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

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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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