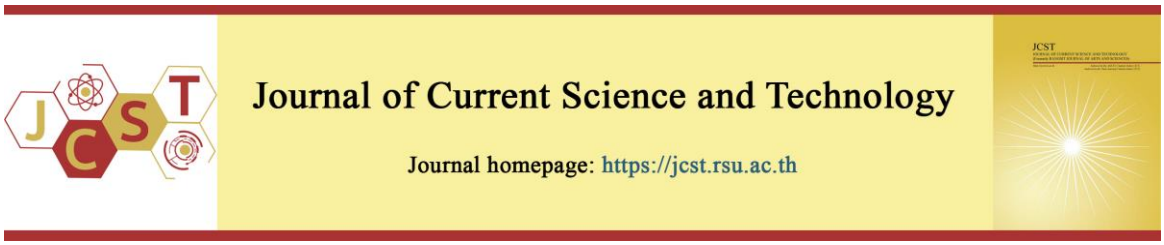
Journal of Current Science and Technology, September-December 2023 Vol. 13 No. 3, pp. 714-724
ISSN 2630-0656 (Online)

Cite this article: Kankasemsuk, S., Tibmanoo, C., Boonsuk, B., Bunma, S., & Suppasat, T. (2023). Total protein content of bee bread in *Apis cerana* combs and *Tetragonula pegdeni* storage pots of different plant sources from Chanthaburi province, Thailand. *Journal of Current Science and Technology, 13*(3), 714-724. https://doi.org/10.59796/jcst.V13N3.2023.1139



Total Protein Content of Bee Bread in *Apis Cerana* Combs and *Tetragonula Pegdeni* Storage Pots of Different Plant Sources from Chanthaburi Province, Thailand

Salinthip Kankasemsuk[1] Chanita Tibmanoo[1] Boonchuang Boonsuk[2] Saowalak Bunma[2] and Tipwan Suppasat[2*]

[1]Demonstration School University of Phayao, University of Phayao, Phayao 56000, Thailand
[2]Program in Biology, School of Science, University of Phayao, Phayao 56000, Thailand

*Corresponding author; E-mail: tipwan.su@up.ac.th

Received 4 May, 2023; Revised 26 June, 2023; Accepted 19 July, 2023;
Published online 30 August, 2023

Abstract

Bee bread is produced from fermented pollen. It is a popular bee product with high levels of protein and nutrients. The bee bread samples were collected from eight indigenous bee colonies in Chanthaburi province: three colonies of *Apis cerana* and five colonies of *Tetragonula pegdeni*. The study aims to compare the total protein content of bee bread using the Bradford assay and to identify the plant families that are food sources for these bees using the acetolysis. The results revealed that the protein content of bee bread from *A. cerana* ranged between 1.48±0.14 and 7.03±0.54 g/100 g, whereas it ranged between 1.78±0.43 and 2.60±0.13 g/100 g in *T. pegdeni*. Moreover, this result reveals a correlation between the food plant diversity and bee foraging. The pollen grains from bee bread of *A. cerana* and *T. pegdeni* were dominant in the family Fabaceae, high-protein plants. Besides, *A. cerana* (AC3) had the highest protein content of the main mixture of Asteraceae, Fabaceae, and Malvaceae pollen. Furthermore, the major plant families in this bee bread were Acanthaceae, Amaranthaceae, Cucurbitaceae, Euphorbiaceae, and Juncaceae. *Tetragonula pegdeni* had a greater pollen diversity of bee bread than *A. cerana*, which was dominant in the families Xyridaceae, Dipterocarpaceae, Fagaceae, Poaceae, and Rutaceae. As a result, the total protein content of the *A. cerana* colonies was higher than that of the *T. pegdeni* colonies. As a result, bee bread may be used as a protein source derived from bee products.

Keywords: *acetolysis; Apis cerana; bee bread; bradford assay; Tetragonula pegdeni; total protein content*

1. Introduction

Bee products are useful for the pharmaceutical or medical industry. In the present, bee bread is a mixture of pollen pellet honey fermentation and microorganisms in the brood cells of honey bees or storage pots in stingless bees. Bee bread is one of the bee by-products made from pollen collected by the bee and combined with nectar and bee salivary enzymes before undergoing lactic acid fermentation in beehives (Kieliszek et al., 2018). In the bee bread nutritive properties, it can be considered a valuable food supplement and nutritional values for human consumption (Urcan et al., 2017). The bee bread contains more major nutrients, such as protein. The effect of high biomolecule proteins in fermented pollen was reported as comprising 63% by mass, followed by carbohydrates with 23% by mass (Urcan et al., 2021). The bee bread is made from pollen fermentation. Its components, nutrients, and

biological compounds varied depending on the pollen collected by honey bees or stingless bees from the food plants in different seasons or areas. The valuable bee product can be obtained from bee bread, including the nutritive properties and biologically active compounds that are correlated with their food source origin (Mohammad et al., 2021). The protein concentration depends on the botanical origin of pollen. For example, in Saudi Arabia, the major floral sources of bee pollens such as alfalfa (Fabaceae), date palm (Arecaceae) and summer squash (Curcubitaceae) were reported as important sources of protein and amino acids for bees and human (Taha et al., 2019). In Malaysia, bee bread from stingless bee (*Heterotrigona itama*) has diverse colors ranging from yellow, orange, brown, black, and purple, which could indicate its botanical origin identified as five plant families; namely, Tymelaeaceae, Rutaceae, Arecaceae, Asteraceae, and Fabaceae. *Bidens pilosa* was recognized as a dominant species present in all bee bread (Mohammad et al., 2020). A stingless bee can carry pollen weighing 10.9 mg (Ramalho et al., 1998), and the pollen load is decreased if the size of the bee increases (Ramalho et al., 2009). The honey bee is larger than a stingless bee; therefore, it only carries about 7.9 mg of pollen (García-García et al., 2004).

The honey bee (*Apis cerana*) and stingless bee (*Tetragonula pegdeni*) are native to Thailand. These bees have several flowering resources and keep pollen pellets in their food brood cells or pots (Biesmeijer & Slaa, 2004). The stingless bees seek food resources within about 300 meters of their nest, which is a shorter distance than the honey bees. Stingless bees can have an average flight range within a radius of 712 meters, varying in species, body size, and food availability (Araujo et al., 2004; Smith et al., 2017; Basari et al., 2018). In Thailand, *A. cerana* is cultured in hives for apiculture and it is popular to raise three types of stingless bees, including *T. pegdeni*, *T. laeviceps*, and *Lepidotrigona terminata*, for meliponiculture (Lumsa-ed et al., 2017). Limited studies have reported on the bee bread's nutritional values, especially from the native bees, *A. cerana* and *T. pegdeni*, in Thailand. Chanthaburi province is a major fruit crop province of Thailand, and *A. cerana* and *T. pegdeni* are important pollinators for plantations in the area. The bee pollen and bee bread will be analyzed for their food plant origins by Erdtman's acetolysis method. The pollen analysis in bee pollen or bee bread grains reveals food plant species. Moreover, the protein degradation has been determined by Bradford assay, based on the equilibrium principle of Coomassie Brillant Blue G-250 and the combination of Coomassie Brillant Blue G-250.

2. Objectives

This study aims to examine the total protein content and food plant origin in bee bread from cultured *A. cerana* and *T. pegdeni* in Chanthaburi province, Thailand.

3. Materials and methods

3.1 Sample collection

A total of eight samples of bee bread were collected from three colonies of *A. cerana* (AC1, AC2, and AC3) and five colonies of *T. pegdeni* (ST6, ST7, ST8, ST10, and ST11). The bee bread of honey bee combs and stingless bee pots was sampled at the same area in Makham district, Chanthaburi province, between October and November 2021. Bee bread pellets were moved from combs or pots. The mixture of bee bread pellets in each vial was dried at 40 °C to constant weight, and the vials were kept at 10 °C for further analysis.

3.2 Total protein content analysis in bee bread

Each of the dried bee bread samples was weighted at 1 g and mashed with a grinding stick in a microcentrifuge tube. Then, the ground powder was weighted at 0.1 g and hydrolyzed in 1 mL distilled water (Westreich & Tobin, 2021). Samples of the bee bread solution were incubated at 55°C and centrifuged at 3,000 rpm for 2 minutes. The supernatant was removed to new microcentrifuge tubes for protein measurement. The total protein content was examined using the Bradford assay (Bradford, 1976) with absorbance measured at 595 nm using a spectrophotometer, the Spectrum SP-UV 200. We used the Bradford reagent, which consisted of Comassie Brilliant Blue G-250 in phosphoric acid and ethanol (Hemedia, HigeneMB, India). The standard curve was obtained from the absorbance of the series of bovine serum albumin (BSA) (Sigma-Andrich, USA) standard protein dilutions. Plots of the standard curve of absorbance at 595 nm on the Y axis versus the concentration of the BSA protein standard were generated using a linear function with a slope and intercept that were

calculated protein concentrations in bee bread samples.

Statistical analysis comparisons of the total protein content of eight colonies measured at 1 g/100 g were generated using a one-way ANOVA of the IBM SPSS program. Additionally, we used a P value <0.05 for statistical significance, which was analyzed by using multiple comparisons of Duncan and Post-Hoc tests (LSD).

3.3 Pollen analysis in bee bread

Each bee bread sample was a mixture of bee bread pellet colors, which were grouped by color as white, yellow, orange, brown, and dark brown. For botanical identification from pollen in bee bread was performed by the acetolysis procedure, adapted from Erdtman (1960) using acetic acid instead of acetic anhydride (it is restricted chemical in Thailand). Each bee bread pellet in each color was sampled, put in a 15 mL centrifuge tube, and mixed with 3 mL of 10% w/v potassium hydroxide. Then, the bee bread pellet was ground with a glass stick, centrifuged at 3,000 rpm for 2 minutes, and the supernatant was discarded. The pollen sediment was added to 3 mL of distilled water and centrifuged at 3,000 rpm for 2 minutes, three times. Furthermore, the sediment was added by 3 mL of acetic acid: sulfuric acid in a 9:1 ratio, boiled at 100°C for 3 minutes, centrifuged at 3,000 rpm for 2 minutes, and washed using 3 mL distilled water as well. Next, 3 mL of each alcohol series (75%, 95%, and absolute ethanol, respectively) was added to the pollen sediment, centrifuged, and discarded. Absolute ethanol was added to the pollen sediment one more time, followed by benzene. The samples were gently mixed and moved to the vials. Then, 0.5 mL of silicone oil was added to each vial. The vials were opened overnight in a fume hood for benzene evaporation and kept the pollen sediment at room temperature.

One drop of pollen sediment from the acetolysis process was mounted on a glass slide and fixed with a cover slide with paraffin at 60 °C on a slide warmer. Palynological analysis and photographs were performed to identify the botanical families using a 100- and 400-magnification compound light microscope (Olympus C21, Japan) connected with the Optika digital camera and Optikam WiFi 7083, WiFi version 1.0 software (Optika, Italy).

Table 1 Color of bee bread pellets from bee bread in *Apis cerana*'s cells and *Tetragonula pegdeni*'s pots

Bee species	Sample no.	Color of bee bread pellets
Apis cerana	AC1	
	AC2	
	AC3	
Tetragonula pegdeni	ST6	
	ST7	

Table 1 Cont.

Bee species	Sample no.	Color of bee bread pellets
	ST8	
	ST10	
	ST11	

Table 2 Plant family identification of pollen acetolysis from bee bread in *Apis cerana*'s cells and *Tetragonula pegdeni*'s pots

Plant families	Bee bread samples							
	Apis cerana			*Tetragonula pegdeni*				
	AC1	AC2	AC3	ST6	ST7	ST8	ST10	ST11
Acanthaceae	+++	+	-	+	+	++	+++	-
Amaranthaceae	+	++	-	-	-	-	-	-
Annonaceae	-	-	+	-	-	-	-	-
Asteraceae	-	-	++++	-	-	+	-	+
Balsaminaceae	+	-	-	-	-	-	-	-
Caprifoliaceae	-	-	+	-	-	-	+	-
Cucurbitaceae	-	-	-	-	-	-	+	-
Dipterocarpaceae	-	+	-	-	+	+	+	+
Euphorbiaceae	-	+	-	+	+	+	+	-
Fabaceae	++++	+++	+	+++	+++	++++	++	++++
Fagaceae	+	+	+	-	-	+	-	-
Ginkgoaceae	-	-	-	+	-	-	+	-
Juncaceae	-	+	+	+	+	+	+	+
Lamiaceae	+	-	-	-	-	-	-	-
Liliaceae	+	++++	-	-	-	-	-	-
Malvaceae	-	+	+	-	+	-	+	-
Meliaceae	-	+	-	-	+	+	-	-
Musaceae	+	+	-	-	-	-	-	-
Oleaceae	-	-	-	-	-	-	+	-
Orobanchaceae	-	-	-	+	+	-	-	-
Papaveraceae	-	-	-	-	-	-	+	-
Poaceae	-	+	-	+	+++	+++	+++	+
Rosaceae	-	-	-	-	+	-	+	-

Table 2 Cont.

Plant families	**Bee bread samples**							
Rutaceae	***Apis cerana***	***Tetragonula pegdeni***	-	+	++	-	++	-
Sapindaceae	**AC1**	**AC2**	**AC3**	**ST6**	**ST7**	**ST8**	**ST10**	**ST11**
Symplocaceae	-	-	-	-	-	+	+	+
Verbenaceae	-	+	-	-	-	-	+	-
Vitaceae	-	-	-	-	++	+	+	+++
Xyridaceae	-	-	-	++++	++++	++++	++++	++++

Remarks: - represented not detected pollen grains.
+, ++, +++, ++++ represented level of relative density (%) of pollen grains in bee bread samples: + (1-25), ++ (26-50), +++ (51-75), ++++ (76-100).

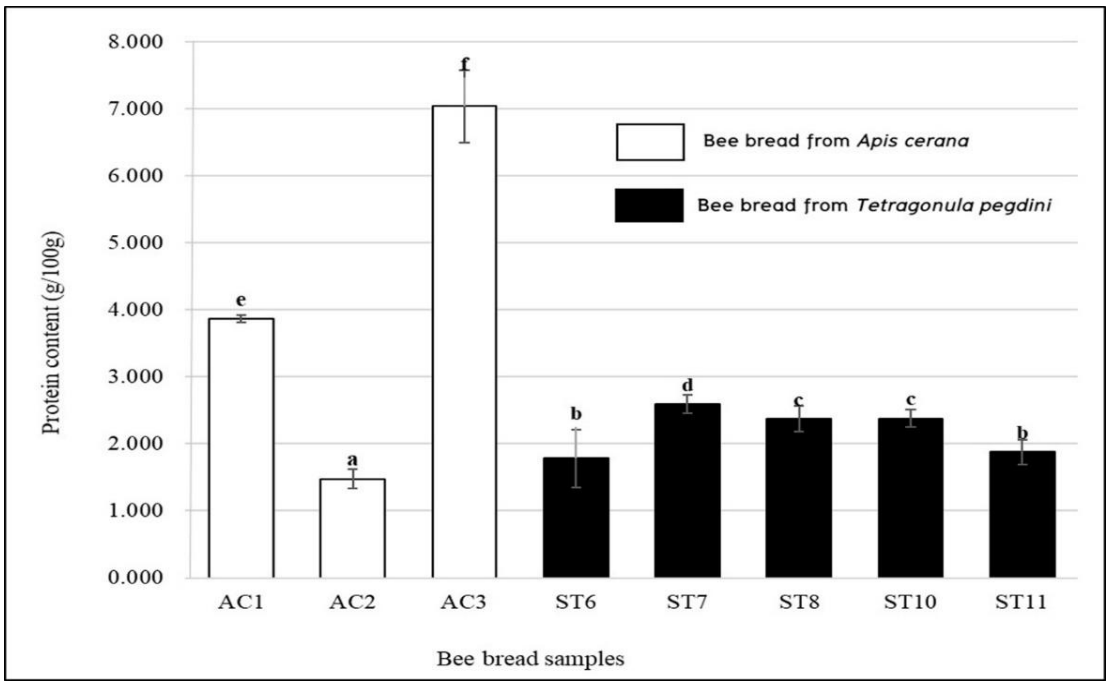


Figure 1 The bar graph shows the average total protein contents (g/ 100 g) of bee bread from three *Apis cerana* (in white color) and five *Tetragonula pegdeni* colonies (in black). Lowercase symbols a, b, c, d, e and f represented statistics significant between colonies at the $P < 0.05$ of the one-way ANOVA and Duncan's test.

4. Results

4.1 Bee bread pellet color of *A. cerana* and *T. pegdeni*

Three bee bread samples of *A. cerana* in AC1, AC2 and AC3 showed the different colors of bee bread pellets in AC2 comprising eight colors. The AC2 bee bread pellets were a diverse pollen grain mixture with yellow, orange, brown, and dark brown colors. In contrast, AC1 samples showed four colors which consisted of yellow to light brown. In addition, the AC3 sample showed two colors but orange color was the dominant one. Five stingless bee samples showed six different colors of bee bread pellets in the ST8 and ST10 samples, five colors in the ST7 samples and three colors in the ST6 and ST11 samples. The bee bread pellets were highly diversified in their colors, of which light-yellow, yellow, red, brown, and dark-brown colors were dominant (Table 1).

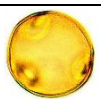
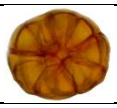
4.2 Total protein contents and botanical identification of *A. cerana* and *T. pegdeni* bee bread

The results of the total protein content of eight bee bread samples were only from the Bradford assay. The results showed the comparison

of the total protein contents of the three samples of bee breads in honey bee combs and five samples of stingless bee pots from the same area in Chanthaburi province. There was a difference in the average of the protein contents among three bee breads from *A. cerana* samples, approximately 4.12±2.79 g/100g which was higher than *T. pegdeni* bee bread, approximately 2.02 ±0.35 g/100g. The highest total protein content was found in the AC3 with 7.03±0.54 g/100g and the AC1 with 3.86±0.05 g/100g, respectively, whereas the AC2 was the lowest protein content with 1.48±0.14 g/100g which was lower than the five stingless bee samples. Regarding *T. pegdeni*, the highest protein content was the ST7 (2.60±0.13 g/100g), and the lowest one was the ST6 (1.78±0.43 g/100g) followed by the ST11 (1.88±0.18 g/100g). The highest and lowest protein contents in the ST6 and ST7 (and ST11), respectively. There were statistically significant with the ST8 and ST10, i.e., the protein content in the ST8 contained 2.37±0.19 g/100g and 2.38±0.12 g/100g in the ST10 (Figure 1 and Table 4).

The pollen characteristics from acetolysis method showed the diversity of food plants of honeybees and stingless bees (Figure 2-4). The light microscopy analysis of pollen grain characteristics from both *A. cerana* (AC) and *T. pegdeni* (ST) bee bread samples showed in Figure 2 and separated only AC samples (Figure 3), and ST samples in Figure 3 and Figure 4, respectively. The plant origin was identified to 28 plant families. There were 21 unknown pollen forms dividing to 9 in AC and 12 in ST bee bread samples. The food plant origin of pollen from all bee bread samples was in Fabaceae which were dominant in AC1, ST8 and ST11. Contrarily, Liliaceae was dominant in AC2 sample, Asteraceae was dominant in AC3 sample, and Xyridaceae was dominant in all of ST bee bread samples. Moreover, Sapindaceae was dominant in ST7 and ST10 bee bread samples. ST10 was also found in the Acanthaceae (Table 2). In terms of plant resources, nine pollen types from the Fabaceae family contain high-protein plant resources. There were 2 major pollen types at the 1st and 2nd types in all samples. The 3rd to 9th pollen types were small amount in samples (Table 3). The AC3 was found only the 2nd pollen type, not found the 1st and other Fabaceae. Nevertheless, the AC3 showed the highest total protein content, found the major family Asteraceae which was a monofloral in bee bread pellets (Table 4). Contrast to the AC2 showed the lowest protein content which was found in 17 plant families and dominant pollen in family Liliaceae. The dominant food plant families for stingless bee were Xyridaceae mixing with the Fabaceae or Sapindaceae. These results showed that *A. cerana* and *T. pegdeni* forage in different food plants, but they overlapped with some plant resources in the same area.

Table 3 The Fabaceae pollen types in bee bread of *Apis cerana*'s cells and *Tetragonula pegdeni*'s pots

Samples	Fabaceae pollen types								
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
									
Apis cerana									
AC1	++++	++	-	+	-	-	-	-	-
AC2	+++	+	+	+	-	-	-	-	-
AC3	-	+	-	-	-	-	-	-	-
Tetragonula pegdeni									
ST6	+++	++	-	-	+	-	-	-	-
ST7	+++	++	-	-	-	+	-	-	-
ST8	+++	++++	-	-	-		+	+	-
ST10	+	++	+	-	+	-	-	-	+
ST11	++++	+	+	-	-	-	-	-	-

Remarks: - represented not detected pollen grains.
+, ++, +++, ++++ represented level of relative density (%) of pollen grains in bee bread samples: + (1-25), ++ (26-50), +++ (51-75), ++++ (76-100).

Table 4 Comparison of the total protein content average (mean±SD) and pollen identification from bee bread

Bee species	Samples	Total protein contents Mean ±SD (g/100g)	Pollen types in bee bread	
			No. of families	Dominant of plant families
Apis cerana	AC1	1.48±0.14	7	Fabaceae
	AC2	3.86±0.05	15	Liliaceae
	AC3	7.03±0.54	7	Asteraceae
Tetragonula pegdeni	ST6	1.78±0.43	8	Xyridaceae
	ST7	2.60±0.13	14	Sapindaceae, Xyridaceae
	ST8	2.37±0.19	12	Fabaceae, Xyridaceae
	ST10	2.38±0.12	19	Sapindaceae, Xyridaceae
	ST11	1.88±0.18	8	Fabaceae, Xyridaceae

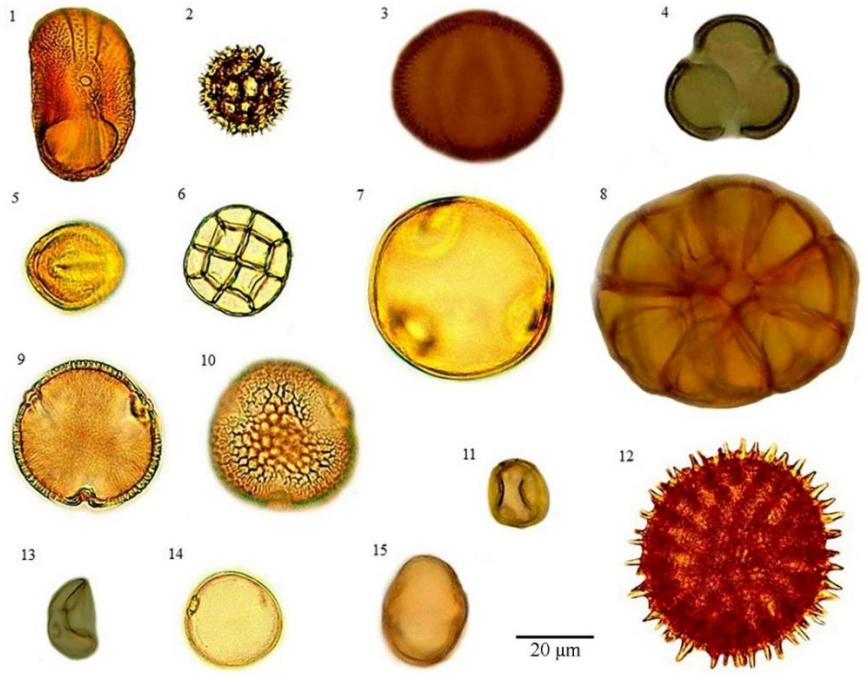


Figure 2 Light microscopy of pollen grains from bee bread of *Apis cerana* and *Tetragonula pegdeni*: 1. Acanthaceae, 2. Asteraceae, 3. Caprifoliaceae, 4. Dipterocarpaceae, 5. Euphorbiaceae, 6-8. Fabaceae, 9-10. Fagaceae, 11: Juncaceae, 12. Malvaceae, 13. Meliaceae, 14. Poaceae, 15. Verbenaceae

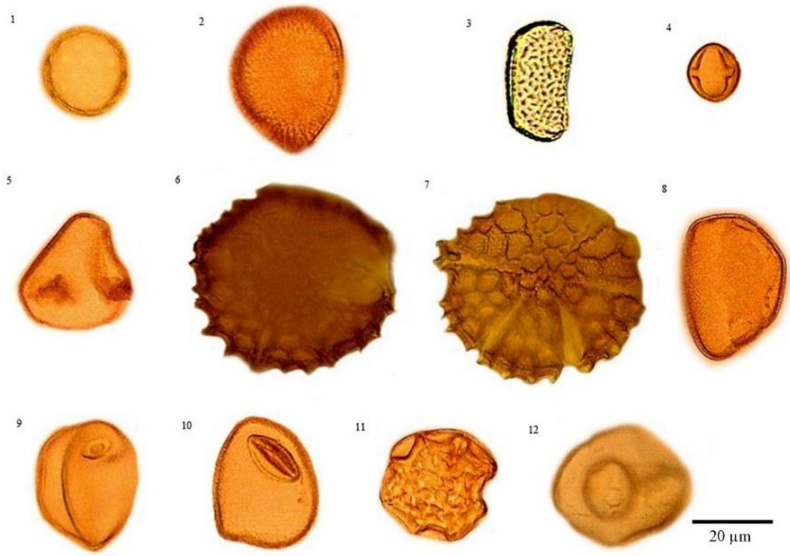


Figure 3 Light microscopy of pollen grains from bee bread of *Apis cerana*: 1. Amaranthaceae, 2. Annonaceae,3. Balsaminaceae, 4. Fabaceae, 5. Juncaceae, 6-7. Lamiaceae, 8-10. Liliaceae, 11. Musaceae, 12. Sapindaceae

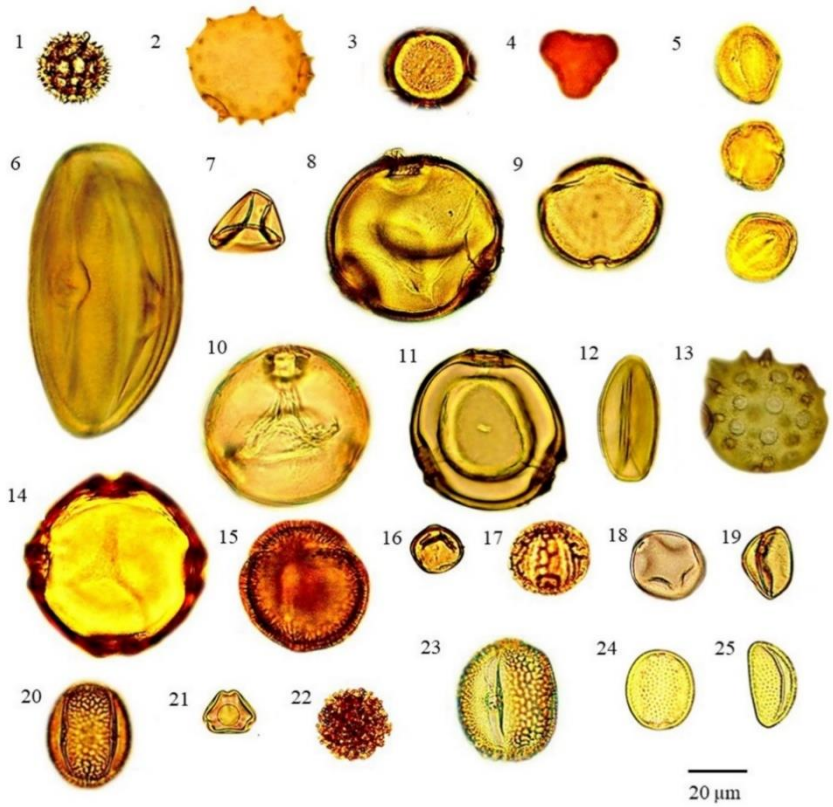


Figure 4 Light microscopy of pollen grains from bee bread of *Tetragonula pegdeni*: 1. Asteraceae, 2. Cucurbitaceae, 3-6. Euphorbiaceae, 7-11. Fabaceae, 12. Ginkgoaceae, 13. Malvaceae, 14. Meliaceae, 15. Oleaceae, 16. Orobanchaceae, 17. Papaveraceae, 18. Poaceae, 19. Rosaceae, 20. Rutaceae, 21. Sapindaceae, 22. Symplocaceae, 23. Vitaceae, 24-25. Xyridaceae

5. Discussion

Almost all of the bee bread of *T. pegdeni* from one pot was the same color. While one cell of *A. cerana* bee bread was a mixture of pollen pellets. Moreover, these results indicated that *A. cerana* and *T. pegdeni* might forage on different flowering plant resources in the same area. Analysis of pollen colors could give an insight into its floral origin of their food sources, but a single pollen color does not indicate the monofloral source (Almeida-Muradian et al., 2005). Bee pollen or bee bread color is also associated with their phenolic content. The pollen color is attributed to the presence of flavonoid which relatively depends on the plant source (Mohammad et al., 2021). Moreover, the protein concentration depends on the botanical origin of pollen. The protein levels are varied across different geographical locations and between bee species (Taha et al., 2019; Mohammad et al., 2021). The results revealed that the protein content of bee bread from *A. cerana* ranged between 1.48±0.14 and 7.03±0.54 g/100g, whereas it ranged between 1.78±0.43 and 2.60±0.13 g/100g in *T. pegdeni.* Belina-Aldemita et al. (2019) quantified the total free amino acids in *T. biroi* stingless bee from the Philippines and found mean values of only 1.83 g/100g bee bread. Although protein content decreases from fresh pollen to bee bread, the amino acid concentration remains unchanged in fresh pollen, bee pollen, and bee bread (Human & Nicolson, 2006; Nicolson & Human, 2012).

A correlation between the food plant diversity and bee foraging, the pollen grains of *A. cerana* and *T. pegdeni* bee bread were identified to 29 plant families (Table 2) with five dominant families, Asteraceae, Fabaceae, Liliaceae, Sapindaceae, and Xyridaceae (Table 4). In honey bee bread, the highest protein content bee was found in the AC3 (Figure 1), correlated with relative density of pollen grains of Asteraceae. The protein content in AC1 was higher than AC2 which the former contained major pollen from Acanthaceae and Fabaceae whereas Fabaceae and Liliaceae were dominant families for the latter (Table 2). In stingless bee bread, the range of protein content was nearby in all samples which found the dominant pollen sources from Fabaceae and monocot families, Liliaceae, Poaceae and Xyridaceae. Pollen morphology is species-specific; therefore, it provides valuable information on the pollen's botanical origin and plants preferentially visited by the bees (Ibrahim et al., 2012; Thakodee et al., 2018). In addition, the analysis of protein from pollen load of honey bee (*A. mellifera*) in Spain is due to the fact that the different species that integrate the pollen type flower on different dates, and thus have a pollen with different characteristics. Honey bees varied their use of the different plants species depending on what flowers were available to them at the time. The pollen of the plant species that reached relatively higher percentages in the pollen spectrum are also those that have the highest protein content (de Sá-Otero et al., 2009). This helps beekeepers plan and manage suitable landscape for bee foraging. Moreover, identifying plant origin also contributes to a better understanding of bee's nutritional source, and bee products made from these plants, eventually, give commercial value to it (de Souza et al., 2019). It is interesting to monitor the bee behaviour and the plant species used as pollen sources and to determine the individual protein content of each plant species (Mohammad et al., 2020; 2021).

6. Conclusion

The results indicated the difference of pollen sources of *A. cerana* and *T. pegdeni* which forage to their colonies. The *A. cerana*, bee bread showed three dominant food plant families, namely Asteraceae, Fabaceae, and Liliaceae. Regarding dicotyledon plants, bee breads of *A. cerana* from Asteraceae contained higher total protein contents than that from Fabaceae. On the contrary, the bee bread from Liliaceae as monocotyledon sources has the lowest protein contents. According to the food plant preference, family Xyridaceae was dominant in stingless bee pots. Moreover, forage pollen of the *T. pegdeni* was major from dicotyledon families such as Fabaceae and Sapindaceae, mixed with Xyridaceae. The total protein contents of *T. pegdeni* bee breads were closely related with *A. cerana* bee bread which had family Liliaceae as its additional food plant resource. It is interesting the bee behaviour and the plant species used as pollen sources. This research suggests that examining the bee foraging behaviour and origin of botanical plant sources play an important role in the future development of the nutrient of bee bread from honey bees and stingless bees in Thailand.

7. Acknowledgements

This project was supported by Science Classroom in University Affiliated School (SCiUS). The funding of SCiUS is provided by

Ministry of Higher Education, Science, Research and Innovation, Jointed by Demonstration School University of Phayao and Economic Insects Research Unit, School of Science, University of Phayao. Also, we would like to thank Mrs. Arlai Jitcharearn for collecting all the samples.

8. References

Almeida-Muradian, L. B., Pamplona, L. C., Coimbra, S., & Barth, O. M. (2005). Chemical composition and botanical evaluation of dried bee pollen pellets. *Journal of Food Composition and Analysis*, *18*(1), 105–111. https://doi.org/10.1016/j.jfca.2003.10.008

Araujo, E. D., Costa, M., Chaud-Netto, J., & Fowler, H. G. (2004). Body size and flight distance in stingless bees (Hymenoptera: Meliponini): inference of flight range and possible ecological implications. *Brazilian Journal of Biology*, *64*(3b), 563–568. https://doi.org/10.1590/S1519-69842004000400003

Basari, N., Ramli, S. N., & Khairi, N. S. M. (2018). Food reward and distance influence the foraging pattern of stingless bee, *Heterotrigona itama*. *Insects*, *9*(4), Article 138. https://doi:10.3390/insects9040138

Belina-Aldemita, Ma. D., Opper, C., Schreiner, M., & D'Amico, S. (2019). Nutritional composition of pot-pollen produced by stingless bees (*Tetragonula biroi* Friese) from the Philippines. *Journal of Food Composition and Analysis*, *82*, Article 103215. https://doi: 10.1016/j.jfca.2019.04.003

Biesmeijer, J. C., & Judith Slaa, E. (2004). Information flow and organization of stingless bee foraging. *Apidologie*, *35*(2), 143–157. https://doi.org/10.1051/apido:2004003

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *7*(72), 248–254. http://doi: 10.1006/abio.1976.9999

de Sá-Otero, M. D. P., Armesto-Baztan, S., & Díaz-Losada, E. (2009). Analysis of protein content in pollen loads produced in north-west Spain. *Grana*, *48*(4), 290–296. https://doi.org/10.1080/00173130903149140

de Souza, R. R., de Abreu, V. H. R., & de Novais, J. S. (2019). Melissopalynology in Brazil: a map of pollen types and published productions between 2005 and 2017. *Palynology*, *43*(4), 690–700. https://doi.org/10.1080/01916122.2018.1542355

Erdtman, G. (1960). The Acetolysis Method—A Revised Description. *Svensk Botanisk Tidskrift*, *54*, 561–564.

García-García, M. C., Ortiz, P. L., & Dapena, M. J. D. (2004). Variations in the weights of pollen loads collected by *Apis mellifera* L. *Grana*, *43*(3),183–192. https://doi.org/10.1080/00173130410020350

Human, H., & Nicolson, S. W. (2006). Nutritional content of fresh, bee-collected and stored pollen of *Aloe greatheadii* var. *davyana* (Asphodelaceae). *Phytochemistry*, *67*, 1486–1492. https://doi.org/10.1016/j.phytochem.2006.05.023

Ibrahim, I. F., Balasundram, S. K., Abdullah, N. A. P., Alias, M. S., & Mardan, M. (2012). Morphological characterization of pollen collected by *Apis dorsata* from tropical rainforest. *International Journal of Botany*, *8*(3), 96–106. https://doi.org/10.3923/ijb.2012.96.106

Kieliszek, M., Piwowarek, K., Kot, A. M., Błazejak, S., Chlebowska-Smigiel, A., & Wolska, I. (2018). Pollen and bee bread as new health-oriented products: A review. *Trends in Food Sciences and Technology*, *71*, 170–180. https://doi.org/10.1016/j.tifs.2017.10.021

Lumsa-ed, J., Prommae, P., & Suthaso, V. (2017). Culturing Stingless Bee in Surat Thani province. *Prawarun Agricultural Journal*, *14*(1), 1–9.

Mohammad, S. M., Mahmud-Ab-Rashid, N. K., & Zawawi, N. (2020). Botanical origin and nutritional values of bee bread of stingless bee (*Heterotrigona itama*) from Malaysia. *Journal of Food Quality*, *2020*, Article 2845757. https://doi.org/10.1155/2020/2845757

Mohammad, S. M., Mahmud-Ab-Rashid, N. K., & Zawawi, N. (2021). Stingless bee-collected pollen (bee bread): chemical and microbiology properties and health benefits. *Molecules*, *26(*4), Article 957. https://doi.org/10.3390/molecules26040957

Nicolson, S. W., & Human, H. (2012). Chemical composition of the 'low quality' pollen of sunflower (*Helianthus annuus*, Asteraceae). *Apidologie*, *44*, 144–152. https://doi.org/10.1007/s13592-012-0166-5

Ramalho, M., Giannini, T. C., Malagodi-braga, K. S., Vera, L., & Imperatriz-fonseca, V. L. (2009). Pollen harvest by stingless bee foragers (Hymenoptera, Apidae, Meliponinae). *Grana*, *33*(4-5), 239–244. https://doi.org/10.1080/00173139409429005

Ramalho, M., Imperatriz-Fonseca, V. L., & Giannini, T. C. (1998). Within-colony size variation of foragers and pollen load capacity in the stingless bee *Melipona quadrifasciata anthidioides* Lepeletier (Apidae, Hymenoptera). *Apidologie*, *29*(3), 221–228. https://doi.org/10.1051/apido:19980302

Smith, J. P., Heard, T. A., Beekman, M., & Gloag, R. (2017). Flight range of the Australian stingless bee *Tetragonula carbonaria* (Hymenoptera: Apidae). *Austral Entomology*, *56*(1), 50–53. https://doi.org/10.1111/aen.12206

Taha, E. K. A., Al-Kahtani, S., & Taha, R. (2019). Protein content and amino acids composition of bee-pollens from major floral sources in Al-Ahsa, eastern Saudi Arabia. *Saudi Journal of Biological Sciences*, *26*(2), 232–237. https://doi.org/10.1016/j.sjbs.2017.06.003

Thakodee, T., Deowanish, S., & Duangmal, K. (2018). Melissopalynological analysis of stingless bee (*Tetragonula pagdeni*) honey in Eastern Thailand. *Journal of Asia-Pacific Entomology*, *21*(2), 620–630. https://doi.org/10.1016/j.aspen.2018.04.003

Urcan, A. C., Criste, A. D., Dezmirean, D. S., Bobiș, O., Bonta, V., Dulf, F. V., ... & Campos, M. G. (2021). Botanical origin approach for a better understanding of chemical and nutritional composition of beebread as an important value-added food supplement. *LWT- Food Science and Technology*, *142*, Article 111068. https://doi.org/10.1016/j.lwt.2021.111068

Urcan, A., Mařghitas, L. A., Dezmirean, D. S., Bobiș, O., Bonta, V., Mureşan, C.I., & Mărgăoan, R. (2017). Chemical composition and biological activities of beebread–review. Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. *Animal Science & Biotechnologies*, *74*(1), 1–9. https://doi.org/10.15835/buasvmcn-asb:12646

Westreich, L. R., & Tobin, P. C. (2021). Comparison of pollen grain treatments without mechanical fracturation prior to protein quantification. *Journal of Insect Science*, *21*(4), 1–4. https://doi.org/10.1093/jisesa/ieab043