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Addition Effects of Methanolic Extract of Propolis against Antioxidant Activity of Ice Cream

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Propolis has been known as a material that contains various compounds. One of propolis source can be obtained from beehives. Propolis has many biological activities and can be used as a food additive due to its composition. In this study, the effect of addition of methanolic extract of propolis into ice cream was studied. The extraction of propolis from beehives has been carried out by maceration method. Total phenolics, flavonoids, and protein were determined using spectrophotometry method. Antioxidant activity was tested using the DPPH method. The result showed the addition of methanolic extract of propolis into ice cream can increase the antioxidant activity, total phenolics, flavonoids, and protein contents. The optimum results of total phenolics, flavonoids, and antioxidant activity on ice cream were obtained on the addition of 10 mL of propolis extract while the optimum result of total protein content was obtained by addition of 30 mL propolis extract.

Keywords: antioxidant; ice cream; methanolic extract; propolis

I. INTRODUCTION

Indonesia is a tropical country that has abundant of natural resources. It consists of 20,000 plant species (Kusmana & Hikmat, 2015) and less than 300,000 of animal species (Wibowo *et al.,* 1996). Honeybee is one type of animal that cultivated in Indonesia, especially *Trigona* sp. species. Honeybee produce honey as main products (Ndip *et al.,* 2011). Honeybee also produce another product called propolis (Yilmaz, 2016).

Propolis is a mixture of resins consisting of plant sap and many compounds secreted from honeybee metabolism (Wagh, 2013). Propolis has a sticky texture at warm temperature and solid texture in cold temperature (Marcucci, 1994). Propolis also called as bee glue that used to protect beehive from external threats (Wardaniati & Pratiwi, 2017).

Propolis has been widely used as traditional medicine (Russo *et al.,* 2004). Propolis contains many compounds such as amino acids, fatty acids, polyphenols, flavonoids, and terpenes (Lotfy, 2006). Propolis also contains cinnamic acid and caffeic acid that act as antibacterial, antiviral, anti-inflammatory, and antioxidant agents (Russo *et al.,* 2002). Propolis also contains various types of vitamins and minerals such as vitamin B1, B2, B6, C, E, magnesium, copper, calcium, iron, and manganese which are very beneficial for health. Similar to honey, propolis also contains several types of sugar such as 1-fructofuranose, 2-fructofuranose, α-D-glycopiranose, and β-D-glycopiranose (Marcucci, 1994). Because of its content, propolis can be used in various fields, for example as a food additive. Propolis can be used to increase the biological activity, quality, and nutrition of foods such as ice cream.

Ice cream is made from milk and other ingredients such as fruits, sweeteners, flavours and emulsifiers and it has a sweet taste (Patil & Banerjee, 2017). The principle of making ice cream is to tie the air into ice cream dough so it will swell and produces a soft texture (Adi *et al.,* 2014). The general composition of ice cream consists of 55-64% water, 9-12% sucrose, 9-12% non-fat solid milk, 10-16% solid fat milk, 0-0,5% stabilisers and 36-45% total solid to produce a stable,

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soft and sweet taste in ice cream (Goff, 1997). Because of its texture and taste, ice cream is very popular as a snack or dessert. Nowadays, a perception about ice cream as a snack/dessert has turned into a functional food that has a health benefit. There is great interest of ice cream enriched with additional nutrients or bioactive substances. These additives were expected to increase the nutritional values, quality, and benefits of the ice cream (Patil & Banerjee, 2017). Based on the description above, this study concern about addition of propolis extract from *Trigona* sp. species as additives in ice cream. Propolis was expected to increase the antioxidant activity of ice cream. Not only that, propolis also expected to increase the nutritional values and quality of ice cream.

II. MATERIALS AND METHODS

A. Materials

The beehives (*Trigona* sp.) was purchased from Petik Madu Farm in Lawang, Malang, East Java. Methanol pa, Folin Ciocalteu reagent, sodium carbonate, sodium nitrite, and sodium hydroxide were purchased from Merck. Gallic acid, quercetin, DPPH (2,2-diphenyl-1-picrylhydrazyl), and Bradford reagents were obtained from Sigma-Aldrich. Sulphuric acid (98%) was obtained from Smartlab Indonesia. Other materials used are universal indicator, Bovine Serum Albumin (BSA), aquabidest, fullcream milk (Ultramilk), powdered skim milk, sweetened condensed milk (Frisian Flag), egg yolks, cornstarch, whipping cream (Anchor), sugar, salt, and vanilla flavour.

B. Propolis Extraction

The procedure for extracting propolis was modified from Nugraheni *et al.* (2016a). Beehives were cut into small pieces, dried and weighed as much as 25 g. Then, 250 mL of methanol was added and stirred using a magnetic stirrer at 450 rpm for 60 minutes at 60°C. The solution then filtered to separate the filtrate and residue. The filtrate was stored in the refrigerator overnight until precipitate formed. Then, the precipitate was filtered and washed using methanol. The filtrate was evaporated at 50°C to produce a brown solution. The solution was kept in the refrigerator at 4°C for the next procedure.

C. Determination of Total Phenolics Content

One milliliter of propolis extract was added with 5 mL of Folin-Ciocalteu reagent (1:50). The mixture was incubated for 2 minutes at room temperature. After that, the mixture was added with 4 mL of Na_2CO_3 solution (0.4 M) and incubated in the dark room for 2 hours. After 2 hours of incubation, the absorbance was measured at 765 nm using a UV-Vis spectrophotometer. The measurement was replicated three times. The results are expressed in the units of µg Gallic Acid Equivalent (GAE)/g dry beehives (Nugraheni *et al.,* 2016a).

D. Determination of Total Flavonoids Content

Determination of total flavonoid content was modified from Lachman *et al.* (2010). A volume of propolis extract (0.4 mL) was added with 9.6 mL of aquabidest, 1.2 mL of H_2SO_4 (0.2 M), 1.2 mL of $NaNO_2$ (3 M), and 1.2 mL of NaOH (10% w/v). The solution was incubated for 15 minutes at room temperature. After 15 minutes of incubation, the absorbance was measured at 395 nm using a UV-Vis spectrophotometer. The measurement was replicated three times. The results are expressed in units of µg Quercetin Equivalent (QE)/g dry beehives.

E. Determination of Total Protein Content

Determination of proteins using the Bradford method was modified from Bradford (Bradford, 1976). One milliliter of propolis extract was added with 0.1 mL of aquabidest and 5 mL of Bradford reagent. The mixture was incubated for 5 minutes at room temperature. After 5 minutes of incubation, the absorbance was measured at 595 nm using a UV-Vis spectrophotometer. The standard used was Bovine Serum Albumin (BSA).

F. Determination of Antioxidant Activity

Measurement of antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method was carried out based on method from Miguel *et al.* (2013). One milliliter of propolis extract was added with 9 mL of DPPH solution (60 µM). The mixture was homogenised and incubated at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The results of

antioxidant activity are expressed as percentage (%) of decolourisation and it can be calculated by Equation (1):

$$\% \text{ Decolourisation} = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100\% \quad (1)$$

G. Fortification of Ice Cream

Full cream milk (200 mL) was added with 60 grams of sugar, ¼ tablespoon of salt and 12 grams of skim milk. The mixture was heated until it dissolves completely. One spoon of dissolved cornstarch then added into the mixture and left it in room temperature. The cooled mixture was stirred using a mixer and added with 150 grams of whipping cream. Six milliliters of sweetened condensed milk, ½ tablespoon of vanilla flavour and whipping cream were added to the mixture by stirring constantly using the mixer. The mixture put in the freezer for 15 minutes, stirred again for 5 minutes, added with 2 eggs, and stir continously. After stirring, the ice cream put in the freezer until it freezes completely.

Fortified ice cream was made with addition of propolis extract(A (10 mL, B (20 mL) and C (30 mL)). The fortified ice cream was stored in the freezer. On the 1st, 5th, 10th, 15th, 20th, 25th and 30th, antioxidant activity and pH of fortifiedice cream were tested (Adi *et al.,* 2014).

III. RESULTS AND DISCUSSIONS

A. Propolis Extraction

Before the extraction process, the beehives were dried for 3 days. The aim of this process is to reduce the water content in the beehives. The beehive was cleaned from impurities such as wood chips and bee pollen. The water content in beehive was 6.46%. The result is in accordance with another research, which the water content is around 4.6 to 9.4% (Cunha *et al.,* 2004). The extraction method used is maceration. The solvent used is methanol with a ratio of 1:10 to beehives. Beehive extraction produces dark brown propolis extract with 40% of recovery.

B. Total Phenolics Content of Propolis Extract

Total phenolic content of the propolis extract is 45.804 ± 0.511 µg AGE/g dry beehives. The amount of total phenolic content in this study is higher than the results of the previous research carried out by Omar *et al.* (2017). In a previous study, the beehive of *Trigona* (*Tetrigona*) *apicalis, Trigona (Heterotrigona) itama* and *Trigona (Geniotrigona) thoracica* from Malaysia was used as the source of propolis extract. They were extracted using ethanol as solvent (1:10). These results indicate that the differences in solvents and site origin of beehives, will affect on the total phenolics content obtained.

C. Total Flavonoids Content of Propolis Extract

The total flavonoid content in propolis extract from *Trigona* sp. is 6.582 ± 0.019 µg QE/g of dry beehive. This result was higher when compared to others. The research of Omar *et al.* (2017) calculated that the flavonoid content of propolis extract from *Trigona* species in Malaysia was 24.27 ± 0.11 mg QE/g dry beehives. The difference of solvent and site origin of beehive affect on the total of flavonoids content.

D. Total Protein Content of Propolis Extract

Total protein of propolis extract obtained is 24.931 µg BSA/g dry beehives. Another result showed that propolis from *Apis mellifera*'s beehives has total protein content as much as 87.04 µg/g (Marcucci *et al.,* 1996). It can be concluded that different extraction method and different site origin of beehives affect the total protein content in propolis extract.

E. Antioxidant Activity by DPPH Method

The study of antioxidant activity was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) method. As a result, % decolourisation of propolis extract is 82,655%. This result was greater than antioxidant activity of ethanolic propolis extract of *Apis mellifera*(57.510 ± 3.027%) (Nugraheni *et al.,* 2016a). It indicates that the difference of solvent and site origin of beehives affect on antioxidant activity (Nugraheni *et al.,* 2016b).

F. Fortification of Ice Cream

1. Production of ice cream

The process of making ice cream produces a different texture of ice cream for each variation. The structure of control was soft and became harder if stored for a long time in the freezer. The control melt slowly at room temperature. Ice cream A

and B has a softer texture than control and melt quickly at room temperature. For ice cream C, it has a softest texture if stored for a longtime in the freezer and melts fastest at room temperature compared to others. Result of physical characteristic observation, including colour and flavour, showed that all ice creams colour was yellowish white and have milk smell. Therefore, it can be concluded that the amount of propolis extract can affect the texture of the ice cream.

2. The measurement of pH

The pH value of four varations of ice cream were measured for a month. The measurement of pH was carried out to determine the effect of the addition of propolis extract to the pH of the ice cream produced. According to research conducted by Bajwa *et al.* (2017), the pH of ice cream is from 6.64 to 6.65. According to the research of Hashim and Al Shamsi (2016), the pH of ice cream was 5.71 ± 0.01. The pH of ice cream obtained from day 1 to day 30 resumed in Table 1.

Table 1. The acidity of Ice Cream (pH)

Sample	pH					
	1	5	12	17	25	30
Control	6	6	6	6	6	6
A	6	6	6	6	6	6
B	6	6	6	6	6	6
C	6	6	6	6	6	6

From the data above, the addition of propolis extract on ice cream does not affect the pH. The pH is still in the range of ice cream pH (Bajwa *et. al.,* 2017; Hashim & Al Shamsi, 2016). In addition, from day 1 to day 30, pH and physical conditions such as colour and flavour of the ice creams did not change.

3. Antioxidant activity of ice cream

The measurement of antioxidant activity was carried out to determine the effect of addition of propolis extract on the antioxidant activity of ice cream. According to research conducted by Shaviklo *et al.* (2011), % decolourisation of ice cream with addition of fish protein was 4.1-4.2%. Percent decolourisation of ice cream in this study was resumed in Table 2.

From Table 2, it was found that % decolourisation of the four ice creams variation tended to increase every day. It can be concluded that the addition of propolis extract affect the antioxidant activity of ice cream. However, there was an exception for ice cream C. Normally, increasing the amount of propolis extract added, % decolourisation will increase too. This is because in ice cream C, the propolis extract and ice cream dough cannot mixed completely. As a result, the propolis extract was not distributed well on the ice cream. It is also supported by the texture of ice cream C that is too soft when compared to others.

On the 25th day, the texture of ice cream B was softer than before. The texture on this ice cream affects the % decolourisation on the 25th day, where % decolourisation of B on the 25th day was lower than ice cream A. This is due to the texture changes that occur on ice cream B that affect the homogenity between ice cream and propolis extract. The texture of ice cream B which was softer than before indicates that the propolis extract did not spread well as before. This statement was supported with decreasing of % decolourisation on the 25th day. Furthermore, the highest antioxidant activity of ice cream was obtained on variation A with the addition of 10 mL of propolis extract.

Table 2. Antioxidant Activity of Ice Cream (%)

Sample	Decolourisation (%)				
	5	12	17	25	30
Control	67.077	72.366	75.864	76.713	80.893
A	81.078	89.983	91.447	93.322	93.562
B	81.312	90.501	91.681	92.973	93.198
C	70.182	89.464	89.865	91.231	91.952

4. Total phenolic and total flavonoid content of ice cream

In this study, total phenolic and total flavonoid content from four variations of ice cream were resumed in Table 3.

Table 3. Total Phenolics and Flavonoids Content in The Ice Cream

Sample	Total Phenolics Content (µg AGE/g of ice cream)	Total Flavonoids Content (µg QE/g of ice cream)
Control	2.356	2.068
A	2.899	2.541
B	0.811	2.525
C	2.572	2.497

From the data above, it was found that the value of the total phenolic and flavonoid content in A, B and C was greater than control (without propolis extract). It can be concluded that the addition of propolis extract affect the value of total phenolics and flavonoids content in cream. Percent of decolourisation increase after addition of propolis extract. The ice cream A had the largest of % decolourisation. According to research conducted by Hashim and Al Shamsi (2016), addition of organic extract in ice cream such as ginger juice (8%) can improve the amount of phenolic content inside. The differences additive added to the ice cream will affect the total phenolic content.

5. Total protein content of ice cream

The addition of propolis extract on ice cream would increase the total protein content. Moreover, the more extract added, the protein content will increase too. The complete result of protein content of ice cream resumed in Table 4. It was found that the largest total protein content was obtained in ice cream C. This result was supported by previous research conducted by Shaviklo *et al.* (2011) whichadded fish protein (30 g/kg) into ice cream. The protein content of ice cream increases up to 69 g/kg of ice cream. So,it can be concluded that the difference in additives added will affect the total protein content in ice cream product.

Table 4. Total Protein Content of Ice Cream

Sample	Total Protein Content (µg AGE/g of ice cream)
Control	11.35
A	15.57
B	17.45
C	19.48

IV. CONCLUSION

Propolis extract was obtained from beehives extraction of *Trigona* sp. with methanol as a solvent. Total phenolics, flavonoids, protein, and antioxidant content of propolis extract were 45.804 ± 0.511 µg AGE/g dry beehives, 6.582 ± 0.019 µg QE/g dry beehives, 24.931 µg BSA/g dry beehive and 82.655%, respectively. Addition of propolis extract can increase the nutritional value of ice cream which is characterised by an increasing of total phenolics, flavonoids, protein content, and antioxidant activity of ice cream. The optimum result of total phenolicS, flavonoids, and antioxidant activity was obtained on ice cream A (addition of 10 mL of propolis extract). While the optimum result for total protein content was obtained in ice cream C (addition of 30 mL propolis extract).

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