

Soybean Dregs as Main Ingredients of Pollen Substitute for *Apis Cerana* Honey Bees

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Abstract

The first objective of this research was to used soybean dregs as pollen substitute. The second objective was test the preferences, preventing abscond and productivity of Apis cerana honey bees colonies consumed the pollen substitute. There were two types of pollen substitute flour (PS) and four types of pollen substitute pasta given to A. cerana honey bee colonies at apiary in Bandung, West Java, Indonesia. The basic ingredients of PS1 flour were soybean dregs, skim milk and instant baker's yeast. The basic ingredients of PS2 flour were soybean dregs, skim milk and tapai (a Indonesia traditional fermented food) starter. The PS1-A paste consisted of PS1 and sugar syrup; PS1-B contained of PS1 and molasses; PS2-A contained of PS2 and sugar syrup; and PS2-B contained of PS2 and molasses. The nutrition of each type of pollen substitute was analyzed. Each type of PS paste was given to five honey bee colonies for five weeks. The best pasta was given to the five honey bee colonies for 15 weeks in dearth periode. The best pasta was given to three colonies for 7 weeks also to test the productivity. The control colonies were not given PS. All of the colonies forage naturally. The analysis of nutrition showed that protein and other nutrients in PS1-A pasta were better than other PS. Honey bee colonies consumed more PS1-A than any other PS. The giving of PS1-A pasta for 15 weeks was able to maintain 5 colonies and they were not abscond. The colonies given PS1-A showed an increase the weight of honey and the volume of honey compared to the control colonies. Honey produced was in accordance with Indonesian National Standard for honey (SNI-01-3545-2004). The conclusion of this research were soybean dregs could be main indegredients of pollen substitute, the PSA-1 was able to prevent abscond and increased productivity of A. cerana colonies.

Keywords: soybean dregs, honey bees, pollen substitute, productivity

BACKGROUND

Maintaining honey bees was very useful for various flowers pollination services and it could get the products such as honey, propolis, royal jelly and bee wax (Sihag and Gupta, 2011). In normal condition, the feed sources of honey bees was nectar and pollen or flower pollen. Nectar was the source of carbohydrates primarily an energy source, and pollen was the main source of protein, fats, minerals, and vitamins (Nicolson, 2011; Somerville, 2000).

The availability of pollen was very important for the health of the colonies. It was significantly affected the ability to construct new honeycomb or nestcomb as one of the colony productivity. For honey bees, building honeycomb was the way to prepare the place for the eggs, larvae and pupa, honey and pollen in the cells of the honeycomb. The availability and quality of flower pollen really determined the condition of growth and health of the colony, especially for the number of eggs, the growth of larvae until adulthood, and the productivity of the colony (DeGrandi-Hoffman et al., 2008). Thus, pollen is a very important feed for honeybees throughout their lives. Creating a pollen substitute was one of the efforts to maintain honey

bee colony to remain productive or to maintain the health of the colony during the dearth period. In Indonesia, pollen substitute has not been produced commercially because the cost production is still high and many beekeepers did not know pollen substitute.

Researchers have tried to make pollen substitute from various ingredients. Many different pollen substitute formulations have been developed for supplement feeding with soybean as the ingredient (Haydak and Tanquary, 1942; Somerville, 2000; Rogala and Szymas, 2004; Widowati et al, 2013, Akbaruddin et al, 2018; Budiaman et al., 2019). Processing soybean for pollen substitute are time consuming and cost. It is necessary to look for other ways that can be applied by beekeeper for make cheap pollen substitute and get lots of honey. Soybean dregs from tofu industry, had process of peeling the epidermis, boiling and mashed it. Another ingredients was molasse from instant yeast industry. The other were skim milk, instant bread yeast and tapai starter.

The use of soybean dregs was based on the research that soybean dregs contained 27.55% protein and 4.93% fat (Nuraini et al., 2009). According to Somerville (2000), this content was in accordance of pollen substitute, it contained of more than 20% protein and less than 7% fat. Nonfat milk or skim milk was used as one of ingredients for pollen substitute based on the US Dairy Export Council (2014). Skim milk contained 36% protein and 0.70% fat. Skim milk also had complete amino acids, including essential amino acids required by honeybees. Skim milk was supplement of protein and amino acids.

Instant bread yeast (*Saccharomyces cerevisiae*) was used in pollen substitute (Somerville, 2000). Instant bread yeast contained up to 50% protein with a balanced amino acid. The given of instant bread yeast made pollen substitute more attractive to honeybees, the instant bread yeast also supplemented the pollen substitute with vitamin B complex (Somerville, 2005). Instant bread yeast also helps to resemble the process of chemical change from pollen to beebread.

Tapai starter for making Indonesian tradisional food glutinous i.e rice tapai and cassava tapai. Tapai starter contained various microorganisms that was capable to hydrolyze starches. Gandjar (2003) stated that the microorganisms in tapai starter contained mold group were *Rhizopus oryzae*, *Amylomyces rouxii* and *Mucor* sp., and from the yeast group were *S. cerevisiae*, *Saccharomycopsis fibuliger*, *Endomycopsis burtonii*, and bacteria *Pediococcus* sp., *Bacillus* sp. Tapai starter had been used as one of the pollen substitute ingredients given to *A. mellifera* colonies Kuntadi (2002). Tapai starter was also used as probiotic in livestock feed (Sianturia et al., 2006).

Molasse was a by-product of sugar cane industry that contained high sugar. Molasse contained 20% water; 35% sucrose; 9% fructose; 7% glucose, 3% reduction sugar; 4% other carbohydrates 4%, ash 12%, and some other substances Paturau (1982). Content high sugar, molasse was used as raw material for monosodium glutamate and animal feed industry. Molasse was also used in making instant bread yeast in industry in Indonesia. It was used as medium for *S. cerevisiae* yeast growth. After yeast was harvested, the molasse became waste. Waste molasse still had high sugar content and contained *S. cerevisiae*.

The first objective of this research was to used soybean dregs as pollen substitute. The second objective was test the preferences, preventing abscond and productivity of *A. cerana* honey bees consumed the pollen substitute.

MATERIALS AND METHODS

Pollen substitute

The ingredients of pollen substitute consisted of soybean dregs, skim milk powder (XX brand), instant baker's yeast (YY brand), tapai starter (ZZ brand), granulated cane sugar (WW brand), molasse from local instant baker's yeast industry, and water. Fresh soybean dregs from the tofu industry located in Ciburial

Village, Bandung. The soybean dregs was squeezed to remove water inside. The soybean dregs was placed on a tray, dried using a fan or the heat of the sun. The drying of soybean dregs lasted up to three days. The dried soybean dregs was milled using a grinder, then sieved using a porous sieve smaller than 200 µm. The flour of soybean dregs was placed in a dry, tightly sealed container. Skim milk, instant baker's yeast, tapai starter and sugar were purchased at traditional markets in Jakarta. Tapai starter was crushed until smooth and placed on a dry container. Molasse was obtained from YY instant baker's yeast industry. During the research, molasse was stored at 4°C. The PS1 flour of pollen substitute was made from 3 parts composition of soybean dregs, 1 part skim milk and 1 part instant bread yeast or one recipe consisted of 36 gram soybean dregs, 12 gram skim milk and 12 gram instant baker's yeast. The PS2 flour was made using 3 parts composition of soybean dregs, 1 part skim milk and 1 part tapai starter, or one recipe consisted of 36 gram soybean dregs powder, 12 gram skim milk and 12 gram tapai ragi. A 50% sugar syrup was prepared by dissolving 80 grams sugar in 80 ml hot water. Pasta PS1-A was made using 60 grams PS1 flour plus 160 ml sugar syrup. PS1-B paste was made using 60 grams of PS1 flour plus 120 ml of molasse. Pasta PS2-A was made using 60 grams of PS2 flour plus 160 ml of 50% sugar syrup. The PS2-B paste was made using 60 grams of PS2 flour plus 120 ml of molasse syrup.

Test 1 : Nutrition test

The nutrition content of flour and pasta pollen substitute was done in Integrated Laboratory of Institut Pertanian Bogor. The verification of nutrition content of pollen substitute used proximate analysis. The results of pollen substitute nutrition tests were compared with each other, either in the form of pollen substitute flour or pasta.

Test 2 : Preference and Productivity

Test 2.1. Testing Preference of Pollen substitute

There were 25 *A. cerana* honey bee colonies in Apiari Assyifa Ciburial Village, Bandung Regency were used in the research to test the preference of four kinds pollen substitute pasta and control (no intervention) groups. The colonies of approximately the same size were conditioned for one month before being treated. One test group used five honey bee colonies. During the intervention, all colonies were allowed to forage naturally. Every week, each colony was given one type of fresh pollen substitute pasta of 50 grams using a digital scales with 0.1 precision. Pollen substitute pasta was placed on the surface of a laminated yellow flat cartoon. The placement of the pollen substitute was just below the colonies inside the beehive (Widowati et al., 2013). The control group were not given pollen substitute pasta. At the end of the week, the remaining pollen substitute pasta was weighed. The preferences of pollen substitute pasta was done for 5 weeks. The percentage of pollen substitute consumed by each colony each week was calculated by the formula:

$$\text{Percentage of colony consumption} = \frac{50 \text{ gram} - \text{the rest of feed (gram)}}{50 \text{ gram}} \times 100\%$$

Test 3: Amino Acid Level

The best pollen substitute pasta of test 1 and test 2 were followed by testing the content of essential amino acid and non essential amino acid. The contents of ten essential amino acid tested were arginine, phenylalanine, histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan and valine. The non-essential amino acid tested were aspartic acid, glutamic acid, serine, glycine, alanine, tyrosine. The amino

acid test on pollen substitute pasta used HPLC method. The tests were conducted at Integrated Laboratory of Institut Pertanian Bogor.

Test 4: Consumption and Productivity

Test 4.1. Consistency of pollen substitute consumption

The best pollen substitute pasta, from the results of test 1 and 2 were given to the honey bee colonies for 15 weeks. The condition of the place where the research conducted in that period was lack of flowers. The honey bee colonies used were equal. Five colonies were given the best pollen substitute pasta and five colonies as control group. During the test, all colonies were allowed to forage naturally. Every week, 50 gram of pollen substitute pasta was given to each test colony, and after 1 week, the rest of the pollen substitute was weighed. The weighing of the pollen substitute paste was done with a digital scale with a precision of 0.1 grams. To know the consistency of the consumption of the given pollen substitute pasta, the remaining residues of the pollen substitute was assumed as follows:

No leftovers: There is no left less

Little : Remaining less than 10%

Medium: Remaining between 10-20%

Much : More than 20%

Test 4.2 Honey production

The best pollen substitute pasta, from the results of test 1 and 2 were given to the honey bee colonies for 7 weeks. The honey bee colonies used were equal. Three colonies were given the best pollen substitute pasta and five colonies as control group. During the test, all colonies were allowed to seek natural feed. After seven weeks, honey was harvested by cutting the honeycomb whose cells contained honey. The honeycomb was then sliced in small pieces and placed on a filter cloth. The honey produced was filtered with two-layer filter fabric. The collected honey was weighed and measured in volume.

Test 4.3 Honey Quality Measurement

The honey harvested from the colonies fed with flour substitute was analyzed in accordance with Indonesian National Standard Honey (SNI 01-3545-2004).

Experiment Design and Data Analysis

The experiment design of pollen substitute pasta preference test used Completely Random Design (RAL) pattern with four treatments, five repetitions each. The treatment consisted of PS1-A, PS1-B, PS2-A, and PS2-B pollen substitute pasta. The observed variable was the amount of pollen substitute pasta consumption per week. The differences in consumption level of each pollen substitute pasta were analyzed by variance (ANOVA). If there was a marked difference between the grade points average, then the analysis was followed by a real honest difference test / Tukey test to find out the different types of treatment. The best pollen substitute pasta among four pollen substitutes tested was followed to Test 3 and Test 4.

RESULTS

Test 1: Nutrition test

There are two kinds of pollen substitute flour examined for its nutritional composition, PS1 consisting of soybean dregs, skim milk and instant baker's yeast and PS2 consisting of soybean dregs, skim milk and tapai starter (PS2). The results of examination of nutritional composition of two types of pollen

substitute flour were presented in Table 1 and nutritional composition of types of pollen substitute pasta were presented in Table 2.

Table 1 Nutritional composition of pollen substitute flour

Nutrient	Flour PS1	Flour PS2
Protein	16.63%	8.13%
Carbohydrate	71.07%	79.99%
Fat	2.22%	1.95%
Ash	2.80%	1.80%

Table 2 The basic composition of nutrient pollen substitute pasta

Nutrient (%)	Pollen substitute Pasta			
	PS1-A	PS1-B	PS2-A	PS2-B
Protein	6.44	6.35	4.47	4.68
Carbohydrate	55.26	21.43	59.20	22.47
Fat	1.16	1.78	0.22	3.35
Ash	0.66	2.62	0.11	2.35
Water	35.84	68.11	35.88	67.45

Test 2: Pollen substitute preference test

The average total consumption all of pollen substitutes pasta by *A. cerana* colonies per week ranges from 59.6% - 97.0% for 5 weeks. The average consumption PS1-A was the highest, ranges between 85.6% - 97.0% per week. The average consumption of PS1-B ranges from 68.1% - 76.4% per week. The average consumption of PS2-A ranges from 75.7% - 91.0% per week, and the average PS2-B consumption ranges from 59.6% - 64.4% per week. Statistical results showed that pollen substitutes PS1-A were consumed more significantly and were significantly different compared to PS1-B and PS2-B ($\alpha < 0.05$), but not significantly different from PS2-A ($\alpha > 0.05$). The average measurements of four types of pollen substitute paste consumed by *A. cerana* honey bee colonies for five weeks were shown in Figure 1.

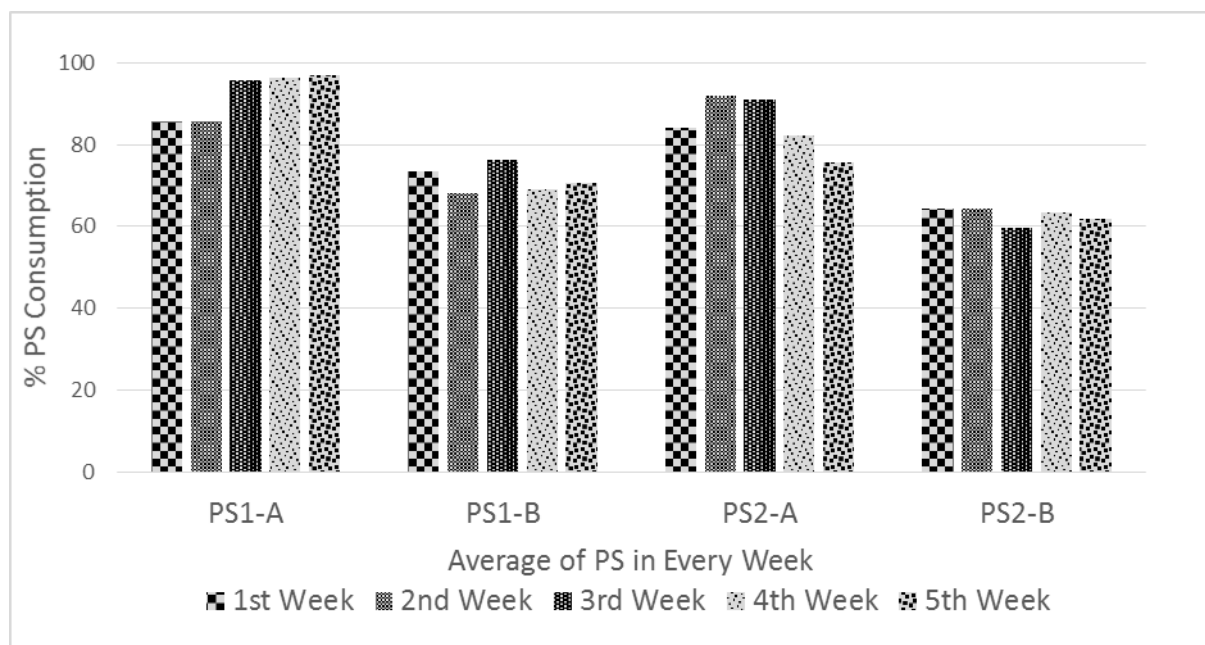


Figure 1 Stem diagram of average consumption of four types of pollen substitute pasta by *A. cerana* honey bee colonies per week.

Test 3: Amino Acid Level

From the results of Test 1 and Test 2, it was found the nutritional composition of pollen substitute pasta, the preference *A. cerana* honey bees and the addition of the widely nesting comb was PS1-A pollen substitute paste. Subsequently, the PS1-A pollen substitute pasta was analyzed for its amino acid composition and the results were shown in Table 3. The results of the analysis were matched with the essential amino acid requirements expressed by De Groot (1953, in Somerville, 2000). There were 10 essential amino acids and six non essential amino acids for honeybees

Table 3 The analysis results of amino acid as PS1-A pollen substitue

No	Amino acid	De Groot	PS1-A
Ten amino acids for honey bees (De Groot, 1953) ¹			
1	Arginine	3,0	0,23
2	Phenylalanine	2,5	0,26
3	istidineI	1,5	0,11
4	Soleusina	4,0	0,26
5	Leusina	4,5	0,39
6	Lisina	3,0	0,22
7	Methionine	1,5	0,04
8	Treonina	3,0	0,16
9	Tryptophan	1,0	0,02
10	Valina	4,0	0,31
Non essential amino acids			
11	Aspartic acid	-	0,50
12	Glutamic Acid	-	0,71

13	Serina	-	0,15
14	Glycine	-	0,25
15	Alanina	-	0,25
16	Tirosina	-	0,08
Total Amino Acid		26	3,94
Total Protein		-	6,44

[Source : ¹ Somerville, 2000]

Test 4: Consumption and Productivity

Test 4.1. Consistency of pollen substitute consumption

The results of the given PS1-A pollen substitute pasta to 5 colonies for 15 weeks were shown in Table 4.

Table 4 Leftover of pollen substitute

Colony	Leftover pollen substitute/Week														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	D	D	D	D	H	H	H	H	H	H	H	H	H	H	H
2	D	H	H	H	H	H	H	H	H	H	H	H	H	H	H
3	D	H	H	H	H	H	H	H	H	H	H	H	H	H	H
4	D	H	H	H	H	H	H	H	H	H	H	H	H	H	H
5	H	H	D	H	H	H	H	H	H	H	H	H	H	H	H

Note :

The remaining feed on pollen substitute pad

H=No leftovers : No leftovers

D=Little : Remain < 10%

S= Medium : Remain between A 10-20%

B= Much : Remain >20%

Test 4.2: Measurement of Honey Production

The giving of PS1-A pollen substitute in the second stage for 7 weeks to three *A. cerana* honey bee colonies resulted the increased of colonies weight as shown in table 5.

Table 5 The weight of honey comb, as well as the weight and volume of honey as the results of the giving PS1-A pollen substitute

Intervention			Control		
Colony	Weight of Honey (kg)	Volume of Honey (ml)	Colony	Weight of Honey (kg)	Volume of Honey (ml)
1	1.52	1,010	1	0.06	50
2	0.30	210	2	0.46	350
3	0.94	820	3	0.40	310
Average	0.92	680	Average	0.31	237

Test 4.3: Honey Quality Measurement

The results of honey quality examination obtained from the colonies given PS 1-A pollen substitute flour based on Indonesian National Standard for honey (SNI-01-3454-2004) were shown in Table 6.

Table 6. The quality of honey harvested based on Indonesian National Standard for honey (SNI-01-3545-2004)

Parameter	Unit	Limit	Test Results
Diastase enzyme activity	DN	3 (min)	6,13
Hydroxymethylfurfural (HMF)	mg/kg	50 (max)	0
Water	%	22 (max)	19,2
Reducing sugar (calculated as glucose)	%	65 (min)	74,4
Sucrose	%	5 (max)	2,55
Acidity	ml NaOH 1N/kg	50 (max)	45,5
The solid insoluble in water	%	0,5 (max)	0,01
Ash	%	0,5 (max)	0,45
Lead Metal contamination (Pb)	mg/kg	1,0 (max)	<0,042
Copper metal contamination (Cu)	mg/kg	5,0 (max)	0,37
Arsenic	mg/kg	0,5 (max)	<0,003
Results			Meet qualification

Note : Min = Minimum; Max = Maximum

Discussion

Test 1: Nutritional Tests

The protein content of PS1 pollen substitute flour was twice (16.63%) compared to PS2 pollen substitute flour (8.13%). The composition that distinguished PS1 and PS2 pollen substitute flour was PS1 used instant baker's yeast and PS2 used tapai starter, while other raw materials were the same. This showed that the use of instant baker's yeast may increase the protein content of the pollen substitute flour compared with the tapai starter. Similarly, the content of fat and ash in PS1 pollen substitute flour was higher than PS2, it was 2.22% and 2.80%, compared with 1.95% and 1.80% ash content. Instant baker's yeast raised the content of fat and ash on the pollen substitute flour, compared to that given by tapai starter.

The content of carbohydrate in PS1 pollen substitute flour was 71.07% and PS2 was 79.99%. The difference between the content of protein, fat, and ash and the pollen substitute flour was the use of instant baker's yeast and tapai starter. This could cause the content of carbohydrate in PS2 pollen substitute powder higher than PS1.

PS1-A and PS1-B pollen substitute pasta showed higher protein than PS2-A and PS2-B in accordance to PS1 pollen substitute flour. Carbohydrate in pollen substitute pasta showed that the addition of sugar syrup increased the content of carbohydrate compared to the addition of molasses. The addition of molasses also

increased the percentage of fat and ash content in the pasta compared to the addition of sugar syrup. Although the giving of the amount of solvent sugar syrup or molasses was not the same as in making paste,

the final result of the water content of the pasta made from the addition of molasses remained higher because the molasses were more dilute than the sugar syrup.

The analysis of pollen substitute was done in the form of flour and not in the form of pasta referring to Saffari et al. (2010a), even though the research gave to the bees *A. mellifera* pasta as pollen substitute. The pollen substitute pasta was made by adding 60% sugar syrup to the pollen substitute flour. Sihag and Gupta (2011) did pollen substitute flour analysis only, though the pollen substitute given to bees *A. mellifera* were solid, semi-solid, and semi-liquid form. In this research, we tried to compare pollen substitute flour and pasta analysis.

The composition of natural protein in pollen in various flowers ranged from 2.5% -61% (Roulston & Cane, 2000). High protein composition in pollen substitute was important for honeybees, because good quality pollen had high levels of protein. A protein composition of more than 20% in the feed of honeybees would make honey bees live longer and the hypopharyngeal gland developed well (Manning, 2008). According to Somerville (2000), 20% protein in pollen is the minimum requirement limit for honeybees could achieve optimal productivity. Protein in PS1 flour (16.63%) and PS2 flour (8.13%) were below 20%,

Protein content in PS1-pollen substitute flour was higher than the PS2 flour. The composition distinguished PS1 and PS2 was used instant bread yeast and tapai starter, while other raw materials were alike. The result showed that use of instant bread yeast was better in increasing protein content compared to tapai starter. For further research or the application of pollen substitute in honeybees, protein content could be increased by used instant yeast.

Carbohydrate content in PS1 pollen substitute flour was 71.07% and in PS2 was 79.99% (Table 1). Carbohydrate was important nutrient for generating energy, especially for flying. Carbohydrate needed by honey bees was usually fulfilled from flower nectar and honey in the cells of honey comb (Brodschneider & Crailsheim, 2010). Apart from nectar and honey, carbohydrate for honey bees was also obtained from pollen (Somerville, 2000). Carbohydrate in pollen was range from 7% -57%. Carbohydrate in natural pollen was sugar such as fructose, glucose, and sucrose; starches and complex carbohydrates such as cellulose (Joseph, 2013). The content of flour in pollen substitute was more than 70%. It was possibly derived from soybean dregs flour which still contained starch, and it needed to be lowered to be more balanced with protein content. The change of the ratio of soybean dregs, skim milk and instant yeast, it was expected to obtain appropriate and better composition for the growth, development and productivity of honey bee colonies.

The result of the analysis of pollen substitute pasta had a very different composition with the pollen substitute flour because of the addition of syrup or molasses. The addition of sugar syrup and molasses caused high water content, while other nutrient composition dropped. In pasta, protein composition dropped dramatically to below 7% due to high water content. Like the composition of pollen substitute flour, the protein content in pollen substitute pasta with instant bread yeast was higher than that with tapai starter.

The carbohydrate content in pollen substitute pasta given sugar syrup (PS1-A and PS2-A) were 55.26% and 59.20% higher than the pollen substitute pasta given molasses (PS1-B and PS2-B), it was 43% and 22.47% (Table 2). This showed that molasses had less carbohydrate content. The low sugar content in molasses was caused sugar was used for growth of yeast in the instant bread yeast industry. The giving of sugar syrup and molasses to pollen substitute flour showed the different water content. It was almost twice as high in PS1-B and PS2-B compared to PS1-A and PS2-A. A small amount of sugar in the molasses made water content in molasses much higher than the 50% sugar syrup.

In contrast, the molasses elevated the ash content in PS1-B and PS2-B compared to the pasta given sugar syrup. The ash in nutrient were inorganic or mineral element, accordance Organic Facts (2015) which stated that molasses contained various minerals such as calcium, manganese, potassium, copper, iron,

phosphorus, chromium, cobalt, and sodium. Somerville (2000); Brodschneider & Crailsheim (2010) stated that pollen is a mineral source for honey bees. The total composition of minerals in various flower pollen varied between 1% -7%.

Fat was essential in honeybee feed because it was the raw material of attractants, as well as an energy producer. Fat was required by all living things, but the fat composition in honey bee feed should be in a low composition. The content of fat in both pollen substitute flour and pasta ranged from 0.22% -3.35%. This fat composition was still in the range of fat composition in natural pollen that was 0.8-18.9% (Roulston & Cane, 2000). The composition of fat in pollen substitute flour was in accordance with Stace (1996), Somerville (2000), and Manning (2008) who suggested fat composition in honey bees feed less than 7%. Stace (1996) stated that the fat composition of more than 7% would be toxic to honey bees.

Test 2: Pollen substitute preference test

The average result of *A. cerana* colonies consumption for five weeks showed that PS1-A pollen substitute pasta was the most consumed compared to other pollen substitute pasta. In addition, it was also seen that in the last three weeks of giving pollen substitute pasta, the amount consumed was more and more. The pollen substitute pasta moltened with molasses showed the lower consumption compared to pollen substitute pasta moltened with sugar syrup.

The consumption of each type of pollen substitute by *A. cerana* honey bees was done for 5 weeks or 35 days. This was done to provide opportunities and to ensure the average level of consumption and the preferences or preferences of the honey bee colonies to pollen substitute. From the amount of pollen substitute consumption per week by the honey bee colonies, the highest was PS1-A pasta that ranged from 81.2% -98.8%.

The statistical results showed that PS1-A was most widely consumed. The second pollen substitute most commonly consumed was PS2-A. PS1-A and PS2-A pollen substitutes containing sugar syrup that seemed to be preferred compared to PS1-B and PS2-B that contained molasses. Molasses had a very strong smell that was probably disliked by honey bees. Adding protein supplement in pollen substitute with baker yeast or tapai starter remains the same as preferred. The amount of pollen substitute consumed was honey bee preference to pollen substitute. In addition, the amount of pollen substitute consumed by honey bees was a good early indication that the pollen substitute served was accepted by honey bees (Brodschneider and Crailsheim, 2010).

Although based on preference tests, PS1-A and PS2-B were not significantly different, but based on the nutritional composition of pollen substitutes, the best pollute substitute chosen to be continued to test 3 and test 4 was pollen substitute PS1-A.

Test 3: Amino Acid Levels

Based on the basic composition of the best nutrients of pollen substitute flour and pasta, pollen pasta PSA was selected for the analysis of amino acid composition as shown in Table 3. De Groot (1953, in Somerville, 2000) described 10 essential amino acids for honey bees. The ten amino acids were also owned by pollen substitute pasta which analyzed arginine, isoleucine, leucine, valine, methionine, phenylalanine, threonine, tryptophan, lysine, and histidine.

Although the content of 10 amino acids were not as high as stated by De Groot, all the essential amino acids needed by the honey bee colony have been fulfilled. It is necessary to look for supplements for pollen substitute that could increase the content of essential amino acids, in order for the needs of the honey bee colonies.

Test 4: Consumption and Productivity

Test 4.1. Consistency of pollen substitute consumption

The amount of pollen substitute consumed was the honey bee preference to pollen substitute. In addition, the amount of pollen substitute consumed by honeybees was a good early indication that pollen substitute presented could be accepted by honeybees (Brodschneider and Crailsheim, 2010).

The consumption of pollen substitute pasta by *A. cerana* honey bee colonies was performed for 15 weeks presented in Table 4. In the first week of giving pollen substitute pasta, the colonies still left less than 10%. After being adaptation, most of *A. cerana* honey bee colonies consumed all the pollen substitute pasta. This showed that pollen substitute given to *A. cerana* bees could replace natural pollen. Data from Table 4 showed that *A. cerana* colonies required pollen substitute for survival. However, consuming a pollen substitute was not matched by the availability of nectar, so no honey was available in the hive.

Nectar and pollen were two very important things in the life of honey bees. Nectar that had been converted into honey as a source of carbohydrate, was needed by worker bees. Paoli et al. (2014) stated that worker bees needed high carbohydrate diets. Carbohydrate was an energy source for adult worker bees that had duties from cleaning the nest, storing food, caring for larvae, to treating queen bees. The essential amino acid diet for honey bees was used for growth, maintenance of somatic cells, and reproduction. However, an excessive diet of essential amino acids for worker bees caused death.

Test 4.2: Measurement of Honey Production

There was weight gain in all *A. cerana* honey bee colonies fed pollen substitute PS1-A for 7 weeks. The average colony weight gain in the colonies given PS1-A was 33.76% more than twice compared to control colonies of 12.58%. The addition of honey bee colony weight after feeding pollen substitute could increase the quantity of eggs, larvae, pupae, and adult bees, or the formation of honeycomb and the collection of honey and bee bread.

The giving pollen substitute to honey bee colonies could increase the number of feed seekers and produce honey (Abd El-Wahab and Gomaa, 2005) and increase the formation of honeycomb, egg hatching, storage of honey and beebread (Prakash et al., 2007). The harvest of honey from *A. cerana* honey bee colonies given pollen substitute showed nearly triple result for honey comb weight, the weight of honey and the volume of honey compared to the harvest of honey from the control colonies. The weight of honey comb was the wax forming honey comb added by the weight of honey. The weight and the volume of honey could indicate the quality of honey. The heavier the honey but the slight volume indicated that the water content of the honey is low, or the honey was so condensed.

Honey production from bee colonies given pollen substitute PS1-A was able to give better result compared to those from the control. This showed that the giving PS1-A pollen substitute could increase the productivity of *A. cerana* honey bee colonies. The productivity of honey bee colonies began with the acceptability of the feed measured by the amount of feed taken from the feeder (feed container). The acceptability of feed was a representation of feed palatability. This is because the honeybees did not take feed that was not palatable for their colonies except in starvation (Saffari et al., 2010a). The acceptability and palatability of the feed should be in accordance with the nutritional value because it could be indicated that the amount of feed consumed would be equivalent to productivity (Saffari et al. 2010b). Pollen substitute given to honey bees was the source of protein, carbohydrates, fats, minerals and vitamins (Somerville, 2000). The giving of pollen substitute was intended for the growth and development of honey bee colonies. Therefore the consumption of pollen substitute should increase the growth and development of honey bee colonies.

Test 4.3: Honey Quality Measurement

The results of honey quality inspection from *A. cerana* honey bee colonies fed pollen substitute PS1-A indicated that the honey met the requirements of Indonesian honey in accordance with SNI-01-3545-2004. The activity of diastase enzyme in honey analyzed showed result of 6,13 DN above minimum 3 DN in accordance to the requirement of honey quality based on SNI-01-3545-2004. Diastase enzyme activity in honey showed the importance of honey quality. Diastase enzyme activity was influenced by honey storage and heating, therefore diastase enzyme was an indicator of the freshness of honey and the heating or overheating on honey. Nevertheless, it was known that unifloral honey had low diastase activity (Bogdanov et al., 2001). Diastase enzyme was an enzyme that functioned to decompose starch into dextrin and maltose. The enzyme would be damaged if it was within 60°C-80°C. The harvested honey diastase enzyme from the colonies given PS1-A higher than 3 DN indicated that no heating of tested honey.

The concentration of Hydroxy Methyl Furfural (HMF) on honey analyzed showed a result of 0 of maximum 50 mg/kg of honey quality requirement in accordance with SNI-01-3545-2004. HMF was the substance of hexose sugar heating, especially fructose which was formed on honey due to heating. Honey heating would produce HMF at high concentration and HMF was known to be toxic to honey bees (Brodschneider and Crailheim, 2010). HMF concentration was the main factor of honey quality as well as freshness indicator of honey and the occurrence of heating or overheating. In fresh honey there was practically no HMF, but would increase in the length of storage, it also depended on the pH honey and on the storage temperature. On storage of honey in warm climate countries the value of HMF was increasing (Bogdanov et al., 2001).

The quality of honey was also determined by the water content. The water content in honey analyzed showed 19.2% below the maximum content of 22% of honey quality requirements in accordance with SNI-01-3545-2004. Water content was the only criterion of honey composition, as part of the Honey Standard had to be fulfilled in the world honey trade (Bogdanov et al., 2001). Water content in honey needed to be adjusted because honey with high water content was more likely for fermentation. To get low water concentration, honey was often heated. The heating of honey could reduce the quality of honey. It was marked by the low or absence of diastase enzymes.

The quality of honey was also determined through the concentration of glucose and surosa sugar. The reducing sugar (calculated as glucose) in honey analyzed showed the outcome of 74.4% above the minimum content of 65% required by honey quality in accordance with SNI 01-3545-2004. Most honey derived from nectar, reducing sugar represented most of the sugar of honey, but in honeydew honey, it is not so because of very different outcome. Most honeydew honey had high value of non-reducing oligosaccharides such as melezitose, maltotriosa and rafinosa. Based on the analysis, rosemary honey from Spain had more than 5% sucrose (Bogdanov et al., 2001).

Sugar was the main component of honey, which achieved to 95% of the dry weight of honey. The main sugar in honey was the hexose fructose and glucose which was the decomposition of disaccharide sugar ie sucrose (Bogdanov, 2009). Sucrose on nectar would naturally break down into glucose in honey. Thus the content of glucose in honey would be high if sucrose was broken down. In other words, high concentration of both sucrose and glucose showed that not all sucrose contained in honey was converted into glucose. However high levels of sucrose did not necessarily mean that the honey was faked or given sugar sucrose. The fake honey usually had a high content of sucrose (Sihombing, 2015).

The result of analysis showed that honey produced by the colonies given PSA pollen substitute that had 45,4 ml NaOH 1N / kg acidity, as required SNI-01-3545-2004 had <50 ml NaOH 1N / kg acidity. Acidity was an important criterion of honey quality. Honey had a pH <7. The acid contained in honey was

relatively low, but it was important to give a sense on honey. Acid was added to honey by bees. The main acid was gluconic acid, it was the outcome from glucose oxidation by glucose oxidase enzyme. In addition, the acids in honey were formic acid, acetic acid, citric acid, lactic acid, maleic acid, malic acid, oxalic acid, pyroglutamic acid and succinic acid (Bogdanov, 2009). The acid in honey was the outcome of fermented honey. Fermentation could be driven due to the high water content.

The result of the measurement of solids insoluble in water on honey analyzed showed 0.01% outcome below 0.5% maximum content required by honey quality in accordance with SNI-01-3545-2004. The measurement of insoluble solids in water was measurement to detect the required honey dung. Honey dung was nest comb wax. The bee bread in the nest comb could become an insoluble solid in water. The result of observation and implementation in the field, the *A. cerana* honeycomb did not contain honey only, often it mixed with bee bread, egg, larvae or pupae of bees. Further efforts to obtain honey quality with low insoluble solids in water, filter was needed to filtrate out the insoluble solids in water.

The result of ash measurement was the mineral content in honey. The result of ash measurements on honey analyzed showed 0.45% outcome below the maximum 0.5% as required by honey quality in accordance with SNI-01-3545-2004. The measurement of ash concentration indicated the honey quality criteria of flower nectar or honeydew honey. The concentration of ash in honey from the nectar of flowers was lower than the concentration of ash from honeydew honey. At present the measurement of ash concentration was generally replaced by the measurement of electrical conductivity. Measurement of ash concentration could be used as a quality factor during the transition period, until electrical conductivity was accepted as a honey standard worldwide (Bogdanov et al., 2001).

Contamination of honey was contamination of metals and substances that was not desired in honey because it could endanger the health of honey users. Metal contamination such as lead and copper and arsenic in honey analyzed showed the following results. Honey lead contamination of 0.042 mg/kg; honey copper contamination of 0.37 mg/kg; arsenic contamination of 0.003 mg/kg of harvested honey from the honey bee colony given pollen substitute PS1-A met the quality requirements of honey in accordance with SNI-01-3545-2004.

The result of the analysis of honey produced by the colony given pollen substitute PS1-A was in accordance with the parameter set by SNI-01-3545-2004. This indicated that pollen substitute PS1-A given to *A. cerana* honey bee colonies had no effect on lowering the quality of honey.

For the next research, it is necessary for add resources of protein to the PS1-A and measurement to the number of larvae and pupae and area of bee bread on nestcomb on colonies given PS1-A.

CONCLUSION

1. The Soybean dregs as waste from tofu industry could be used as raw material of pollen substitute given to *A. cerana* honey bee colonies.
2. Pollen substitute PS1-A was the most preferred pollen substitute in *A. cerana* honey bee colonies.
3. The giving of pollen substitute of PS1-A when no feed source for *A. cerana* honeybees was to prevent abscond.
4. The giving of pollen substitute PS1-A to *A. cerana* honey bee colonies increased the weight and the volume of honey from the control colonies.
6. The giving of pollen substitute PS1-A to *A. cerana* honey bee colonies gave the quality of honey produced in accordance with Indonesian National Standard for honey or SNI-01-3545-2004.

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