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Original research article

Chemical Compositional Characterization on Five Samples for Development of Artificial Bee Feed

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Abstract The western honey bee, *Apis mellifera* L. is an essential pollinator for high yield in agriculture and provides products of economic values. Recently, the sudden decline of the bee population by numerous causes has occurred, which is called colony collapse disorder (CCD). One of the reasons in CCD, the limited nutrition in colonies declines brood rearing and shortens the lifespan of adult workers. Beekeepers need to provide artificial bee feed regularly to maintain health colony and continuity of bee-related products on apiculture. This study focuses on development of artificial bee feed, through investigation of different contents in nutritional components on five samples, namely canola pollen, mixed pollen, bee bread, MegaBee, and Test A. Among them, Test A was developed as artificial bee feed and was compared with other samples. The five samples were analyzed on free sugar, organic acid and amino acid. Sucrose content showed the largest amounts with 46.15% and 59.60% on Megabee and Test A, respectively. The Test A showed the largest account for citric acid with 92.33%. A total of 21 amino acids were detected on five samples. Among the detected 21 amino acids, proline was accounted the highest content in canola pollen, mixed pollen and bee bread with 1159.21 mg/L, 998.90 mg/L and 783.73 mg/L, respectively. Among the five samples, Test A showed the highest amount of lysine with 471.30 mg/L. Nutritional content, balance and efficiency needs to be considered for the development of artificial bee feed. This study will contribute to provide future directions on development of artificial bee feed.

Keywords Artificial bee feed, Nutrition, Free sugar, Organic acid, Amino acid

INTRODUCTION

The Western honey bee, *Apis mellifera* L., is an essential partners of pollination for high yield in agriculture. It also provides honey, pollen, bee bread, propolis, royal jelly and bee venom and these products offer economic value (Kieliszek *et al*., 2018). The recent declines in honey bee populations and happened the sudden loss of honey bee in a colony is called colony collapse disorder (CCD) (Vanengelsdorp *et al*., 2015). The causes for CCD have been attributed to pesticide, parasitic mite (Amdam *et al*., 2004) and disease, but perhaps the reasons for bee-population declines is inadequate nutrition (van der Steen, 2007). A steady supply of pollen stimulates brood rearing and provides growth of colonies. Whereas, low nutrition contributes to reduced brood rearing and early transition in workers from nursing to foraging (DeGrandi-Hoffman *et al*., 2010). This early transition in workers negatively affect the longevity of honey bees. The premature foragers die faster than normal bees. Thus, colonies with limited nutrition will decline brood rearing and a shorter lifespan in adult workers. If parasitic mites and viruses are present, the colony decline can be even more serious and could be happened CCD.

Bees usually consume pollen after it is fermented, in

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the form of bee bread (Morais *et al*., 2013) Bee bread is the result of lactic fermentation of pollen collected by bees from flowers, mixed by their digestive enzymes and honey (Kieliszek *et al*., 2018). It differs from freshly collected pollen, in having a lower pH and less starch content (Herbert and Shimanuki, 1978; Ellis and Hayes, 2009). It is considered to have a higher nutritional value than pollen, better digestibility, and richer chemical composition. Bee bread is the main food in the hive especially for larva and young bees and it is the main source of protein in the diet of bees, determines their good health. It is mainly composed of proteins, vitamins, minerals, amino acids, fats and carbohydrates. The bee bread is considerably larger amount of peptides and free amino acids, because the proteins in bee bread are more biological active they are easily absorbed. Because of the proportions of ingredients in bee bread, it is a perfect supplementary nutrient.

Beekeepers is the necessity to maintain honey bee colonies in good condition during times when pollen is in short supply or not available (Morais *et al*., 2013). To maintain continuity of bee related products, beekeepers need to provide artificial bee feed regularly. But in Korea, beekeepers normally use pollen collected by other honey bee colonies is not a good option considering the health of the hive. Because of the risk of introducing disease into the colony (van der Steen, 2007). Therefore, artificial bee feed development is of vital importance for maintaining a healthy colony and increasing the productivity in apiculture. The purpose of this study is to investigate the nutritional value of canola pollen which is representative pollens that are widely used, mixed pollen, bee bread, MegaBee (commercial bee diet supplement) and our developed product which is named Test A that are replacing pollen components. The nutritional value was analyzed contents of free sugar, organic acid, and amino acids. This study will contribute towards providing a future direction on development of better artificial bee feed.

MATERIALS AND METHODS

1. Free sugar analysis

The free sugars from samples were analyzed using Dionex ultimate 3000 (Dionex). Free sugars were identified using a refractive index detector (Ri-101, Shodex) and a Sugar-pak column (Waters). The mobile phase was deionized water at a flow rate of 0.5 mL/min and the oven temperature was set to 80°C.

2. Organic acids analysis

The organic acids were analyzed using Dionex DX500 ion chromatograph (Dionex). Organic acids were identified using an electro conductivity detector and an amount to 20 μL of each sample was injected into the ICE-AS6 column. The mobile phase was 0.4 mM heptafluorobutyric acid at a flow rate of 1 mL/min with Anion-ICE micromembrane suppressor as a suppressor and 5 mM tetrabutylammonium hydroxide as a regenerant.

3. Amino acids analysis

The amino acids from each sample were analyzed using o-phthalaldehyde (OPA)/mercaptopropionic acid (MPA) and fluorenylmethyl chloroformate (FMOC) derivatization. The samples were mixed in borate buffer with OPA and FMOC. Samples were then analyzed using an Agilent 1200 series HPLC instrument (Agilent Technologies). After derivatization, an amount to 1 μL of each sample was injected into the INNO C18 column, 4.6 mm × 150 mm, 5 μm (Youngjin Biochrom) at 40°C. Ultraviolet rays were detected at λ = 338 nm by connected UV detectors. The emission and excitation wavelengths measured using fluorescence were 450 and 340 nm for the OPA derivative and 305 and 266 nm for the FMOC derivative. The mobile phase was comprised of solutions A (10 mM Na_2HPO_4 and 10 mM $Na_2B4O_7 \cdot 10H_2O$, pH 8.2) and B (water : acetonitrile (ACN) : methanol (MeOH) = 10 : 45 : 45, v/v%) with a flow rate of 1.5 mL/min. The gradient of A: B was initially set to 100 : 0 (v/v%), 55 : 45 at 26~28 min, 0 : 100 at 28~30.5 min, and 100 : 0 from 30.5 min.

RESULTS AND DISCUSSION

1. Protein sources for development of artificial bee feed and Test A

The nutritional value of pollen often is evaluated by

protein concentrations and amount of essential amino acids (Brodschneider and Crailsheim, 2010). To find protein sources for development of artficial bee feed, we searched twelve canditate protein sources which were industrial by-products. The sources were analysied for crude fiber, crude protein and essential amino acids. Depending on the results, brewer's yeast and soybean oil meal were chosen for protein sources of substitute diet, which is named as Test A. The contents of Test A and the price of each ingredient was listed in Table 1. In test A, brewer's yeast and soybean oil meal was mainly used for protein suppliment. Soybean oil meal is suitable and most used feed for all livestock. The protein content is 50% after removing the skin of soybean, but 44% if the skin is not removed. Soybean oil meal is the standard for comparing the price or quality of different protein feeds. In soybean oil meal, the protein content or amino acid composition is uniform compared to other plant protein feeds. But soybean oil meal lacks of amino acids such as methionine, lysine and cystine, making it a limited source of amino acid for unit animals. Thus we mixed Brewer's yeast in Test A, it contain a lot of proteins, nucleic acids, carbohydrates, minerals, and vitamins.

Table 1. Contents and ingredient prices of Test A

Content	Content per 1 kg	Price (won)
Brewer's yeast	450 g	4500
Sucrose	500 g	655
Soybean oil meal	25 g	75
Egg Yolk	25 g	682.5
Vegetable oil	12 mL	122.7
Water	25 mL	
Total	Powder: 1 kg	About 6035.2

2. Carbohydrates

Carbohydrates are primary energe source of honey bees (Brodschneider and Crailsheim, 2010). Nectar provides main source of carbohydrates for most bee species (Vaudo *et al*., 2015). Therefore sucrose is added in Teast A. Free sugar composition of honey accounts for an average of 38% fructose, 31% glucose which are the only monosaccharides in honey and other di and trisaccharids (Doner, 1977). Bee larvae require carbohydrates for normal development, often in the form of bee bread, however, a large quantity of carbohydrate-rich nectar is required by adult foragers (Brodschneider and Crailsheim, 2010). An adult honey bee worker require approximately 4 mg of utilizable sugars per day for survival (Barker and Lehner, 1974). Workers at foraging age have the enzymes necessary to use polysaccharides (starch) for flight metabolism (Hrassnigg *et al*., 2005). A lack of carbohydrates limits the number of larvae

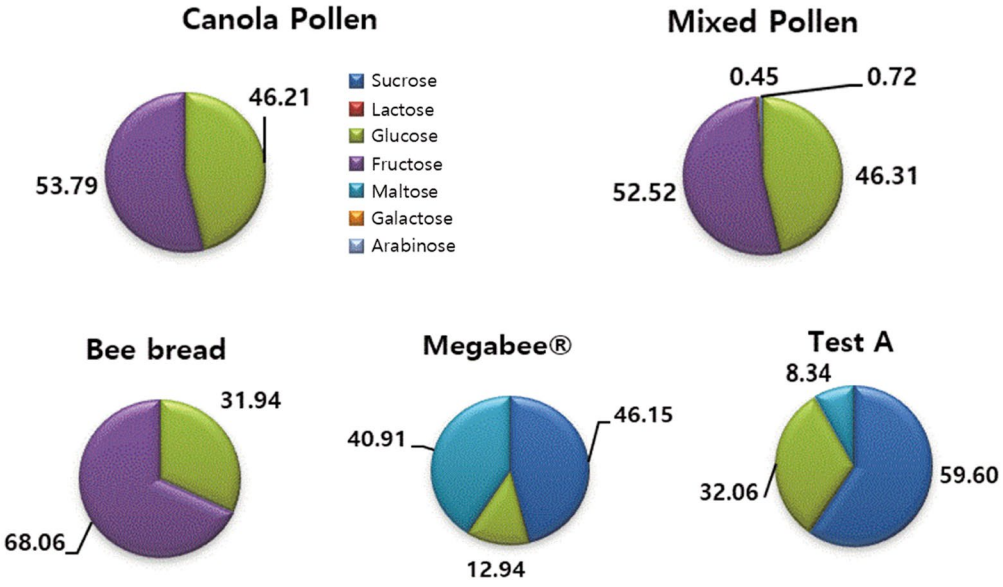

Fig. 1. Contents of free sugar on five samples (%).

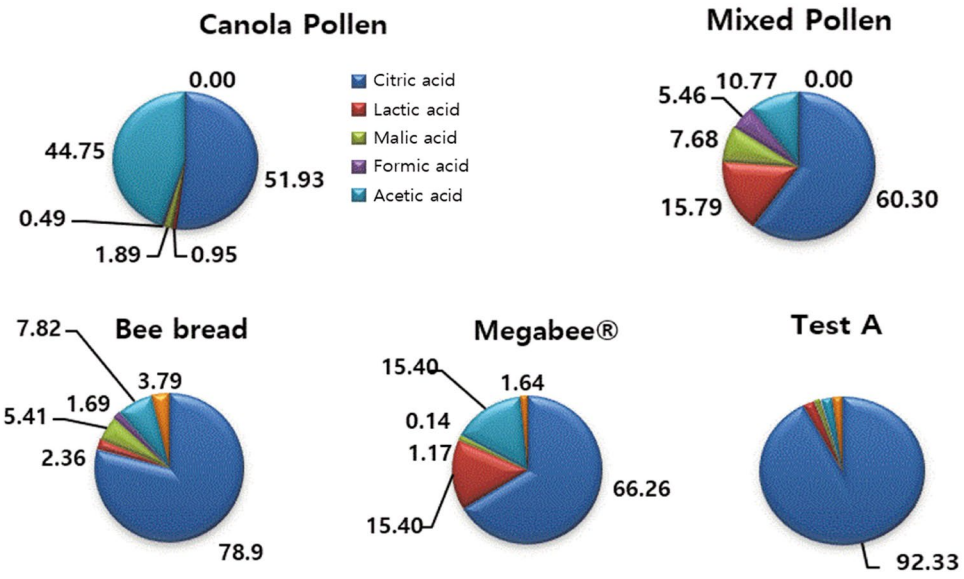

Fig. 2. Contents of organic acid on five samples (%).

reared, when nectar sources are poor and winter storage has already depleted, or after harvesting the honey without adequate replacement of carbohydrates. Thus, carbohydrates are fed with sucrose solution to colonies in routine (Brodschneider and Crailsheim, 2010).

3. Sugar and organic acid

Canola pollen, mixed pollen, bee bread, Megabee and Test A were analysed for free sugar and organic acid concentration (Fig. 1 and Fig. 2). In Megabee and Test A, sucrose content showed the largest amounts with 46.15% and 59.60%, respectively. Sucrose was not found in canola pollen, mixed pollen and bee bread. Sucrose can not be used by honey bees and it's molecule must be broken into two simple free sugars of glucose and fructose. This is done by an enzyme called sucrase, found in the digestive cavity stomach. Glusoce, as free sugar, plays a vital role in the functioning of brain, providing muscle energy for locomotion and body cells funtioning. Canola pollen and mixed pollen has a high content of glucose with 46.21% and 46.31%, respectively. Fructose, although an important free sugar for providing energy, can cause a problem like hydroxymethylfurfural (HMF) which is kown to be toxic for honey bees. Acids, like acetic acid (vinegar), citric acid (lemon juice) and tartaric acid (cream of tartar), produces HMF when mixed fructose. Bee bread showed the largest content of fructose with 68.06% among the 5 samples. In canola pollen and mixed pollen accounted for the largest content of fructose with 53.79% and 52.52%, respectively. However, fructose was not found in Megabee and Test A.

Table 2. Contents and functions of essential amino acids for honey bee

Essential amino acid	Function	Rate of essential amino acid for honey bee (%)
Arginine	Essential, growth period	3
Histidine	Essential, growth period	1.5
Isoleucine	Essential	4.5
Leucine	Essential	4
Lysine	Essential, muscle health	3
Methionine	Essential	1.5
Phenylalanine	Essential	2.5
Threonine	Essential	3
Tryptophane	Essential	1
Valine	Essential, muscle synthesize	4

In the result of organic acid analysis, ctric acid accounted for the largest content in all five samples. Among them, Test A showed the highest amount of citric acid with 92.33%. Citric acid is a white powder used to give a sour taste to beverages and food products. It is also used as a preservative to prevent spoilage because it

Table 3. The composition of amino acids in five samples (mg/L)

Amino Acid	Canola-pollen	Mixed-pollen	Beebread	Megabee	Test A
Lysine	23.40	13.62	50.56	49.02	471.30
Leucin	43.84	69.44	70.19	442.74	1244.29
Phenylalanine	300.34	76.07	89.42	330.92	958.34
Isoleucine	34.67	40.57	52.08	175.78	889.03
GABA	106.94	205.00	208.28	655.29	1772.25
Alanine	307.45	366.42	404.85	1379.75	3384.16
Arginine	796.42	120.13	485.77	1257.79	3751.45
Aspartate	251.47	262.25	405.28	243.79	1325.78
Glutamate	616.67	269.26	239.97	710.95	4707.36
Asparagine	774.00	299.11	585.97	329.88	1690.43
Serine	143.59	185.73	166.94	165.91	1249.74
Glutamine	97.58	93.84	85.18	82.51	222.81
Histidine	226.66	145.14	74.58	191.07	729.54
Glycin	36.78	38.34	93.87	243.45	923.41
Threonine	52.71	49.81	98.80	103.98	750.25
Proline	1159.21	998.90	793.73	124.28	288.57
Hydro proline	49.24	143.21	101.70	–	–
Tyrosine	169.14	75.97	101.01	93.40	854.33
Valine	165.53	123.94	159.97	343.67	1376.98
Methionine	42.83	9.63	20.95	81.57	198.26
Tryptophan	182.10	14.57	9.56	60.90	332.32

increases the acidity of products and restricts the bacterial growth responsible for food spoilage. It is also used in dietary supplements, as it enhaces the bioavailablity of the minerals.

4. Amino acids

De Groot reported in 1953 that arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine are the essential amino acids for honeybees (De Groot, 1953). Table 2 enlists the functions and requirements of each essential amino acid for honey bee. Among them, isoleucine accounted the highest content with 4.5%. Dietary sources of essential amino acids are used for growth, somatic maintenance and reproduction. Nicolson and Human in 2013, reported the essential amino acid concentrations in bee-pollens, expressed as the percentage of the total amino acids ranges from 34.59% to 48.49%. The most abundant amino acids in pollen proteins are proline, leucine, lysine, glutamic acid and aspartic acid (Nicolson and Human, 2013). The results of amino acid content in five samples are listed in Table 3. Canola pollen, mixed pollen and bee bread accounted for the highest content of proline with 1159.21 mg/L, 998.90 mg/L and 783.73 mg/L, respectively. Proline, a non-essential amino acid, is an important amino acid for the laying of queen bees and also necessary for wing muscles development in insects. It is swiftly metabolized and produces several nicotinamide adenine dinucleotide phosphate. Thus, prolin is considered as a fovourable ingredient of honeybees (Kim *et al*., 2020). Among the five samples, Test A showed the highest amount of lysine with 471.30 mg/L. Lysine is directly involved in nitric oxide synthesis, a known neurotransmitter to affect memory in bees and moths (Gage *et al*., 2020).

CONCLUSION

Development of artificial bee feed is important in order to build and maintain healthy colonies and consequently increase the productivity. In bee bread nutritional contents such as free sugar, organic acid, and amino acids are of vital importance. Along with the nutritional content, economic efficiency also needs to

be considered for the development of artificial bee feed. However, the development of a super artficial bee feed, depends on considering several aspects and requires multicentric studies.

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