


図１５　世界の EC 市場規模の推移

４　国内外で市販される抹茶の特性
4.１ 粒度分布特性
4.２ 測色特性

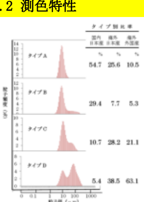


図１６　国内外から購入した抹茶のタイプ別比率

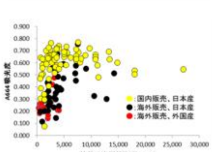


図 17　国内外から購入した抹茶 A_{664} 吸光度

４　国内外で市販される抹茶の特性
4.２ 測色特性
4.３ 化学成分特性

抹　茶
（市販茶5,200円）

食材用抹茶
（秋冬番茶）

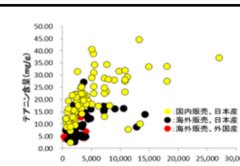


図２０　国内外から購入した抹茶のテアニン含量

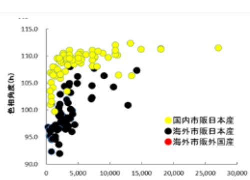


図18　国内外から購入した抹茶の色相(h)角度

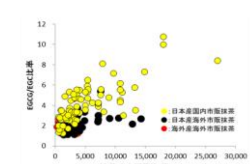


図２１　国内外から購入した抹茶の EGCG/EGC 比率

4　国内外で市販される抹茶の特性
4.4 中国産抹茶との差別性

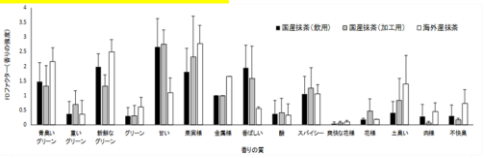


図２２　香気エキス希釈分析により解析された国産抹茶と海外産抹茶の香りの質とその強度

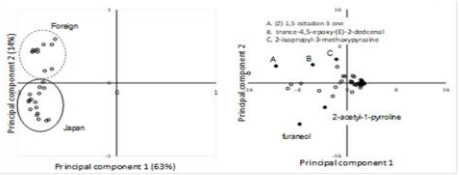


図２３　香気エキス希釈分析結果の主成分分析による国産抹茶と海外産抹茶の区別

4　国内外で市販される抹茶の特性
4.4 中国産抹茶との差別性

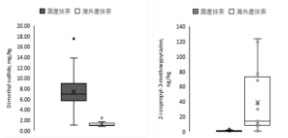


図２４　国産抹茶と海外産抹茶を区別する香気成分含有量

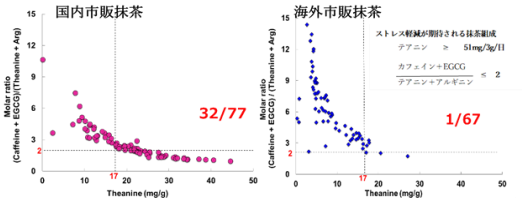


図２５　国内外から購入した抹茶におけるストレス軽減の推測

５．輸出国別特性とSWOT解析
5.1 輸出国別特性
5.2 SWOT解析

表６　主要な輸出対象国別の特徴

対象国・地域	特性
米国	・輸入量も多く、比較的高価な抹茶が使用され、知名度も高い ・健康イメージ、中国産抹茶も多く、飲食素材としての広がりも多い
カナダ	・比較的輸出単価の高いものの需要が高い ・日本茶に対して好意的で、茶専門店での購入が多い
イギリス	・輸出単価も高く、抹茶市場も大きい ・残留農薬規制が厳しい
ドイツ	・輸出単価も高く、抹茶市場も大きい ・自宅飲用比率が高く、残留農薬規制も厳しい
フランス	・日本茶は伸び悩みがあるが、抹茶比率は比較的小さい ・日本産の要好者も多く、抹茶の文化性に着目
イタリア	・輸出単価も高く、抹茶の輸出要が高い ・現状での茶消費量は少なく、今後の伸びが期待される
ロシア	・茶消費量は多く、抹茶への興味も増大化 ・抹茶は比較的安価なものが好まれ、Net 環境は整備されている
シンガポール	・需要量は多い、比較的安価、自宅での飲用が多く、知名度も高い。 ・輸出障壁は比較的低く、Net 環境は整っている
香港	・市場は大きく、抹茶単価も比較的高い ・輸出障壁も比較的低く、日本への興味も高い
台湾	・市場は非常に大きいが、抹茶単価は低い ・日本茶、抹茶の知名度高く、和食とのセットや文化性で評価されている
タイ	・抹茶需要が増加し、市場は堅調に伸び、開心も高い ・抹茶単価は低く、飲料への活用が多い、Net 環境は整備されている
ベトナム	・茶の生産国でもあるが、日本茶への関心が高い ・抹茶需要も徐々に浸透しているが、その単価は低い
マレーシア	・日本茶需要は順調な高まりを見せているが、抹茶単価は低い ・お茶はスーパーでの購入も多く、健康イメージが高い
中国	・輸出障壁が非常に高いが、日本産抹茶市場はある ・中国産抹茶との差別性が重要
オーストラリア	・日本茶需要は多いが、抹茶単価はやや低い ・在住日本人も多く、日本食レストランも多く今後の伸びが期待される

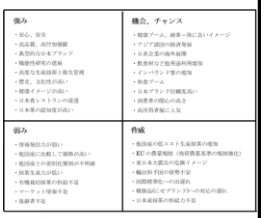


図２４　抹茶輸出に係る SWOT 解析

表８　抹茶市場の分類とその概略

分類	概略		抹茶国
定着市場	日本茶の浸透度が高く、輸出制約も比較的小さく実績の高い国	輸出単価の高い国	米国、香港、カナダ、オーストラリア、マカオ
		輸出単価の安い国	台湾、タイ
制約市場	日本茶の認知度、要望は比較的高いが、輸出障壁が高い国	残留農薬規制	ドイツ、フランス、イギリス、イタリアなどEU諸国
		ハラール	インドネシア、マレーシア、
有望市場	日本茶や抹茶への関心を急速に高め、今後の伸びが期待される国		シンガポール、ベトナム、ロシア、インドネシア、ロシアなど

６．輸出戦略
6.2 抹茶市場の分類と輸出戦略
① 定着市場
　1)ブランディングの強化
　2)高品質抹茶の低コスト生産
② 制約市場
　1)欧米向け有機栽培抹茶
　2)ムスリムに対するハラール認証
③ 有望市場
　1)ECの活用
④ インバウンド市場
　1)海外からの来日者への対応
　2)体験型「コト」消費への対応

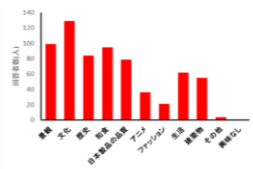


図２８　インバウンドの日本への興味点

図２６　各国・地域の有機認証マーク

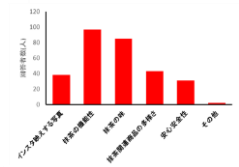
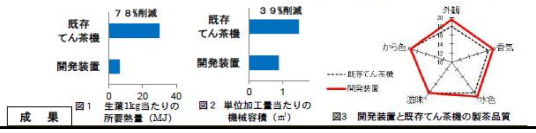


図２９　インバウンドに開いた抹茶の PR 方法

６．輸出戦略
6.2 抹茶市場の分類と輸出戦略
① 定着市場
　2)高品質抹茶の低コスト生産

新構造の高品質てん茶機の開発
☆製茶に要する熱量の７８％、製造コスト３０％削減
開発装置の構造と効果



成　果
図１　生葉1kg当たりの所要熱量（MJ）
図２　単位加工量当たりの機械容積（㎥）
図３　開発装置と既存てん茶機の製茶品質

カフェインレス茶の需要予測について

目次

2．カフェインレス(デカフェ含)飲料の推移

表1　世界の国々におけるデカフェコーヒーの消費率(%)

国　名	2000 %	2009 %
オーストリア	12	9
ベルギー	12	10
デンマーク	Low	3
フィンランド	1	1
フランス	9	7
ドイツ	11	8
ギリシャ	Low	1
イタリア	3	7
オランダ	12	14
ノルウエイ	Low	1
ポルトガル	Low	4
ロシア	Low	1
スペイン	10	17
スウェーデン	Low	1
スイス	5	4
イギリス	13	13
カナダ	—	8
アメリカ	—	16
ブラジル	—	Low
日本	—	Low

Source;Various trade publications and estimates

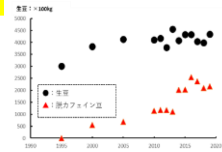


図1　国内におけるコーヒー生豆と脱カフェイン豆」の輸入の推移


写真1　国内で市販されているカフェインを低減化した茶商品

3．カフェインに対する感受性について

3.1　カフェインに対する感受性

3.2　カフェインに対する各国の基準

表2　世界各地のADORA2A(アデノシンレセプター)表現型の割合

	n	CC(%)	CT(%)	TT(%)
ミャンマー	93	20 (21.5)	44 (47.3)	29 (31.2)
日本(茨城)	77	19 (24.7)	38 (49.4)	20 (26.0)
モンゴル	79	19 (24.1)	39 (49.4)	21 (26.6)
中国(上海)	95	23 (24.2)	58 (61.1)	14 (14.7)
アメリカ-白人	92	32 (34.8)	41 (44.6)	19 (20.7)
コートジボアール	47	20 (42.6)	22 (46.8)	5 (10.6)
カメルーン	64	33 (51.6)	24 (37.5)	7 (10.9)
コロンビア	72	34 (47.2)	33 (45.8)	5 (6.9)

表3　飲料別カフェイン摂取量(1995)[7]

	Coffee mg/日	Tea mg/日	Cocoa mg/日	All sources mg/日
ドイツ	292	9	12	313
イタリア	198	3	8	210
カナダ	180	18	12	210
フランス	215	8	16	239
アメリカ	143	12	12	168
オーストラリア	202	29	0	232
イギリス	92	96	14	202
日本	119	44	5	169
マレーシア	49	27	4	81
中国	2	14	0	16
ロシア	26	40	7	72

※　エナジードリンク、ソフトドリンクを除く

表4　各国におけるカフェインの最大摂取量

悪影響のない最大摂取量			飲料換算	機関名
妊婦		300mg/日		世界保健機関 (WHO)
		200mg/日		欧州食品安全機関 (EFSA)
		300mg/日	コーヒー マグカップ2杯(237ml/杯)	カナダ保健省
		300mg/日		オーストリア保健・食品安全局(AGES)
		200mg/日		英国食品基準庁(FSA)
授乳中の女性		200mg/日 注1		欧州食品安全機関 (EFSA)
健康な子供及び青少年	子供、青少年	3mg/kg体重/日		欧州食品安全機関 (EFSA)
	子供(4〜6歳)	45mg/日		カナダ保健省
	子供(7〜9歳)	62.5mg/日		
	子供(10〜12歳)	85mg/日		
	13歳以上の青少年	2.5mg/kg体重/日		
	子供(5〜12歳)	95mg/日(3mg/kg体重/日)	コーラ2缶	欧州・ニュージーランド
健康な成人		400mg/日 (3mg/kg体重/1回 注2)		欧州食品安全機関 (EFSA)
		400mg/日	コーヒー マグカップ3杯(237ml/杯)	カナダ保健省
		400mg/日	・3〜5カップ/日 ・カフェインとエナジードリンクを一緒に摂取すべきでない	米国保健福祉省(DHHS)及び農務省(USDA)
		210mg/日	インスタントコーヒー約3カップ	欧州・ニュージーランド

注1)　乳児に健康リスクは生じない
注2)　1回当たり摂取量約3mg/kg体重以下(例:体重70kgの成人で、約200mg以下)であれば急性毒性の懸念は生じない

4．カフェインに対するアンケート結果

表５　アンケートの質問概要

・性別(男性549名、女性559名)	
・年齢層(〜20、21〜39、40〜49、50〜)各280名程度	
・地域	・カフェインに対する知識
・世帯内容	・カフェイン含有飲料の選択基準
・年収	・カフェインレス茶の飲用体験
・お茶の飲用頻度	・カフェインレス茶の飲用時間帯
・茶種ごとの飲用頻度	・カフェインレス茶の飲用場所
・飲料選択時の重視点	・カフェインレス製造特性と購入期待度
・緑茶の購入場所	・カフェインレス緑茶の需要予測
・緑茶の飲用場所	・カフェインレス緑茶の購入希望価格
・緑茶への期待点	・カフェインレス緑茶への期待点

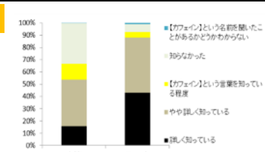


図２　カフェインに対する知識

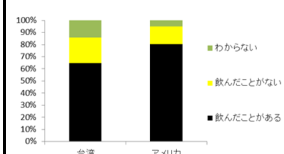


図３ カフェインレス飲料の飲用経験

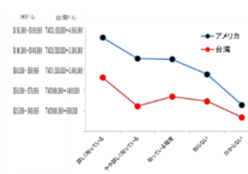


図４　カフェインの知識と収益との関係

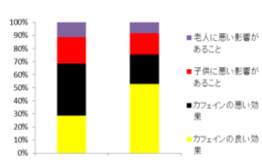


図５ カフェイン飲料選択時に気にする点

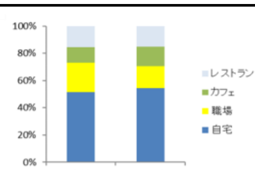


図６　カフェインレス茶の飲用場所

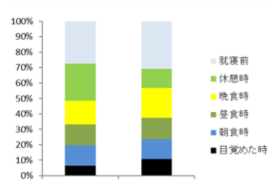


図７　カフェインレス茶の飲用時間帯

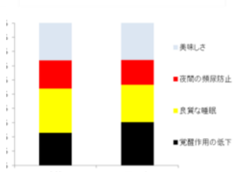


図８ 新カフェインレス緑茶への期待点

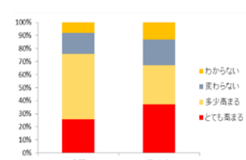


図10 夜間の頻尿防止に対するカフェインレス茶の今後の需要との関係

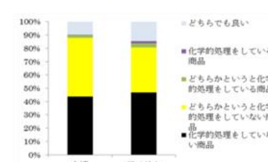


図11 ナチュラルカフェインレス飲料の選択志向

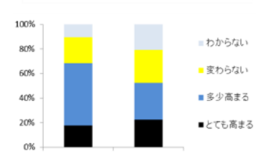


図12 カフェインレス飲料の需要予測

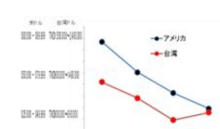


図13 カフェインレス茶の需要予測と収入との関係

令和2年　茶学入門

令和2年 後期

世界の茶の生産と加工

茶学総合研究センター　中村順行

チャの分類

Genus Camellia
- Subgen. Protocamellia
- Subgen. Camellia
  - Sect. Oleifera
    - C.oleifera
    - C.sasanqua
  - Sect. Camellia
    - C.japonica
- ***Subgen. Thea***
  - ***Sect. Thea***
    - ***C.sinensis***
      - ***var.sinensis***
      - ***var.assamica***
    - C.taliensis
    - C.irawadiensis
  - Sect. Chrysantha
    - C.chrysantha
- Subgen. Metacamellia

おチャとは

1935年　第6回世界植物学会議
チャ属とツバキ属をツバキ(Camellia)属とする
1958年 Sealy「ツバキ属の改訂」:チャをツバキ属チャ節とする。
引用文献　Sealy,J.R.:
A revision of the genus Camellia, Royal Horticultural Society, London, p.239 (1954)

お茶の木はツバキの親戚？

ツバキ(山茶)科に属する永年性常緑樹
- チャ節(Section *Thea*)
  - チャ(C. *sinensis* (L.) O. kuntze)
    - 中国種(C. *sinensis* var. *sinensis*)
    - アッサム種(C. *sinensis* var. *assamica*)

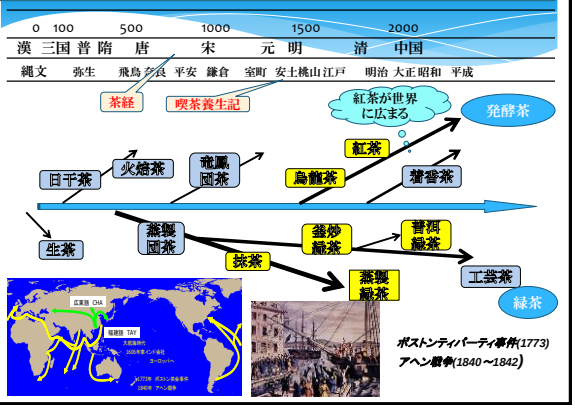
表　中国種とアッサム種の性状

性状＼種類	中国種（日本種も含む）	アッサム種
木の形	灌木、樹高が低く、地際より多くの枝幹が伸びる	喬木、主幹は１本
葉の大きさ	小さい	大きい
葉先	とがっていない	細長くとがっている
葉面	濃緑色でなめらか	淡緑色で葉脈と葉脈の間の部分が盛り上がる
耐寒性	強い	弱い
用途	緑茶向き	紅茶向き

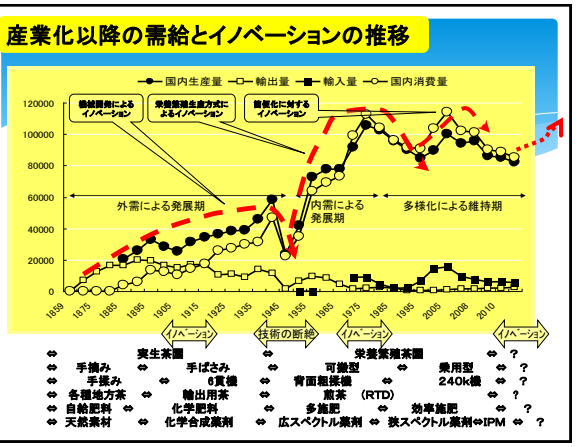
チャの特質

チャが他の植物と異なる点
- ☆カフェイン
- ☆ガレート型のカテキン
- ☆テアニン
- ☆その他(フッ素、アルミ等)

茶には2000年の歴史。時代とともに多様に進化してきた



産業化以降の需給とイノベーションの推移



緑茶生産方法の推移


手摘み


可搬型摘採機


乗用型摘採機


手揉み


機械揉み


FA対応機械揉み

摘採は手摘みから機械摘みに変わり、著しく摘採能率を向上してきた。製造は手揉みから機械化され、徐々に投入量を増加させるとともに最近ではコンピュータ制御による自動化に技術革新したことで、日本独自の生産加工技術を確立し、品質の高位平準化に貢献してきた

緑茶消費方法の推移

現在の生活の中では
お茶を飲む風景も激変しています
⇒ 生産されるお茶も変わります






番茶 ⇒ せん茶 ⇒ 茶素材

日本における茶の生産

平成25年度

県 名	面積 (ha)	生産量 (1000t)
静岡県	18,300	32,200
鹿児島県	8,660	25,600
三重県	3,150	7,130
熊本県	1,640	1,300
京都府	1,580	3,020
福岡県	1,570	2,290
宮崎県	1,540	4,100
埼玉県	925	537
佐賀県	953	1,510
その他	7,082	5,113
合 計	45,400	82,800

茶栽培の年間スケジュール

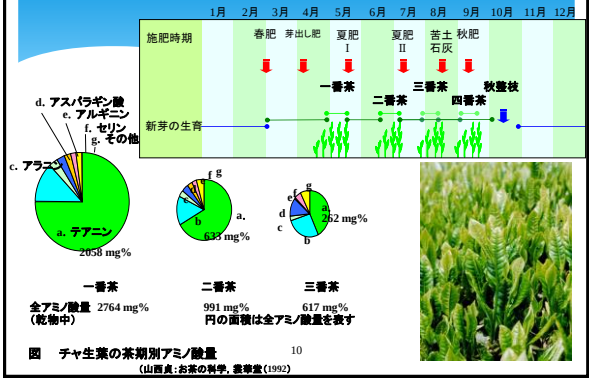
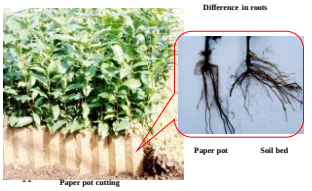


図 チャ生葉の茶期別アミノ酸量
（山西貞：お茶の科学，養賢堂(1992)）

チャの育苗


挿し木


挿木１年目

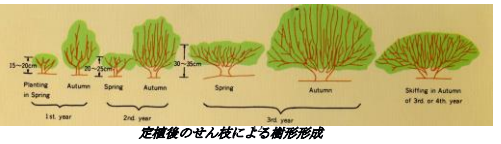


チャの定植と仕立


苗の植え付け


植え付け後のせん枝


定植1年後の茶園



定植後のせん枝による樹形形成

代表的な摘採方法

Hand plucking

摘採方法と一人当たり摘採量

摘採方法	一人、一日当り摘採量
手摘み	10 ～ 15 kg
手はさみ	100 ～ 200
機械摘採	
二人用可搬型摘採機	700 ～ 1,000
乗用型摘採機	4,000 ～ 5,000
レール走行式摘採機	2,000 ～ 3,000

Hand-shear plucking

Portable machine for two persons

Riding-type plucking machine

防霜対策

First crop injured by frost

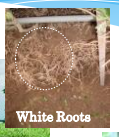
Anti-frost fan

Sprinkler

14

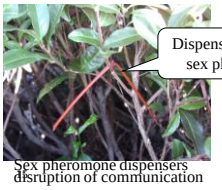
持続型茶業を目指した施肥と防除

Deep plow subsailer



White Roots

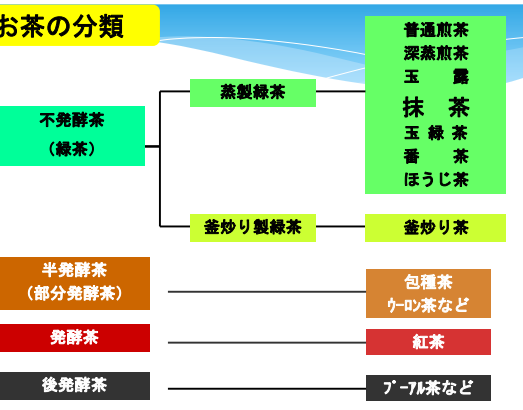
Natural enemies



Dispenser releasing sex pheromone

Sex pheromone dispensers disruption of communication

お茶の分類



煎茶(荒茶)製造工程

Cooperative tea factory

Primary drying tea roller

Secondary drying tea roller

Tea steaming machine

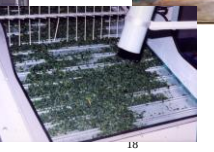
Tea roller

Finally drying tea roller

てん茶(抹茶原料)製造工程

冷却散茶機

レンガ造り、てん茶機(乾燥機)



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釜炒り茶製造工程

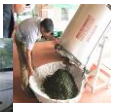
手作り用釜

炒葉機

締炒機

半発酵茶(ウーロン茶)製造工程

生葉 → 日光萎凋 → 室内萎凋 → 殺青 → 揉捻 → 乾燥



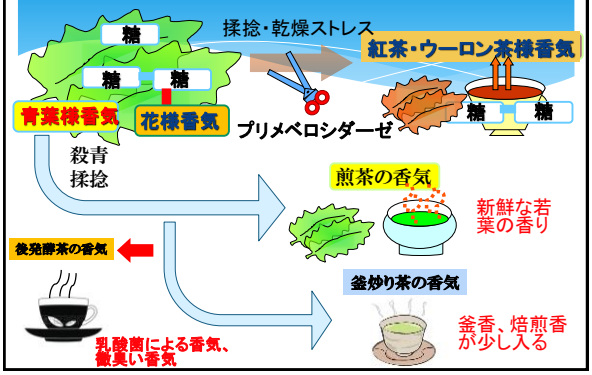
発酵茶(紅茶)製造工程

生葉 → 萎凋 → 揉捻 → 発酵(酸化) → 乾燥

同じ茶葉から様々なお茶が作れ、成分も変わる

生葉	緑茶	紅茶
カテキン類	カテキン類 ⇒	テアフラビン、テアルビジン
クロロフィル	クロロフィル ⇒	フェオフイチン
ビタミンC	ビタミンC ⇒	消失(酸化物、分解物)
香り	青葉様香気 ⇒	花様香気

茶種の違いによる香気の発揚

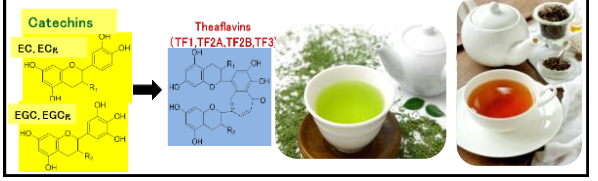


発酵によりカテキンがテアフラビンに重合する

The synthesis of Theaflavins from Catechins

Leading body				%1)
(-)-EC	＋(-)-EGC	⇒	TF1 Theaflavin	8.0
(-)-ECG(	＋(-)-EGC	⇒	TF2 A Theaflavin 3-o-gallate	30.0
(-)-EC	＋(-)-EGCG	⇒	TF2 B Theaflavin 3'-o-gallate	20.0
(-)-ECG	＋(-)-EGCG	⇒	TF3 Theaflavin 3,3'-di-o-gallate	40.0

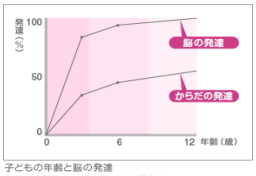
1) The ratio in Total Theaflavins of Black tea



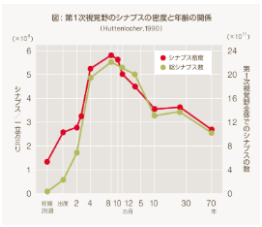
幼児期に感性を磨く‼ ‼

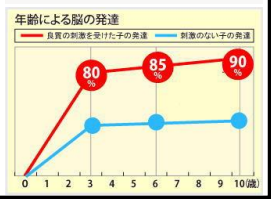
「三つ子の魂100まで」
3歳までのしつけや教育は大切

胎児から 3歳の間に、脳は急速に発達する。脳のシナプスは生後2カ月～4カ月で急激に増え、8カ月で最大となり、3歳頃には大人とほぼ同数に。４歳の時点で脳の発達の８割が完成する。



子どもの年齢と脳の発達
参考文献「脳と保育」（時実利彦）岩崎社





食育の重要性

食事を通して
☆体の健康
☆こころの健康
☆感謝の気持ちを持つこと

食育の基本
食材本来の味を教えること、
食材の旬がわかる季節のものを利用すること
家族と食卓を囲む楽しさを伝えること


ジャンクフードの落とし穴


彩、香、味、季節、自然を愉しむ

味覚形成にとっても幼児期は重要

何故、味覚形成が重要なの！？
1. 健康のため⇒バランスの取れた食生活
2. 豊かな生活を楽しむため
3. より美味しいものをたべるため⇒味覚音痴は淋しい
4. 日本の自然(家庭の味、田舎の味)を感じるため

乳幼児に濃い甘味・旨味・塩味に慣れさせることは、正常な味覚と健全な脳の発達を損ねる可能性があります！

『いろいろな食材を食べる経験』を通して、脳が味覚の情報を蓄積し、好き嫌いや食べ物に対するイメージが完成していく。

子供にとって苦手な渋味、苦味、辛味 ⇒ 体の健康

渋味は、生理学的定義に基づく味覚のいわゆる五原味（甘・酸・塩・苦・旨味）には含まれず、辛味と同様、渋味は痛みや触覚に近い感覚で舌や口腔粘膜のたんぱく質の変性によっ生じると考えられている。

良薬 口に苦し

大人の味？？

体の健康にとって
苦み成分であるポリフェノール
は重要

第5回
サタデーセミナー(11月21日)

お茶の抗酸化力

静岡県立大学
茶学総合研究センター

お茶って、な~に

チャ節(Section *Thea*)
チャ(C. *sinensis* (L.) O. kuntze)
中国種(C. *sinensis* var. *sinensis*)
アッサム種(C. *sinensis* var. *assamica*)
ツバキ属(genus *Camellia*)
ツバキ節(Section *Camellia*)
サザンカ節(Section *Paracamellia*)等

チャが他の植物と異なる点
☆カフェイン
☆ガレート型のカテキン
☆テアニン
☆その他(フッ素、アルミ等)


中国種


アッサム種



チャはツバキの仲間、でも飲用されるのは茶樹だけ

お茶の機能性成分



20~30%
70~80%

不溶性成分
☆食物繊維(20~30%):便秘予防、大腸がん予防、心疾患予防
☆たんぱく質(24%):栄養
☆βカロテン(20mg%):抗酸化、抗がん、抗糖尿、抗心疾患、免疫活性
☆ビタミンE(25~70mg%):抗酸化、抗がん、免疫活性
☆クロロフィル(0.80%):がん予防、抗突然変異、抗腫瘍、免疫活性

水溶性成分
☆カテキン類(10~18%):抗酸化、抗菌、抗がん、生活習慣病予防、消臭、抗アレルギーなど
☆カフェイン(3~4%):眠気防止、強心、二日酔い防止
☆フラボノール(0.6~0.7%):抗酸化、抗がん、免疫活性
☆ビタミンC(200mg%):抗酸化、免疫活性
☆ビタミンB(1.4mg%):抗酸化
口内炎予防
☆サポニン(0.1%):抗喘息、抗菌、血圧効果
☆テアニン(0.6~2%):リラックス、血圧効果
などなど

酸化ストレスと抗酸化作用

酸化ストレス
外部刺激により発生した活性酸素が体内で細胞を傷つけ老化などの原因になる事

抗酸化作用
酸化ストレスから自己を守るための防御システム

薬剤や、酸化した食べ物、化学物質の摂取
↓
体内で「活性酸素」が発生

バランスが大事

●活性酸素の発生を抑制
●活性酸素によって生じたダメージの修復、再生などの防御システム

抗酸化作用を持つ物質のことを「抗酸化物質」といいます。

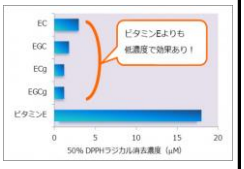
4

茶の抗酸化能に与える要因

茶の主要な抗酸化成分
カテキン、フラボノール、ビタミンC、ビタミンE、βカロテン 等々

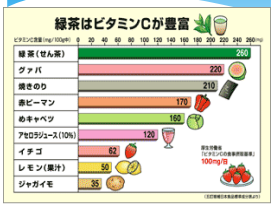
抗酸化成分の含有量を左右する要因
品種、茶期、摘採時期、部位、被覆の有無、加工法、貯蔵法 等々

カテキンには強い抗酸化活性がある



http://www.mitsui-norin.co.jp/ochalabo/power/power20150206.html

ビタミンCの含有量



ビタミンEの含有量

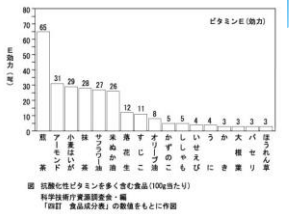
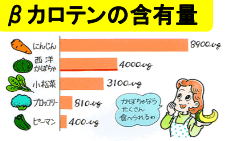


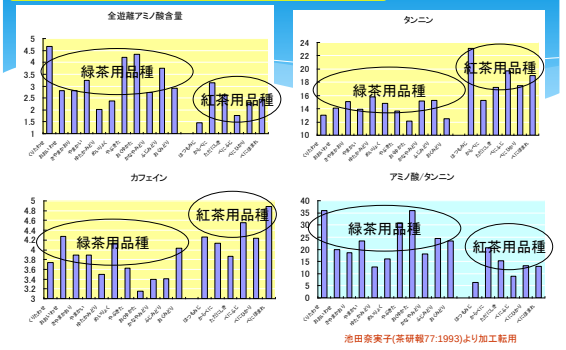
図 抗酸化性ビタミンを多く含む食品(100g当たり)
科学技術庁資源調査会・編
「四訂 食品成分表」の数値をもとに作図

βカロテンの含有量



6

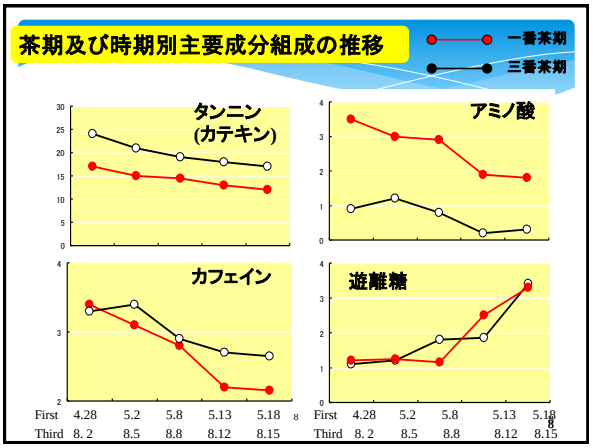
日本の品種間における成分量の違い



池田奈実子(茶研報77:1993)より加工転用

概して、紅茶用品種は緑茶用品種に比較し、アミノ酸含量が低く、タンニン含量が高い

茶期及び時期別主要成分組成の推移



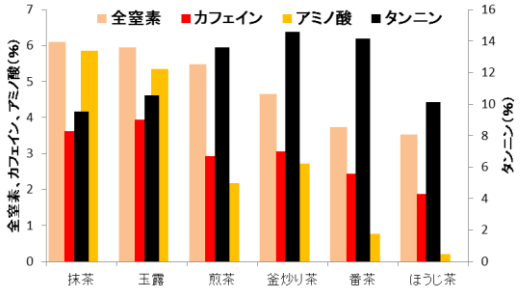
茶芽の葉位別成分含量

茶葉の葉位別主要成分含量(%)

葉位	Tannin	Caffeine	Amino acids	Free Sugars
一心1葉	14.45	3.50	3.11	0.77
第2葉	13.02	3.00	2.92	0.81
第3葉	12.79	2.65	2.34	1.02
第4葉	12.69	2.37	1.95	1.59
茎	6.23	1.31	5.73	2.61

茶種別主要成分含量

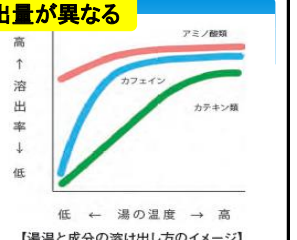
・茶種によっても内容化学成分が異なる
・旨味のもとであるアミノ酸は抹茶や玉露で多い
・タンニン(カテキン)は釜炒り茶や番茶で多い



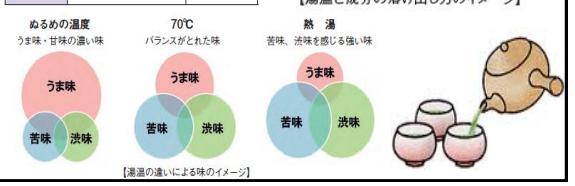
茶の成分は温度の違いで浸出量が異なる

主な味成分とその味

分類	成分	味
アミノ酸類	テアニン	甘味、うま味
	グルタミン酸	うま味、酸味
カテキン類	エピカテキン	苦味
	エピガロカテキン	苦味
	エピカテキンガレート	渋味、苦味
	エピガロカテキンガレート	渋味、苦味
カフェイン		軽い苦味



【湯温と成分の溶け出し方のイメージ】



【湯温の違いによる味のイメージ】

抗酸化活性の様々な測定法

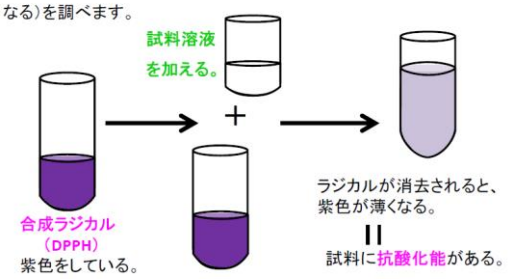
Table 3 抗酸化活性測定法の反応機構および特徴

分析方法		簡便性	分析機器の汎用性の有無	生体への利用可能性	測定メカニズム
略称	正式名称				
ORAC	Oxygen Radical Absorbance Capacity	+ +	+	+ + +	HAT-based method
TRAP	Total Radical-trapping Antioxidant Parameter	− − −	− −	+ + +	HAT-based method
FRAP	Ferric Reducing Ability of Plasme	+ + +	+ + +	− −	SET-based method
CUPRAC	Copper Reduction Assay	+ + +	+ + +		SET-based method
TEAC	Trolox Equivalent Antioxidant Capacity	+	+	−	SET-based method
DPPH	1,1,-diphenyl-2-picrylhydrazl radical scavenging assay	+	+	−	SET-based method
TOSC	Total Oxidant Scavenging Capacity	−	−	+ +	HAT-based method
LDL oxidation	Low-Density Lipoprotein Oxidation	−	+ + +	+ + +	HAT-based method
PHOTOCHEM	Photochemiluminescence	+	− −	+ +	?

AOU 研究会ウエブサイト (http://www.antioxidant-unit.com/) より抜粋

DPPH法を用いた抗酸化活性の測定法

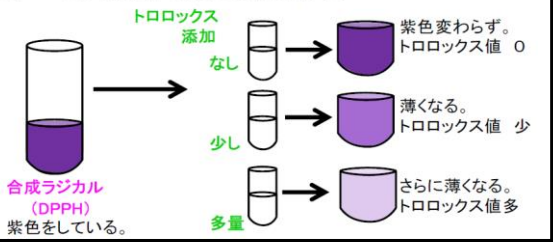
従来から用いられている方法の1つに、DPPH法があります。DPPHは、合成ラジカルの略称です。一定量のDPPHと、抗酸化能を測定したい試料をまぜ、試料がどれだけDPPHを消去するか（ラジカルでなくなる）を調べます。



DPPH法を用いた抗酸化活性の測定法

DPPH溶液は紫色をしており、DPPHが少なくなると紫色も薄くなります。この紫色の濃さを、吸光度で測定します

一方で、指標として、合成ビタミンE剤であるトロロックスという物質によるDPPHの消去能を調べます。 食品など試料の抗酸化能は、このトロロックス添加量に換算して数値化します。



食品素材としての茶の利用

サプリを喰べて
Happy感じますか？

県大　茶学総合研究センター　中村順行

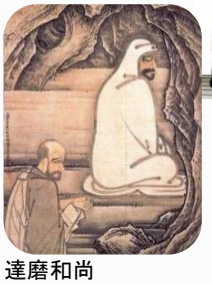
お茶の始まり

HPより引用

お茶を飲んでよかった

神　農

西暦500年前後に陶弘景(452-536)がまとめた『神農本草経』に**「神農嘗百草、日遇七十二毒、得茶而解之**



達磨和尚

修行のとき、眠気を覚ますため、まゆげをそぎ落としたのが湯に入り、お茶になったと言われる。

お茶の別名は
めざまし草

日本にも仏教とともに伝来し、文化的にも大きく育て上げてきた

お茶の波及

HPより引用

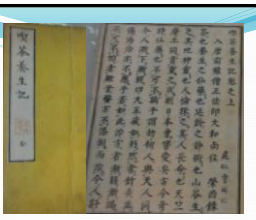
陸　羽

陸羽の時代の「茶」は、粉末状にしたものを、主に葱や生姜等と一緒に煮て飲む、「スープのような茶」に使われていた。陸羽はそれを、「湯の捨て水」として非難し、「茶経」を記し、茶だけで愉しむように提案した。

茶は中国西南国境の三日月地帯が原産地

喫茶養生記

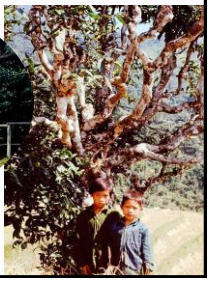
茶者養生之仙藥也　延齢之妙術也



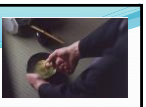
①茶は身体衰弱、意志消沈のときは、気力を強くする。
②茶は人を愉快な気持ちにさせ、酒の酔いを醒まし、睡気を起こさない。
③茶は小便の通じが良く、喉の渇きをとりさり、消化不良をなくす。
④茶は身を軽くし、脚気によい。
⑤茶は精神を整え、内臓を和らげ、身体の疲労をやすらかに除く。

チャの特質

Camellia sinensis　から作られる多様なお茶は、カフェイン、カテキン、テアニンなどの特異成分を含むが故に世界中の人々を虜にした



現在のお茶に期待されるものは？



栄養供給

栄養バランス

健康・こころ

Happy

食品　飢餓からの脱出⇒体の維持生長　⇒

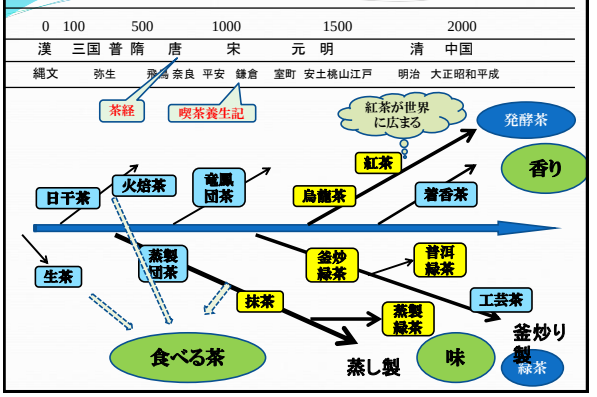
健康性

生活を豊かにするお茶

喉の渇きを満たすだけなら水でも良い
心の渇きを癒すためにはお茶が良い

体の健康補助にはサプリでも事足りるが
それでHappyになれますか？

茶には2000年の歴史。時代とともに多様に進化

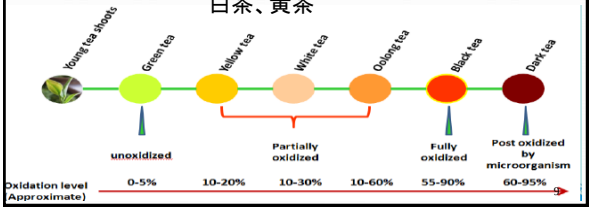


同じ茶葉から様々なお茶が作れ、成分も変わる

生葉	緑茶	紅茶
カテキン類	カテキン類 ⇒	テアフラビン、テアルビジン
クロロフィル	クロロフィル ⇒	フェオフィチン
ビタミンC	ビタミンC ⇒	消失(酸化物、分解物)
香り	青葉様香気 ⇒	花様香気

世界のお茶

緑茶（不発酵茶）
　蒸し製緑茶(日本式)
　釜炒り製緑茶(中国式)
青茶「ウーロン茶」（半発酵茶）
紅茶（発酵茶）
　※発酵：葉の酵素による酸化反応
黒茶「後発酵茶」（堆積茶）
　※発酵：微生物発酵
白茶、黄茶



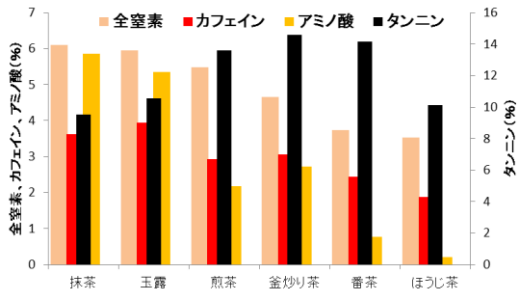
茶種によって成分量が異なる

安徽農業大学
Xiaochun Wan

	Green tea (n=344)	Black tea (n=387)	White tea (n=109)	Oolong tea (n=134)	Yellow tea (n=31)	Dark tea (n=89)
EGC	2.43±1.15	0.40±0.42	0.53±0.39	2.13±0.79	1.36±0.87	0.45±0.40
+C	0.09±0.06	0.21±0.28	0.15±0.19	0.07±0.03	0.08±0.04	0.03±0.03
EGCG	6.96±1.69	0.35±0.54	3.98±1.90	4.47±1.71	4.54±1.67	0.36±0.51
EC	0.84±0.23	0.24±0.27	0.28±0.16	0.60±0.19	0.51±0.20	0.17±0.15
ECG	1.99±0.67	0.55±0.45	1.46±0.64	1.08±0.36	1.81±0.91	0.15±0.18
Caffeine	3.26±0.69	3.05±0.82	3.95±0.54	2.28±0.57	3.06±0.62	2.70±0.93
Theanine	1.05±0.44	0.83±0.37	1.20±0.59	0.21±0.16	1.11±0.68	0.04±0.05
Total catechins	12.30±2.58	1.75±1.44	6.4±2.83	8.35±2.49	8.30±3.46	1.16±1.05

茶種別主要成分含量

・茶種によっても内容化学成分が異なる
・旨味のもとであるアミノ酸は抹茶や玉露で多い
・タンニン（カテキン）は釜炒り茶や番茶で多い



白葉茶

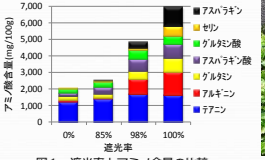


図1　遮光率とアミノ含量の比較

強遮光処理をすることで白い芽の品種と同様な白葉茶の生産が可能です。

低カフェイン茶

H.Pより引用

若い女性や高齢者は睡眠阻害、妊娠時には乳児への影響を避けるため、茶の飲用を遠慮する人が多い

最近では、様々な低カフェイン茶が販売されるようになってきました

ギャバロン茶

ギャバロン茶は嫌気処理で作成され、血圧上昇抑制に効果が高い

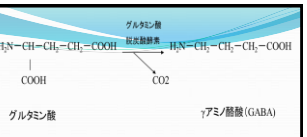


図 嫌気条件下におけるGABAの生成

生葉収穫 → 嫌気処理 → 蒸熱処理 → 深蒸煎茶製造に準じた工程 → 火入れ → 製品

微生物制御発酵茶

HPより引用

煎茶成分の組成と溶出比率

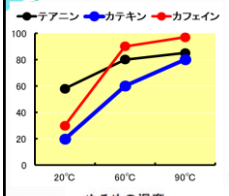
水溶性成分

カテキン，
カフェイン，
ポリサッカライド、
アミノ酸、
サポニン、
GABA、
ビタミンC、
ビタミンB、
など

不溶性成分

粗繊維、タンパク質、カロテン、ビタミンE、クロロフィル、その他

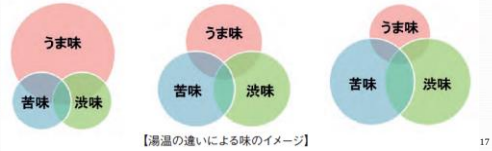
湯温と溶出



ぬるめの温度
うま味・甘味の濃い味

70℃
バランスがとれた味

熱 湯
苦味、渋味を感じる強い味



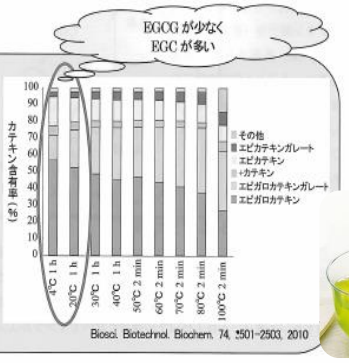
【湯温の違いによる味のイメージ】

湯温によりカテキンの種類も異なる

免疫力の向上にも効果的!!!!

緑茶浸出液中に含まれる各種カテキンの割合

エピガロカテキンとエピガロカテキンガレートは、お茶を淹れる時の水の温度の違いによって、含まれる割合が変わってきます。



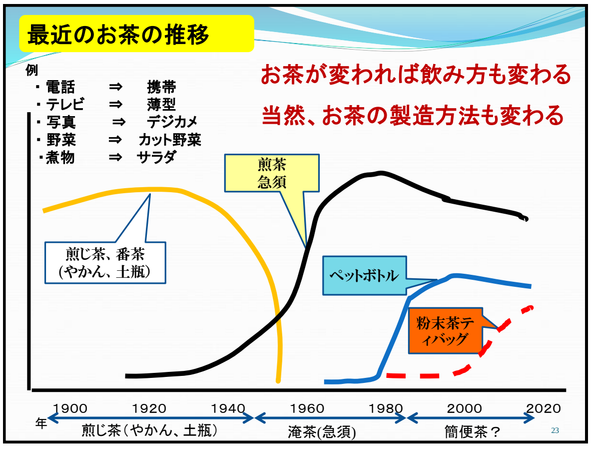
Biosci. Biotechnol. Biochem. 74, 2501-2503, 2010


飲み方も変化している
もう都会では茶殻は出せない
⇒　ベンディングマシン、粉末茶、ペットボトルで対応
飲み方が変われば、茶種も変わる
せん茶だけでは対応できない
需給の歪み
番茶　⇒　せん茶　⇒　茶素材
19


ますますの簡便化志向
美味しくて簡便に飲める茶
ポスト急須も必要では
急須いらずの新規本格的緑茶
煎茶ラテ
粉末茶
ティバッグ
高級ボトリング茶
20


高級茶の飲用の場を広げよう
～ボトルティは面白い～
☆最高の旨味抽出が可能
☆誰でもが同じ味で出せる
☆演出が可能
☆付加価値向上


ティーバッグも増加（遊び心、高級化路線が重要）
ANIMAL TEA PARTY
Green tea
Tea Bag
Tbag


最近のお茶の推移
例
・電話　⇒　携帯
・テレビ　⇒　薄型
・写真　⇒　デジカメ
・野菜　⇒　カット野菜
・煮物　⇒　サラダ
お茶が変われば飲み方も変わる
当然、お茶の製造方法も変わる
煎茶
急須
煎じ茶、番茶
（やかん、土瓶）
ペットボトル
粉末茶テ
ィバッグ
1900
1920
1940
1960
1980
2000
2020
年
煎じ茶（やかん、土瓶）
淹茶(急須)
簡便茶？
23


茶のすべてを利用
お茶の新しい文化
「飲む」から
「食べる」へ
NPO法人 日本食茶の会

世界の「食べる」茶

H.Pより

ミエン(ラオス、タイ)、ラペソー(ミャンマー)など



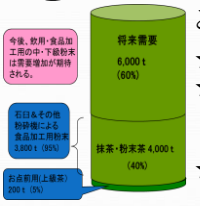
擂茶(中国)



その他
打油茶(中国)
油茶
など

抹茶は世界のスーパーフード

抹茶は、
急須で淹れたお茶では摂取できない、プロビタミンA(βカロテン)、ビタミンE(トコフェノール)、食物繊維なども摂取可能。



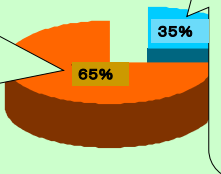
☆抹茶は美味しい
☆急須で淹れた場合、カテキンの40%程度は茶殻に残るが、抹茶では全てを摂取可能。
☆緑色が美しいのみならず、食品添加素材としても有用。

食品業界もお茶の注目

各種食材への利用も急増

茶の機能に関する代表的書籍

機能性を主体とした茶成分とその特性



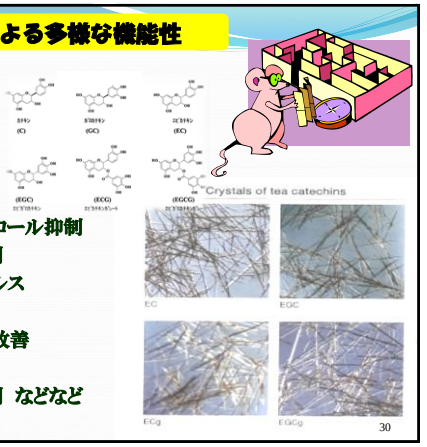
水溶性成分
☆**カテキン類**(10～18%):抗酸化、抗菌、抗がん、生活習慣病予防、消臭、抗アレルギーなど
☆**カフェイン**(3～4%):眠気防止、強心、二日酔い防止
☆**フラボノール**(0.6～0.7%):抗酸化、抗がん、免疫活性
☆**ビタミンC**(200mg%):抗酸化、免疫活性
☆**ビタミンB**(1.4mg%):抗酸化、口内炎予防
☆**サポニン**(0.1%):抗喘息、抗菌、血圧効果
☆**テアニン**(0.6～2%):リラックス、血圧効果
などなど

不溶性成分
☆**食物繊維**(20～30%):便秘予防、大腸がん予防、心疾患予防
☆**たんぱく質**(24%):栄養
☆**βカロテン**(20mg%):抗酸化、抗がん、抗糖尿、抗心疾患、免疫活性
☆**ビタミンE**(25～70mg%):抗酸化、抗がん、免疫活性
☆**クロロフィル**(0.80%):がん予防、抗突然変異、抗腫瘍、免疫活性

茶はカテキンを始め多くの特異的な成分を含有し、それぞれ機能性をもつため、その機能性を活かした商品も数多く開発されている

カテキン類による多様な機能性

- 抗酸化
- 抗突然変異
- 抗がん
- 酸化防止
- 抗動脈硬化
- 血中コレステロール抑制
- 脂肪吸収抑制
- 抗菌、抗ウイルス
- 虫歯予防
- 腸内フローラ改善
- 消臭
- 血圧上昇抑制　などなど



カテキン類による抗がん作用

HPより引用

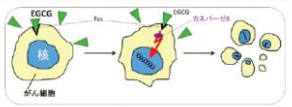
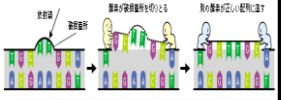


図1　EGCGによるアポトーシス誘導のメカニズム
EGCGが細胞表面のFasタンパク質に結合すると、その信号によりカスパーゼが活性化され、最終的にDNAの分解、細胞の断片化が起こり、細胞が死ぬ。



カテキンなどによるDNA修復作用

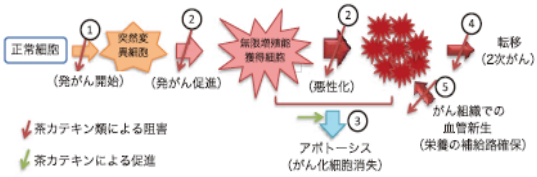


図1　茶カテキン類による発がん過程の抑制効果

がんと緑茶に関する疫学的調査研究のまとめ

表1　がんと緑茶に関する疫学調査研究(伊勢村護)

	前向きコホート研究		症例対照研究	
がんの部位	リスク軽減あり	リスク軽減なし	リスク軽減あり	リスク軽減なし
大腸	3	6	4	3
肺	0	4	2	3
胃	2	6	8	8
食道	0	2	4	5
乳房	3	5	3	0
前立腺	2	1	2	0
卵巣	1	0	2	0
すい臓	0	2	2	1
腎臓、膀胱	0	1	1	4
肝臓	1			
子宮内膜			2	1
甲状腺	1	1		
血液	1			

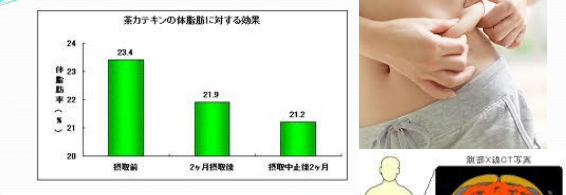
データは、～緑茶と健康のメカニズム～　機能効用ナビゲーション　2010
(静岡県経済産業部農林業局茶業農産課)

前向きコホート研究:
まだ病気になっていない人達を対象に調査し、数年後の追跡で発病を調査する方法

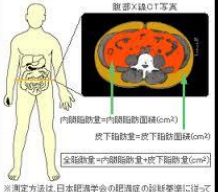
症例対照研究:
特定の病気が発症した人を対象に、健常人との比較調査する方法

カテキン類による抗体脂肪抑制作用

HPより引用



茶カテキンの継続摂取による体脂肪低減効果[6]



※測定方法は、日本肥満学会の肥満症の診断基準に従って測定しました。

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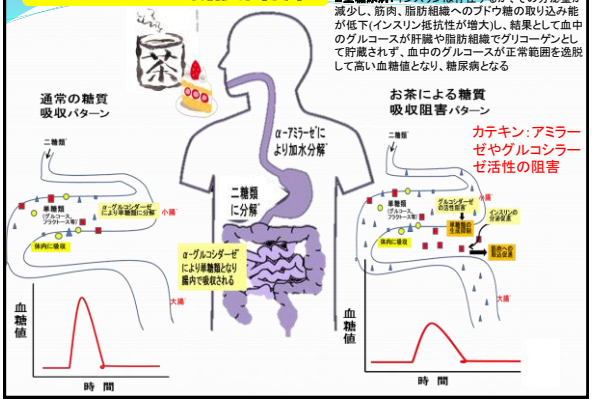
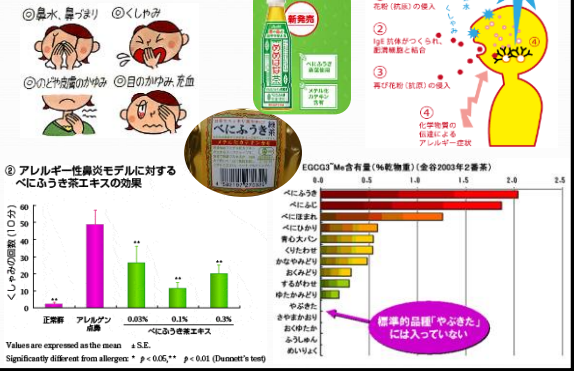
カテキンによる抗糖尿作用

1型糖尿病: 脾臓のβ細胞の破壊によるインスリン欠乏により、高血糖、糖尿病へと至る。
2型糖尿病: インスリンは存在するが、その分泌量が減少し、筋肉、脂肪組織へのブドウ糖の取り込み能が低下(インスリン抵抗性が増大)し、結果として血中のグルコースが肝臓や脂肪組織でグリコーゲンとして貯蔵されず、血中のグルコースが正常範囲を逸脱して高い血糖値となり、糖尿病となる

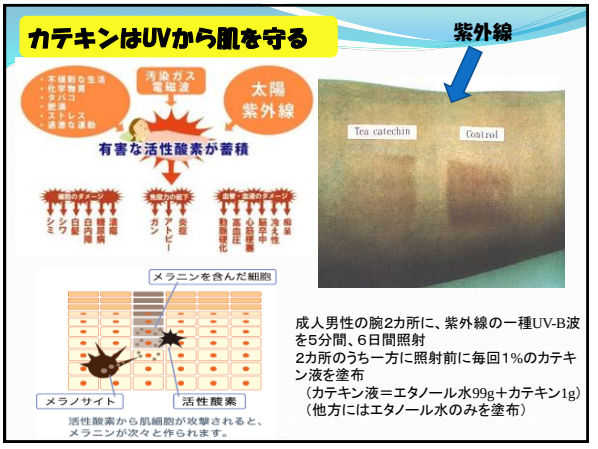


抗アレルギー効果

HPより引用



カテキンはUVから肌を守る



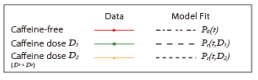
成人男性の腕2カ所に、紫外線の一種UV-B波を5分間、6日間照射
2カ所のうち一方に照射前に毎回1%のカテキン液を塗布
（カテキン液＝エタノール水99g＋カテキン1g）
（他方にはエタノール水のみを塗布）

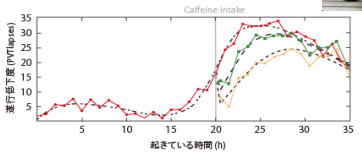
カフェインの機能性

- 覚醒作用
- 大脳刺激作用
- 疲労回復
- 強心作用
- 利尿作用

カフェインの別名は
「目覚まし草」

カフェインの覚醒効果（遂行の改善）
20時間断眠後の睡魔による遂行低下を改善





出典元：「Journal of Theoretical Biology」Volume 358: I（2014 年 Ramakrishman 他）

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カフェインによる運動機能の向上

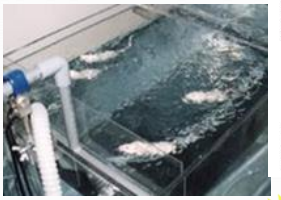
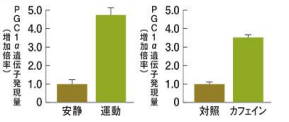


図4 カフェインは筋肉に対して運動に似た作用を及ぼす



運動によるマウス骨格筋およびカフェイン処理による骨格筋培養細胞における PGC1α遺伝子の発現量増加

マウスを強制的に運動させると骨格筋ではミトコンドリア機能の活性化や脂肪酸燃焼に重要な役割を担う遺伝子である PGC1α遺伝子発現量が増加する。PGC1α遺伝子発現量が増加すると代謝が活性化して脂肪が燃焼し、インスリン感受性が強まる。培養骨格筋細胞をカフェインで刺激しても、PGC1α遺伝子発現量が増加する。

テアニンの機能性

- 血圧降下
- 脳神経機能調整
- 血管性痴呆症予防作用
- 抗ストレス作用
- 記憶学習行動促進作用

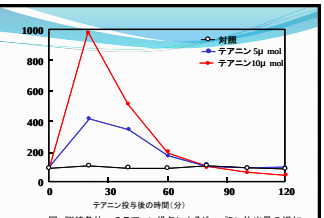
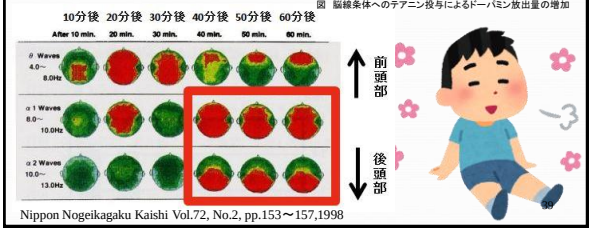


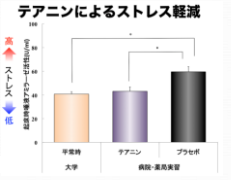
図 脳線条体へのテアニン投与によるドーパミン放出量の増加



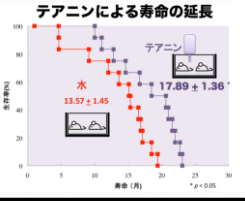
Nippon Nogeikagaku Kaishi Vol.72, No.2, pp.153～157,1998

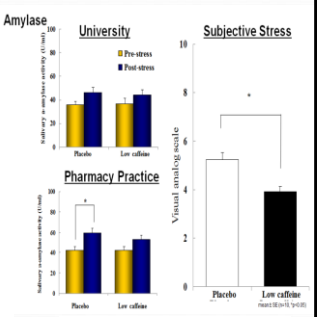
テアニンにはストレス解消、寿命の延伸効果も

テアニンによるストレス軽減



テアニンによる寿命の延長





ヒト試験による低カフェイン茶の抗ストレス効果

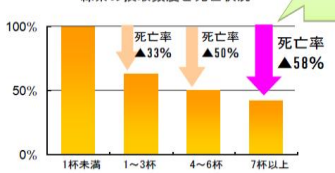
お茶は長寿の秘訣

多く緑茶を飲む高齢者ほど、長生き

Q（この1ヶ月で）緑茶を1日に何杯くらい飲みましたか？

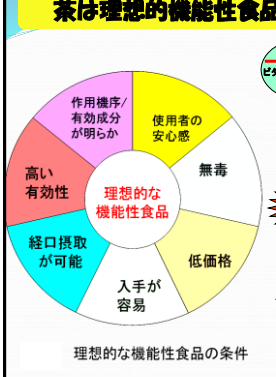
緑茶を「1日に7杯以上」飲む人は 長生き 死亡が半分に!!

緑茶の摂取頻度と死亡状況

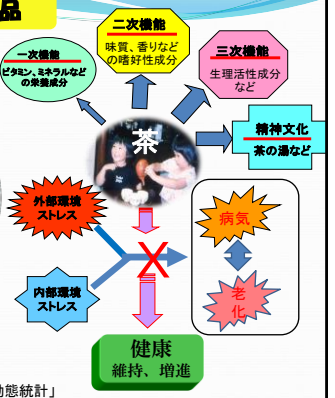


出典：静岡県高齢者コホート調査
Suzuki(2009) Annual Epidemiology

茶は理想的機能性食品



理想的な機能性食品の条件



資料：厚生労働省「平成22年人口動態統計」

茶の多用途利用

表　茶の新需要の事例

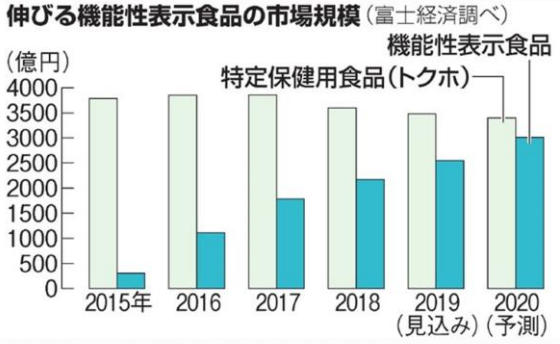
区　分	需　要　分　野　と　応　用　例
茶として利用	水出し茶、各種発酵茶、新香味茶、ギャバロン茶、低カフェイン茶、濃縮茶、混合茶　など
飲用・形態を変えて利用	ドリンク茶、ティバッグ、インスタントティ、粉末茶、微粉末茶(食用、即席飲用、酒割用)、カード茶、錠剤茶、カプセル茶、茶ワイン、緑茶酒、スポーツ飲料、カテキン粉末など
食品・食用として利用	☆ 形態を変えてそのまま食用として利用 ☆ 食品素材として利用 「素材」「食品」「菓子類」「その他」健康補助食品
飲食料以外に利用	☆ 衣料用など ☆ 医療用 ☆ 化粧品、石鹸用など ☆ 消臭剤、脱臭剤など ☆ 日用品など ☆ 建材、家具、家電用品など ☆ 家畜、ペット用品 ☆ 植物活性用 ☆　その他

様々な飲食料以外にも利用され、新しいビジネスを創造している

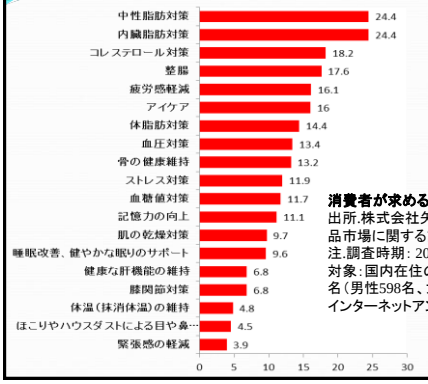
食品の分類(HPより引用)

機能性表示食品の市場規模　特定保健用食品

⇒機能性表示食品に変化



消費者が求める機能性の項目



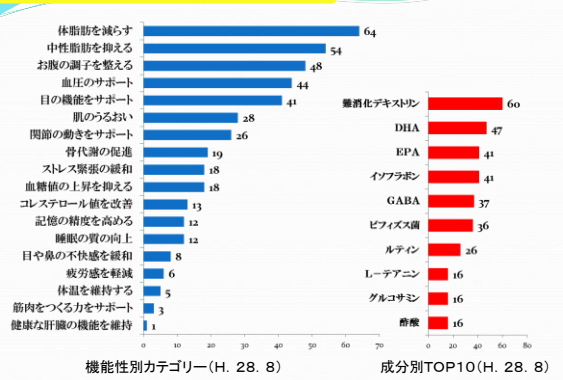
消費者が求める機能性は？

出所.株式会社矢野経済研究所「健康食品市場に関する調査（2017年）

注.調査時期：2016年12月、調査（集計）

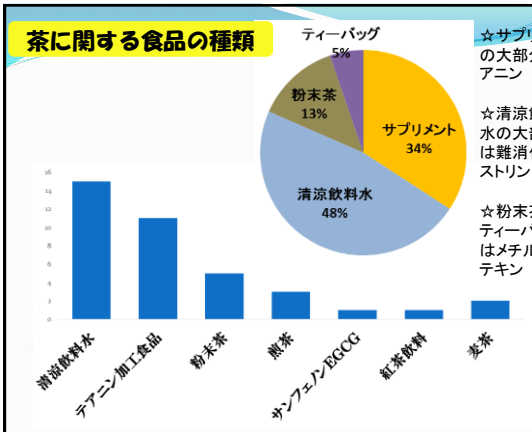
対象：国内在住の30代以上の男女1,193名（男性598名、女性595名）、調査方法：インターネットアンケート、複数回答

機能性のカテゴリーと成分



機能性別カテゴリー（H. 28. 8）　成分別TOP10（H. 28. 8）

茶に関する食品の種類



☆サプリメントの大部分はテアニン

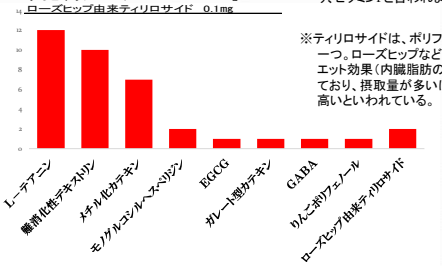
☆清涼飲料水の大部分は難消化デキストリン

☆粉末茶、ティーバッグはメチル化カテキン

茶関係の機能性表示食品も増加 （各メーカーHPより引用）

茶に関する機能性関与成分と含有量

機能性関与成分	含有量
L－テアニン	200mg
難消化性デキストリン	5g
メチル化カテキン	最小34mg
モノグルコシルヘスペリジン	170mg
EGCG	最小300mg
ガレート型カテキン	394mg
GABA	28mg
りんごポリフェノール	110mg
ローズヒップ由来ティリロサイド	0.1mg



※モノグルコシルヘスペリジンは、近年流行っている中性脂肪を下げる働きのあるポリフェノールの一種です。
ヘスペリジンは、オレンジなどの柑橘類全般に含まれているポリフェノールであり、ビタミンPと言われます。

※ティリロサイドは、ポリフェノール成分の一つ。ローズヒップなどに含まれ、ダイエット効果（内臓脂肪の減少）が見られており、摂取量が多いほどその効果が高いといわれている。

機能性表示食品　茶カテキン

成分名	茶カテキン
届出件数	12件
名称(カテゴリ)	粉末飲料【6件】 清涼飲料水【3件】 栄養補助食品【3件】
届出機能	体脂肪を減らす【9件】 記憶の精度を高める【2件】 疲労感を軽減,体脂肪を減らす【1件】
機能性の評価方法	SR(成分)【12件】
安全性の評価方法	喫食実績の評価【6件】 既存情報による安全性試験結果【5件】 喫食実績の評価;既存情報による安全性試験結果【1件】

機能性表示食品　エピガロカテキンガレート

成分名	エピガロカテキンガレート
届出件数	23件
名称(カテゴリ)	清涼飲料水【10件】 栄養補助食品【8件】 粉末飲料【5件】
届出機能	体脂肪を減らす【7件】 血糖値の上昇を抑える【5件】 目や鼻の不快感を緩和【4件】 体脂肪を減らす;中性脂肪を抑える;血糖値の上昇を抑える【2件】 中性脂肪を抑える;血糖値の上昇を抑える【1件】 コレステロール値を改善;体脂肪を減らす【1件】 お腹の調子を整える;体脂肪を減らす【1件】 お腹の調子を整える;体脂肪を減らす;中性脂肪を抑える;血糖値の上昇を抑える【1件】 口腔内環境を良好に保つ【1件】
機能性の評価方法	SR(成分)【17件】 RCT【3件】 RCT;SR(成分)【3件】
安全性の評価方法	既存情報による安全性試験結果【10件】 喫食実績の評価【8件】 喫食実績の評価;既存情報による安全性試験結果【4件】 喫食実績の評価;既存情報による食経験の評価【1件】

機能性表示食品　GABA

成分名	GABA
届出件数	368件
名称(カテゴリ)	栄養補助食品【125件】 清涼飲料水【72件】 菓子類【51件】 粉末飲料【36件】その他
届出機能	血圧のサポート【73件】 ストレス・緊張の緩和【69件】 睡眠の質の向上【33件】 ストレス・緊張の緩和,疲労感を軽減【21件】 ストレス・緊張の緩和,血圧のサポート【14件】 血圧のサポート,中性脂肪を抑える,血糖値の上昇を抑える【9件】 ストレス・緊張の緩和,睡眠の質の向上【9件】 疲労感を軽減,血圧のサポート【7件】 ストレス・緊張の緩和,疲労感を軽減,睡眠の質の向上【6件】 血圧のサポート,コレステロール値を改善【5件】 疲労感を軽減【4件】 ストレス・緊張の緩和,疲労感を軽減,血圧のサポート【3件】 血圧のサポート,血糖値の上昇を抑える【3件】 疲労感を軽減,脳の機能をサポート【2件】 疲労感を軽減,睡眠の質の向上【2件】 ストレス・緊張の緩和,疲労感を軽減,睡眠の質の向上,血圧のサポート【2件】 コレステロール値を改善【1件】 ストレス・緊張の緩和,記憶の精度を高める【1件】 血圧のサポート,体脂肪を減らす【1件】 ストレス・緊張の緩和,肌のうるおい【1件】 ストレス・緊張の緩和,睡眠の質の向上,脳の機能をサポート【1件】 血圧のサポート,体脂肪を減らす,中性脂肪を抑える【1件】 血糖値の上昇を抑える【1件】 血圧のサポート,中性脂肪を抑える,体温（末梢体温）を維持する【1件】 記憶の精度を高める【1件】 中性脂肪を抑える,血糖値の上昇を抑える【1件】 睡眠の質の向上,血圧のサポート【1件】 ストレス・緊張の緩和,睡眠の質の向上,血圧のサポート【1件】 疲労感を軽減,脳の機能をサポート,血圧のサポート【1件】 肌のうるおい,肌の弾力【1件】
機能性の評価方法	SR(成分)【365件】

ありがとうございました

主催：静岡市（地域福祉共生センター「みなくる」）
事業受託：静岡県立大学（「ふじのくに」みらい共育センター）
協力：静岡県立大学茶学総合研究センター

地域健康オープンカレッジ2020

お茶の文化で世界を巡る

～地域の風土とともに発達したお茶～

講師：中村　順行　茶学総合研究センター長
（静岡県立大学食品栄養科学部　特任教授）
亀岡　葉子　先生（日本茶インストラクター）

難易度
★☆☆
（どなたでも）

お茶って、な~に

- チャ節（Section *Thea*）
  - チャ（*C. sinensis*　(L.) O. kuntze）
  - 中国種（*C. sinensis* var. *sinensis*）
  - アッサム種（*C. sinensis* var. *assamica*）
- ツバキ節（Section *Camellia*）
- サザンカ節（Section *Paracamellia*）等　11節

ツバキ属（genus *Camellia*）

ツバキ

サザンカ

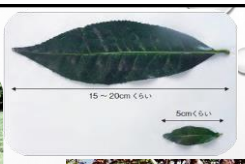
チャが他の植物と異なる点

☆カフェイン
☆ガレート型のカテキン
☆テアニン
☆その他（フッ素、アルミ等）

チャはツバキの仲間、でも飲用されるのは茶樹だけ

チャの特質

中国種



アッサム種

表　中国種とアッサム種の性状

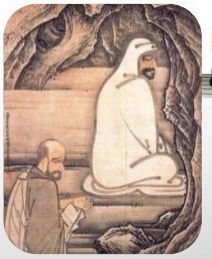
種類 / 性状	中国種（日本種も含む）	アッサム種
木の形	灌木、樹高が低く、地際より多くの枝幹が伸びる	喬木、主幹は1本
葉の大きさ	小さい	大きい
葉先	とがっていない	細長くとがっている
葉面	濃緑色でなめらか	淡緑色で葉脈と葉脈の間の部分が盛り上がる
耐寒性	強い	弱い
用途	緑茶向き	紅茶向き

お茶の始まり

HPより引用

神　農

西暦500年前後に陶弘景（452-536）がまとめた『神農本草経』に「神農嘗百草、日遇七十二毒、得茶而解之



達磨和尚

修行のとき、眠気を覚ますため、まゆげをそぎ落としたのが湯に入り、お茶になったと言われる。

お茶の別名は
めざまし草

日本にも仏教とともに伝来し、文化的にも大きく育て上げてきた

トピックス　お茶は薬草？

薬草 ⇒ 嗜好品

最古の薬書
（後漢1～2世紀）

Wikipediaより引用

茶は上薬

陶弘景は「神農本草経集注」により苦菜を茶とした

茶の味は味は苦く、性質は寒。効能は五臓（肝、心、脾、肺、腎）の病気、食べ過ぎによる胃もたれを治し、長く服用すれば気分を安らかにし、元気をまし、身を軽くし、老化にも耐えうる

お茶の波及

HPより引用

陸　羽

茶経

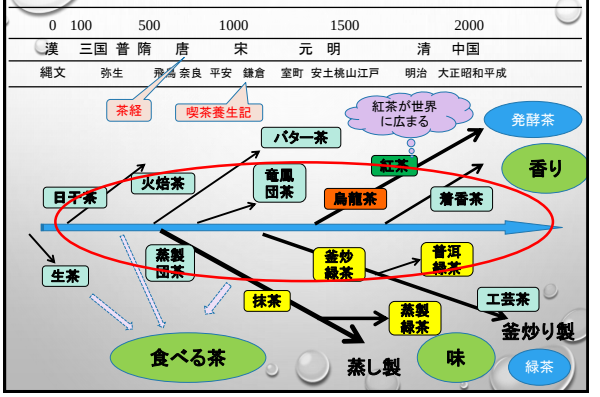
陸羽の時代の「茶」は、粉末状にしたものを、主に葱や生姜等と一緒に煮て飲む、「スープのような茶」に使われていた。陸羽はそれを、「清の捨て水」として非難し、「茶経」を記し、茶だけで愉しむように提案した。

薬草 ⇒ 嗜好品

食べる ⇒ 飲む

お茶の発祥と伝搬　紀元前(2000年以上前～)

茶には2000年の歴史。中国から世界中に多様に進化



中国茶の変遷

唐代
「餅茶」固形茶

宋代
「片茶」精巧細微な貢納用の固形茶


餅茶


片茶(団茶)

明代
貢納用の固形茶の献上を一律葉茶にした。中国本土での固形茶飲用の歴史は終焉

蒸製から釜炒製へ
明代以前、固形茶、葉茶ともに「蒸す」製法が中心。明代に釜で炒って製茶する技術が生まれ、飲み方も茶壺(急須)を使用し、葉を湯に浸して出汁を飲む方法が普及。現代風の喫茶の始まり

清代以降の多様な茶類
釜炒り散茶の種類の多様化。伝統の緑茶と黒茶以外、紅茶、白茶、青茶の多様な発酵茶と再加工茶である花茶(ジャスミン茶)が現れた。
青(烏龍)茶は清代の1855 年頃
白茶は1857 年頃
紅茶は明末・清初(1643 年頃)に発明

清代には、地域による喫茶の嗜好性の違いも顕著になり、福建や広州では烏龍茶が愛飲され、長江の中下流域では緑茶、四川や雲南ではプーアル茶(黒茶)、茶の栽培が難しい北方では、北京中心に花茶の消費が圧倒的に多くなった。

トピックス
「茶」の文字の起源は？

中国　唐時代(7～10世紀)
茶の呼び名は五種類
「茶(と)　:早い時期に摘むもの
「檟」(か)　:苦いお茶
「蔎」(せつ):西南地方の人の呼び名
「茗」(めい):遅い時期に摘むもの
「荈」(せん):遅い時期に摘むもの

茶
↓
茶

Q1 「茶」という字の起源は？

中国には、「茶」に似た漢字に「荼」があります。「荼」は、もともとはキク科の苦みのある野草のことを指していましたが、紀元前1世紀ごろにはそれがお茶の意味にも使われるようになりました。
お茶を飲む風習が広まってみんなが飲むようになると、お茶だけを指す文字が必要になりました。そこで唐の時代(618～907年)に新しく「茶」という漢字ができたと考えられています。
日本では、奈良時代(8世紀)の史料である正倉院文書などに「茶」の文字が見られますが、これはお茶ではなく苦菜のことだといわれます。日本で「茶」の字が使われるのは、平安時代初めからで、当時の歴史書や漢詩集に出てきます。
なお、お茶の意味で使われる漢字には、「茗」という字もあります。

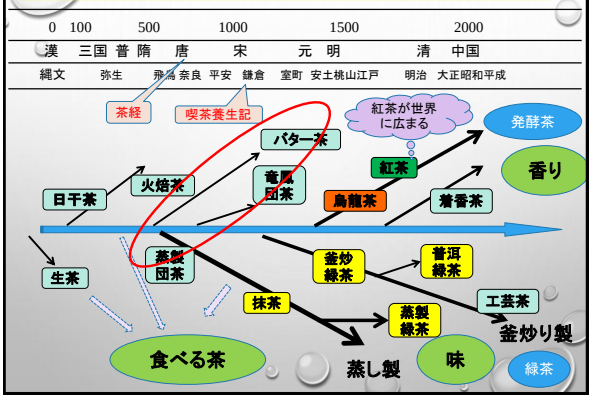
めざせ！お茶博士こどもお茶小事典より引用

最近では中国式の6茶種に分類されることが多い

茶の分類
- 緑茶(不発酵茶)
  - 蒸し製緑茶(日本式)
  - 釜炒り製緑茶(中国式)
- 青茶「ウーロン茶」(半発酵茶)
- 紅茶(発酵茶)
  - ※発酵:葉の酵素による酸化反応
- 黒茶「後発酵茶」(堆積茶)
  - ※発酵:微生物発酵
- その他
  - 白茶
  - 黄茶
  - 二次加工茶

お茶の種類の多様性

茶馬古道によるチベットへの茶の波及（7世紀頃～）



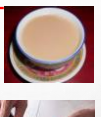
トピックス 茶馬古道

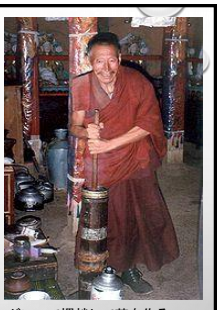
雲南省で取れた茶（磚茶）をチベット人および馬で運んだことから名付けられた交易路である。7世紀の唐や吐蕃の時代にはすでに交易が始められ、20世紀中ごろが流通の絶頂期と言われる。

雲南省南部の易武が起点。景洪市やプーアル、那柯里などのメコン川沿いに雲南省を北上し、虎街では町の真ん中を茶馬古道が通っている。

トピックス チベットではバター茶

中国内モンゴルにおける磚茶文化
―茶馬交易が結んだ乳と茶―

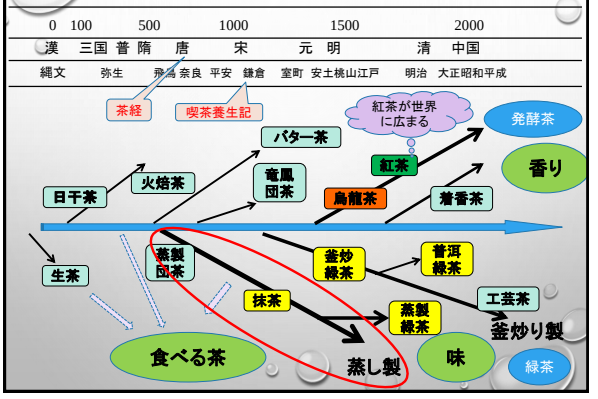




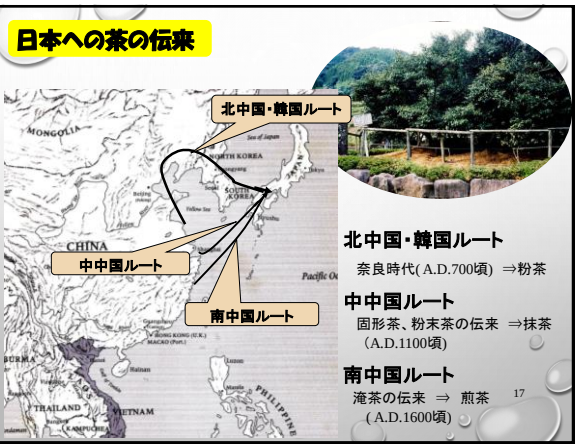
ドンモで撹拌して茶を作る

茶はチベット人の生活には欠かせない飲料。
茶馬古道で入った黒茶の磚茶を削りお茶を煮出し、牛乳やラクダ乳などの乳、ヤク乳から作られたギー(ヤクのバター)などと塩を加え、沸騰させないように加熱し、ドンモで撹拌して飲む

日本には仏教とともに波及（8世紀頃～）



日本への茶の伝来



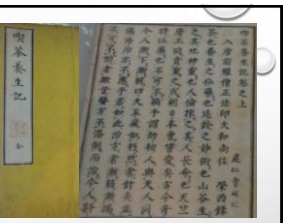
北中国・韓国ルート
奈良時代(A.D.700頃) ⇒粉茶

中中国ルート
固形茶、粉末茶の伝来 ⇒抹茶
(A.D.1100頃)

南中国ルート
滝茶の伝来 ⇒ 煎茶
(A.D.1600頃)

喫茶養生記（栄西：1211）

茶者養生之仙薬也 延齢之妙術也



①茶は身体衰弱、意志消沈のときは、気力を強くする。
②茶は人を愉快な気持ちにさせ、酒の酔いを醒まし、睡気を起こさない。
③茶は小便の通じが良く、喉の渇きをとりさり、消化不良をなくす。
④茶は身を軽くし、脚気によい。
⑤茶は精神を整え、内臓を和らげ、身体の疲労をやすらかに除く。

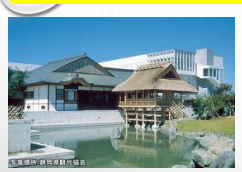
日本における主要な茶の推移

時代とともに飲用茶も大きく変化してきた

茶種の変遷

平安時代	団茶	上流階級
鎌倉時代	抹茶	武士、上流階級
江戸時代	抹茶、煎茶、釜炒り茶	上流階級
	番茶	庶民
明治時代	煎茶、番茶	
	輸出用各種茶	輸出用
現在	機械製煎茶	国内用

日本における茶の文化の醸成



日本文化には仏教と茶を切り離して語れない

一茶 茶筒 茶飯事
茶うけ 茶碗 茶化
す 茶話会 茶菓
茶番 茶色 茶寿
茶堂 海老茶 茶飲
仲間 茶目っけ 浮
世茶屋 目茶目茶
茶托 滅茶苦茶 無
茶苦茶 日常茶飯事
茶番劇 茶髪 茶巾
茶目 お茶の子 茶
腹 お茶の間 茶箪
笥 茶尻 茶坊主

緑茶生産方法の推移

お茶の産業化は明治期以降

手摘み

可搬型摘採機

乗用型摘採機

手揉み

機械揉み

FA対応機械揉み

摘採は手摘みから機械摘みに変わり、著しく摘採能率を向上してきた。製造は手揉みから機械化され、徐々に投入量を増加させるとともに最近ではコンピュータ制御による自動化に技術革新したことで、日本独自の生産加工技術を確立し、品質の高位平準化に貢献してきた

緑茶消費方法の推移

庶民がお茶を飲めるようになったのは江戸時代中期以降

煎じ茶 ⇒ 煎茶に

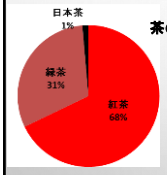
番茶 ⇒ せん茶 ⇒ 茶素材

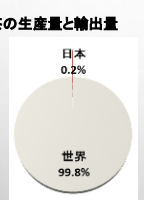
トピックス 日本茶はガラパゴス化？

日本の茶は中国から伝わり、湯通しや蒸気により酵素を不活化する方法。中国ではその後すべて釜炒りに変化

日本茶＝蒸し製

緑茶＝中国茶＝釜炒り茶

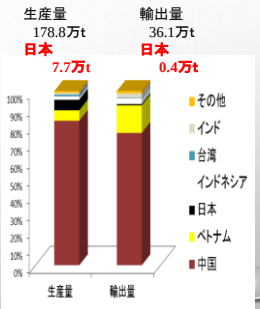




生産量
556万t
日本
約 7.7万t

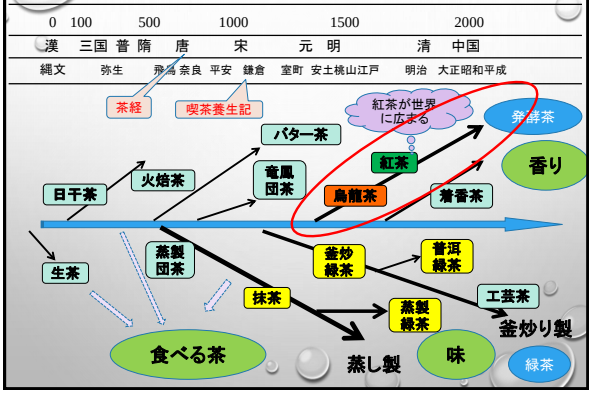
輸出量
179.8万t
日本
0.4万t

世界の茶の生産量と輸出量（2016）



世界における緑茶の生産量と輸出量

ヨーロッパへの茶の波及（16世紀～） そして世界中に



ヨーロッパへのティーロード

お茶が初めてヨーロッパに伝えられたのは16世紀中頃。17世紀初期に緑茶から紅茶に変化し、19世紀初期にインドで紅茶生産。19世紀中期にセイロンで生産開始。

広東語系（陸路）		福建語系（海路）	
広　東	チャ	福　建	テ
朝　鮮	チャ	スリランカ	テーイ
日　本	チャ、サ	南インド	テイ
モンゴル	チャイ	オランダ	テー
イラン	チャ	イギリス	ティー
トルコ	チャイ	ドイツ	テー
ギリシャ	チャイ	フランス	テ
ロシア	チャイ	イタリア	テ
ポルトガル	チャ	スペイン	テー
アラビア	シャー	デンマーク	テ

ヨーロッパにおける紅茶文化の成立

中国からは茶の機能性　シノワズリー（中国趣味）

茶の効能論争　ミルク

コーヒーハウスからティハウスに

コーヒーから紅茶に

緑茶 ⇒ 紅茶 ⇒ アフタヌーンティ

エール(ビールの一種)
肉食文化

東洋文化への憧れから自国の文化に

日本からはもてなしの文化

砂糖

茶の湯の文化

26

紅茶文化の定着

産業革命、植民地政策により豊かな社会に

大英帝国 ⇒ 世界に波及

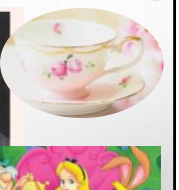
把手のないカップから
把手のあるカップに

家庭への回帰
朝食文化の成立
マナーの成立

トピックス　ティーカップにはなぜ把手が？

取っ手のないティーカップ
島田お茶

銀製のティーセットを使うイギリスの家族
ヴィクトリア・アンド・アルバート美術館
画像提供　八坂書房



中国から伝来したお茶
当初は緑茶であり、同時に飲み方や道具類も伝来

紅茶の開発とともに、自国でも紅茶の飲み方に適した茶の道具類を製造するようになった

トピックス　なぜ紅茶をブラックティーと呼ぶの？

イギリスにお茶が中国から輸入されたのは17世紀後半

なかには、福建省厦門から武夷山周辺のお茶も輸出された

武夷山のお茶は緑茶から茶の色が黒い半発酵茶も混在
当然、浸出液も黒みを帯びていた

そのお茶をブラックティーと呼んだ

やがてイギリスにおいて茶の主流になり、さらに好みに応じて発酵度をあげた製品づくりや、製法を綿密にした「工夫紅茶」が中国で開発され、現在の紅茶のもととなり、色は変われどブラックティーと呼ばれている

ちなみに、日本では紅茶が世界的に広まって以降に知られるようになったため、色から紅茶と呼ぶ

トピックス　お茶は世界の歴史を変えた

ボストンティパーティ事件(1773)

アメリカがイギリスから独立するきっかけとなった事件

イギリスがアメリカに課した茶への重税に抗議する人々がボストン湾に茶を投げ捨てた

アメリカの南北戦争の一端にもなった事件

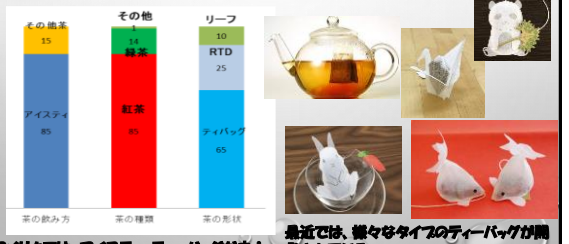
アヘン戦争(1840～1842)

イギリスで飲茶の習慣が浸透し、茶の輸入が激増するに伴い大量の銀が清(中国)に流れたのでその赤字貿易解消のためにアヘンを輸出したことがきっかけとなった事件

トピックス
ティーバッグとアイスティー

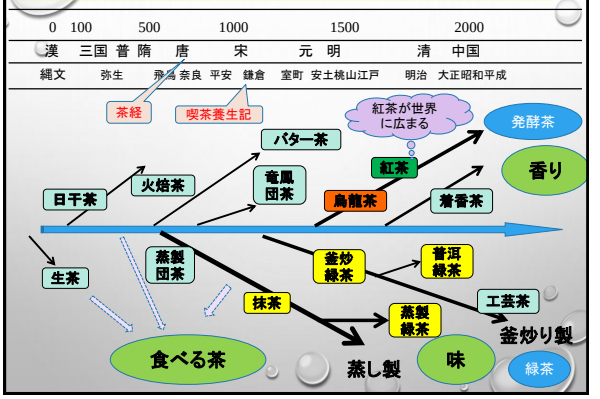
ティーバッグは、1908年にコーヒー貿易商であるトーマス・サリヴァンによって偶然に発明されたというのが定説となっている

アイスティーは、1904年にアメリカ・セントルイスで開かれた万国博覧会でイギリス人の紅茶商が提供したのがアイスティーの始まりだといわれます



アメリカでは、アイスティ、ティーバッグが中心　最近では、様々なタイプのティーバッグが開発されている

茶には2000年の歴史。時代とともに多様に進化



世界のチャの栽培地域

温暖地、緑茶産地　；中国種
亜熱帯地、紅茶産地；アッサム種

日本茶の輸出量と金額の推移

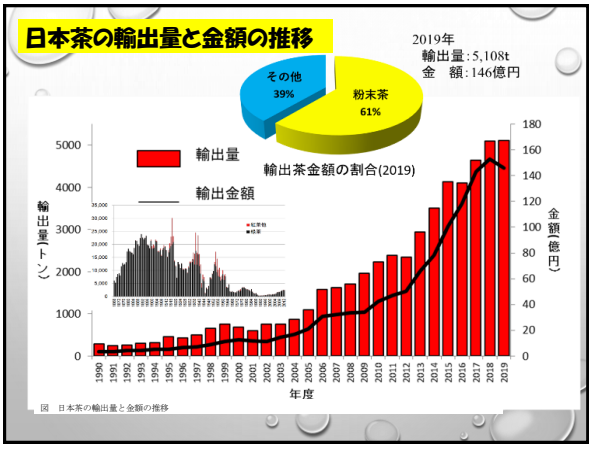


図　日本茶の輸出量と金額の推移

日本茶の特質は

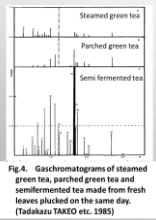
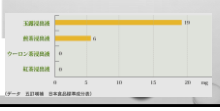


Fig.4.　Gaschromatograms of steamed green tea, parched green tea and semifermented tea made from fresh leaves plucked on the same day. (Tadakazu TAKEO etc. 1985)



世界の茶のなかでの日本茶の特質は!!

- 浸出液が緑色であること
- 蒸熱処理のため、浸出液中に成分が溶出しやすいこと
- 旨味のアミノ酸含量が高く、カテキン類の濃度が低いこと
- 香りに若葉の新鮮香があること
- 針状のお茶であること
- ビタミンCを多く含むこと
- 歴史、文化性が高いこと

抹茶は世界のスーパーフード

抹茶は　美味しさと健康性で世界の関心事

抹茶は、
急須で淹れたお茶では摂取できない、プロビタミンA(βカロテン)、ビタミンE(トコフェノール)、食物繊維なども摂取可能。

☆抹茶は美味しい
☆急須で淹れた場合、カテキンの40％程度は茶殻に残るが、抹茶では全てを摂取可能。
☆緑色が美しいのみならず、食品添加素材としても有用。

2020,12.19

茶は養生の仙薬
その魅力と機能性

静岡県立大学　茶学総合研究センター　中村順行

茶の栽培加工から
機能性、販売、経営手法まで総合的に科学する!!

茶学総合研究センター

目的及び組織体制

学内の各教員が専門性を活かして実施する茶に関する情報を一元化するとともに、相互に連携した取組みを行う。また、県内の他大学や公設試験研究機関をはじめ行政・茶業界とも連携して茶業振興に貢献する。

センター4本柱

1. 緑茶の機能性及び疫学に関する研究
2. 茶学教育と人材育成
3. 茶葉及び茶飲料の嗜好特性の解明
4. 茶の抗付加価値化とマーケティング

目　次

茶は養生の仙薬、その魅力と機能性

☆ お茶の特質

☆ チャの主要成分とその機能性

☆ 機能性に特化した品種や栽培・加工法

☆ 茶の特定保健用食品と機能性表示食品

お茶って、な~に

ツバキ属 (genus *Camellia*)
- チャ節（Section *Thea*）
  - チャ（C. *sinensis* (L.) O. kuntze）
    - 中国種（C. *sinensis* var. *sinensis*）
    - アッサム種（C. *sinensis* var. *assamica*）
- ツバキ節（Section *Camellia*）
- サザンカ節（Section *Paracamellia*）等　11節

ツバキ

サザンカ

チャが他の植物と異なる点
- ☆カフェイン
- ☆ガレート型のカテキン
- ☆テアニン
- ☆その他（フッ素、アルミ等）

チャはツバキの仲間、でも飲用されるのは茶樹だけ

お茶の始まり

HPより引用

神　農

西暦500年前後に陶弘景(452-536)がまとめた『神農本草経』に「**神農嘗百草、日遇七十二毒、得茶而解之**」

達磨和尚

修行のとき、眠気を覚ますため、まゆげをそぎ落としたのが湯に入り、お茶になったと言われる。

お茶の別名は
めざまし草

日本にも仏教とともに伝来し、文化的にも大きく育て上げてきた

トピックス
お茶は薬草？

薬草 ⇒ 嗜好品

最古の薬書
（後漢1～2世紀）

Wikipediaより引用

茶は上薬

陶弘景は「神農本草経集注」により苦菜を茶とした

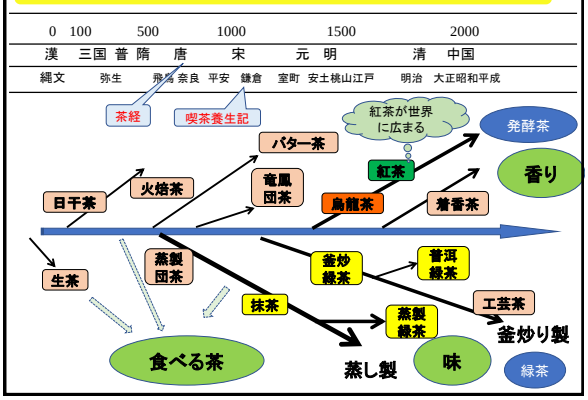
茶の味は味は苦く、性質は寒。効能は五臓（肝、心、脾、肺、腎）の病気、食べ過ぎによる胃もたれを治し、長く服用すれば気分を安らかにし、元気をまし、身を軽くし、老化にも耐えうる

喫茶養生記

茶者養生之仙藥也延齡之妙術也

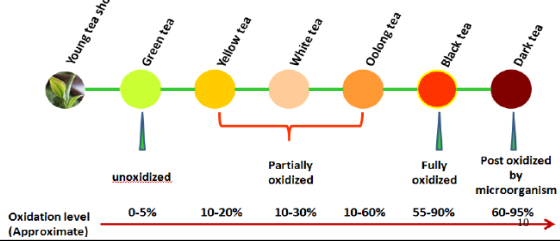
①茶は身体衰弱、意志消沈のときは、気力を強くする。
②茶は人を愉快な気持ちにさせ、酒の酔いを醒まし、睡気を起こさない。
③茶は小便の通じが良く、喉の渇きをとりさり、消化不良をなくす。
④茶は身を軽くし、脚気によい。
⑤茶は精神を整え、内臓を和らげ、身体の疲労をやすらかに除く。

茶には2000年の歴史。時代とともに多様に進化



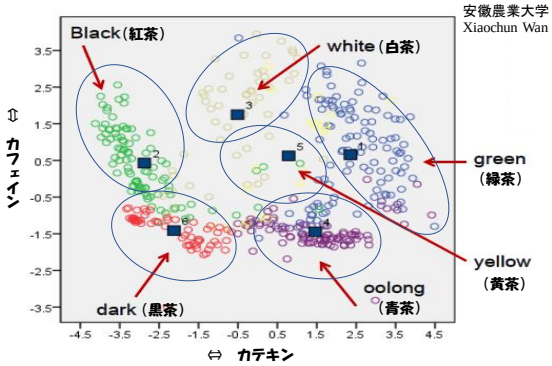
お茶の種類

緑茶（不発酵茶）
　蒸し製緑茶(日本式)
　釜炒り製緑茶(中国式)
青茶「ウーロン茶」（半発酵茶）
紅茶（発酵茶）
黒茶「後発酵茶」（堆積茶）
白茶、黄茶



成分量により茶種の分類がおおまかに可能

安徽農業大学
Xiaochun Wan



目　次

茶は養生の仙薬、その魅力と機能性

☆ お茶の特質

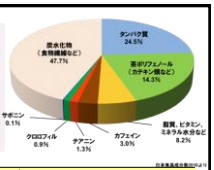
☆ チャの主要成分とその機能性

☆ 機能性に特化した品種や栽培・加工法

☆ 茶の特定保健用食品と機能性表示食品

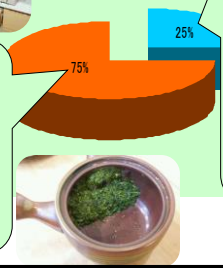
茶のもつ機能性

＋ 精神機能



一次機能	**栄養性**	ビタミン	ビタミンC、ビタミンE、β カロテンなど
		ミネラル	カリウム、リン、微量必須元素など
二次機能	**嗜好性**	味	テアニン、遊離アミノ酸、カテキン、カフェインなど
		香り	テルペン、アルコール、カルボニール、エステルなどの精油
		色	フラボノール、テアフラビン、クロロフィルなど
三次機能	**体調調節**	カテキン、カフェイン、テアニン、ビタミン類、γアミノ酪酸、微量元素など	

お茶の機能性成分



水溶性成分
- ☆**カテキン類**(10～18%):**抗酸化**、抗菌、抗がん、生活習慣病予防、消臭、抗アレルギーなど
- ☆**カフェイン**(3～4%):眠気防止、強心、二日酔い防止
- ☆**フラボノール**(0.6～0.7%):**抗酸化**、抗がん、免疫活性
- ☆**ビタミンC**(200mg%):**抗酸化**、免疫活性
- ☆**ビタミンB**(1.4mg%):**抗酸化**、口内炎予防
- ☆**サポニン**(0.1%):抗喘息、抗菌、血圧効果
- ☆**テアニン**(0.6～2%):リラックス、血圧効果

などなど

不溶性成分
- ☆**食物繊維**(20～30%):便秘予防、大腸がん予防、心疾患予防
- ☆**たんぱく質**(24%):栄養
- ☆**βカロテン**(20mg%):**抗酸化**、抗がん、抗糖尿、抗心疾患、免疫活性
- ☆**ビタミンE**(25～70mg%):**抗酸化**、抗がん、免疫活性
- ☆**クロロフィル**(0.80%):がん予防、抗突然変異、抗腫瘍、免疫活性

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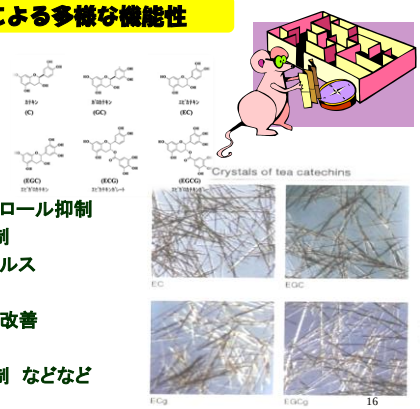
茶の機能に関する代表的書籍

カテキン類による多様な機能性

- 抗酸化
- 抗突然変異
- 抗がん
- 酸化防止
- 抗動脈硬化
- 血中コレステロール抑制
- 脂肪吸収抑制
- 抗菌、抗ウイルス
- 虫歯予防
- 腸内フローラ改善
- 消臭
- 血圧上昇抑制　などなど



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カテキン類による抗がん作用

HPより引用

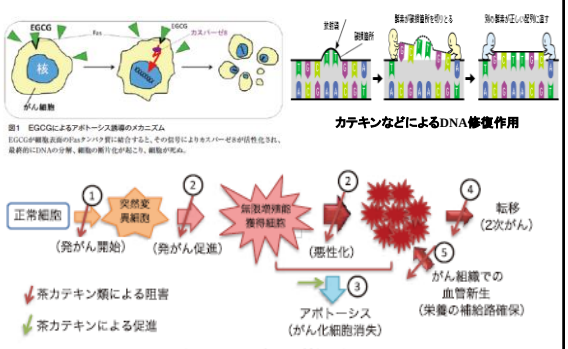


図1　茶カテキン類による発がん過程の抑制効果

がんと緑茶に関する疫学的調査研究のまとめ

表１　がんと緑茶に関する疫学調査研究（伊勢村護）

	前向きコホート研究		症例対照研究	
がんの部位	リスク軽減あり	リスク軽減なし	リスク軽減あり	リスク軽減なし
大腸	3	6	4	3
肺	0	4	2	3
胃	2	6	8	8
食道	0	2	4	5
乳房	3	5	3	0
前立腺	2	1	2	0
卵巣	1	0	2	0
すい臓	0	2	2	1
腎臓、膀胱	0	1	1	4
肝臓	1			
子宮内膜			2	1
甲状腺	1	1		
血液	1			

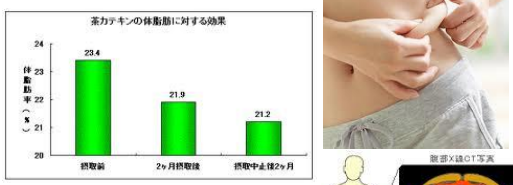
データは、～緑茶と健康のメカニズム～　機能効用ナビゲーション 2013
（静岡県経済産業部農林業局茶業農産課）

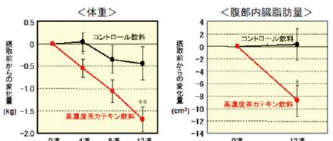
前向きコホート研究:
まだ病気になっていない人達を対象に調査し、数年後の追跡で発病を調査する方法

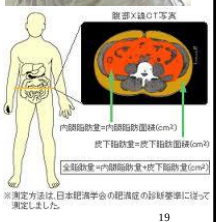
症例対照研究:
特定の病気が発症した人を対象に、健常人との比較調査する方法

カテキン類による抗体脂肪抑制作用

HPより引用







茶カテキンの継続摂取による体脂肪低減効果[4]

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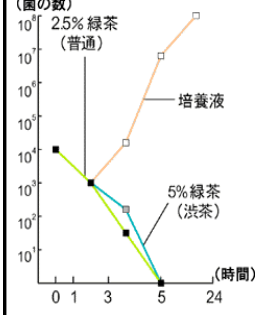
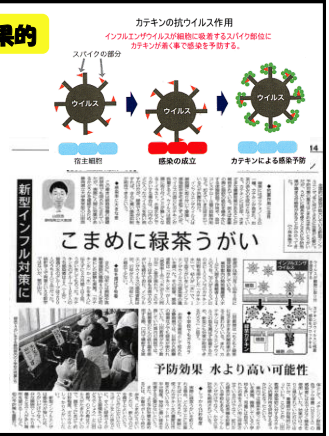
インフルエンザにも効果的





COVID-19に対する研究

2020 春先

分子ドッキングシュミレーション(Molecular docking simulation)

EGCGに効果:インドのMohammad Faheem Khanら

テアフラビンに効果：Manish Manish(インド)、Jrhau Lungら(台湾)

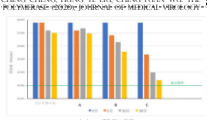
① MOHAMMAD FAHEEM KHAN, MOHSIN ALI KHAN, ZAW ALI KHAN, TANVEER AHAMAD, WASEEM AHMAD ANSARI IDENTIFICATION OF DIETARY MOLECULES AS THERAPEUTIC AGENTS TO COMBAT COVID-19 USING MOLECULAR DOCKING STUDIES (2020). COMPUTATIONAL CHEMISTRY

② MANISH MANISH. STUDIES ON COMPUTATIONAL MOLECULAR INTERACTION BETWEEN SARS-COV-2 MAIN PROTEASE AND NATURAL PRODUCTS (2020). CHEM RXIV. ORE PRINT

③ JRHAU LUNG, YU-SHIH LIN, YAO-HSU YANG, YU-HIN, CHOU, LI-HSIN, SHU, YU-CHING, CHENG, HUNG, TE, LIU, CHING-YUAN, WU. THE POTENTIAL CHEMICAL STRUCTURE OF ANTI-SARS-COV-2 RNA-DEPENDENT RNA POLYMERASE. (2020). JOURNAL OF MEDICAL VIROLOGY. WILEY. 92. 693-697.

2020 11月27日

奈良県立医科大学は、茶に新型コロナウイルスを不活化する効果があるとプレスリリース



Significant inactivation of SARS-CoV-2 by a green tea catechin, a catechin-derivative and galloylated theaflavins *in vitro*

Eriko Ohgitani[a]*, Masaharu Shin-Ya[a]*, Masaki Ichitani[b], Makoto Kobayashi[b], Takanobu Takihara[b], Masaya Kawamoto[c], Hitoshi Kinugasa[c] and Osam Mazda[a]

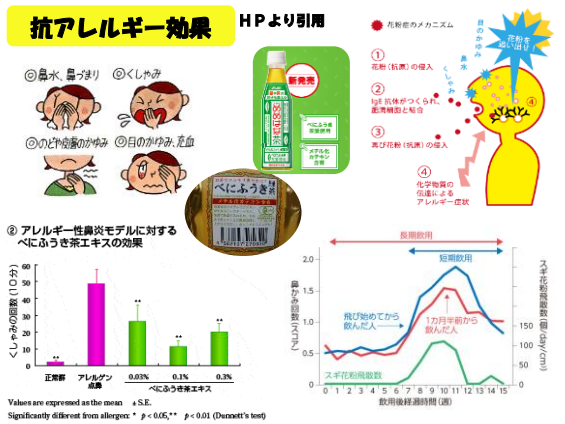
2020 12月6日

京都府立医科大学、(株)伊藤園

テアフラビン、EGCGに高い効果ありと Bio Rxiv Preprint doiに公開

抗アレルギー効果

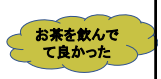
HPより引用



カフェインの機能性

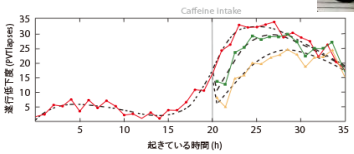
- 覚醒作用
- 大脳刺激作用
- 疲労回復
- 強心作用
- 利尿作用

茶の別名は「目覚まし草」



カフェインの覚醒効果（遂行の改善）

20時間断眠後の睡魔による遂行低下を改善



出典元：「Journal of Theoretical Biology」Volume 358: I（2014 年 Ramakrishman 他）

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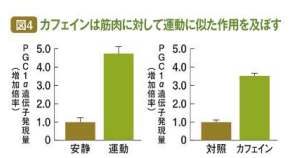
カフェインによる運動機能の向上



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テアニンの機能性

- 血圧降下
- 脳神経機能調整
- 血管性痴呆症予防作用
- 抗ストレス作用
- 記憶学習行動促進作用

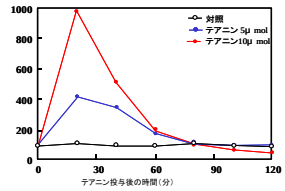
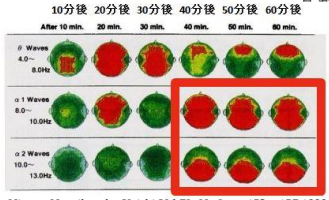


図 脳線条体へのテアニン投与によるドーパミン放出量の増加



Nippon Nogeikagaku Kaishi Vol.72, No.2, pp.153～157,1998

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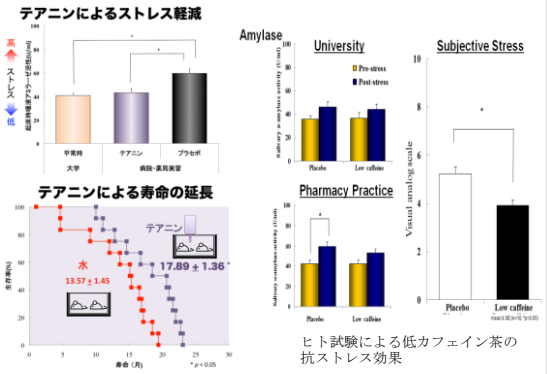
テアニンにはストレス解消、寿命の延伸効果も



ヒト試験による低カフェイン茶の抗ストレス効果

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茶は養生の仙薬、その魅力と機能性

☆ お茶の特質

☆ チャの主要成分とその機能性

☆ 機能性に特化した品種や栽培・加工法

☆ 茶の特定保健用食品と機能性表示食品

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べにふうき

べにふうき（紅富貴）は、べにほまれと枕Cd86を交配した後代のアッサム種に近い茶品種である。紅茶、半発酵茶の用途として開発

サンルージュ

C. taliensis ---------- 茶中間母本農6号 ---------- サンルージュ
（自然交雑）（旧系統名：P9518）（自然交雑）（旧系統名：枕飼03-1384）

サンルージュ

左：サンルージュ 右：やぶきた

図2「サンルージュ」の一番茶新芽(左)と水色(右)

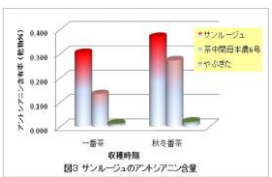


図3 サンルージュのアントシアニン含量

新芽中のアントシアニン含量が高い茶品種で炭疽病や輪斑病に比較的強い抵抗性を示し、芽数が多く、仕立てやすいなど栽培特性に優れています。アントシアニンは抗酸化作用や抗眼精疲労作用が期待できる植物由来機能性成分として注目されている。

農研機構茶業研究所成果集より引用

白葉茶

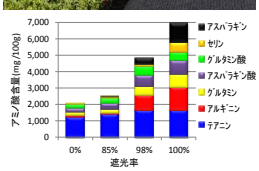


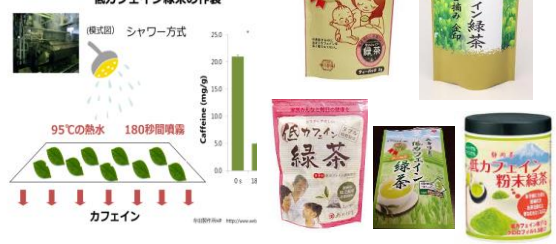
図1 遮光率とアミノ含量の比較

強遮光処理をすることで白い芽の品種と同様な白葉茶の生産が可能です。

低カフェイン茶

H.Pより引用

若い女性や高齢者は睡眠阻害、妊娠時には乳児への影響を避けるため、茶の飲用を遠慮する人が多い



最近では、様々な低カフェイン茶が販売されるようになってきました

ギャバロン茶

ギャバロン茶は嫌気処理で作成され、血圧上昇抑制に効果が高い

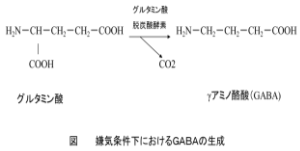


図　嫌気条件下におけるGABAの生成

生葉収穫 → 嫌気処理 → 蒸熱処理 → 深蒸煎茶製造に準じた工程 → 火入れ → 製品

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☆ 茶の特定保健用食品と機能性表示食品

機能性表示食品の市場規模

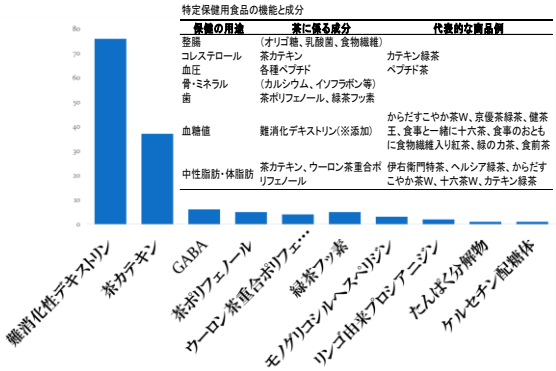
特定保健用食品
⇒機能性表示食品に変化

伸びる機能性表示食品の市場規模（富士経済調べ）

茶に関する特定保健用食品の用途と成分

特定保健用食品の機能と成分

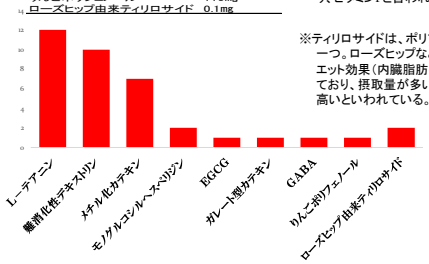
保健の用途	茶に係る成分	代表的な商品例
整腸	（オリゴ糖、乳酸菌、食物繊維）	
コレステロール	茶カテキン	カテキン緑茶
血圧	各種ペプチド	ペプチド茶
骨・ミネラル	（カルシウム、イソフラボン等）	
歯	茶ポリフェノール、緑茶フッ素	
血糖値	難消化デキストリン(※添加)	からだすこやか茶W、京優茶緑茶、健茶王、食事と一緒に十六茶、食事のおともに食物繊維入り紅茶、緑の力茶、食前茶
中性脂肪・体脂肪	茶カテキン、ウーロン茶重合ポリフェノール	伊右衛門特茶、ヘルシア緑茶、からだすこやか茶W、十六茶W、カテキン緑茶



茶を中心とした特定保健用食品例
最近では　W効果の商品が多い

茶に関する機能性表示食品とその成分

機能性関与成分	含有量
L－テアニン	200mg
難消化性デキストリン	5g
メチル化カテキン	最小34mg
モノグルコシルヘスペリジン	170mg
EGCG	最小300mg
ガレート型カテキン	394mg
GABA	28mg
りんごポリフェノール	110mg
ローズヒップ由来ティリロサイド	0.1mg



※モノグルコシルヘスペリジンは、近年流行っている中性脂肪を下げる働きのあるポリフェノールの一種です。
ヘスペリジンは、オレンジなどの柑橘類全般に含まれているポリフェノールであり、ビタミンPと言われます。

※ティリロサイドは、ポリフェノール成分の一つ。ローズヒップなどに含まれ、ダイエット効果（内臓脂肪の減少）が見られており、摂取量が多いほどその効果が高いといわれている。

機能性表示食品例

L－テアニン

難消化デキストリン

メチル化カテキン

EGCGなど

機能性表示食品　エピガロカテキンガレート

成分名	エピガロカテキンガレート
届出件数	23件
名称(カテゴリ)	清涼飲料水【10件】 栄養補助食品【8件】 粉末飲料【5件】
届出機能	体脂肪を減らす【7件】 血糖値の上昇を抑える【5件】 目や鼻の不快感を緩和【4件】 体脂肪を減らす;中性脂肪を抑える;血糖値の上昇を抑える【2件】 中性脂肪を抑える;血糖値の上昇を抑える【1件】 コレステロール値を改善;体脂肪を減らす【1件】 お腹の調子を整える;体脂肪を減らす【1件】 お腹の調子を整える;体脂肪を減らす;中性脂肪を抑える;血糖値の上昇を抑える【1件】 口腔内環境を良好に保つ【1件】
機能性の評価方法	SR(成分)【17件】 RCT【3件】 RCT;SR(成分)【3件】
安全性の評価方法	既存情報による安全性試験結果【10件】 喫食実績の評価【8件】 喫食実績の評価;既存情報による安全性試験結果【4件】 喫食実績の評価;既存情報による食経験の評価【1件】

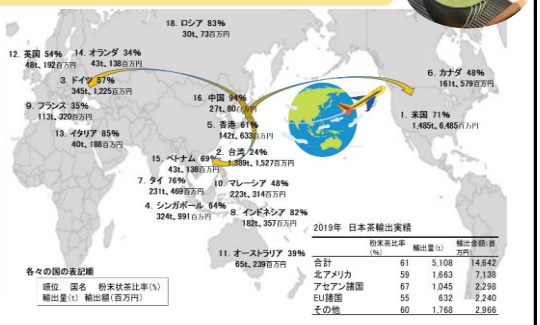
茶の多用途利用

表　茶の新需要の事例

区　分	需　要　分　野　と　応　用　例
茶として利用	水出し茶、各種発酵茶、新香味茶、ギャバロン茶、低カフェイン茶、濃縮茶、混合茶　など
飲用・形態を変えて利用	ドリンク茶、ティバッグ、インスタントティ、粉末茶、微粉末茶(食用、即席飲用、酒割用)、カード茶、錠剤茶、カプセル茶、茶ワイン、緑茶酒、スポーツ飲料、カテキン粉末など
食品・食用として利用	☆ 形態を変えてそのまま食用として利用 ☆ 食品素材として利用 「素材」「食品」「菓子類」「その他」健康補助食品
飲食料以外に利用	☆ 衣料用など ☆ 医療用 ☆ 化粧品、石鹸用など ☆ 消臭剤、脱臭剤など ☆ 日用品など ☆ 建材、家具、家電用品など ☆ 家畜、ペット用品 ☆ 植物活性用 ☆ その他

茶は飲用だけでなく、食品素材として、さらには機能性成分を活かした様々な飲食料以外にも利用され、新しいビジネスを創造している

海外市場の特性と輸出戦略



2019年 日本茶輸出実績

	粉末茶比率(%)	輸出量(t)	輸出金額(百万円)
合計	61	5,108	14,642
北アメリカ	59	1,663	7,138
アセアン諸国	67	1,045	2,298
EU諸国	55	632	2,240
その他	60	1,768	2,966

世界各国への日本茶の輸出状況(2019)

静岡県立大学
茶学総合研究センター　中村順行

日本茶の輸出量と金額の推移

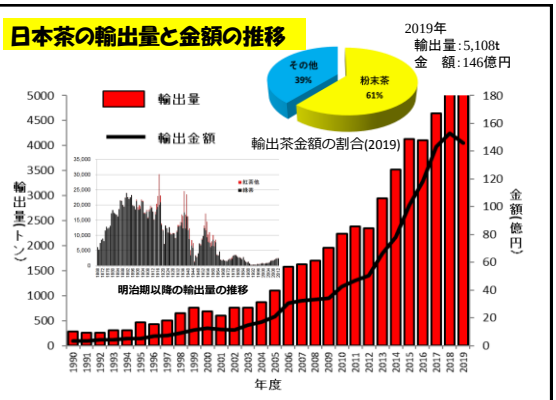
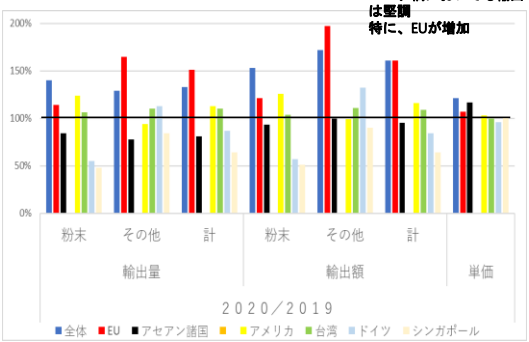


図1　　日本茶の輸出量と金額の推移

コロナ禍における日本茶の輸出状況

コロナ禍においても輸出は堅調
特に、EUが増加



1月から8月期までの2019年と2020年の輸出比較

抹茶は世界のスーパーフード

抹茶には
美味しさと健康性が求められる

抹茶は、
急須で淹れたお茶では摂取できない、プロビタミンA(βカロテン)、ビタミンE(トコフェノール)、食物繊維なども摂取可能。

☆抹茶は美味しい
☆急須で淹れた場合、カテキンの40%程度は茶殻に残るが、抹茶では全てを摂取可能。
☆緑色が美しいのみならず、食品添加素材としても有用。

海外で市販されている抹茶

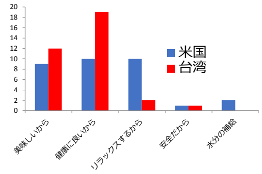
抹茶道具販売(イギリス)

有機日本茶専門店
(オランダ)

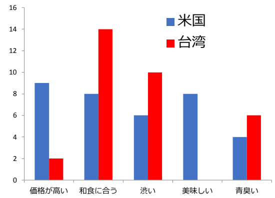
抹茶は食材としての利用が急増

輸出国のニーズは？

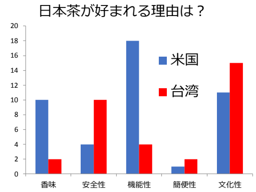
輸出対象国により異なる!?
- 飲食材としての利用
- リーフ茶を飲む国
- ティバッグが主体の国
- 有機栽培茶が好まれる環境
- 機能性に対する評価の高低



日本茶が好まれる理由は？



日本茶に対する評価は？

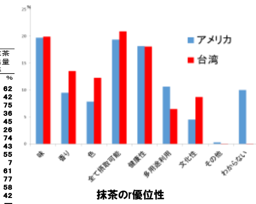


日本茶に期待する点は？

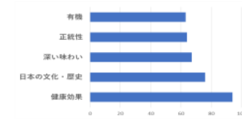
抹茶の輸出と優位性

各国への日本茶の輸出概要

項　目	茶消費量	一人当たり消費量	日本茶輸出量	日本茶輸出金額	輸出kg当り価格	粉末茶輸出量比率
	千トン	kg	トン	百万円	円	%
アメリカ	129	0.40	1,595	6,811	4,271	62
カナダ	17	0.47	205	688	3,347	42
ロシア	253	0.89	15	39	2,555	75
イギリス	110	1.67	35	195	5,570	36
ドイツ	31	0.80	374	1,397	3,740	45
フランス	14	0.21	104	310	2,971	26
イタリア	7	0.12	36	139	3,862	74
オーストラリア	11	0.44	87	280	3,221	43
シンガポール	—	—	307	925	3,008	55
台湾	37	1.37	1,216	1,407	1,157	7
香港	11	1.51	172	862	4,992	61
タイ	—	—	291	541	1,862	77
ベトナム	—	—	72	121	1,688	58
マレーシア	25	0.79	178	323	1,822	42
中国	1,956	1.42	53	124	2,362	—
資料統計年度	2015～2017平均	2015～2017平均	2018	2018	2018	2019．1～8月



抹茶のr優位性



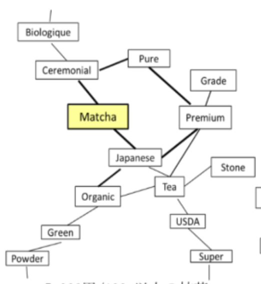
日本産抹茶の魅力(回答者140人)

各国のネットで市販される抹茶の価格

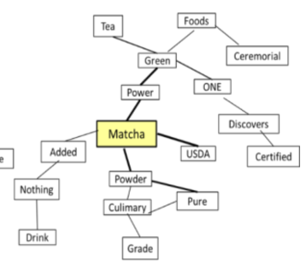
国名	抹茶のみ 点数	抹茶のみ 比率(%)	混合抹茶 点数	混合抹茶 比率(%)	平均価格(円/100g) 全体	平均価格(円/100g) 上位10点	平均価格(円/100g) 下位10点
イギリス	44	88.0	6	12.0	4,266	9,812	1,185
アメリカ	44	88.0	6	12.0	3,636	9,586	609
フランス	41	82.0	9	18.0	4,795	11,412	1,346
ドイツ	45	90.0	5	10.0	4,459	10,808	1,050
台湾	8	26.7	22	73.3	787	1,445	295
シンガポール	32	84.2	6	15.8	2,532	6,850	362
日本	34	77.3	10	22.7	2,042	5,330	304

注 各々の国のアマゾンネットで市販されているMatchaあるいは抹茶の掲出順位別50点(台湾、シンガポールは掲出されたもの全て)程度を調査対象とした。

輸出国の購買行動



5，000円/100g以上の抹茶



1，000円/100g以下の抹茶

価格の異なる抹茶のトレンドサーチ解析

抹茶市場の分類とその概略

分類	概略		該当国
定着市場	日本茶の浸透度が高く、輸出制約も比較的小さく実績の高い国	輸出単価の高い国	米国、香港、カナダ、オーストラリア、マカオ
		輸出単価の安い国	台湾、タイ
制約市場	日本茶への認知度、要望は比較的高いが、輸出障壁が高い国	残留農薬規制	ドイツ、フランス、イギリス、イタリアなどEU諸国
		ハラール	インドネシア、マレーシア、
有望市場	日本茶や抹茶への関心を急速に高め、今後の伸びが期待される国		シンガポール、ベトナム、インドネシア、ロシアなど

定着市場

各々の市場においてさらに輸出を増強するために

1)　ブランディングの強化

・抹茶品質の差別化による優位性の保持

海外産抹茶と日本産高級抹茶との違いは、
・色相角度が高い　　・クロロフィル含量が高い　　・EGCG/EGC比率及びテアニンやアルギニン含量が高い　　・香りが異なること

・機能性エビデンスの付与

抹茶はスーパーフードとして健康機能性が期待される部分も多いため、抗ストレス効果の認められる、テアニンや（(カフェイン＋EGCG)／(テアニン＋アルギニン)）比率などから健康機能性をPRしていくことも重要となる。

・本物志向への対応、情報の発信

ブランド力を強化するためには本物の抹茶を志向する消費者の期待を裏切らない抹茶の供給はもとより、確たるエビデンスを持った健康効果などをプロモーション素材として活用し発信していくことも重要である。

2)　高品質抹茶の低コスト生産

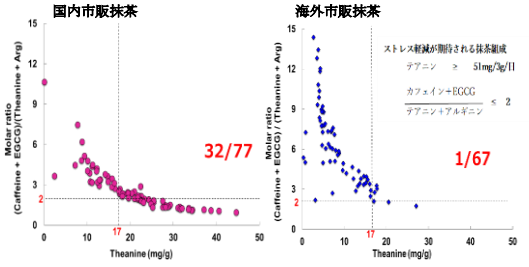
抹茶の需要が拡大するにつれ、従来型のれんがで作られた碾茶炉が最近では効率化を目指したネット型碾茶炉や機械化碾茶炉などへの開発も進んでいる。

日本産抹茶のブランディング強化のために

1．色
　⇒被覆技術、貯蔵技術
2．旨味（テアニン、アルギニン）
　⇒茶期、品種、被覆技術、施肥技術など
3．香り（ジメチルスルファイド、ピラジン　など）
　⇒被覆技術、加工技術
4．機能性（テアニン、アルギニン、EGCG/ EGC）
　⇒茶期、被覆技術、施肥技術
5．その他
　⇒文化性、ヒストリー、保管法、利用方法など

日本産抹茶の機能性での優位性

国内産でも、二番茶期中盤以降の新芽から作られる抹茶ではなかなか優位に立てない



国内外から購入した抹茶におけるストレス軽減の推測

制約市場

1) 欧米向けには、有機栽培抹茶

EU諸国やアメリカなどへの輸出は、残留農薬基準の遵守や、それ以上に有機栽培抹茶の供給が重要となる。

様々な国においてオーガニックの認定制度、認定機関があるが、日本においては有機JASが一般的である。有機JASは、EU諸国やアメリカ、カナダはそれぞれの国の有機制度と同等と認められている。

2) ムスリムに対するハラール認証について

今後、抹茶の輸出が伸びているイスラム諸国ではハラール認証の取得の是非が問われるが、現状では大きな問題はない。

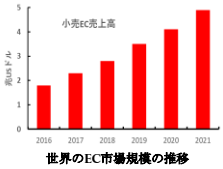
しかしながら、抹茶を食品加工用に使用する場合には、最終商品での認証が必要となる場合もあるので事前確認しておいた方が良い。

各国・地域の有機認証マーク

有望市場

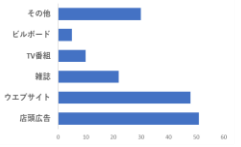
1) ECの活用

最近では、世界的にEC(電子商取引)の市場規模が拡大し、コロナ禍により一気に加速している。対人販売ではないため、パッケージのデザイン性はもとより、商品説明など消費者が知りたいことを端的に表現し、他商品との差別化を図る必要があるため、消費者目線をより重要視した商品企画が重要となる。

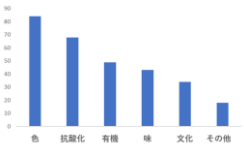


世界のEC市場規模の推移

広告⇒オンライン広告「リスティング広告、ディスプレイ広告、SNS広告など」



抹茶製品の広告を目にした場所(回答者109人)

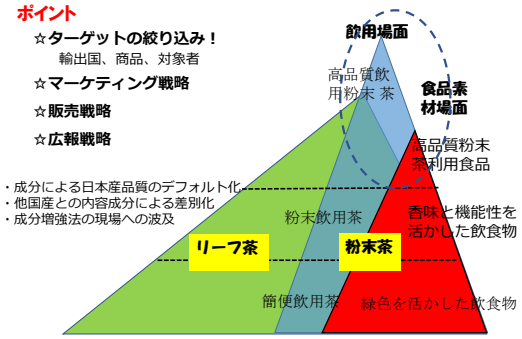


抹茶製品の広告、パッケージで印象に残っていること(回答者　141人)

海外市場に対応したマーケティング戦略

ポイント

- **☆ターゲットの絞り込み！**
  輸出国、商品、対象者
- **☆マーケティング戦略**
- **☆販売戦略**
- **☆広報戦略**



海外茶市場の構造モデルの提示

４．令和２年度　雑誌、新聞記事など

２０２０年７月２日　静岡新聞　朝刊

緑茶効能 研究着手

コロナ感染阻害か確認

県議会答弁

県議会6月定例会は1日、自民改革会議の増田享大氏（掛川市）、ふじのくに県民クラブの四本康久氏（富士宮市）、自民改革会議の中谷多加二氏（浜松市天竜区）が一般質問を行った。志村信明農林水産担当部長は、緑茶に新型コロナウイルスの感染を抑える効能があるかを調べる研究に着手したと明らかにし「本年度中をめどに感染阻害効果とそのメカニズムを明らかにする」と述べた。増田氏への答弁。

県お茶振興課によると、緑茶は県立大の研究でインフルエンザウイルスの感染予防効果が確認されている。新型コロナウイルスに対しては、茶の研究実績が豊富な県立大教授ら専門家が参画し、県環境衛生科学研究所で5月から、培養細胞を使って感染抑制効果を確かめる研究に取り組んでいるという。県茶業研究センターがカテキンなど茶の成分の提供や分析を担う。

志村部長は研究成果が出た時点で学術専門誌に論文を投稿すると表明し「科学的エビデンス（根拠）を有する正確な情報として、県民や茶業関係者に情報発信する」と強調した。海外に向けても、県立大と連携協定を結んでいるカリフォルニア州立大デービス校で研究成果を発表する予定だと説明し「効能を広く伝えて静岡茶の需要拡大を図り、本県茶業を再生する」と意欲を示した。（政治部・宮嶋尚顕）

２０２０年１０月２１日　静岡新聞　朝刊

オンラインで学ぶお茶の入れ方講座

来月6・17日、県茶商

県茶商工業協同組合は11月6、17の両日、インターネットを通じてお茶の入れ方を学ぶ「オンラインお茶講座」を開く。受講無料。

ビデオ会議アプリ「Ｚｏｏｍ（ズーム）」を活用し、日本茶インストラクターがお茶の入れ方やリーフ茶の魅力を伝える。両日とも午前10時半からと午後2時からの全4回。各回定員12人。

希望者は10月22日までに、同組合に電子メールで申し込む。受講者には事前に急須と飲み比べ用の茶を送付する。

問い合わせは同組合へ電０５４（２５４）２５１８〉へ。

２０２０年１１月１８日　静岡新聞　夕刊

茶況

18日

オンラインで茶の特徴紹介

県茶商など講座

オンラインでおいしいお茶の入れ方を伝えた講座の一場面

県茶商工業協同組合と県茶業会議所は17日、インターネットを通じてお茶の入れ方を学ぶ「オンラインお茶講座」を初開催した。4部制で約50人が参加し、静岡茶への理解を深めた。

事前にテキストや一人用急須、飲み比べ用の茶を郵送した。川根や掛川、御前崎など各産地の茶を並べて見せ、それぞれの特徴を紹介したり、品種の違いや適切な茶葉の量、抽出時間を解説したりした。

講師を務めた日本茶インストラクターの岡村由紀子さんは「一煎目は甘みやうま味が強いから低い温度で入れて」と説明。おいしく飲むポイントについては「湯を入れて茶葉が開きかけたころがおすすめ。熱い湯を入れると苦みや渋みが増す」と解説した。

２０２０年７月１１日　静岡新聞　朝刊

県立大の国際的オンライン講義

浜松湖南高生が体験

日本茶テーマ 英語で視聴

浜松市西区の県立浜松湖南高は9日の授業で、県立大が実施しているオンライン講義を視聴した。大学の国際的な講義を体験するのが狙いで、今回が初の試み。英語科の2年生約40人が参加した。

県立大などで行われている講義を視聴する生徒ら＝浜松市西区の浜松湖南高

オンライン講義は文部科学省指定の「大学の世界展開力強化事業」の一環。県立大のほかに上智大、米国の大学が参加している。

この日は日本茶の販売戦略についての講義が進められた。生徒は英語のみの講義映像を視聴しながらノートに内容を書き写すなどした。岩崎典子副校長は「今年は新型コロナウイルスの影響で校外での活動や国際交流が難しい。オンラインならではの教育活動を今後も推進したい」と話した。同校は本年度から2年間、県教委のグローバル・ハイスクールの指定を受けている。（浜松総局・足立健太郎）

２０２０年７月１２日　静岡新聞　朝刊

甘い粉末茶葉 商品化へ

東海大静岡翔洋高 授業始まる

静岡市清水区の東海大静岡翔洋高で11日、甘味付き粉末茶葉の商品開発に取り組む授業が始まった。1年生約80人が来年2月の商品化を目指す。

ウス茶糖と抹茶ミルクの飲み比べをする生徒＝静岡市清水区の東海大静岡翔洋高

〝家族で楽しむ〟テーマ

高校生に地元の特産品である茶の価値を見直してもらい、消費を次世代につなげるのが目的。生活協同組合パルシステム静岡の担当者を講師に、「家族で楽しめる県産のお茶を使った粉末茶葉」を開発する。完成品は地産地消の商品を集めた同社のチラシに掲載する。

初回授業では、原料の茶葉を提供する掛川市の製茶販売会社「山英」の担当者が茶の歴史や販売状況などを説明した。生徒は市販の「ウス茶糖」を水で割ったものと、ミルク成分の入った「抹茶ミルク」を牛乳で割ったものを飲み比べ、味の感想を話し合った。

生徒は今後、市場調査や意見交換を重ね、味や内容量のほか、価格やパッケージデザインも考える。（清水支局・石岡美来）

２０２０年１０月２日　静岡新聞　朝刊

茶況　1日

茶の薬膳スープ 掛川・山英発売

掛川産茶葉など29品目を使った「ホッと、スープ。」

掛川市日坂の製茶販売「山英」は今春、いずれも掛川産の茶葉とクズを使った薬膳野菜スープ「ホッと、スープ。」を発売した。

茶とクズで〝健康長寿のまち掛川〟を目指す加工用原料茶開発促進協議会のプロジェクトで、掛川カレーに続くレトルト2作目。薬膳の手法で29品目を組み合わせ、体の冷えを補い熱をこもらせない効能があるという。監修はホテルクラウンパレス浜松の中華料理店総料理長岡部悟さん。

山英の自社技術で粉末にした茶葉やクズの茎葉はポリフェノールやプエラリンなど健康成分が豊富。山崎元郷専務は「特に冷え性に悩む女性から好評。茶の健康効能に触れ、親しみを感じてほしい」と話す。税抜き480円。

（掛川支局・宮坂武司）

2020年8月26日　静岡新聞　夕刊

NEXT特捜隊
あなたの疑問調べます

夕食の習慣で困った！

夕食時に緑茶を飲むと夜何時間も眠れないことがあります。睡眠と上手に付き合えるお茶の飲み方を知りたいです。

緑茶と睡眠 良い関係は

読者の日常の困り事や疑問を取材、調査する本社「NEXT特捜隊」に、浜松市中区の50代女性から声が寄せられた。睡眠に差し支えのないお茶の飲み方を調べてみた。

女性が眠れないと感じ始めたのは新茶シーズンの5月ごろ。夕食と一緒に緑茶を飲んだら、床に就いても眠れなかったり、夜中に起きた後ぐっすり休めなかったりする日々が続いた。周囲に相談すると、20代や40代の友人が同様の経験があると答えたという。

女性の悩みを解決しようと、県立大茶学総合研究センター（静岡市駿河区）を訪ねてみた。海野祐子客員准教授によると、緑茶には眠気防止につながる成分のカフェインが含まれている。夕方に緑茶を飲むと、カフェインが、眠りにつながる物質「アデノシン」を押しのけるように、脳内にたまっていく。このため、体は疲れているが眠りにくい状態になるという。

体内に吸収されたカフェインは、約5～6時間で半分ほどに減る。海野客員准教授は「就寝の6時間前までに緑茶を飲むと、眠りにくさは解消されるのでは」との見方を示す。カフェインは茶の新芽の部分に多く含まれていることから、「新茶はカフェインの含有量が多い」とも指摘する。

カフェインの抽出量を調整することはできないだろうか。カフェインは入れる湯の温度が高いほど溶け出しやすい。低い温度で入れると、量を比較的抑えることができる。リラックス効果のある成分「テアニン」を含むアミノ酸は、低温度の方が比較的多く溶け出す。

ふじのくに茶の都ミュージアム（島田市）の白井徹副館長は「低い温度の湯や水で入れると、テアニンがカフェインよりも多く溶け出し、快適な眠りにつながる」との見解を示し、「眠れないのが気になるのならば、濃いお茶を避けて、カフェインレスのお茶か水出し茶を選んでみては」と提案する。

ただ、お茶を飲むと眠れなくなるかどうかは個人差もある。

これらの解決策を女性に伝えた。早速試してみたという女性は「就寝6時間前に濃いお茶を飲んだら眠れました。水出し茶も良かったです。効果はありますね」と話した。

（福田雄一）

〈随時調査します〉

情報や調査依頼はLINEや記者コンシェルからお寄せください。
▲LINEの友だち追加

カフェイン半減に6時間

水出しでリラックス効果アップ

英語での実施科目「Japanology」を県内の複数高校に配信　（静岡県立大学　ＨＰ）

海外との交換留学を推進する観点から、本学の外国人留学生の受講を想定して、2016年度より小林裕和客員教授（当時：副学長）が代表教員となり、英語で日本を紹介する「Japanology」を開講しました。本科目は、これまでに米国カリフォルニア大学デービス校、同マルケット大学、上智大学などとオンラインで結び、双方向的な授業を実施しています。今回は、県立浜松湖南高校および県立三島北高校とオンラインで結んで情報を共有し、浜松湖南高校では英語科2年生約40名が参加しました。

中村順行茶学総合研究センター長の尽力により、今回の講師にはStephane Danton氏をお迎えしました。Danton氏はフランス・リヨン生まれ。フランスでソムリエの資格を取り、フランスワインの販売を目的にして、約30年前に来日しました。その際に、日本のお茶に出会い、その魅力に取りつかれ、15年前に日本茶専門店「おちゃらか」を東京に開店しています。Danton氏は、果物、花、ヨモギ、やきいも、昆布といった日本独特の香りを緑茶に付けたオリジナルフレーバーティーを開発しました。「目・鼻・口」で味わう新たなジャンルのフレーバーティーは、若者を中心に大いに受けています。今回は、Danton氏がなぜフレーバーティーを日本で販売しているのか、またそのマーケティング戦略について話していただきました。

Danton氏の講義概要：

マーケティング戦略の中で、一番重要なことは相手とのコミュニケーションを図ること。コミュニケーションなくしては、商品の紹介、相手の好み、どこでどのようにだれとお茶を飲みたいかも分からず、それに適したお茶の紹介もできない。コミュニケーションは母国語で図れるとは限らず、身振り手振りを交えても良いから意思の疎通が重要。これがマーケティングのベースとなる。そしてお茶に付随するそれぞれのストーリーが、付加価値を高める素材となる。お茶を顧客に知っていただくためには、顧客以上の知識が必要であり、猛勉強の日々。それを持続するには、①ゆっくりとあせらないこと、②いつも新しいことを見つけること、③自分で試してみること、④自己満足も含め納得すること、⑤学ぶこと。

英語での質疑応答では、世界販売戦略およびコミュニケーション術について議論しました。欧米およびイスラム社会への売り込みには、日本茶をそのまま輸出するのではなく、彼らの好みを模索し合わせること、お茶に興味を持ってくれれば、ワインのように高級品の購入欲に繋がることなどが話し合われました。

県内高校生に対し、英語を伝達手段として、茶を含む本県主要産業への関心を喚起すべく、本学ではこのような活動を継続していきたいと考えています。

オンライン講義の様子

講師 Stephane Danton氏

浜松湖南高校での視聴の様子

２０２０年５月１０日　産経新聞　朝刊

初夏の香り　安らぐ甘さ

新茶

おいしい新茶のいれかた

1 お湯を沸かした後、70度くらいに下げる

2 急須に茶葉大さじ1杯（6㌘）を入れ、お湯を少しずつで1分蒸らす

3 お湯を200㏄、7~8分目、急須に注ぐ

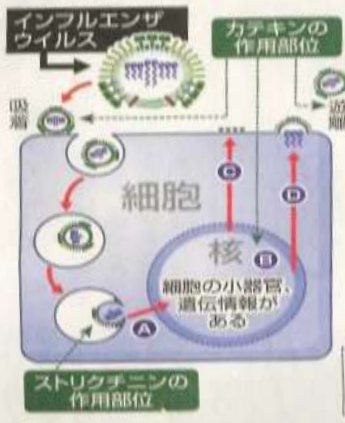
4 1分ほどたったら、湯飲みに注ぐ（最後の一滴まで）

ご感想・情報　▷Eメール　▷FAX 03・5255・6634

２０２０年１１月１３日　中日新聞

緑茶の効能　追究進む

対コロナ 県立大など検証



インフルエンザウイルスの感染の流れと成分の作用部位

県立大・鈴木隆教授監修

県薬剤研究センターと県環境衛生科学研究所、県立大が五月から、新型コロナウイルスの感染を防ぐ効果が緑茶にあるかを共同で研究し、来年三月までの論文発表を目指している。緑茶に含まれるカテキンがインフルエンザウイルスの予防に役立つとする研究成果が多いことなどから、コロナへの予防効果が期待されている。ウイルスに詳しい県立大薬学部の鈴木隆教授にウイルスや緑茶の感染を抑制する作用を解説してもらった。

「カテキン」の働き　鈴木隆教授の解説

人の病気に関連するウイルスは数百種類あるといわれている。大きさや形はまちまちだが、非常に小さい。人の目で見ることができない細菌と比べてもおおむね十分の一以下。人の血液細胞の赤血球がメロン大とすれば、インフルエンザウイルスはゴマ粒くらい。

ウイルスは増殖方法に特徴があり、細胞分裂して増える細菌に対し、ウイルスは単体で増えることはできない。細胞に侵入し、細胞の中にあるものを使って増殖する。細胞を自動車工場に例えれば、工場内で部品を集めて組み立てる仕組みで、「子ウイルス」という新車をつくる。子ウイルスは別の細胞に移って侵入し、増殖を繰り返す。

ウイルスや緑茶の予防効果を解説する県立大薬学部の鈴木隆教授＝静岡市駿河区の県立大で

カテキンはインフルエンザウイルスが細胞に侵入する時に使う突起（スパイク）に作用し、細胞への吸着を防ぐ。細胞内で増えた子ウイルスが細胞から出やすいようにしている酵素の働きも抑えるなど、複数の働きがある。

乾燥茶葉中に0・5％程度含まれるストリクチニンという成分も、インフルエンザウイルスの感染を抑える。この成分はウイルス膜と細胞膜が結合し、二つの膜になる「膜融合」を阻害する。融合しないと、遺伝子が細胞に入れないので感染を防ぐことができる。

ウイルスは遺伝子の構造などにより大きく七グループに分けられる。インフルエンザとコロナはグループが異なるが、ウイルスの表面が脂質の膜で覆われている点は同じ。ウイルス膜と細胞膜の膜融合で感染するのも同じで、似たところがある。

カテキンはインフルエンザとは異なるグループのウイルスでも感染を防ぐ力があることが、試験管による基礎研究で分かっている。カテキンは多くのウイルスの感染を阻害することが報告されていることから、コロナにも働く可能性がある。

Export strategy of "matcha"

日本産抹茶の特性を活かした輸出戦略 第1回目

抹茶の輸出と海外市場の特性

静岡県立大学 茶学総合研究センター
中村 順行

はじめに

リーフ茶の消費減少等により荒茶価格が低下するとともにコロナ禍により、我が国の茶業経営は一層厳しくなっている。一方、海外マーケットに目を向けると緑茶の輸出は増加し、特に、抹茶はスーパーフードとして世界中の関心事であり、日本茶輸出の牽引役ともなっている。海外における抹茶に対するニーズの高まりは他国産抹茶の増大化も含め、飲食物への加工用など多用途への利用が急激に広がり、新たな輸出戦略の構築も必要となっている[1]。

そこで、今回から3回シリーズで、まず「抹茶の輸出と海外市場の特性」を概観し、2回目は「国内外で市販される抹茶の科学的特性」を紹介し、3回目には日本産抹茶の科学的特性や消費国の嗜好、消費、購買特性を踏まえ輸出をさらに堅調に拡大するため「海外における抹茶市場の特性と輸出戦略」を考えたい。

なお、データの大部分は、抹茶の海外市場の飛躍的拡大を目指し、外国産の抹茶・粉末緑茶との違いを明確にし、日本茶ブランドとしての高級抹茶の輸出戦略を構築することを目的とした農林水産省「革新的技術開発・緊急展開事業（先導プロ）」「海外市場の飛躍的拡大を目指す高品質抹茶の低コスト製造技術およびカフェインレス茶系統の開発」（平28～令2）で実施したものである。

抹茶生産と輸出の推移

(1) 抹茶の生産量の推移

近年、抹茶は飲用のみならず加工用など多用途に利用され抹茶の原料であるてん茶の生産量は10年前に比較し倍増の3,464t（2019）となっている[2]。また、世界的に消費が増大するなか、中国、台湾、韓国、ベトナムなど諸外国でも生産されるようになり、「STiR（コーヒーと茶のビジネス雑誌）」（2018）では抹茶は少なく見積もっても2023年までにはアメリカ内で6,000億円市場になると予想している[3]。さらに、正確なデータはないが中国では抹茶生産に本腰が入れられ、ひとつの地域で4,000t

の生産が見込まれるなど、拡大基調が続いている[4]。

(2) 輸出の推移

このような中、日本からの抹茶の輸出も堅調で、全体の輸出量5，108ｔ、146億円（2019）のうち粉末茶が60％程度を占め、日本茶輸出を牽引し（図1）、本年度のコロナ禍においても全体的には昨年以上の伸びを示している。輸出先としては、アメリカを中心に台湾、ドイツ、シンガポール、香港などと続くが、アメリカと台湾で輸出量の56％、金額の55％と大半を占める（図2）。また、

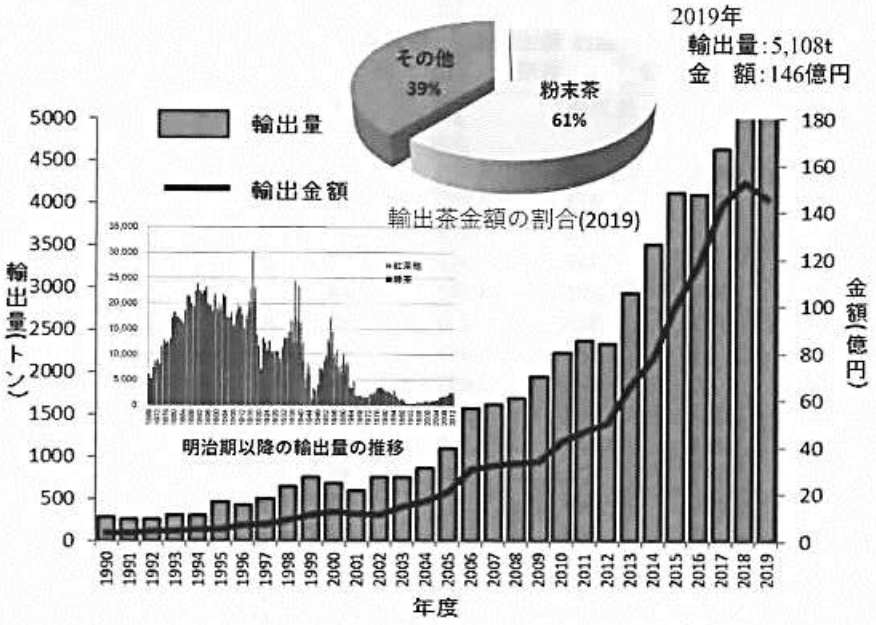
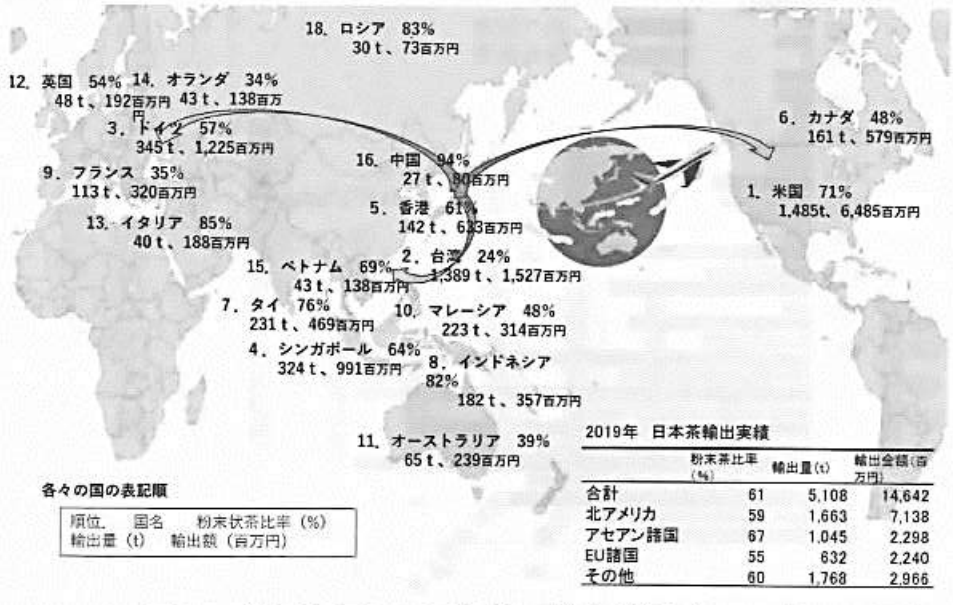


図1　日本茶の輸出量と金額の推移

粉末茶に牽引され輸出国は少なくとも世界70ヵ国以上にのぼり、アセアン諸国での伸びが大きい[1]。輸出の大半を占めるアメリカと台湾では、輸出の様相が異なり、量的にはアメリカが1，485ｔ、台湾が1，389ｔであるが、金額的にはアメリカが6，485百万円（平均単価4，368円／kg）に比較し、台湾は1，527百万円（平均単価1，100円／kg）と少ないのみならず、粉末茶比率もアメリカ71％、台湾21％と大きく異なっている（2019）。世界的にはＥＵ諸国で平均単価が高く、アセアン諸国では安価な傾向にある[2]。



	粉末茶比率（%）	輸出量(t)	輸出金額(百万円)
合計	61	5,108	14,642
北アメリカ	59	1,663	7,138
アセアン諸国	67	1,045	2,298
EU諸国	55	632	2,240
その他	60	1,768	2,966

図2　世界各国への日本茶の輸出状況（2019）

海外市場の特性

(1) マーケットの特性

日本茶の主要な海外マーケットは、表1に示す通り、アメリカ、台湾、ドイツ、シンガポール、タイ、カナダなどであり、それらの国の一人当たりの茶消費量はアメリカ、カナダ、ドイツなどでは380g～480g程度であり、イギリス、台湾、香港などでは1,370g～1,670g程度と国によって大きく異なる[1,2]。

表1　各国への日本茶の輸出概要

項　目	茶消費量	一人当たり消費量	日本茶輸出量	日本茶輸出金額	輸出kg当り価格	粉末茶比率
	千トン	kg	トン	百万円	円	%
アメリカ	129	0.40	1,485	6,485	4,368	71
カナダ	17	0.47	162	579	3,580	42
ロシア	253	0.89	30	73	2,429	89
イギリス	110	1.67	48	192	3,993	57
ドイツ	31	0.80	346	1,225	3,543	43
フランス	14	0.21	113	320	2,833	33
イタリア	7	0.12	40	188	4,679	83
オーストラリア	11	0.44	65	239	3,687	46
シンガポール	—	—	324	991	3,058	52
台湾	37	1.37	1,389	1,527	1,100	21
香港	11	1.51	143	633	4,430	46
タイ	—	—	231	469	2,027	73
ベトナム	—	—	60	105	1,742	92
マレーシア	25	0.79	223	314	1,410	35
中国	1,956	1.42	27	80	2,982	92
資料統計年度	2015～2017平均	2015～2017平均	2019	2019	2019	2020. 1～8

日本茶飲用経験者はアセアン諸国では70%以上と非常に高いが、欧米諸国においては30～40%程度と低く、飲用時に砂糖など何も入れない人はアセアン諸国で60%程度、欧米では30%程度である。また、抹茶のことを知っている人の割合もアセアン諸国では70%以上であるが、欧米諸国では40～50%程度である。日本から輸出される粉末茶比率はアメリカ、ロシア、イタリア、香港、タイなどは60%以上と高いが、フランス、台湾などは30%以下と低い[5-7]。

近年、抹茶はスムージーやラテ、さらには食材としての利用も国内外で増え、加工用抹茶と言われるものも多くなっている。そのため、価格的にも100g当たり1,000円以下のものから茶道

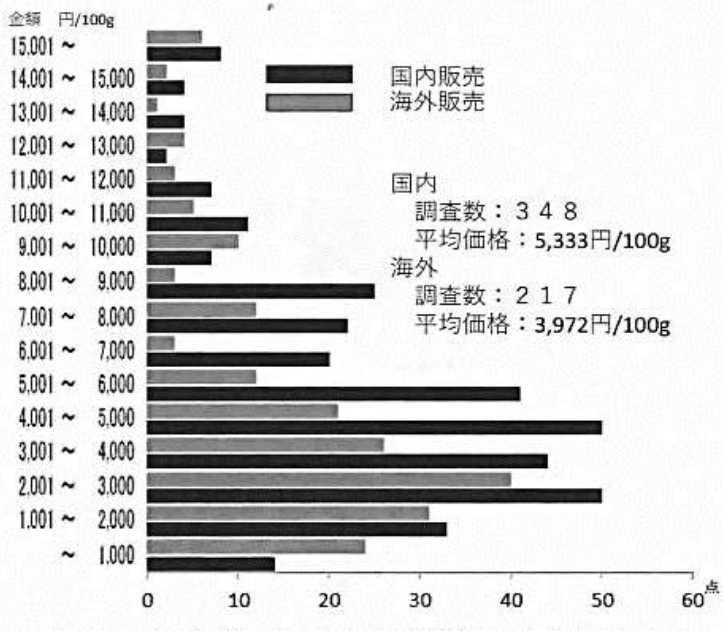


図3　国内外で市販される抹茶の金額別比率

用の2万円以上のものまで幅広い価格帯で販売され、国内では2,000~6,000円／100g程度の比率が高く、調査した348点の平均単価は5,333円／100gだった。一方、海外では3,000円／100g以下の安価なものが多く、平均単価も3,972円／100g（n＝217）だった（図3）。

概して、国内外で市販されている高価格帯の抹茶は20~30gの缶入りのものが多く、その大部分は日本産であるが、低価格帯のものはアルミ袋入りのものが多く、中国産や韓国産のものもある。さらに、低価格帯の抹茶には粉末茶などの混合物や甘味料の添加されたものも数多くみられる。

(2) 嗜好、消費、購買特性の違い

・嗜好性

アメリカや台湾において抹茶を選ぶ際には、香味や価格が重視される。なかでも、他のお茶に対して抹茶は「香味に優れる」「すべてを摂取可能」「健康に良い」などとともに「色が綺麗」「多用途利用ができる」「文化的」などの利点を持ち、高い嗜好性を有している（図4）。また、抹茶と粉末茶との違いを認識できる人も約半数と多く、その見分け方として、味（41%）、香り（23%）などの香味で評価する人が多い。

・消費特性

抹茶の飲用法としては、アメリカ・台湾とも水や湯に溶いて飲む人が約半数程度と多く、牛乳などの他の飲料に混ぜて飲む人も30%以上あり（図5）、これらの人は、「とても美味しい」「美味しい」と評価していた。また、抹茶を使用した飲食物についてもクッキーやチョコレートなどの菓子類、スムージーやラテなどの飲料を70%以上が知っていて、半数以上が食べたり、飲んだりしたことがあった。また、そのような抹茶を利用した菓子類、飲料、食べ物に対して「とても好き」「やや好き」が90%以上と高かった。

日本茶の飲用場所は欧米諸国では概して自宅が多い

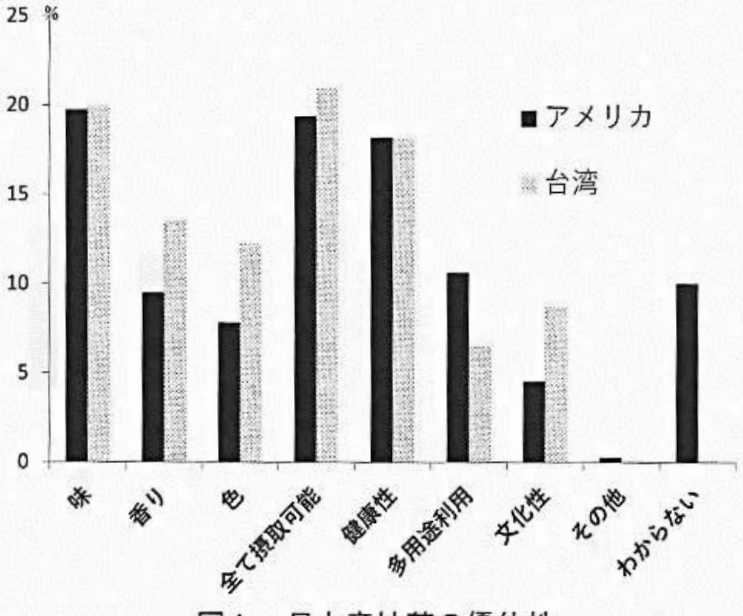


図4　日本産抹茶の優位性

購入希望額であったが、台湾では538円、310円、267円といずれも米国に比較して低かった（表2）。

アメリカで販売されている高価格帯（5，000円以上／100g）と低価格帯（1，000円以下／100g）の抹茶とのトレンドサーチでは、高価格帯では

表2　抹茶関連商品の購入期待価格

	米国	台湾
	円	円
抹茶のみ(円/100g)	4,739	538
抹茶ラテ	599	310
抹茶アイス	631	267

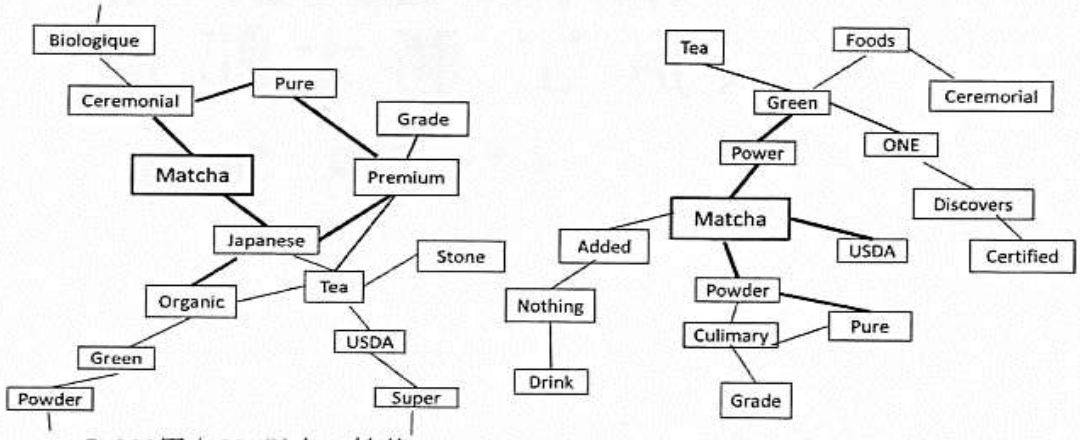


図7　米国で市販される抹茶のパッケージに見られるキーワード
（※パッケージに記載されている文章のトレンドサーチ解析、太線ほど関連性が高い）

「Ceremonial」、Japanese」、Premium」、Pure」などが、低価格帯では「Power」、Green」、Powder」、USDA」が、重要なキーワードとして解析され、価格の違いによる商品への期待感の違いが明らかであった（図7）。また、国内において販売されている抹茶のトレンドサーチでも高級抹茶と加工用抹茶の二種類に大きく分類された。高級抹茶には宇治や西尾の産地名や缶入り、有機栽培などが、加工用抹茶には食品、業務用、袋入りなどがキーワードとして上げられ、明らかに用途も別物と考えられた。

次号では、国内外で市販されている抹茶の科学的特性を紹介する。

（なかむら　よりゆき）

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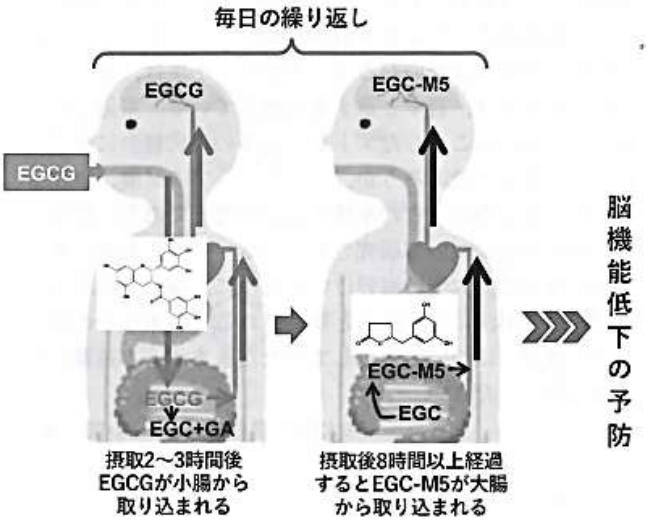
2）公益社団法人日本茶業中央会(2020)、令和2年度版茶関係資料．
3）STiR Matcha (2018)、http://stir-tea-coffee.com/tea-report/matcha-what-is-it/ (April 11, 2018)
4）Zhejiang Online Reporter (2018)、"争"出抹茶産業新高度
5）日本茶輸出促進協議会(2016)、輸出先国での日本茶消費実態調査
6）JETRO(2013)、日本食品に対する海外消費者意識アンケート調査（中国、香港、台湾、韓国、米国、フランス、イタリア）7か国・地域比較
7）日本茶輸出促進協議会(2017)、米国における抹茶消費動向調査報告書
8）農林水産省(2006)、平成18年度調査農林水産物・食品輸出マニュアル

カテキン
緑茶カテキンが脳の老化を予防する

海野けい子
静岡県立大学茶学総合研究センター



キーワード：カテキン，エピガロカテキンガレート，認知機能，加齢，カテキン代謝物

はじめに

緑茶を毎日飲むことが認知症や軽度認知障害の予防につながることが，これまでの疫学調査の結果から明らかとされてきている[1]．緑茶の主要な成分はカテキン類，カフェインおよびテアニン等のアミノ酸類であるが，緑茶中のどのような成分がどのように脳に作用しているのかについてはまだ明らかとなっていない．ここでは緑茶カテキンの脳に対する作用について，われわれが行ってきた研究の概要や最近の研究動向を紹介する．さらにカテキン類の代謝分解物も生体機能に重要な作用を及ぼしている可能性が報告されていることから，あわせて概説する．

緑茶に含まれるカテキン

カテキン類は緑茶葉（乾物）中10～18%を占める成分である．その中で緑茶カテキンとして最も量が多いのがエピガロカテキンガレート（EGCG）で，全体の50～60%を占める．次いでエピガロカテキン（EGC），エピカテキンガレート（ECG），エピカテキン（EC）等が含まれている（図1）．カテキン類は強い抗酸化作用を示すことから，活性酸素による酸化的傷害から生体を防御する食品成分として主にEGCGを中心に多くの研究が行われてきており，種々のがんに対する作用，糖尿病や肥満，心血管疾患，ウィルス等の感染症に対する作用等について多くの論文および総説が出されている．脳に対する作用では，加齢に伴う機能低下やアルツハイマー病等の神経変性疾患に対するカテキンの作用等がこれまでに報告されその有効性が明らかにされつつあるが[2]，その作用機序の解明にはまだ至っていない．

連載開始にあたって：お茶成分の脳における作用

ヒトの脳は地球上の生物の中で最も発達している．約145億個の神経細胞から成り，酸素やエネルギーとしてブドウ糖を多く消費し，呼吸，体温調整，睡眠といった生命活動はもちろんのこと，運動や言葉，感覚，意識など，あらゆる活動をつかさどる器官である．大脳新皮質は他の生物に比べ大脳の中でも大きな割合を占め，人間の進化過程で巨大化した新しい脳の部分である．また，大脳新皮質の中でも前頭連合野は，人間が人間らしい能力を発揮する主要な部分で，五感から集まった情報を整理・統合，理解し，それに基づきさまざまな価値を判断，次にどう行動するかといった高機能な司令塔の役割をする．

大脳が発達したヒトは高度な社会生活をおくるがゆえに，多様な外的要因に右左と振り回されている．外的要因には良い刺激もあれば，悪い刺激もあり，おおむね悪いほうが多いのが現実ではなかろうか．ヒトは悪い外的要因を受け続けると心が病み，その対応策として“こころの健康”といったケアが必要となる．国の政策の一つである“健康日本21”においても，国民の“休養・こころの健康”について健康な生活を確保するための取り組みについて述べられている．こころの健康とは，いきいきと自分らしく生きるために重要な条件である．さらに，人生の目的や意義を見いだし，主体的に人生を選択すること（人間的健康）も大切な要素であり，「生活の質」に大きく影響するものである．こころの健康には，個人の資質や能力の他に，身体状況，社会経済状況，住居や職場の環境，対人関係など，多くの要因が影響し，なかでも，身体の状態とこころは相互に強く関係している．個人をとりまく外界が変化すると，それまでと違ったやり方で新たに対応することが要求される．このような外界の変化はストレスと呼ばれ，さまざまな面で変動の多い現代は，ストレスの多い時代であるといえる．外界に起きた変化に適応しようとして内部にストレス反応とよばれる緊張状態が誘起される．ストレスの影響を強く受けるかどうかには個人差があるが，過度のストレスが続くと，精神的な健康や身体的な健康に影響を及ぼすことになり，その結果睡眠不足を引き起こすことにもなる．また近年の高齢化社会が進行するなか，生活習慣病のみならず，アルツハイマー病などの認知症の増加が社会問題となっている．長期縦断的な認知症の有病率調査を行った平成26年度厚生労働科学研究費補助金特別研究事業「日本における認知症の高齢者人口の将来推計に関する研究」のデータでは，認知症と診断された人は2012年で462万人（有病率15.0％）であったのが，各年齢の認知症有病率が一定と仮定しても2025年では675万人（有病率19.0％）となり，40年後の2060年には4人に1人の850万人と推計され，想像するとおぞましい現実に向き合うこととなる．もし，自分が認知症になったらと思うとゾッとするし，やはり“ピンピンコロリ”が理想の人生の終焉であると思う．

お茶は鎌倉時代に中国から日本に伝わったとされ，栄西禅師による喫茶養生記には「茶也養生之仙薬也延齢之妙術也」と記されており，健康に役立つことが経験的に知られている．小生は30歳ごろから20年来茶道を習っているが，お茶の先生は健康で若々しく，心穏やかで，また認知症といった病気を患ったことをあまり聞いたことがない．これもお茶の効果ではないだろうかと思う．小生は，自分に足らない栄養素は，自ずと欲すると感じている．茶道を長く続けているのはこうしたストレス過多の現代社会に生活しているためなのであろうか．カテキン，アミノ酸，カフェインなどはお茶に含まれる機能成分の代表であり，数多くの生理的作用について研究されている．今回，お茶の産地である静岡県立大学の海野けい子先生に，現代をとりまく社会問題の解決に寄与すると期待される“お茶成分の脳における作用”について解説していただきます．本連載にどうぞご期待ください．

（太陽化学株式会社　小関　誠）

カテキン摂取による脳機能ならびに寿命への影響

カテキンの脳に対する作用を調べるため，加齢に伴い脳機能が低下しやすい老化促進モデルマウスSAMP10を用い，飲水としてカテキンを自由摂取させ，このマウスにとって初老期に相当する11月齢の時点で学習能への影響を調べた．ここで用いたカテキン（EGCG, EGC, ECG, EC等の混合物）の濃度は，私たちが通常飲んでいる緑茶の1/3程度の濃度である0.02％とした．ただし，マウス（体重30～35g）の摂水量が多かった（10mL/日）ことから，これは60mg/kgに相当した．マウスが暗いところを好む性質を利用し，暗室に入ったら弱い電気ショックを与えて，暗室が安全な場所ではないことを学習させる（受動回避による記憶獲得）試験では，通常の水を摂取していたコントロールマウスに比べ，緑茶カテキンを摂取していたマウスでは加齢に伴う学習能の低下が有意に抑制されていた[3]．

そこで次に緑茶カテキンの脳機能と寿命への影響を，1～60mg/kgの濃度で比較した．カテキン1mg/kgは，ヒトでは緑茶1杯程度に相当する．これまでにカテキンによる寿命延長効果がハエや線虫で報告されているが，マウスではこれまで報告されていなかったことからSAMP10マウスを用いて調べた．その結果マウスが1mg/kgの緑茶カテキンを飲水として毎日摂取した場合，通常の水を

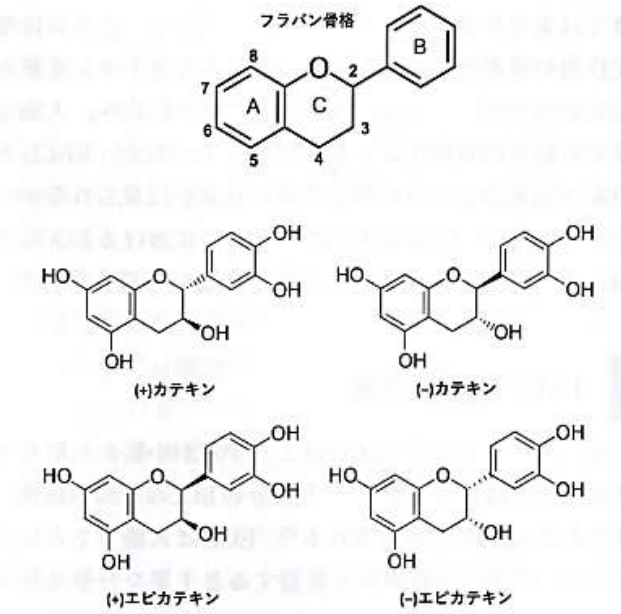
◇◇◇ コラム ◇◇◇

カテキンは，フラボノイドと呼ばれる植物が生産する二次代謝産物の一種である．茶のカテキンとしては，1929年に辻村みちよ*らによって初めて緑茶抽出物よりエピカテキンが結晶状に単離された．カテキンの化学構造の特徴は，フラバン骨格C環の2位と3位の炭素が不斉炭素となっていることであり，このためたとえばカテキンであれば，(+) カテキン，(−) カテキン，(+) エピカテキン，(−) エピカテキンの4種の立体構造が異なる形をとることができるが(図)，緑茶に含まれるカテキンはほとんどは (−) エピ体である．

またB環の水酸基が2個の場合と3個の場合があり，2個のものはカテキン，3個のものはガロカテキンと呼ばれる．カテキンやガロカテキンの多くは3位の水酸基に没食子酸が結合して存在し，カテキンガレートやガロカテキンガレートと呼ばれる．カテキン類は通常のフラボノイドと異なり，糖と結合した配糖体としては天然にはほとんど存在しない．

カテキンは茶葉に特異的に多く含まれるが，カカオや，ブドウ，リンゴ，モモ，ナシ，マンゴー，ネクタリン，プラム，ラズベリーなどの樹木性果実にも含有されている．ただし，(+) カテキンや (−) エピカテキン，(−) エピガロカテキン等の没食子酸を含まない遊離型カテキンが主体となっている．

* 日本初の女性農学博士



図■カテキンの構造

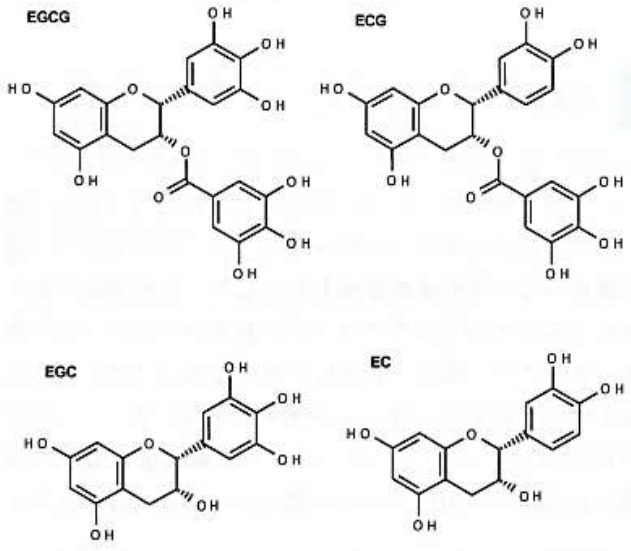


図1■緑茶中に含まれるカテキン類

飲んでいたコントロール群に比べ生存期間中央値 (MST) が有意に延長することが認められたが，カテキンの濃度が高くなるとむしろ寿命延長効果は減少したことから[4] (表1)，SAMP10の寿命延長に関しては高用量のカテキンは必要ではないことが示された．

一方脳機能に関しては，受動回避試験による記憶獲得においてはコントロール群に比べカテキン1mg/kg以上で有意な効果が認められたが，15mg/kgで最も効果が認められ，長期記憶保持能は60mg/kgで有意に高まっていた．Y迷路を用いた空間作業記憶に関しては30mg/kg以上で有意に高まっていた．カテキン類の脳内への移行は血液脳関門により制限されていることから，脳内のカテキン量は末梢に比べかなり低いと考えられる．加齢に伴う認知機能の低下抑制に関しては，カテキンを少なくとも1mg/kg以上毎日摂取する必要があり，濃度に依存してより高まることが示唆された[4]．

カテキン各成分間で作用を比較すると，EGCGは脳機能の低下抑制効果があったが，同じ濃度のEGCでは改善効果が見られなかった．この両者の違いはガレート基

表1■緑茶カテキン摂取による生存期間の変化

緑茶カテキン (mg/kg)	Median survival time (months)		*p* Value
	Month	Ratio	
0	10.8	1.00	—
1	17.2	1.59	0.027*
5	15.3	1.42	0.272
15	15.3	1.42	0.082
30	15.3	1.42	0.364
60	13.6	1.26	0.880

(*p*-Value is based on Log-rank test.) (文献4より)

の有無であるが，ガレート基をもつ没食子酸（GA）だけでは効果がなかった[5]．カテキンの作用としては抗酸化作用の重要性が指摘されている．実際カテキンを摂取していたマウスではコントロールマウスに比べ，大脳皮質での脂質の過酸化が有意に低下していたが，EGCGとEGCでは脳における抗酸化作用には違いは見られなかったことから[5]，抗酸化作用だけでは脳におけるEGCGとEGCの作用の違いを十分に説明できないと考えられた．

EGCGの吸収と代謝

経口的に摂取したEGCGはごく少量が小腸から取り込まれ血中に移行するが[6, 7]，大部分のEGCGは腸内細菌によりEGCとGAに分解される[8]．EGCは大腸でさらに分解され，摂取後8時間以上経過すると主要な分解産物として5-(3′,5′-dihydroxyphenyl)-γ-valerolactone（EGC-M5）や5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactoneが生成することがヒトやラット，マウスで報告されている[8, 9]．これらはさらにグルクロン酸抱合体（EGC-M5-GlcUA）や硫酸抱合体（EGC-M5-Sul）となる（図2）．GAはピロガロール（PG）を経てグルクロン酸抱合体（PG-GlcUA）となる．これまでの研究から，EGCGの代謝分解物は大腸から吸収され血流を介して体内を循環したのち，尿中に排泄されているものと考えられている．

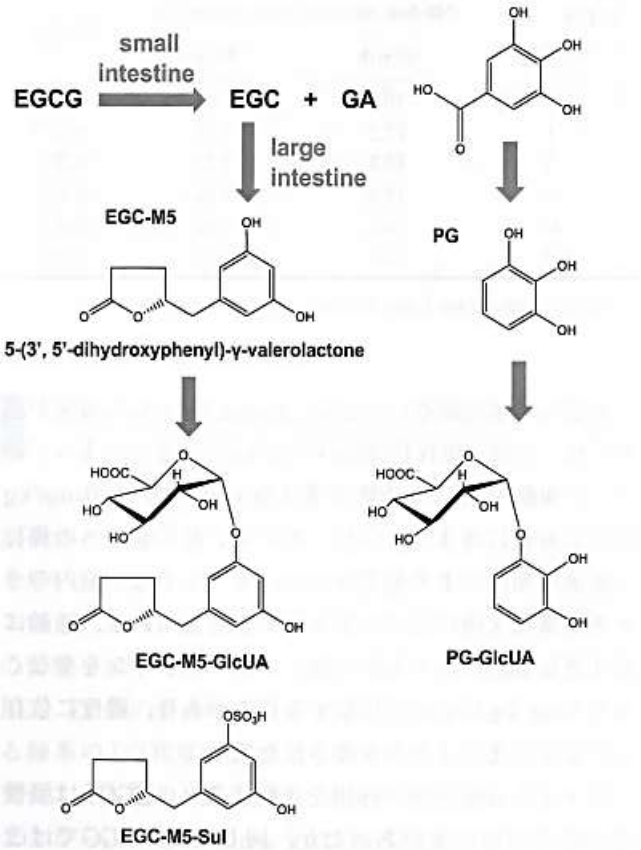


図2■EGCGおよびその代謝分解物

カテキンの血液脳関門透過性

カテキンが脳で作用するためには血液脳関門（BBB）を通過する必要があるが，これまでにEGCGがBBBを通過して実際に脳実質に至っていることは確かめられていなかった．そこでインビトロのBBBモデル（RBT-24，ファーマコセル(株)）を用い，カテキン類が血管側から脳実質側にどの程度移行できるか調べた．その結果EGCGは30分間で4.0%，EGCは5.0%移行することがわかった[5]（表2）．これはGAやカフェインに比べれば低いが，確かにEGCGもEGCも脳実質に入ることが示唆された．これまでに同様なインビトロのBBBモデルを用い（+）Catechinの1時間当たりの透過率が7.4%，ECが15.4%であると報告されており[10]，3位の水酸基の立体構造の違いがBBB透過性に大きく影響することが示されている．EGCGはGAが高い透過性を示すことから，分子が大きいにもかかわらずEGCと同程度の透過性を示すのではないかと考えられる[5]．一方，EGCにGAを共存させた場合は，単独のときに比べEGCの透過率はかなり低下した．またEGC-M5はEGCGやEGCよりやや高い透過率を示し，抱合体になると透過率がやや低下した[11]．

培養細胞を用いた検討

脳内に取り込まれたカテキン類の作用を検討するため，ヒト神経芽細胞腫のSH-SY5Y細胞にEGCG等を作用させた．その結果50nMで作用させたとき，神経細胞の分化の指標となる神経突起が最も長くなり，神経突起の数も多くなることが見いだされ，その作用はEGCGで最も顕著であった[5]（図3）．EGCGに次いでEGC-M5も神経突起を伸ばす作用を示すことが明らかとなった[11]．EGCGのBBB透過率に基づくと，マウスの脳機能に効果が認められたときの脳内のEGCG濃度は50nM程度になると

表2■カテキンおよびその代謝分解物の血液脳関門透過性

Catechin & its metabolites	BBB permeability（%）(0.5h)
EGCG	4.00±0.17
EGC	4.96±0.55
GA	9.42±1.01
EGC-M5	5.34±0.23
EGC-M5-Sul	4.34±0.40
EGC-M5-GlcUA	3.72±0.01
Caffeine	13.43±1.31

（文献5, 11の値を修正）

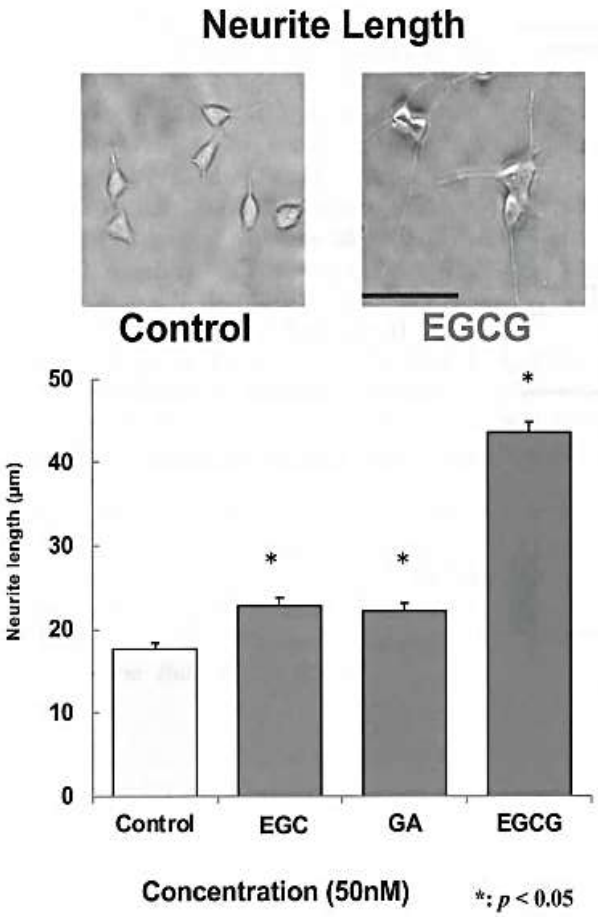


図3■カテキンによる培養神経細胞の分化誘導（文献5より）

推測されたことから，マウスの脳内においてEGCGは神経細胞の分化を促しているのではないかと考えられた．

脳内の遺伝子発現変化

実際に緑茶カテキンを1カ月間摂取していた2月齢のSAMP10マウスについて海馬での遺伝子発現を，通常の水を摂取していたコントロール群のマウスと比較してみた．その結果，最初期遺伝子と呼ばれる一連の遺伝子の発現が有意に高まっていることが明らかとなった[4]．たとえば*Nr4a1*（nuclear receptor subfamily 4, group A, member 1），*Fos*（FBJ osteosarcoma oncogene），*Egr1*（early growth response 1），*Npas4*（neuronal PAS domain protein 4），*Cyr61*（cysteine-rich with EGF-like domain 2）などである．最初期遺伝子は細胞への刺激に応答して速やかに発現が誘導される遺伝子であり，そのmRNAやタンパク質は神経活動の分子マーカーとして広く利用されていることから，緑茶カテキンを摂取していたマウスでは神経活動が若齢時に高まっていたことが示唆された．FosやEgr-1, Npas4はシナプス可塑性に関与すること，Nr4a1はシナプスや樹状突起の分布や密度を調節していること，Cyr61は海馬ニューロンの分枝に必要であることなどがこれまでに報告されている．これら遺伝子の発現は6月齢では緑茶カテキン摂取群でコントロール群より高い傾向が見られたが，12月齢では両群に違いは認められなかった[4]（図4）．神経が発達する若齢時にこれら遺伝子の発現が高まることが重要ではないかと考えられる．では緑茶カテキンがどのようにしてこれら遺伝子の発現を高めているのだろうか？これら最初期遺伝子の転写誘導が生ずる共通の因子として，神経細胞においては細胞外からのカルシウムイオンの流入が考えられている．一方，EGCGは海馬においてカルシウムシグナルを調節することが報告されている[12]．これらのことを併せて考えると，海馬内に取り込まれたEGCGは細胞内へのカルシウムの流入を増加させて最初期遺伝子の発現を高め，結果的に加齢時の脳機能の低下を抑制しているのではないかと考えられる．

EGCGとその代謝分解物の脳に対する作用

経口的に摂取したEGCGは摂取後2～3時間のところではごく少量が小腸から取り込まれ血流を介して脳に至り，海馬内の遺伝子発現を変化させ神経細胞の分化を促進していることが示唆された．その後EGCGは速やかに排泄されてしまうが，8時間以上経つと，EGCGの代謝分解産物であるEGC-M5が大腸から吸収され，血流を介して脳に至り，これも神経細胞の分化を促進するものと考えられる．これまではカテキン類のバイオアベイラビリティは低い（2～8%）と言われていたが，腸での分解物を含めて考えると40%ほどになることが報告されている[13]．EGCGとその代謝分解物の脳機能に対する作用が実際どの程度であるかまだ比較することはできないが，EGCGに次いでEGC-M5も神経突起を伸ばす作用を示すことが明らかとなったことから，EGCGとその代謝分解物であるEGC-M5の両方が時間差をもって作用することにより，加齢に伴う脳機能の低下を抑制しているのではないかと考えられる．実際に，カテキン類に共通した代謝分解物として5-hydroxyphenyl-γ-valerolactone-sulfateが実験動物の脳内で見いだされたことが報告されており[14]，カテキン類（Flavan-3-ols）に共通した代謝分解産物としてphenyl-γ-valerolactoneやphenylvaleric acidsの重要性が指摘されている[15]．カテキン代謝物の作用については，ここで示した神経細胞に対する作用以外に血圧[16]，免疫能[17]，糖代謝[18]などで重要な作用を及ぼしていることがこれまでに報告されており，今後さらにその生理作用が明らかになってくるものと期待される．

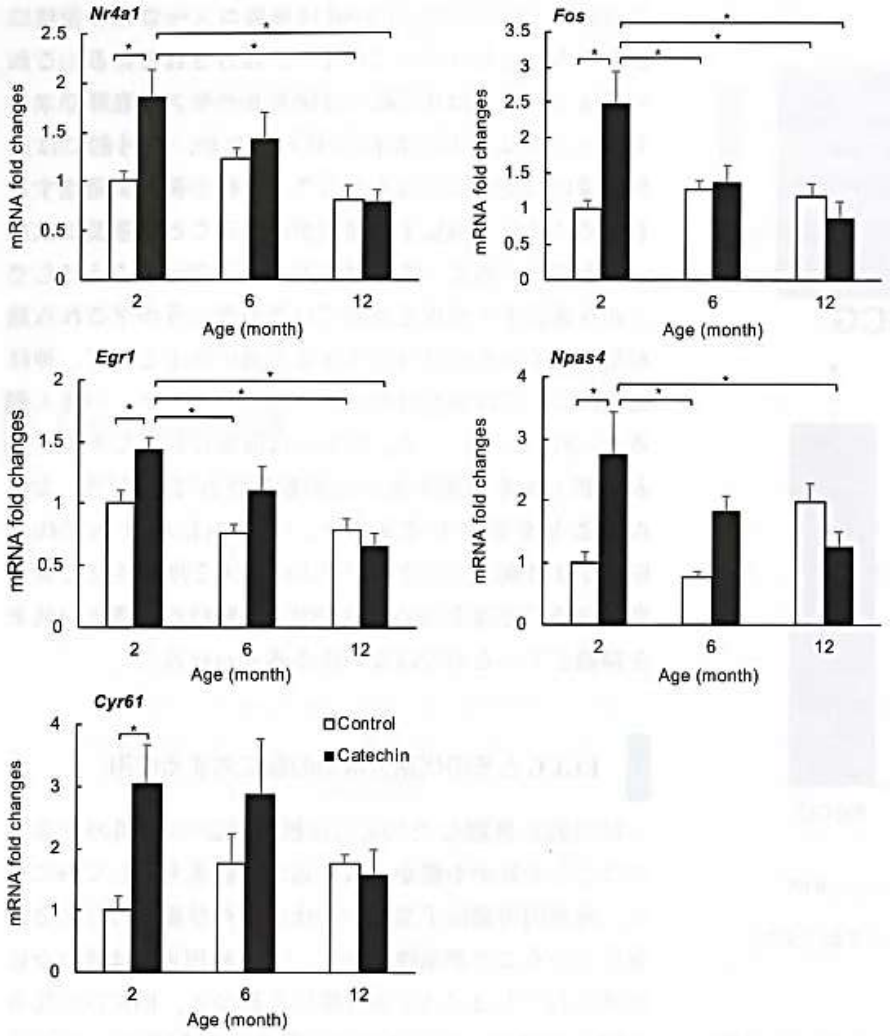


図4■海馬における最初期遺伝子の発現（文献4より）

一方，カテキン類のバイオアベイラビリティには個人差が大きいことも報告されている[13]．腸内細菌の組成には個人差が大きいことから，腸内細菌叢の違いがカテキンの吸収・代謝に大きく影響を及ぼすとともに，カテキンを連続的に摂取することにより腸内細菌叢の組成も変化すると考えられる．緑茶の脳機能に対する作用を明らかにするためには，代謝分解物を含めたカテキンの作用についてさらに検討する必要があると考えられる．

まとめ

緑茶中のカテキンは主にEGCGとその代謝分解物が主要な成分として脳に作用し，神経細胞の分化を高めることにより脳の老化を予防しているものと考えられる．今後，カテキンの代謝分解物の作用を含めた更なる解析が必要である．脳機能に対する食品成分の作用に関しては，腸内細菌叢との関連を視野に入れた幅広い研究が必要となってくるものと考えられる．

謝辞：カテキン代謝物に関する成果は，三井農林(株)食品総合研究所の南条文雄，高垣晶子，原(寺脇) 彩との共同研究により得られたものです．

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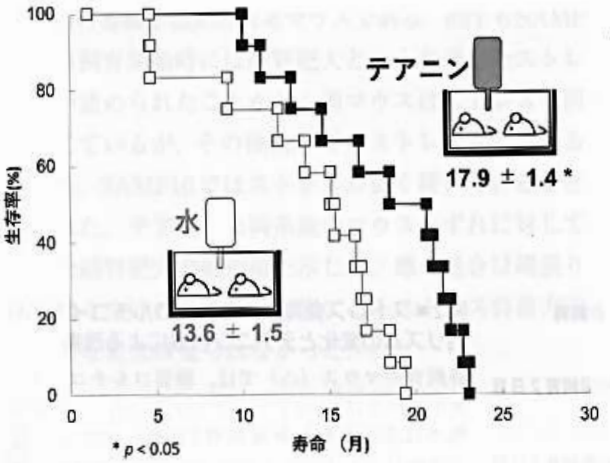
SEMINAR ROOM

セミナー室　　お茶成分の脳における作用-2

テアニン
テアニンによるストレス軽減

海野けい子
静岡県立大学・茶学総合研究センター



キーワード：テアニン，ストレス，寿命短縮，脳萎縮，海馬

はじめに

テアニン（図1）は脳内の神経伝達物質の一つであるグルタミン酸と構造が似ていることから，脳内で何らかの生理作用を示すと考えられ多くの研究が行われてきた．実験動物を用いた研究で，テアニンが腸から吸収された後，血液脳関門を介して脳内に取り込まれることや[1]，ドーパミン等の脳内神経伝達物質に影響を及ぼすこと[2]，記憶力改善作用，カフェインによる興奮作用の抑制作用，脳神経細胞保護作用等[3]が見いだされ，また培養細胞を用いて神経細胞新生への関与が報告されている[4]．ヒトにおけるテアニンの作用としてはこれまでにリラックス作用[3]，ストレス軽減作用[5,6]，うつ病・統合失調症の症状軽減作用[7]等が報告されている．ここではテアニンのストレス軽減作用に焦点を当て，その作用機構についてこれまでに明らかとなったことを紹介する．

社会心理的ストレスの負荷による副腎の肥大とテアニンによるその抑制

適度なストレスは必要であり良い効果をもたらすと考えられているが，ストレスが長期にわたり負荷された場合，「うつ」や気分障害，心血管系疾患，加齢関連疾患などさまざまな疾患の発症や悪化をもたらすと考えられている[8,9]．オス動物の縄張り意識を利用した「対面飼育」という方法でのストレス負荷は，互いの存在がストレスとなることから，ヒトでのストレスに近い社会心理的ストレスがマウスにおいても負荷されることとなる[10]．

生体にストレスが負荷されると興奮性のシグナルを経て視床下部，下垂体，副腎皮質からなるHPA軸（hypo-

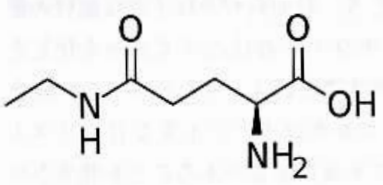
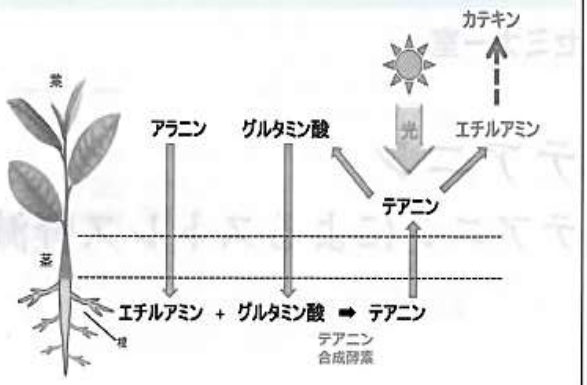


図1■テアニンの構造

◇◇◇ コラム ◇◇◇

テアニンは玉露から発見されたアミノ酸の一種で，1950年に酒戸弥二郎により化学構造がγ-glutamylethylamideであることが明らかとされた．茶に含まれるアミノ酸であることから，茶の旧学名“*Thea sinensis*”にちなんで“Theanine（テアニン）”と命名されたと言われている．茶以外には，キノコの一種や近縁のサザンカに僅かに含まれているだけであり，テアニンは茶に特有のアミノ酸である．乾燥茶葉中でアミノ酸類は1～8%に相当するが，その中でテアニンは約半量を占め，また茶葉が含有する窒素の過半を占める．テアニンは根で生成され幹を経由して葉に蓄えられるため(図)，吸収したアンモニア態窒素を茶樹にとって安全な形態にして蓄積するためにテアニンを合成していると考えられている．テアニンは太陽光の下でカテキンを中心としたポリフェノールに代謝転換されるので，玉露や抹茶の原料となる茶葉は，収穫の前（最低2週間程度）日光を遮る被覆を施すことにより，テアニンの分解が抑制され高い状態に維持される．先端の若芽ほどテアニン含有量は高く，良質の春茶・一番茶は夏茶の二番茶，三番茶より多い．テアニンは緑茶の主要な旨味成分であり，テアニン含量と緑茶の品質には正の相関が見られる．日本では1964年に食品添加物として指定されている．



図■テアニンの合成と分解

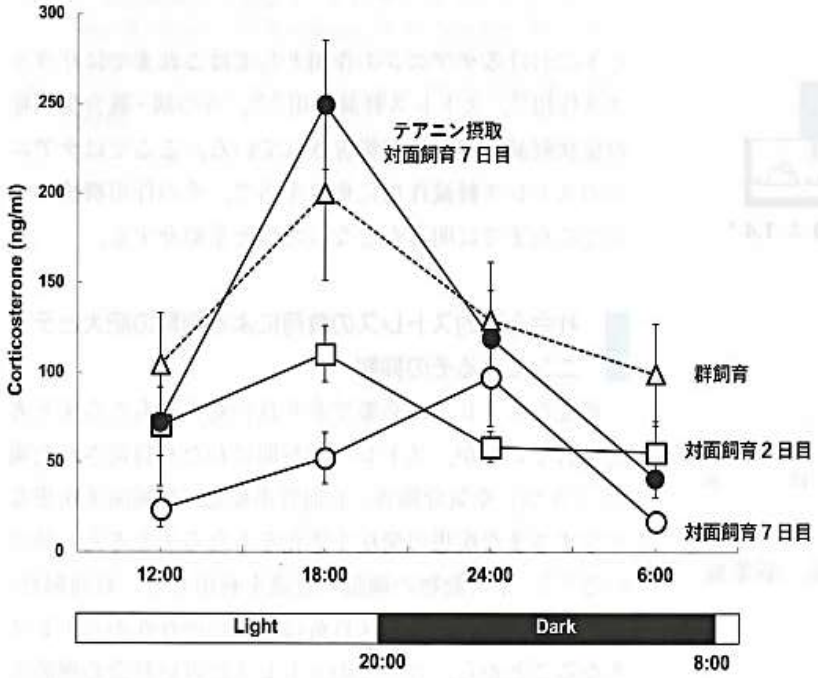


図2■ストレス負荷による糖質コルチコイドの日内リズムの変化とテアニン摂取による改善

群飼育のマウス（△）では，糖質コルチコイドの血中レベルは18:00をピークとする日内リズムが観察される．しかし対面飼育2日目のマウス（□）では糖質コルチコイドの血中レベルが低下し，対面飼育7日目のマウス（○）ではピーク時間に変化が生じた．一方，対面飼育条件下でもテアニンを摂取していたマウス（●）では正常な日内リズムが観察された．（文献10より）

thalamic-pituitary-adrenal axis）の活性化によりホルモン分泌の変化や副腎肥大といったストレス応答反応が見られる．実験で一般的に使用されているddYという系統のマウスを用いたとき，対面飼育条件下では副腎の肥大とともに糖質コルチコイドの日内リズムが変化したが，テアニンを摂取することによりそれらが正常状態となった（図2）．糖質コルチコイドの正常な日内リズムは脳におけるシナプス形成に重要であることが報告されており[11]，ストレスによるホルモンの日内リズムの乱れは脳機能低下の一因と考えられる．テアニンはHPA軸の正常化を介してストレス軽減作用をもたらしていることが示された．

対面飼育による副腎の肥大は，これまでに調べた雄マウスすべての系統で観察されたが，ddYマウスの場合は対面飼育10日目以降で有意な肥大が見られなくなった[10]．一方，老化促進モデルマウスSAMP10では対面飼育開始7カ月の時点でも有意な副腎の肥大が認められた[12]．SAMP10は通常のマウスに比べ寿命が短く，加齢に伴い脳機能の低

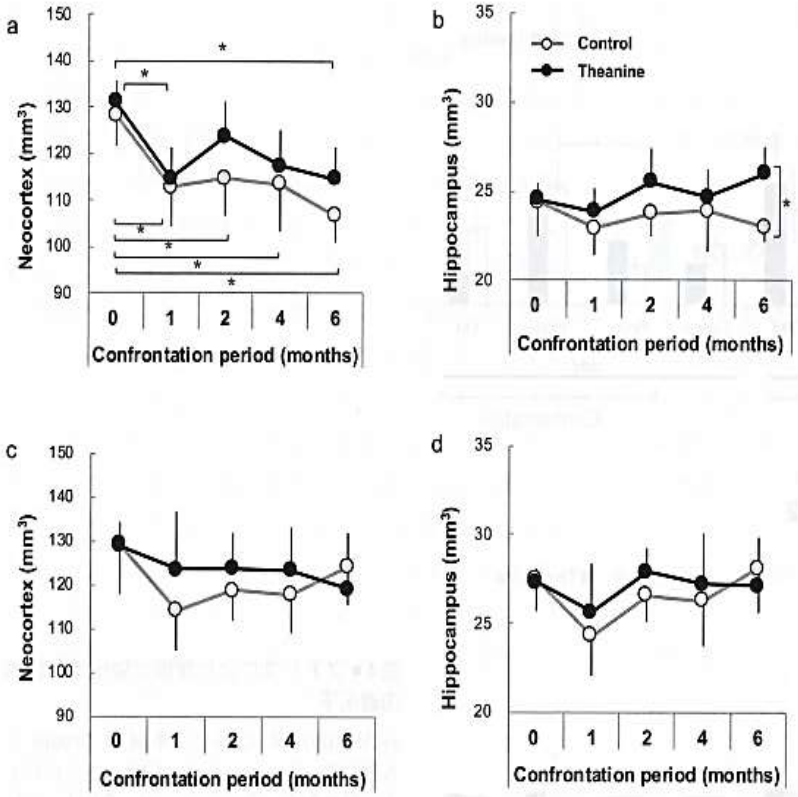


図3■対面飼育下のマウスの脳容積の変化

SAMP10の大脳皮質（a）および海馬（b），ddYの大脳皮質（c）および海馬（d）について，対面飼育を0，1，2，4および6カ月間行ったときの変化を示す．テアニン（6mg/kg）は飲水として毎日自由摂取させた．コントロール群は水を摂取した．*，$p<0.05$．（文献18より）

下や脳の萎縮が認められるマウスである．ddYもSAMP10も対面飼育開始時には副腎肥大という共通したストレス応答が認められたことから，両マウスはストレスを同様に感じているが，その後ddYではストレスが軽減するのに対し，SAMP10ではストレスが長く続いていることが示された．テアニンは両系統のマウスいずれに対しても優れた副腎肥大抑制作用を示した．雌の場合は縄張り意識が強くないので，対面飼育によるストレス負荷方法では顕著な変化は見られなかった．

ストレスの負荷による寿命短縮とテアニンによるその抑制

SAMP10マウスの平均生存期間は，通常の飼育条件である群飼育のとき（17.6±1.2月齢）に比べ対面飼育条件下では有意に減少し（13.6±1.5月齢），ストレス負荷により生存期間が3/4に短縮された[12]．しかし同じストレス条件下でもテアニンを含む水を摂取していたマウス（6mg/kg）では，生存期間が群飼育の場合と同程度まで延長した（17.9±1.4月齢）[12]．群飼育のSAMP10にテアニンを与えても平均生存期間に変化は認められなかったことから（16.9±1.4月齢），テアニンはストレスを軽減することにより寿命の短縮を抑制したと考えられた．一方ddYでは寿命の短縮は認められないことから，SAMP10はストレスに脆弱な系統であり，ddYはストレス耐性（レジリエント）の系統であると考えられる．

ストレスに対する感受性には個人差があり，同じ条件下でもストレスを強く感ずる場合とそうでない場合があることはよく知られているが，ストレス感受性の違いが何に起因するのかについてはまだ十分には解明されていないことから，この2系統はストレス感受性の違いを研究するのに適した実験動物であると考えられる．

ストレス負荷による学習能低下の促進と脳の酸化傷害の蓄積

SAMP10は11月齢以降になると有意に学習能が低下するが，8月齢の時点ではまだ学習能の低下は観察されない．しかし対面飼育条件下では8月齢の時点で既に脳機能が低下しており，ストレスにより脳機能の低下も促進されることが見いだされた[12]．一方テアニンを摂取していた対面飼育群のマウスでは，脳機能の低下は認められなかった．

脳は大量の酸素を消費することから，代謝の過程で多くの活性酸素種（ROS）を産生するため酸化傷害を受けやすい[13]．9月齢の時点で大脳皮質DNAの酸化傷害の程度（酸化傷害のマーカーとして8-oxodeoxyguanosineのレベル）を比較した結果，対面飼育群では同じ月齢の群飼育のマウスに比べ有意に酸化傷害が高まっていることが見いだされた[12]．SAMP10では通常のマウスに比べ若齢時から脳内で産生されるROSが多いこと[14]，ならびに抗酸化酵素の中のグルタチオンパーオキシダーゼの

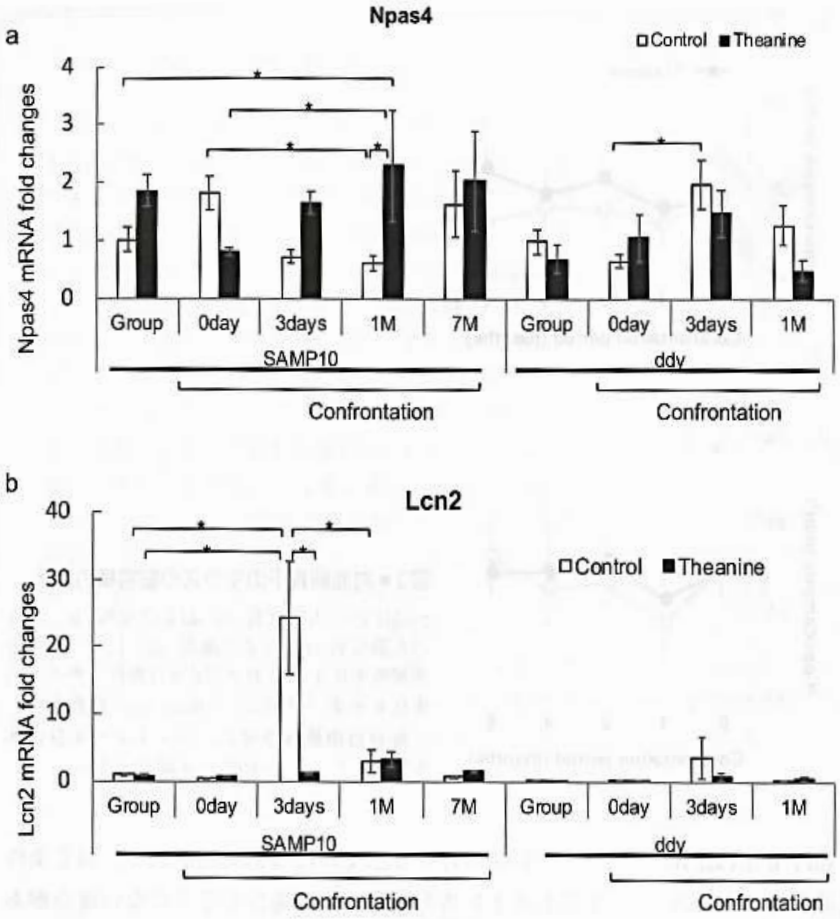


図4■ストレスにより発現が変化する脳内の遺伝子

SAMP10の海馬において，群飼育（Group）の水摂取群（Control, □）の発現レベルに対する，対面飼育（Confrontation）を0日，3日，1カ月および7カ月間行ったときの変化を，テアニン（6mg/kg）摂取群（Theanine, ■）ならびにddYと比較した．*, $p<0.05$.（文献18より）

活性が高齢マウスで低下していること[15]等によりDNAの酸化傷害が加齢に伴い蓄積しやすい．しかしテアニンはカテキンのような直接的な抗酸化作用を示すとは考えられず，テアニン摂取によるDNAの酸化傷害抑制作用は，脳内におけるROS産生／消去のバランス保持に対し間接的に作用しているものと考えられる．

ストレスによる脳の萎縮とテアニンの作用

強いストレスを経験したヒトほど脳の萎縮が生じていることや[16]，虐待を受けた子供で脳の萎縮が生じていること[17]が報告されている．これまで脳の萎縮に関しては適切な実験モデルは確立されていなかったが，SAMP10では加齢に伴い大脳が萎縮するだけでなく，ストレス負荷により脳の萎縮がさらに促進することが見いだされた[12]．そこでストレスによる脳の萎縮がいつ頃から始まり，またどのような部位で生ずるのか明らかにすることを目的として，核磁気共鳴装置（MRI）を用いて対面飼育を1カ月，2カ月，4カ月および6カ月行ったマウスを用い脳の詳細な検討を行った[18]．その結果，SAMP10では対面飼育によるストレス負荷開始1カ月後に大脳皮質において顕著な萎縮が生じており，その後，さらに萎縮が進行することが明らかとなった．テアニンを摂取していた場合もストレス負荷1カ月後に顕著な萎縮が観察されたが，2カ月後の時点で萎縮がいったん回復した（図3a）．海馬においてはストレス負荷1カ月後に萎縮する傾向が認められたが，テアニンを摂取していた場合はその後萎縮が回復し，6カ月後の時点でテアニンを摂取していなかったコントロール群に比べ，有意に海馬が大きくなっていた（図3b）．一方ddYの大脳皮質ではコントロール群で対面飼育開始1カ月後に萎縮の傾向が認められたが，その後回復した．テアニンを摂取していた場合は萎縮が認められなかった（図3c）．海馬でも同様であった（図3d）．これらのことからストレスによる脳の萎縮は早期に生じ，テアニンはストレスによる脳の萎縮の抑制および回復に関与していること，ならびにSAMP10とddYではストレスによる脳の萎縮にも違いがあることが示された．

脳内の遺伝子発現変化

ストレスの脳に対する影響が早期に認められたことから，脳内でどのような変化が起きているのかを調べるた

め，ストレス負荷3日目の時点での海馬での遺伝子発現の変化をDNAマイクロアレイにより網羅的に解析し，その結果を基にリアルタイムPCR法で経時的な変化等の詳細な比較検討を行った．その結果コントロール群のSAMP10の海馬では，*Npas4*（neuronal PAS domain protein 4）の発現が有意に低下していたが，テアニンを摂取していた群ではその低下が抑制されていた[18]（**図4**a）．一方ddYではコントロール群でむしろ*Npas4*の発現は増加し，テアニン摂取群ではそれがやや低下する傾向が見られた．最初期遺伝子の一つである転写因子Npas4は神経活動依存的なシナプス形成に重要な役割を果たすとともに，不安，抑うつ様行動や学習行動に密接に関与していると考えられている[19]．ラットに慢性的ストレスを負荷したとき海馬での*Npas4*の発現が低下するが，ストレスに脆弱な個体に比べてストレスに強い（レジリエント）個体では*Npas4*の発現が回復したことが報告されている[20]．SAMP10はストレスに脆弱であるが，テアニン摂取で*Npas4*の発現低下が抑制されたことによりストレス耐性が高まったと考えられる．

主要な興奮性神経伝達物質であるグルタミン酸は，視床下部の室傍核にあるグルタミン酸受容体を介してHPA軸を調節している[21]．これまでに，脳内に取り込まれたテアニンはグルタミントランスポーターに強力に作用し，グルタミン酸の供給を抑制することにより過剰興奮を抑制していることが考えられている[22]．Npas4も神経の興奮／抑制の調節に関与していることから[23]，主要な興奮・抑制の神経伝達物質であるグルタミン酸やγ-アミノ酪酸（GABA）がテアニン摂取により実際に脳内で変化しているのか今後明らかにする必要がある．

Lcn2（lipocalin 2）はストレス負荷によりSAMP10では顕著にその発現が増加し，テアニン摂取により発現が抑制されていた[18]（図4b）．一方ddYではその発現増加はわずかであった．Lcn2はさまざまな脳の病態において変化した活性化アストロサイトから放出され，神経に傷害を引き起こしたり炎症を増大させたりすることから，神経細胞死が高まることが報告されている[24]．過剰な*Lcn2*の発現増大は，SAMP10における脳萎縮の重要な要因となっていると考えられる．テアニンによる*Lcn2*の発現抑制は，加齢や神経変性疾患に伴う脳内の慢性的な炎症の抑制においても重要かもしれない．

ヒトにおけるストレス軽減効果

テアニンが実際にヒトでもストレスを軽減できることは，これまでに明らかとなっている[5, 6]．ストレスの指標として心拍数や唾液免疫グロブリンA，あるいは唾液アミラーゼ活性の変化を基に調べたところ，軽いストレスが負荷されるようなときにテアニンを飲んでいるとストレスが軽減されることが明らかとなっている．

まとめ

ストレス感受性が高いマウスでは，ストレス負荷により寿命の短縮に加え，脳の萎縮，脳機能の低下促進等，脳の老化が促進されることが明らかとなった．テアニンは海馬において*Npas4*や*Lcn2*の発現を変化し，ストレス軽減・脳の萎縮抑制をもたらしていることが示唆された．ストレスに対する感受性には個人差があるが，テアニンの適切な摂取は心身の健康維持に有用であると考えられる．

謝辞：MRIを用いたストレス負荷による脳の萎縮に関する成果は，東北大学の住吉　晃博士（現放射線医学総合研究所）らとの共同研究により得られたものです．

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緑茶
緑茶のストレス軽減および抗うつ作用

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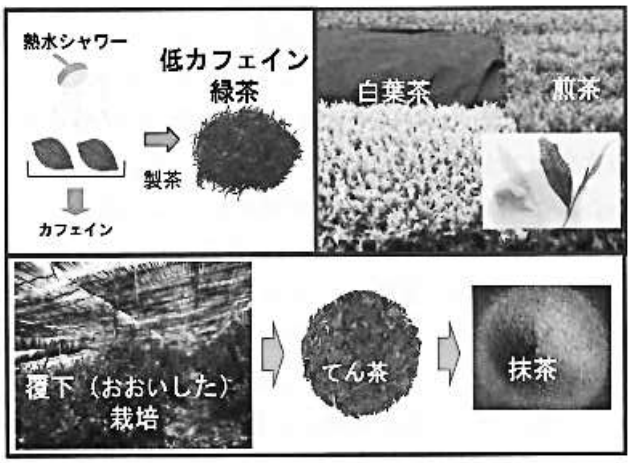

キーワード：抗ストレス作用，抗うつ作用，低カフェイン緑茶，抹茶，白葉茶

はじめに

これまでに緑茶中の主要なアミノ酸であるテアニンに着目し，そのストレス軽減作用について実験動物を用いた研究や臨床研究で明らかにしてきたが，緑茶を摂取した場合にもテアニンと同様のストレス軽減効果が期待できるか検討した．その結果テアニンの抗ストレス作用は，緑茶の主要成分であるカフェインやエピガロカテキンガレート（EGCG）の共存により大きく阻害されることや[1]，テアニンについで多く含まれるアミノ酸のアルギニンがテアニンの作用に協同的に作用することを見いだした[1]．そこで緑茶中のカフェインを低下させた「低カフェイン緑茶」を水で淹れることによりEGCGの溶出を低下させた場合や，テアニンを多く含む抹茶や白葉茶についてストレスに対する作用を検討したので紹介する．

緑茶成分間の相互作用

生体にストレスが負荷されると典型的なストレス応答反応として副腎肥大が生ずることを利用して，実験動物を用い緑茶成分のストレス軽減作用ならびに成分間の相互作用を検討した．マウスを用いてオスの縄張り意識を利用したストレスを負荷したとき，テアニンによる副腎肥大抑制作用はカフェインあるいはEGCGの共存で強く抑制されることが明らかとなった[1]．一方ガレート基をもたないエピガロカテキン（EGC）は，低濃度ではストレスを軽減する作用を示すとともに，テアニンに対しては何ら妨害作用を示さないことが明らかとなった[1]．またテアニン以外の遊離アミノ酸について検討したところ，グルタミン酸やグルタミンにはストレス軽減効果は認められなかったが，アルギニンは優れたストレス軽減効果を示すことが明らかとなった[1]．そこでテアニン，カフェイン，EGCG，EGCおよびアルギニンについて共存による影響を検討した．ここで用いたテアニンの投与量（3.2mg/kg）は，ヒトでは成人で200mgに相当し，

◇◇◇ コラム ◇◇◇

緑茶には主要な成分としてカテキン類，カフェイン，テアニンなどの遊離アミノ酸類が含まれており，それらが渋み，苦味，旨みの成分として緑茶の味を決めている．これらに加え，茶葉には食物繊維，ビタミンC，カロテン，ビタミンE，ミネラルなどの栄養素も豊富に含まれている．

緑茶は，摘み取った茶葉を加熱によって酵素活性を失活させて成分の酸化を防ぎ，緑色を保たせた不発酵茶である．一方同じ茶葉を原料としているが，茶葉中の酸化酵素の働きにより発酵を完全に行ったものが紅茶であり，一部を酸化させ緑茶と紅茶の中間の半発酵の状態のものがウーロン茶である．さらに緑茶には一般的な茶葉を用いた煎茶と，収穫前の20日程度を遮光した玉露やてん茶がある．てん茶はその後石臼で微細粉末にして抹茶となる．煎茶，玉露および抹茶では，上級茶と呼ばれるものほど全窒素，カフェイン，全遊離アミノ酸類およびテアニン含有量が多い．また煎茶に比べ遮光処理を行う玉露や抹茶では，カフェインおよびテアニンを含む遊離アミノ酸量が多い．表に緑茶中の一般的な化学成分含有量を示した．番茶にはきちんとした定義はないが，ここで示した番茶は一番茶，または二番茶以降に摘み取られたお茶，または一番茶・二番茶の新芽を摘んだのち，茶樹の下の方に残っている葉を摘んだものを言う．ほうじ茶は番茶や煎茶を約200℃で数分間焙焼したもので，一般的な緑茶に比べ香ばしい香りをもつ．ほうじ茶では加熱によりカフェインが一部昇華するが，ゼロになるわけではない．番茶などを材料としているので，煎茶や玉露に比べればカフェイン量は低くなっている．番茶，ほうじ茶にはテアニンはほとんど含まれていない．

表■緑茶中の化学成分含有量（乾物中）

茶の種類	全窒素（%）	カフェイン（%）	全遊離アミノ酸（g/100g）	テアニン（g/100g）	カテキン類（%）
煎茶	4.45~6.03	2.77~3.49	1.46~3.53	0.61~1.98	14.5~12.9
玉露	5.18~6.31	2.90~4.04	2.64~5.36	1.34~2.65	14.1~10.8
抹茶	5.38~6.36	3.23~3.85	3.40~5.80	1.17~2.26	6.5~6.2
番茶	3.83	2.02	0.77	—	12.45
ほうじ茶	3.46	1.93	0.20	—	10.37

（煎茶・玉露・抹茶は下級～上級茶の値，番茶・ほうじ茶は中級茶の値である）
（茶の科学，村松敬一郎　編，朝倉書店より引用）

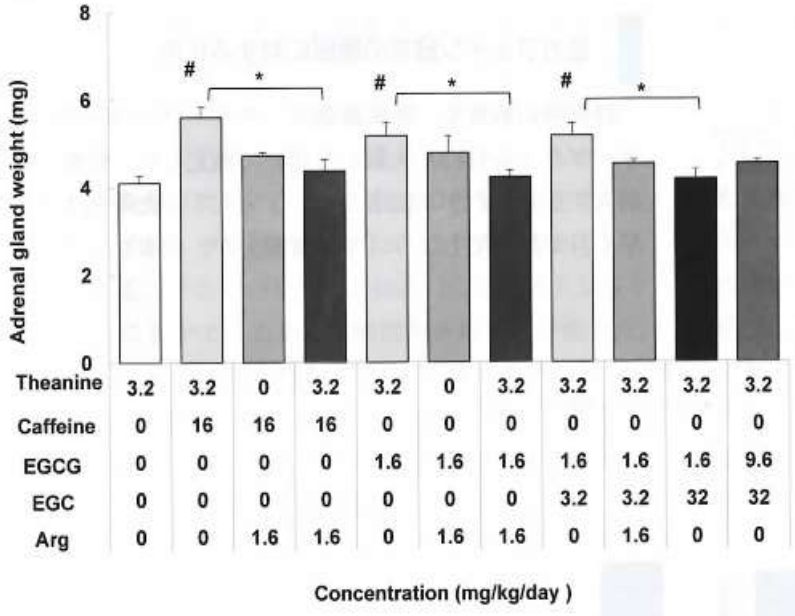

図1■テアニンの副腎肥大抑制効果に対するカフェイン，EGCG，EGCおよびアルギニンの作用

データはmean±SDとして示した．#, $p<0.05$（テアニン（3.2mg/kg）と比較），*, $p<0.05$.（文献1より）

これはサプリメントとしてテアニンが一般的に用いられている用量である．アルギニン量は，緑茶中におけるアルギニンがテアニン量の1/2程度であることに基づき設定した．その結果，テアニンに対して5倍量のカフェインが共存した場合，テアニンによる副腎肥大抑制効果が打ち消されてしまうことが示された（**図1**）．カフェイ

ンに対し1/10量のアルギニンだけでは十分ではないものの，テアニンとアルギニンの両者の共存においてカフェインの作用が打ち消され副腎肥大が抑制されることが示された（図1）．EGCGに対しても，テアニンとアルギニンが協同的に作用することが明らかになった．またEGCもテアニンと協同的に作用することが見いだされた（図1）．

低カフェイン緑茶によるストレス軽減効果

テアニンのストレス軽減作用に対し，カフェインやEGCGが抑制的に作用することから，緑茶中のカフェインおよびEGCGを低下させることにより相対的にテアニンの効果が高まることが期待された．そこで，摘み取った茶葉を熱水で処理することにより茶葉中のカフェインを当初の1/3～1/4に低下させた「低カフェイン緑茶」を作製した[1]．カフェインやガレート基を有するEGCGは低温では顕著に溶出量が低下するが，アミノ酸類やEGCは水温の影響がほとんど見られないことから[2]，水で淹れる「水出し」とすることによりEGCGの溶出量は低下し，相対的にテアニン，アルギニン，EGCの溶出量は高まった状態となる．このようにして作製し製茶した低カフェイン緑茶を用い，20代，40～50代（中高齢者）および80～90代（高齢者）の参加者に低カフェイン緑茶の「水出し」を飲んでいただき，ストレスや睡眠への影響を観察した[3-5]．ストレスの程度は唾液中のアミラーゼ活性を測定することにより評価するとともに（唾液アミラーゼモニター，ニプロ(株)，大阪），アンケートにより主観的ストレスや疲労感の程度を調べた．

その結果，いずれの年代においても対照群に比べ低カフェイン緑茶を摂取していた群で，唾液アミラーゼ活性により評価したストレスが軽減していることが明らかとなった（図2）．また20代では主観的なストレスの程度をVAS（Visual Analogue Scale）を用いて数値化して評価した場合に，低カフェイン緑茶摂取群で有意にストレスの程度が低下していた[3]（図3）．中高齢者では疲労の程度を，厚生労働省が策定した「労働者の疲労蓄積度診断チェックリスト」を用いて評価した結果，月曜の朝の疲労感が低カフェイン緑茶摂取群で有意に低下していた[4]（図3）．金曜日の夕方の疲労感には違いは認められなかった．月曜の朝に不調を感ずる人は多くいることから，低カフェイン緑茶のこの抗疲労効果は重要であるかもしれない．

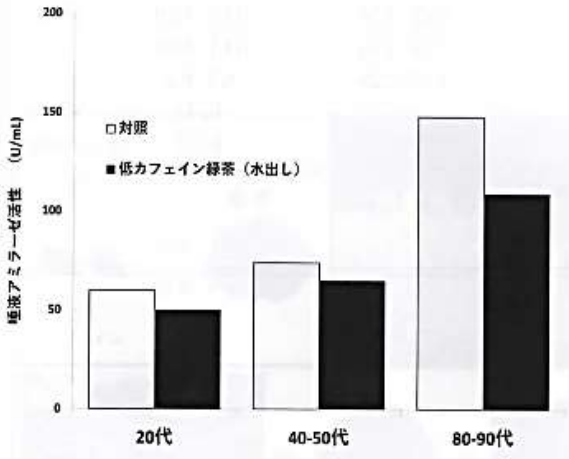

図2■低カフェイン緑茶によるストレス軽減効果

低カフェイン緑茶摂取群では，対照群に比べ唾液アミラーゼ活性の増加が抑制されていた．低カフェイン緑茶（3g）に水500mLを加えた．対照群として20代では麦茶（水出し），40～50代では一般煎茶（水出し），80～90代では煎茶（湯）とした．（文献3～5のデータを改編）

低カフェイン緑茶の睡眠に対する作用

睡眠時の脳波を，簡易睡眠計（スリープスコープ，スリープウェル(株)，大阪）を用いて測定した．睡眠には個人差があったが高齢者では，予定していた時間よりも早く目が醒めてしまう「早朝覚醒」が，低カフェイン緑

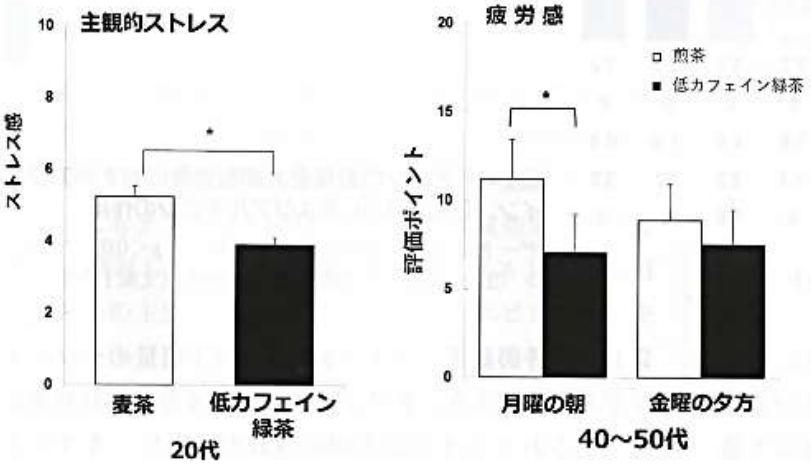

図3■低カフェイン緑茶による主観的ストレスと疲労感への効果

データはmean±S.E.M.として示した（*, $p<0.05$）．（文献3, 4より）

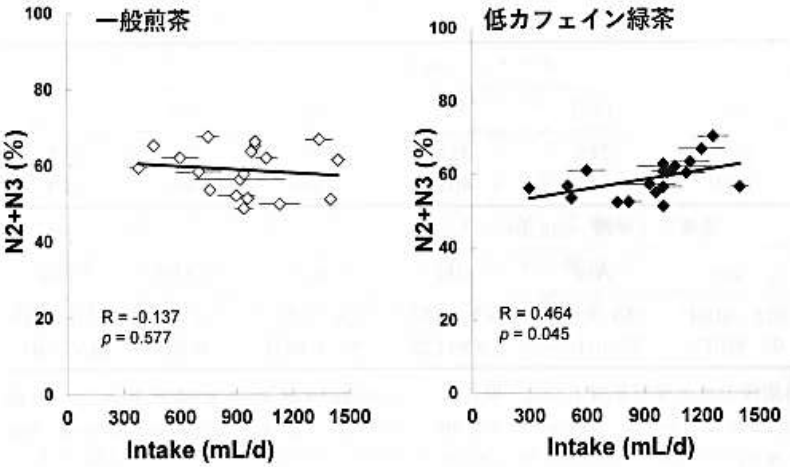

図4 ■ 低カフェイン緑茶によるノンレム睡眠の増加
睡眠時におけるノンレム睡眠の割合が高いほど，深い眠りとなっていることを意味する．N2およびN3はノンレム睡眠を示す．（文献4より）

茶摂取群で抑制されていた[5]．中高齢者でも時間は短いが同様の効果が認められた[4]．また高齢者では，通常の緑茶を摂取していたときに比べ低カフェイン緑茶に切り替えたことによりストレスが軽減していた参加者ほど，睡眠に関する種々のパラメーター（睡眠時間，中途覚醒，睡眠効率，入眠潜時，ノンレム睡眠）に改善が認められた[5]．また中高齢者では，低カフェイン緑茶の摂取量が多い参加者ほど，睡眠の質の高さを示すノンレム睡眠の割合が高まっていた[4]（**図4**）．この試験はクロスオーバー試験という同一被験者での試験であったことから，統計的精度は高いと考えられる．

抹茶のストレス軽減作用

抹茶は，収穫前の約20日間遮光を行うことにより茶葉中のテアニン量の低下を防いでいることから，煎茶に比べ茶葉に含まれるテアニン量が多くカテキンは少ないが，カフェインを多く含んでいる緑茶である．しかし栽培条件や収穫時期などにより，抹茶に含まれるテアニンやカフェイン，カテキンの量にはかなり違いがあるのも事実である．そこでいくつかの抹茶についてストレスを軽減することができるか，実験動物を用いて評価した．その結果抹茶中に含まれるテアニン（T）とアルギニン（A）の和に対する，カフェイン（C）とEGCG（E）の和のモル比を示す「CE/TA比」が2以下の抹茶では，マウスのストレスによる副腎肥大を抑制することができた．一方，2より大きい抹茶ではストレスによる副腎肥大を抑制することができないことが明らかとなった[6]．抹茶中のカフェイン量には抹茶の種類による違いはあまり見られなかったが，テアニンとEGCGの割合は大きく変動していた[6]．またアルギニン量はテアニンに比例して変動したが，品種間や茶種間で差が大きい成分であることが報告されている[7]．日本国内ならびに海外で販売されている抹茶についてCE/TA比を比較した結果，日本国内で販売されていた76銘柄中32銘柄はCE/TA比が2以下であった[6]．一方海外で販売されていた67銘柄ではCE/TA比が2以下のものは1銘柄のみであった[6]．

実際にヒトにおいてCE/TA比が2以下の抹茶でストレスが軽減されるのか調べた結果，CE/TA比が1.79の抹茶3gを水に懸濁して摂取した場合ストレス軽減効果が認められたが，CE/TA比が10.79の抹茶ではストレス軽減効果が認められなかった[6]．現代の日本においては抹茶を飲む習慣はそれほど一般的ではないことから，次に抹茶を含む菓子類として摂取した場合も同様の効果が認められるか検討した．その結果クッキーとして摂取した場合も，CE/TA比が1.79の抹茶ではストレス軽減効果が認められたが，CE/TA比が10.79の抹茶ではストレス軽減効果が認められなかった[8]．抹茶の臨床研究では，茶席での抹茶1〜2杯分を想定して1日に抹茶3〜6gを被験者に摂取していただいた．水に懸濁した場合，多くの被験者の抹茶摂取量はほぼ3gであったが，クッキーの場合はほぼすべての被験者が抹茶として4.5g相当を摂取した結果となった．今後はストレス軽減効果を期待して抹茶を摂取する際に，量的にどの程度の抹茶が必要であるのか検討する必要があるが，これまでの研究から少なくとも抹茶のCE/TA比には注意を払う必要があると考えられる．一般に，上級〜中級の抹茶はCE/TA比が2以下のものが多かった．

白葉茶の抗うつ作用

茶樹を2週間ほど完全に遮光すると，一般の煎茶に比べてアミノ酸量を6〜7倍に増加させることができる．このようにして作製された緑茶を「白葉茶」と言い，旨

表1■白葉茶および煎茶中の成分比較

緑茶	カフェイン（mg/L）	カテキン（mg/L）						
		EGCG	EGC	ECG	EC	GC	CG	(+) C
白葉茶	209.8	150.4	135.2	24.6	41.0	5.0	2.8	3.4
煎茶	112.0	134.2	229.0	21.0	46.6	13.6	4.6	2.0

緑茶	遊離アミノ酸（mg/L）								
	Thea	Arg	Gln	Asn	Asp	Glu	Ser	GABA	Total
白葉茶	140.2 (0.388)	69.9 (0.194)	51.7 (0.143)	33.8 (0.094)	33.5 (0.093)	19.3 (0.053)	12.6 (0.035)	0 (0)	361.0 (1.00)
煎茶	28.8 (0.538)	5.4 (0.101)	3.9 (0.073)	0.7 (0.013)	5.5 (0.103)	6.9 (0.129)	2.2 (0.041)	0 (0)	53.5 (1.00)

白葉茶または煎茶3gに水500mLを加え，時々撹拌し3時間後の溶出成分を測定した．EGCG, (−)-epigallocatechin gallate; EGC, (−)-epigallocatechin; ECG, (−)-epicatechin gallate; EC, (−)-epicatechin; CG, (−)-catechin gallate; (+)C, (+)-catechin; Thea, theanine; Arg, arginine; Gln, glutamine; Asn, asparagine; Asp, aspartic acid; Glu, glutamic acid; Ser, serine; GABA, γ-aminobutyric acid.（文献10より）
遊離アミノ酸のカッコ内の数値は，全アミノ酸量に対する割合を示す．

味の強い緑茶として注目されている[9]．白葉茶では一般煎茶に比べテアニンが約5倍，アルギニンが13倍と増加しており（表1），CE/TA比は1.12であったことからストレス軽減効果が期待された．そこで白葉茶について臨床研究を行なった結果，予想に反し一般煎茶と比べ有意なストレス軽減効果は認められなかった[10]．そこで実験動物を用いてその原因の解明を試みた．

白葉茶では完全な遮光を2週間ほど行うことにより，可溶性タンパク質の分解が起こり，遊離アミノ酸組成が一般煎茶の場合と大きく異なる[10]（表1）．テアニンは量的には増加するが，一般煎茶に比べ全アミノ酸に占める割合はむしろ低下し，一方アルギニンやグルタミン，アスパラギン，アスパラギン酸などの割合が増加した[10]．

そこでテアニンのストレス軽減効果に対するアミノ酸の比率の違いの影響を検討した結果，アスパラギンやアスパラギン酸の増加はテアニンの作用を抑制することが見いだされた[10]．次に白葉茶および一般煎茶に含まれる割合でテアニン，アルギニン，カフェイン，EGCGを溶解した水をマウスに与えた．白葉茶ではCE/TA比が1.12であり，また一般煎茶のCE/TA比は4.47であったが，いずれもストレスを軽減できなかった．そこでカフェインとEGCGを減少してCE/TA＝0.42とした場合，有意なストレス軽減効果が認められた[10]．また，カフェインやEGCGに比べるとアスパラギン酸のテアニンに対する影響は小さいことも見いだされた．低カフェイン緑茶などのデータと合わせて考えると，茶溶出液の場合はCE/TA比が0.54以下では効果があるが，0.9以上ではストレス軽減効果が見られないことが示唆された．これらのことから，抹茶の場合と茶溶出液の場合ではストレス軽減効果が期待されるCE/TA比に違いがあることが明らかとなった．茶溶出液と抹茶との作用の違いについては今後さらなる検討が必要である．

ところでうつ病は最も一般的な精神疾患で，ストレスはその重要な危険因子である．これまでに緑茶の摂取がうつ病を予防することが報告されており[11]，その要因として緑茶中のカフェインやカテキンの関与が示唆されている．白葉茶では一般煎茶に比べてカフェインが多いことから，実験動物を用い白葉茶の抗うつ効果を検討した．その結果白葉茶を摂取していたマウスではうつ様行動が有意に低下することが明らかとなった[10]．一般煎茶にも有意ではないが同様な効果が認められた[10]．これらのことから，白葉茶の場合はストレス軽減効果よりむしろ抗うつ効果が期待されることが明らかとなった．実験動物を用いた検討で，テアニンは神経の興奮／抑制の調節に関与している*Npas4*（neuronal PAS domain protein 4）の発現に影響を及ぼすことが見いだされている[12]．またカフェインやEGCGは脳内の主要な興奮性神経伝達物質であるグルタミン酸の発現を高める一方，抑制性の神経伝達物質であるγ-アミノ酪酸（GABA）に対しては抑制的に作用することが報告されている[13, 14]．アルギニンは一酸化窒素（NO）やポリアミンなどの重要な代謝物へと変換されることから，中枢の機能調節に重要な役割を担っていると考えられる[15]．これらが作用したときに総合的に脳がどのような興奮／抑制の状態となるのか，さらなる検討が必要である．

まとめ

ストレス軽減効果に着目して緑茶の脳に対する作用を評価したとき，緑茶溶出液ではテアニンとアルギニンの和に対する，カフェインとEGCGの和のモル比であるCE/TA比は0.5以下であることが望ましいことが明らかとなった．抹茶の場合はCE/TA比が2以下であればストレス軽減効果が認められたことから，抹茶の微細粉末

の状態が吸収や代謝に及ぼす影響について今後さらなる検討が必要である．

一方，ストレスが負荷されたときの生体の応答として，興奮状態になる場合もあれば抑うつ状態になる場合もある．これにはストレスの状況や個人による感受性の違いなど複雑な要因が関与するが，いずれにおいても脳内の興奮と抑制のバランスを保つことは，心身の健康を保つうえで非常に重要であると考えられる．緑茶は食品であるが，緑茶に含まれるテアニンやカフェイン，EGCG，アルギニンの組成の違いにより実際にストレスが軽減されたり睡眠に影響が生じたりすることなど，その機能性が明らかとなった．さらに，うつ状態が軽減される可能性も示唆された．したがってストレスを抱えたときには抹茶，夕方には良い睡眠のために低カフェイン緑茶，起床時には気持ちを高める煎茶や白葉茶など，状況に合わせた緑茶の選択が心身の健康増進に寄与するものと考えられる．今後食品成分がもつ機能性について，さらなる解明が進むことが期待される．

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お茶で快眠！

じめじめと暑くて寝苦しい日本の夏。皆さん、ぐっすり眠れていますか？
今月は快眠をサポートする上手なお茶の取り入れ方をご紹介します。

お茶は睡眠の敵!?

特集タイトルを見て、「お茶が睡眠を助ける!? むしろその逆では？」と思った方も少なくないかもしれません。確かに、カフェインの入った紅茶や緑茶、烏龍茶などの「お茶」は、就寝の3時間前くらいから飲むのを控えた方がよいとされます。

ただ、良質な睡眠は寝る前の行動だけで得られるものではありません。大切なのは、朝起きてから夜寝るまでの生活習慣。一日の中で上手にお茶を楽しみながら、ぐっすり眠ってスッキリ目覚める体づくりを目指しませんか？

カギは体内時計

快眠に向けた第一歩は、毎朝起きる時間を一定にすること。人の体内時計は、朝、太陽の光を浴びるとリセットされ、活動状態に導かれます。脳は光が届いてから14～16時間後に眠気がくるようにコントロールされているので、毎朝起きる時間を一定にすると、夜には自然と眠れるようになるのです。起きたらまずはカーテンを開けて、朝の光を浴びましょう。

朝の眠気覚ましには、温かい緑茶や紅茶がおすすめです。高温のお湯でいれるとカフェインをしっかり引き出せます。特に緑茶には、体内時計を整えるといわれるポリフェノール「エピガロカテキンガレート」も多く含まれているので、朝の一杯に最適です。

よい昼寝のコツ

昼食後、ひどい眠気に襲われたら、思い切って仮眠をとった方が効率的です。20分程度の昼寝は脳のパフォーマンスを高める効果があることから「パワーナップ（power nap）」と呼ばれ、世界的企業でも積極的に導入されています。

睡眠専門医の坪田聡（つぼたさとる）先生によると、「昼寝で頭がスッキリすると、午後の活動量が増えるので、夜の寝つきもよくなる」といいます。ただ、問題なのは昼寝の仕方。30分以上寝ると深い眠りに入ってしまうので、その前に短時間で起きることが重要です。そこで注目されているのが、昼寝前に飲むお茶の効果。

「お茶のカフェインが効いてくるのは、飲んでから20～30分後。だから昼寝の前に飲むと、ちょうど目覚めたい時間にカフェインの効果が表れて、スッキリ起きることができます」（坪田先生）

6:00

起きたらまずは朝日を浴びて。**朝食と一緒に濃いめのお茶を飲んで、**いざ活動モードへ。

12:00

散歩など、軽く体を動かすとGOOD！適度な疲労は夜の寝つきをよくしてくれます。

14:00

眠気を感じたら20分以内の昼寝を。**直前にお茶を飲むのを忘れずに！**

「快眠生活」のススメ

質のよい睡眠は免疫力を高め、健康な体づくりにつながります。

教えてくださった方
睡眠専門医 医学博士
坪田聡 先生

香りでリラックス

夜、スムーズに寝つくには、交感神経優位の活動モードから、夕方に向けて副交感神経優位のリラックスモードに体を切り替える必要があります。交感神経は緊張や興奮ですぐに活性化しますが、副交感神経は緩やかにしか働きが上がりません。そのため寝る3時間ほど前から意識的にリラックスを心がけることが大切です。

例えば、いい香りで心を落ち着かせるのもその一つ。おすすめは温かい**麦茶**(P8~9)や焙じ茶。あの独特のこうばしい香りは、焙煎の過程で生じる「ピラジン」という成分で、脳への鎮静効果が認められています。特に麦茶はノンカフェインなので、寝る直前に飲んでも安心です。

ルピシアには他にも様々なノンカフェインティーがあります。好きな香りの**フルーツティー**(P4~5)や**ルイボス**(P6~7)をのんびり楽しめば、癒やし効果も抜群！　カモミールやローズ、レモンバームなどリラックス効果のあるハーブがブレンドされたお茶もお休み前にぴったりです。

よい睡眠とよい目覚めは表裏一体。その時々に合ったお茶のパワーを活用して、快適なリズムを手に入れましょう。

睡眠の質を高める

「水出し緑茶」とは？

近年の研究で、緑茶に含まれるテアニンにはストレス軽減や、睡眠の質を改善する効果があることがわかり、話題を呼んでいます。

テアニンの働きは、エピガロカテキンガレート(EGCG)やカフェインが混在すると弱められてしまうため、テアニンの効果を十分に発揮させるには、茶葉から溶け出すEGCGやカフェインをいかに抑えるかがポイントです。

そこでおすすめなのが、水出し緑茶(P15)。テアニンは冷水にも簡単に溶け出しますが、EGCGやカフェインは水温が低くなるほど溶け出しにくくなるからです。より低温の氷水で作る「氷水出し緑茶」なら、テアニンの効果がさらに期待できます。

緑茶のストレス軽減効果などを研究している静岡県立大学の海野(うんの)けい子先生によると、低カフェイン処理した水出し緑茶を飲んだ場合、サプリメントでテアニンを摂取するより少ないテアニン量で高い睡眠効果が得られるのだそう。「科学的に証明しきれてはいませんが、テアニンの働きを助けるアルギニンなど、テアニン以外の緑茶成分との相加的効果と考えられます」

まだまだ寝苦しい暑さが続く夏、水出し緑茶を習慣にしませんか。

教えてくださった方
静岡県立大学
茶学総合研究センター客員准教授
海野けい子 先生

WEBでもっと詳しく！
ウェブサイトでは氷水出し緑茶の魅力や作り方について詳しくご紹介しています。
ルピシア 氷水出し緑茶　検索

19:00

夕食は寝る3時間前までに。アルコールやカフェインもこの辺りで控えましょう。

21:00

入浴は寝る1~2時間前に、38~40度のお湯に10~20分浸かるのがおすすめ。

22:00

ノンカフェインのお茶を飲みながらリラックス。パソコンやスマホはそろそろOFFに！

23:00

部屋の照明は消すか豆電球程度の明るさにして。今日もぐっすりお休みなさい！

特集　第1部

茶は養生の仙薬なり

健康を保ち、和をもたらす

茶はそもそも〝薬〟として渡来した。爾来、日本人が長年親しんできた茶が健康に資するメカニズムは、近年、科学的に解明されつつある。茶研究の第一人者に「茶の健康力」を教わる。

総論

お茶と健康

●解説　中村順行（なかむらよりゆき）さん（静岡県立大学茶学総合研究センター長、特任教授・67歳）

⬆岩手大学大学院修士課程修了。静岡県農林技術研究所部長、静岡県茶業研究センターを経て現職。著書に『日本茶の起源を探る』他。

「お茶はそもそも〝薬〟として日本に渡来しました。感染症の予防にも効果があります」

茶は2000年以上昔から飲み継がれてきた、優れて文化的な嗜好飲料だ。緑茶、紅茶、烏龍茶など、世界には様々な茶が誕生したが、チャノキの原産地は中国西南地域が有力といわれている。

日本茶研究の第一人者である中村順行さんが解説する。

「中国から日本に茶が伝わったのは奈良時代。その後、唐から帰国した近江の僧・永忠が嵯峨天皇に茶を献じた記録も残っています。その喫茶の習慣を確たるものにしたのが、臨済宗の開祖、栄西禅師です。鎌倉前期に南宋から飲茶法である抹茶法をもたらし、自ら『喫茶養生記』を著して〝日本の茶祖〟とうたわれています」

栄西は、『喫茶養生記』の序文に「茶は養生の仙薬、延齢の妙術也」と書き記して、茶の生理学的な薬効を様々に説いている。

実際、茶にはカテキン、テアニン、カフェイン、ビタミンが多く含まれている。近年、その機能性が科学的に明らかになってきた。

「緑茶に10～18％含まれているカテキン類は、エピカテキン、エピカテキンガレート、エピガロカテキン、エピガロカテキンガレート

⬆茶はツバキ科の常緑樹チャノキの葉を摘み取って加工する。紅茶や烏龍茶とは違い、茶葉を発酵させずに作るのが緑茶である。

今回訪れた無農薬茶の名産地

⬇昔ながらの無農薬栽培法で育てる茶園が増えている。安心・安全な茶を作る農家の「家飲み茶の極意」を次ページからお送りする。

取材・文／佐藤俊一　写真提供／鎌倉国宝館、無農薬茶の杉本園

←『喫茶養生記』は、禅師・明庵栄西(1141～1215)が茶の効能や製法を伝えた上下2巻からなる医書。将軍・源実朝に献上されたといわれる。左に掲げたのは現存する最古の写本。ここには写っていないが、上巻の冒頭では「茶は養生の仙薬、延齢の妙術也」と記す。重要文化財。寿福寺蔵

など主に4種。このカテキン類には抗酸化作用、抗がん、抗動脈硬化、血中コレステロール抑制、抗菌、抗ウイルス、血圧上昇抑制など、多様な機能性を持つことがわかってきました」

カテキン類がコレラ菌に効力を持つことは早くから知られていた。感染型の腸炎ビブリオ菌、毒素型のブドウ球菌やボツリヌス菌、それから腸管出血性大腸菌O・157に対しても、茶はごく薄い濃度でも大きな効力を発揮するという。

風邪やインフルエンザの感染予防にも、カテキン類は有効だ。

「カテキン類のなかでも、とくにエピガロカテキンは免疫力を高める力を持っています。また、緑茶でうがいをすることにより、エピガロカテキンガレートがインフルエンザウイルス表面の突起に結合し、のどの粘膜細胞にウイルスを吸着できなくして、予防効果を示します」

抽出温度で味・成分が変わる

茶には20種ほどのアミノ酸が含まれているが、その50%以上を占めるのがテアニンだ。

「テアニンは茶の甘みや旨味の成分。コーヒーやココアには含まれない特有のアミノ酸です。飲むとホッとするリラックス効果、抗ストレス作用、血圧低下作用、血管性認知症予防効果などがあります」

茶の成分の浸出度は、抽出温度で変わる。甘み・旨味成分のテアニンは低温でもよく抽出されるが、渋み成分であるカテキン類や香り成分は高温のほうが多く出る。

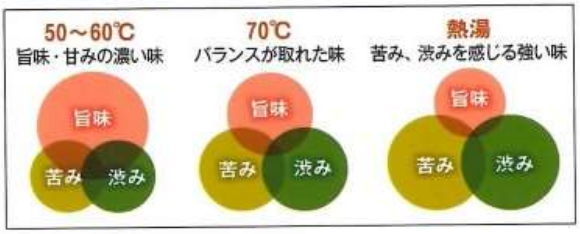
「旨味を優先する玉露や上級煎茶は少し冷ました温かめの湯で淹れますが、紅茶・烏龍茶・ほうじ茶など芳香を大事にする茶は、熱湯で淹れます。番茶も熱湯で淹れ、爽快な渋みと香りを愉しむものです」

免疫力を高める上では、水出し茶もよいという。前出のエピガロカテキンは低温でも多く抽出され、他方、免疫作用を抑制する働きもあるエピガロカテキンガレートは低温では抽出されにくいからだ。

昨今は、抗アレルギー作用の高いメチル化カテキンが豊富な紅茶品種「べにふうき」や、抗眼精疲労に着目した高アントシアニン含有茶「サンルージュ」など、機能性の高い品種も注目を集めている。

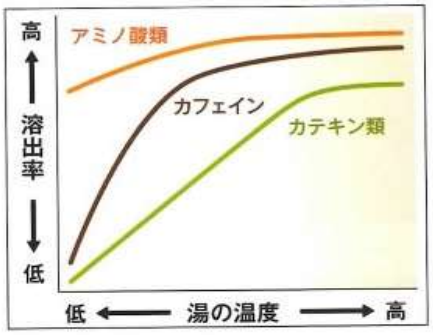
「日常茶飯に摂取できる茶は、健康寿命を延ばす上でも〝天与の配剤〟といっていいでしょうね」

「旨味」はぬるめの湯でよく出る



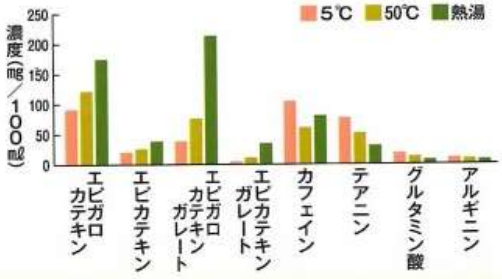
↑緑茶の甘み・旨味成分のテアニンは、ぬるめの湯で溶け出す。一方、苦み・渋みのもとになるカテキン類は、熱湯で多く溶け出す。

「テアニン」は、水出しでも多く溶け出す



←アミノ酸類のテアニンは、水出しでも容易に溶け出す。カテキン類は湯温が低いと多くは溶け出さない。カフェインは両者の中間だ。

抽出温度による茶の浸出成分の違い



↑煎茶を5℃の冷水、50℃の湯、熱湯で順次浸出。冷水では濃厚な旨味と甘み、50℃では調和の取れた渋み、熱湯では強い苦みを愉しめる。

●掲載図表は静岡県立大学茶学総合研究センター資料と「茶業研究報告」(日本茶業学会)を参照し編集部で作成。

茶は養生の仙薬、その魅力と機能性

中村順行
静岡県立大学 茶学総合研究センター

【目的】茶は2000年以上前から養生の仙薬として飲み続けられ、各地域の風土に適した各種各様の茶が開発され嗜好品として世界中の人々を虜にしてきた。この養生の仙薬の機能性が科学的に明らかにされるとともに、最近では、機能性を強化した加工法や機能性を表示した商品が数多く見られるようになってきたので、その概要を紹介する。

【結果】チャには他の植物には稀なカテキン類、アミノ酸の一種であるテアニン、カフェインなどが含まれ、様々な機能性をもつ。また、最近では機能性に特化した品種や栽培・加工技術の開発も見られたり、機能性を活かした特定保健用食品や機能性表示食品も販売されている。

① チャの主要成分とその機能性

チャは、カテキン類、テアニン、カフェインをはじめとする多くの機能性成分をもつ。なかでも、カテキン類は茶葉中に10～18%含まれ、抗酸化、抗突然変異、抗がん、抗動脈硬化、血中コレステロール抑制、抗菌、抗ウイルス、虫歯予防、腸内フローラ改善、消臭、血圧上昇抑制など多くの機能性をもつ。テアニンは、チャのアミノ酸中の50%程度を占め、お茶を飲むとホッとするリラックス効果、抗ストレス作用、血圧低下作用、血管性痴呆症予防効果などがある。カフェインは、中枢神経の興奮作用を示し、覚醒効果や強心作用、利尿作用などがある。その他、チャは各種のビタミン類を豊富に含み、ビタミンA(カロテン)には抗酸化、抗ガン作用が、ビタミンB群には口角炎予防、抗酸化作用が、ビタミンCには抗酸化作用、ストレス解消作用、風邪予防、美肌効果が、ビタミンE(トコフェノール)には抗酸化や老化抑制作用などがある。

② 機能性に特化した品種や栽培・加工法

近年、健康志向の高まりから特定の機能性成分を増強したお茶が開発されている。品種として、抗アレルギー作用の高いメチル化カテキンを豊富に含む“べにふうき”、抗眼精疲労に着目した高アントシアン含有茶である“サンルージュ”が育成されている。また、収穫後の加工工程中に嫌気処理することで血圧降下作用を持つγアミノ酪酸含量を高めたギャバロン茶や殺青時に熱湯浸漬処理しカフェインを低減した低カフェイン茶なども開発されている。

③ 茶の特定保健用食品や機能性表示食品

茶の特定保健用食品として、ペットボトルではカテキン類を主体に体脂肪の減少やコレステロール吸収抑制などが、粉末茶では難消化デキストリンが添加され血糖値の上昇抑制を表示した商品が多い。また、機能性表示食品としては、カテキン類による目や鼻の不快感の軽減、体脂肪の減少やコレステロールの値を減らす機能、テアニンによる睡眠の質の向上、ストレスの緩和、GABAによる血圧のサポートや難消化デキストリンによる食後の血糖値上昇を穏やかにする機能などが表示された多くの商品群が開発されている。

【考察】チャは多くの成分を含有し、その機能性について数多くのエビデンスが報告されている。しかしながら、疫学調査ではヒトにより茶飲用時の濃度、遺伝的背景、腸内フローラ、生活習慣などが異なるため、必ずしも好結果がもたらされるばかりではない。今後、茶の機能性をより強く発揮させるためには効果的な飲用方法や濃度などの検討も必要と考えられる。

【結論】茶は養生の仙薬としてクローズアップされている。文化的で嗜好飲料でもある茶は、日常茶飯事に摂取可能な予防薬として超高齢社会の中でも重要な役割を果たす逸材である。

睡眠の質を高める「水出し緑茶」とは？

近年の研究で、緑茶に含まれるテアニンにはストレス軽減や、睡眠の質を改善する効果があることがわかり、話題を呼んでいます。

テアニンの働きは、エピガロカテキンガレート（ＥＧＣＧ）やカフェインが混在すると弱められてしまうため、テアニンの効果を十分に発揮させるには、茶葉から溶け出すＥＧＣＧやカフェインをいかに抑えるかがポイントです。

そこでおすすめなのが、水出し緑茶。テアニンは冷水にも簡単に溶け出しますが、ＥＧＣＧやカフェインは水温が低くなるほど溶け出しにくくなるからです。より低温の氷水で作る「氷水出し緑茶」なら、テアニンの効果がさらに期待できます。

静岡県立大学
茶学総合研究センター客員
准教授 海野けい子 先生

緑茶のストレス軽減効果などを研究している静岡県立大学の海野（うんの）けい子先生によると、低カフェイン処理した水出し緑茶を飲んだ場合、サプリメントでテアニンを摂取するより少ないテアニン量で高い睡眠効果が得られるのだそう。「科学的に証明しきれてはいませんが、テアニンの働きを助けるアルギニンなど、テアニン以外の緑茶成分との相加的効果と考えられます」

まだまだ寝苦しい暑さが続く夏、水出し緑茶を習慣にしませんか。

お茶の成分でストレスによる脳の萎縮を予防できる可能性、静岡県立大学茶学総合研究センター発表

2020.02.11　ライフスタイル　#ヘルスデーニュース

お茶の成分がストレスによる脳萎縮を予防――マウスでの研究

ストレスに長期間さらされていると、脳が萎縮したり認知機能が低下することが、動物実験で示されている。

またヒトにおいても、たび重なるストレスと脳の前頭前野などの容積に関連が見られるとする研究報告がある。

このような影響を避けるにはストレスがかからない環境に移ることが一番だが、それを簡単に実行できる人はあまりいない。

が、ひょっとしたら、お茶を飲むことが脳の萎縮の予防につながるかもしれない――という研究結果が「Nutrients」1月8日オンライン版に掲載された。

静岡県立大学茶学総合研究センターの海野けい子氏らは、お茶に最も多く含まれているアミノ酸で緑茶の旨味成分の1つである「テアニン」の機能性に着目し、これまでにテアニンがマウスのストレスを軽減し認知機能低下を抑制することなどを報告してきている。

今回の研究では、テアニンがストレスによる脳萎縮を抑制するかを、東北大学加齢医学研究所の住吉晃氏らとの共同研究により磁気共鳴画像法を用いて検討した。

4週齢のマウスを5日間グループで飼育し環境に慣れさせた後、そのままグループで飼育する群と、仕切板により1匹ずつ個室で飼育した後に途中から仕切板を外して2匹の相部屋に移す群に分けた上で、さらにそれぞれを2分し、一方は通常の水、もう一方はテアニンを20μg/mL含む水溶液を与えるという計4条件で飼育した。

個室から2匹相部屋に移す条件では、2匹のマウスが互いに相手を侵入者と見なしストレスがかかった状態になる。

このストレス状態の期間は、0、1、2、4、6カ月の5パターン設定した。一連の実験は、ストレスに対する感受性が強い「SAMP10」というマウスと、比較対照として動物実験で一般的に使われる「ddY」という計2種類のマウスを用いて行った。

まずSAMP10マウスの脳の容積を前記の4条件別に見ると、ストレスを負荷し通常水で飼育した群は、ストレスを負荷しテアニン水溶液で飼育した群や、ストレスを負荷せずに通常水で飼育した群に比較して、海馬（記憶を司り、アルツハイマー病では初期から萎縮する部位）や新皮質（脳の高次機能を司る部位）が有意に小さいことがわかった。

次にこの変化を経時的に見ると、ストレス負荷1カ月の時点で新皮質が有意に萎縮したが（112.75±8.26mm3）、テアニン水溶液で飼育した群では２カ月目で回復した（123.75±7.57mm3）。

また海馬ではストレスを6カ月間負荷した時点で、通常水で飼育した群（23.01±0.79mm3）とテアニン水溶液で飼育した群（26.02±1.46 mm3）に有意差が生じていた。

これらの結果は、ストレスによってSAMP10マウスの海馬や新皮質で萎縮が生じるが、テアニンがその抑制や回復に寄与したものと考えられる。

なお、グループ環境で8カ月間飼育した（ストレスを負荷しなかった）群では、通常水（25.75±1.69 mm3）、テアニン水溶液（25.54±1.91 mm3）の違いによる海馬の容積に差がなかった。

一方、ddYマウスではストレス負荷1カ月時点で、通常水で飼育した群とテアニン水溶液で飼育した群のいずれも海馬容積が有意でないながら軽度に縮小する傾向が見られたが、2カ月目以降、両群ともに回復した。

グループ環境で8カ月間飼育した群では、通常水で飼育したマウス（23.93±1.04 mm3）はテアニン水溶液で飼育したマウス（27.81±1.16 mm3）に比べ、海馬の容積が有意に小さかった。

ddYマウスの検討では、海馬で見られたこれらと同様の変化が新皮質においても認められた。

上記のほか研究グループでは、ストレス負荷がSAMP10マウスの遺伝子に及ぼす影響を検討した。

その結果、Npas4やLcn2といった遺伝子の発現がストレスの影響を受けて変化し、テアニン水溶液で飼育したマウスではそれらの変化が抑制されることがわかった。

海野氏らはこれらの結果を踏まえ、「ストレス負荷によって、ストレスに敏感なマウスの脳容積が減少する。

これに対し、茶葉の主要アミノ酸であるテアニンは、ストレス応答遺伝子発現を修正することで脳萎縮を防ぐことが示唆される」とまとめている。（HealthDay News 2020年1月27日）

Abstract/Full Text
https://www.mdpi.com/2072-6643/12/1/174

構成／DIME編集部

「緑茶で養生」

日々の緑茶で
心を癒して、
元気な体を。

健やかな一年を。

宇治田原製茶場・月刊『茶の間』は、緑茶を通じて、皆様に健康と幸せをお届けしています。
昨年は未曾有の苦難に見舞われ、大変な一年でした。そのようななか、急須で淹れる「一杯のお茶」が、
心身の癒しや健康に役立ち、お茶の大切さが改めて見直された一年だったと思います。
お茶の歴史は古く、二千年以上も前から、途絶えることなく飲み継がれてきました。
日本には奈良時代に中国から初めて伝わってきたと言われていますが、
喫茶の習慣が見られるようになったのは、鎌倉時代とされています。その頃"日本の茶祖"と言われる
栄西(ようさい)禅師が『喫茶養生記』のなかで著しているように、お茶はそもそも薬として伝わっていたのです。
江戸時代でも、貝原益軒が『養生訓』で茶の効用を記し、茶の養生を説いていました。
当時、お茶は文字通り茶色でしたが、京都・宇治田原の茶農・永谷宗円が緑の水色(すいしょく)を味わえるように
「青製(あおせい)煎茶製法」を開発し、緑茶を生み出したことで、急須で淹れて一服する日常が日本全国に
広まっていきました。このように先人たちは、心と体にいいものとして様々な緑茶を嗜み、緑茶の
色・味・香りを楽しみ続けてきたのです。また新しい年が始まりました。今年は今日まで受け継がれてきた
「緑茶で養生」を心がけ、毎日おいしい緑茶を飲み続けることで、健康で幸せな一年を過ごしましょう。

新春 特別コラム

心と体によいものとして飲み継がれてきた 緑茶の健康力を信じて、今こそ毎日飲み続けましょう。

もとより日本の緑茶は旨みや甘み、渋みや苦みに、香りを楽しむ飲み物として、親しまれてきました。昨年からは生活様式の変化とともに、家で過ごす時間が増え、緑茶を楽しむことで、おいしさだけでなく、癒しやストレス解消といった緑茶の力が注目されています。また、薬として伝来した緑茶は、健康によいものとして飲み継がれてきましたが、近年は緑茶の健康成分や機能性が科学的に明らかになり、緑茶の持つ力が確かなこととして享受できるようになっています。新年を迎え、家族が集い緑茶を囲めば、楽しいステイホームカフェになります。急須で緑茶を淹れ、家族みんなで一服しながら、今年一年、緑茶の健康力を体に摂り入れてください。

静岡県立大学 茶学総合研究センター
センター長 特任教授 中村順行(なかむらよりゆき)先生

農学博士。岩手大学大学院農学研究科修士課程修了。静岡県茶業研究センター長を経て現職。茶の生産・加工、機能性、マーケティングなど幅広く活動中。

真冬の今こそ、

「緑茶で養生」を

緑茶は、心と体の養生に良きものなり

宇治田原製茶場・月刊『茶の間』は、緑茶を通じて、皆様に健康と幸せをお届けしています。2月は、寒くて衣服をさらに着重ねる"衣更着"という言葉から、如月と名付けられたとされており、一年で一番寒い季節です。冷えを感じると、体調を崩しやすいと言われますが、毎日の暮らしのなかで、熱い緑茶を飲むことは、体を温め、心をほっこりほぐしてくれるので、まさに今の季節には大切なことだと思います。お茶は、中国から日本へ健康のためのものとして伝わってきたとされています。鎌倉時代初期に、"日本の茶祖"と言われる栄西禅師がお茶の健康作用や種類、栽培や製法などをまとめた『喫茶養生記』のなかで書き記しているように、当時のお茶は飲み物というよりも、健康のための貴重なものだったようです。それから800年が経ち、緑茶は嗜好品としておいしさを楽しむとともに、文字通り"一服する"ことで、心のやすらぎや癒しを感じる、日常になくてはならないものとして浸透しました。また、この数年は、緑茶の健康成分や機能性が科学的に明らかになり、再び健康によい飲み物として注目を浴びています。健康に気をつけたい真冬の今こそ、「緑茶で養生」を。毎日熱い緑茶を飲んで、温かい心と体で、厳しい寒さを乗り切りましょう。

▲喫茶養生記
（早稲田大学図書館所蔵）

如月 特別コラム

この冬は、熱湯で淹れた緑茶を飲んで、多様な機能性を持つカテキンを、しっかり摂り入れましょう。

緑茶はカテキンやテアニン、カフェインなど、健康によい成分を含むことで、人々に親しまれてきました。中でもカテキンは、緑茶に最も多く含まれる成分で、外敵に対する力など、特に多様な機能性を持つことがわかっています。また、緑茶に多彩に含まれる健康成分ですが、それぞれの浸出度合いはお湯の温度で変わり、カテキンは高温のお湯で淹れる方が多く溶け出すため、熱湯で淹れることをおすすめしています。ちょうど今は、極寒の季節。熱湯で淹れた熱い緑茶が、とてもおいしく感じるものです。湯呑を手に、淹れたての緑茶の温もりを感じながら、カテキンを中心とする緑茶の健康力をしっかり摂り入れることを習慣にして、この冬を元気に過ごしましょう。

静岡県立大学 茶学総合研究センター
センター長
教授 中村順行（なかむらよりゆき）先生

農学博士。岩手大学大学院農学研究科修士課程修了。静岡県茶業研究センター長を経て現職。茶の生産・加工、機能性、マーケティングなど幅広く活動中。

広報しずおか　No.346　静岡気分　令和３年１月号

静岡市の1 やぶきた原樹

始まりのやぶきた原樹！ やぶきた茶の歴史や効能

静岡県立大学
薬学部５年
五十嵐 弦さん

やぶきた茶発祥の地
出典：「静岡市の特産品（静岡茶の歴史とやぶきた茶）」JA静岡市

やぶきた原樹

やぶきた原樹について知っていますか？ やぶきた原樹は、静岡鉄道「県立美術館前駅」から静岡県立大学へ行く坂の途中で見ることができます。やぶきた原樹の芽を使ったお茶は、やぶきた茶と呼ばれ、なんと全国のお茶の栽培面積の約76％を占めるほど広く親しまれている品種で、静岡県内でも、約93％も占めるほどの品種なのです。

「やぶきた」とは、もともとやぶきた原樹が竹やぶの北にあったことから名付けられ、現在の各地の茶産地にはやぶきた原樹の分身が植えられています。やぶきた原樹は杉山彦三郎翁によって品種改良を重ねた結果作られた品種であり、杉山彦三郎は茶の葉を食べたり、匂いを嗅いだりしてやぶきた原樹の開発に努めてきたとも言われています。（羽野）

僕はお茶の効能について紹介したいと思います。お茶の作用として、よく知られているものに覚醒作用があります。お茶を飲むと目が覚めますよね？ これは「カフェイン」という緑茶の苦み成分によるものです。一方で、お茶を飲むとホッとします。これは「テアニン」と呼ばれるうまみ成分によるものです。テアニンを摂取すると「α波」が増えることが知られています。カフェインは高温で多く抽出され、テアニンは50～60℃で多く抽出されます。このようにお茶を抽出する温度で味や成分が変わります。

また、お茶には美肌効果があると言われています。これは苦渋味成分であるカテキンによって、メラニンの産生が抑制され、そばかすやシミができにくくなるという研究報告があるようです。市販のローションや化粧品に緑茶成分が入っているのも納得できますね。塗るだけでなく飲むことでも効果が得られるとも言われます。

今回の取材によって、何気なく飲んでいるお茶に多くの作用があることがわかりました。これからもお茶を飲んで、元気に過ごしていきたいと思います。ぜひ皆さんもお茶を飲んできれいに元気になりましょう！（五十嵐）

静岡県立大学薬学部４年
羽野 蘭さん

「静岡県立大学 茶学総合研究センター長の中村さん」にお話を伺いました

富岳館高校　ホームページ

■学校の様子（トップページ） >> 記事詳細

2020/09/24　**9月２４日　静岡県立大学　出張講座　「お茶の健康効果」を受講しました**　by: 総務課HP係

9月２４日（木）、子ども地域福祉系列２年次生が、静岡県立大学食品栄養科学部の斉藤貴江子先生による「お茶の健康効果」の高大連携出張講座を受講しました。

「茶には抗酸化作用、抗がん作用、抗メタボ作用、ストレス軽減効果、抗アレルギー効果、認知症予防といった作用がある」ことを説明してくださいました。

また、「茶は飲用だけでなく、食品素材として活用されており、機能性成分を活かし、さまざまな飲食料以外に利用され、新たなビジネスを創造している」と茶の多岐にわたる利用についても紹介してくださいました。

講義のあと、湯の温度や抽出時間の異なるお茶を飲み比べ、茶の魅力に触れていました。

皆さんが日常的に飲んでいる、紅茶/緑茶/烏龍茶は全て同じツバキ科の常緑樹である〝カメリアシネンシス“というお茶の木から作られている事をご存じですか？それぞれ味も水色も香りも違いますが、これは葉を摘み取った後の発酵の度合いによって生まれているのです。その中でも高い香りが特徴の紅茶は、世界中で一番飲まれている“お茶”です。

世界三大紅茶は、ダージリン（インド）・キーマン（中国）・ウバ（スリランカ）ですが、紅茶は、品種やそれぞれの国の気候風土で味や香りが異なります。

最近ブームの国産紅茶（和紅茶）は、温暖な気候の鹿児島や静岡で多く作られています。海外の紅茶に比べて渋みが少なくすっきりとし、甘い香りで食事に合わせやすいのが特徴です。季節のフルーツや炭酸、生姜やシナモン等のスパイスでアレンジをして、自分が好きな味を見つけて愉しむのもお勧めです。

また、温かい紅茶は血流を良くし体を温める作用があります。ウイルスに負けない免疫力の高い体作りのためにも、“紅茶のある生活”を愉しみたいですね。（静岡県立大学 茶学総合研究センター）

【静岡県立大学　茶学総合研究センター】

大学内の茶に関する研究情報を一元化するとともに、産学民官と連携して茶を総合的に科学し、茶業振興に寄与することをめざし、茶の栽培加工から機能性、販売、経営手法まで総合的に科学する静岡県立大学食品栄養環境科学研究院附属のセンターです。

静岡市地域福祉共生センター「みなくる」
静岡市駿河区南八幡町3-1　南部図書館2階
TEL:054-201-9010　FAX:054-201-9020
MAIL:mina.ccrc@u-shizuoka-ken.ac.jp

共生事業受託：静岡県立大学
（「ふじのくに」みらい共育センター）

静岡のお茶で元気に！

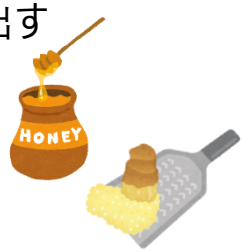
静岡県産 和紅茶

～おいしい飲み方と
ミニ情報～

～これからの季節に❀～

温かいジンジャーミルクティー

1. 牛乳300mlとティーバック1袋を鍋に入れ火にかける
2. 沸騰直前で弱火にして3分～5分火にかける（ふきこぼれないように注意）
3. 火を止め、ティーバックを取り出す
4. 砂糖orハチミツとおろした生姜を加える
5. 数回混ぜてからカップに注ぐ



～気軽に ❀～

水出しアイスティー

1. 容器に水500mlとティーバック1袋を入れる
2. 冷蔵庫で一晩　常温なら3時間
3. ティーバックを取り出す

1日で飲みきってくださいね。

❀香りが良いうちにお早めにお飲みください❀

茶学総合研究センター　ホームページがリニューアルされました

５．茶学総合研究センター
研究業績一覧

茶学総合研究センター

	所属学会
特任 教授：中村　順行・博士（農学）	日本茶業学会、茶の湯文化学会
助　　教：斎藤　貴江子・博士（農学）	日本栄養・食糧学会、日本未病システム学会、植物環境工学会、日本酸化ストレス学会、The Oxygen Society
客員准教授：海野　けい子・博士（薬学）	日本薬学会、日本基礎老化学会、日本抗加齢医学会、茶学術研究会、老化促進モデルマウス(SAM)学会
客員 教授：伊勢村　護・博士(理学)	

研究センター概要

食品栄養科学部、薬学部、経営情報学部などがそれぞれ進める茶に関する研究情報を一元化するとともに、茶の栽培加工から機能性、販売、経営手法まで総合的に科学することを目的に相互に連携した取り組みを行う。また、県内の他大学や公設試験研究機関をはじめ行政・茶業界と連携して、茶業振興に寄与することを目的に、日本の大学では初めて開設した茶の総合研究センターとして幅広く活動している。

主要研究題目

1. 緑茶の機能性及び疫学に関する研究
   緑茶の機能性の強化と各種疾病との関連を調査する
2. 茶学教育と人材育成
   茶の都を牽引し、お茶の総合的知見を有する人材を育成する
3. 茶葉及び茶飲料の嗜好特性の解析
   茶の品質特性の評価と嗜好性の解析により販売促進戦略を構築する
4. 茶の高付加価値化とマーケティング
   消費者の視点に立った緑茶のマーケティング戦略を調査研究する

研究業績

【原著論文】

1. **Sumio Hayakawa, Tomokazu Ohishi, Noriyuki Miyoshi, Yumiko Oishi, Yoriyuki Nakamura, Mamoru Isemura.** Anti-Cancer Effects of Green Tea Epigallocatchin-3-Gallate and Coffee Chlorogenic Acid. Molecules, 25(19), 4553 (2020).
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6. **Keiko Unno, Akira Sumiyoshi, Tomokazu Konishi, Michiko Hayashi, Kyoko Taguchi, Yoshio Muguruma, Koichi Inoue, Kazuaki Iguchi, Hiroi Nonaka, Ryuta Kawashima, Sanae Hasegawa-Ishii, Atsuyoshi Shimada, Yoriyuki Nakamura:** Theanine, the Main Amino Acid in Tea, Prevents Stress-Induced Brain Atrophy by Modifying Early Stress Responses. Nutrients. 12, E174 (2020)
7. **Keiko Unno,, Yoshiichi Takagi, Tomokazu Konishi, Mitsuhiro Suzuki, Akiyuki Miyake , Takumi Kurotaki , Tadashi Hase , Shinichi Meguro , Atsuyoshi Shimada , Sanae Hasegawa-Ishii , Monira Pervin, Kyoko Taguchi , Yoriyuki Nakamura.** : Mutation in Sodium-Glucose Cotransporter 2 Results in Down-Regulation of Amyloid Beta (A4) Precursor-Like Protein 1 in Young Age, Which May Lead to Poor Memory Retention in Old Age. *International Journal of Molecular* Sciences, **21**, 5579, 2020
8. **Masakazu Hashimoto Tsuyoshi Nakai, Teruaki Masutani, Keiko Unno, Yukihiro Akao.**: Improvement of Learning and Memory in Senescence-Accelerated Mice by S-Allylcysteine in Mature Garlic. *Extract 9.***12**, 1834, 2020
9. **Keiko Unno,, Yoshio Muguruma, Koichi Inoue, Tomokazu Konishi , Kyoko Taguchi , Sanae Hasegawa-Ishii , Atsuyoshi Shimada , Yoriyuki Nakamura** : Theanine, Antistress Amino Acid in Tea Leaves, Causes Hippocampal Metabolic Changes and Antidepressant Effects in Stress-Loaded Mice. *International Journal of Molecular* Sciences, **22**, 193. (2020)
10. **Keiko Unno, Monira Pervin, Kyoko Taguchi, Tomokazu Konishi, Yoriyuki Nakamura.** : Green Tea Catechins Trigger Immediate-Early Genes in the Hippocitive Declampus and Prevent Cognine and Lifespan Shortening *Molecules*. **25**, 1474, 2020
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【総 説】

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3. **海野けい子**: セミナー室 お茶成分の脳における作用・1 カテキン 緑茶カテキンガ脳の老化を予防する 化学と生物 （日本農芸化学会会誌） Vol.58. No.11 621-627 2020
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2. **海野けい子**: 老化予防 茶の健康効果 20 選 日本茶業体制強化推進協議会, pp.29-30 (2020)
3. **海野けい子**: 抗ストレス作用 茶の健康効果 20 選 日本茶業体制強化推進協議会, pp.35-36 (2020)
4. **中村順行**:茶の機能性成分の変異　茶の健康効果 20 選 日本茶業体制強化推進協議会, pp.5-6 (2020)
5. **海野けい子**:緑茶 抗疲労・抗ストレス・睡眠改善食品の開発 シーエムシー出版，pp.106-112 (2020)
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［報告書］

1. **中村順行**:令和元年度 茶学総合研究センター　実績報告書
2. **中村順行**:令和元年度　茶産地確立支援事業実績報告書
3. **中村順行**:農水省革新的技術開発・緊急展開事業「海外市場の飛躍的拡大を目指す高品質抹茶の低コスト製造技術」報告書（令和元年度）
4. **海野けい子、中村順行**:緑茶の機能性及び疫学に関する研究 : 2)抹茶がヒトの抗ストレス性に与える影響の解明　～クッキーに抹茶を入れた場合の抗ストレス性の評価～ 令和元年度茶学総合研究センター実績報告書、p.6-7.
5. **海野けい子、林美智子、田口今日子、中村順行**: 緑茶の機能性及び疫学に関する研究 : 3)ストレスによる脳の萎縮とテアニンによるその抑制機構 令和元年度茶学総合研究センター実績報告書、p.8-9.
6. **海野けい子、パービン・モニラ**:緑茶の機能性及び疫学に関する研究 : 4）緑茶カテキンによる認知症の予防 令和元年度茶学総合研究センター実績報告書、p.10-11.
7. **海野けい子**:長期ストレス負荷と脂質摂取量の増加が脳に及ぼす影響：テアニンとアルギニンの作用の違い 第 6 回小林国際奨学財団研究助成報告書

【学会発表（口頭、ポスターなど）】

1. **下位香代子**:ポリフェノールはどれくらい摂ったら体にいいのか，日本農芸化学会 2020 年度大会，シンポジウム、ポリフェノール～第 7 の栄養素を目指して～， 2020 年 3 月 27 日
2. **斎藤貴江子、中村順行**：*Lactococcus lactis subsp. cremoris* を用いた後発酵茶の作製とその特徴 日本未病学会 2020 年 11 月（online）
3. **海野けい子、パービン・モニラ、田口今日子、小西智一、中村順行**: SAMP10/TaSlc における緑茶カテキン摂取による寿命延長と脳機能改善 第 35 回老化促進モデルマウス（SAM）学会学術大会（岐阜）、抄録集、p.30、2020 年 6 月 20 日
4. **海野けい子、住吉　晃、小西智一、林美智子、田口今日子、中村順行**:ストレスによる脳の萎縮とテアニンによるその抑制機構 第 20 回日本抗加齢医学会総会（東京）、2020 年 9 月 25-27 日
5. **Monira Pervin1, Keiko Unno, Yoriyuki Nakamura**:Anti-stress and neuroprotective effect of theanine and arginine in chronic psychosocially stressed mice and SH-SY5Y cells 第 20 回日本抗加齢医学会総会（東京）、2020 年 9 月 25-27 日
6. **海野けい子**:茶の主要成分の機能性　その 1～茶成分全般、カテキン～オンラインによる茶の機能性成分を活かした輸出戦略を考える（静岡）、2020 年 11 月 30 日
7. **海野けい子**:茶の主要成分の機能性　その２～カフェイン、テアニン～オンラインによる茶の機能性成分を活かした輸出戦略を考える（静岡）、2020 年 12 月 3 日
8. **海野けい子**:国内外で市販される抹茶や白葉茶の機能性の評価　オンラインによる茶の機能性成分を活かした輸出戦略を考える（静岡）、2020 年 12 月 10 日

9. **海野けい子**:お茶のテアニンの抗ストレス作用　お茶の健康機能性に関するウェブセミナー（東京）、2020 年 12 月 11 日
10. **海野けい子**:緑茶の機能性について　第２回宇治茶アカデミー、2020 年 12 月 23 日
11. **中村順行**:　教育講演　茶は養生の仙薬、その魅力と機能性　第 15 回日本禁煙科学学術会議総会（静岡）2020 年 12 月 19 日

対外活動

【講演】

1. **中村順行**:2020. 1. 次世代に展開する茶の魅力
2. **中村順行**:2020. 2. セイロン紅茶のブラディング
3. **中村順行**:2020. 2　静岡抹茶の特質
4. **中村順行**:2020. 2　茶の官能評価
5. **中村順行**:2020. 8. お茶の淹れ方
6. **中村順行**:2020. 8. ホットプレートを用いたお茶づくり
7. **中村順行**:2020. 8. 茶の機能と多用途利用
8. **中村順行**:2020. 10 高級抹茶の輸出戦略
9. **中村順行**:2020. 10　世界の茶の生産と加工
10. **中村順行**:2020. 10 幼児期のお茶
11. **中村順行**:2020. 11　サタセミ　茶の抗酸化力
12. **中村順行**:2020. 11　機能性成分を活かしたマーケティング
13. **中村順行**:2020. 11　食品素材としてのお茶
14. **中村順行**:2020. 11　他国産茶と日本産茶の区別性
15. **中村順行**:2020. 11　茶の機能性成分の変異
16. **中村順行**:2020. 12 お茶の文化で世界を巡る
17. **中村順行**:2020. 12 茶は養生の仙薬～その魅力と機能性

その他刊行物や新聞報道

【刊行物】

1. **海野けい子**:睡眠の質を高める「水出し緑茶」とは？　LUPICIA ルピシアだより　No. 295　2020 年 8 月
2. **中村順行**:特集　茶は養生の仙薬なり　総論　お茶と健康　サライ　2020 年 7 月号

【新聞報道等】

1. **斎藤貴江子**:2020 年 9 月 25 日「2 年生がお茶の効能に理解深め」　岳南朝日新聞
2. **中村順行**:2020 年 5 月 10 日　産経新聞　朝刊「初夏の香り　安らぐ甘さ　新茶」
3. **中村順行**:2020 年 7 月 11 日　静岡新聞　朝刊　「日本茶テーマ　英語で視聴　県立大学の国際的オンライン講義　浜松湖南高校生が体験
4. **海野けい子**:2020 年 8 月 26 日　静岡新聞　夕刊　「ＮＥＸＴ特捜隊　あなたの疑問調べます　夕食の習慣で困った！緑茶と睡眠の良い関係はカフェイン　半減に 6 時間　水出しでリラックスアップ
5. **中村順行**:2020 年 7 月 12 日　静岡新聞　朝刊「甘い粉末茶葉　商品化へ　東海大静岡翔洋高　授業始まる“家族で楽しむ”テーマ」
6. **中村順行**:2020 年 7 月 12 日　静岡新聞　朝刊「茶況　茶の薬膳スープ　掛川・山英発売」

7. **中村順行**: 広報しずおか 2020 年 1 月号 静岡市のナンバー１　オンリー１　始まりのやぶきた原樹！やぶきた茶の歴史や効能

【委員会等活動】

1. **斎藤貴江子**:富士山麓アカデミック＆サイエンスフェア実行委員
2. **斎藤貴江子**:静岡市環境審議会委員 （2016 年 11 月～）
3. **斎藤貴江子**:静岡県環境影響評価審査会委員 (2017 年～)
4. **斎藤貴江子**:食と農が支える豊かな暮らしづくり審議会委員 (2017 年～2020 年)
5. **海野けい子**:老化促進モデル(SAM)学会　幹事
6. **海野けい子**:基礎老化学会　評議委員
7. **海野けい子**:抗加齢医学会　評議委員
8. **中村順行**:日本茶業学会理事
9. **中村順行**:茶の湯文化学会理事
10. **中村順行**:ふじのくに茶の都ミュージアム収集保管事業評価委員
11. **中村順行**:国際銘茶品評会評茶委員
12. **中村順行**:日本茶アワード審査委員長
13. **中村順行**:日本茶新評価運営委員会委員
14. **中村順行**:ふじのくに茶の都運営委員
15. **中村順行**:ふじのくに茶の都ミュージアム客員研究員
16. **中村順行**:日本茶インストラクター認定委員
17. **中村順行**:日本茶アワード実行委員会委員
18. **中村順行**:茶産地確立支援事業推進委員
19. **中村順行**:日本茶アドバイザー専任講師
20. **中村順行**:全国手もみ保存会全国手もみ茶品評会審査委員
21. **中村順行**:静岡県茶手揉保存会審査員
22. **中村順行**:静岡市茶輸出推進協議会委員長
23. **中村順行**:掛川市茶振興計画策定委員長
24. **中村順行**:茶新需要検討委員会委員
25. **中村順行**:農水省普及指導員資格試験委員
26. **中村順行**:加工用原料茶開発促進協議会委員
27. **中村順行**:静岡県農林技術研究所茶業研究センター整備基本計画策定委員
28. **中村順行**:HACCP 手引書作成等(製茶)作業部会委員
29. **中村順行**;種苗法　出願品種現地調査員
30. **中村順行**:農林水産祭中央審査委員会委員
31. **中村順行**:ChaOI プロジェクト戦略推進委員
32. **中村順行**:第 8 回世界お茶まつり実行委員会委員
33. **中村順行**:茶成分近赤外分光分析方法 JAS 開発に関する準備委員会委員長

[研究・教育・社会活動]

1. **斎藤貴江子**:茶の効能，高大連携事業，静岡県立富岳館高等学校, 2020 年 9 月 24 日

令和元年度　茶学総合研究センター　メンバー

センター長	中村順行(特任教授)
副センター長	熊澤茂則(教授)
センター研究員	岩崎邦彦(教授)、斎藤貴江子(助教)
センター客員研究員	下位香代子(客員教授)、海野けい子(客員准教授)、伊勢村護(客員教授)、
センター事務員	林美智子、Monira Pervin(博士)、亀岡葉子、田口今日子

令和３年３月