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The antibacterial properties of stingless bee honey in Malaysia and its effects as food preservative

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ABSTRACT *Nowadays consumers are more anxious about the usage of artificial preservatives as it poses a number of serious health risks. Thus, the demands of using natural preservatives in food are on the rise. The knowledge on the preservative effect of Malaysian stingless bee honey is limited. Hence, the objectives of this research are to evaluate the ability of stingless bee honey to inhibit the growth of bacteria and to investigate the effectiveness of stingless bee honey as a natural food preservative. This research was conducted by characterizing honey based on physicochemical analysis including pH, moisture content and sugar profile (glucose, fructose and sucrose), and examining the antimicrobial properties of stingless bee honey. The antibacterial property of stingless bee honey and commercial honey was examined by agar well diffusion where the commercial honey demonstrated a lower antibacterial property [Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus)] due to processing and storage conditions. Three samples of food which are milk, bread and commercial sambal hitam (spicy black sauce mainly made from bilimbi, Averrhoa bilimbi) were selected to evaluate the preservative nature of honey by comparing the microbial growth in all food samples with and without the presence of honey. By evaluating the turbidity of food samples at 550nm, the result showed that microbial growth count of samples with stingless bee honey is lower compared to samples with commercial honey and without honey. Besides that, stingless bee honey successfully inhibited 53% of bacterial growth and it was discovered that stingless bee honey was more effective against the gram - negative bacteria. Thus, due to the antibacterial properties, stingless bee honey can be used as a natural food preservative.*

Keywords: Stingless bee honey, preservatives, antibacterial properties, bacteria, honey

Preservatives either natural or artificial are referred to as any substances that are added to fruits, vegetables, ready-to-eat food items, cosmetics and pharmaceuticals with the aim to prolong their shelf life, retain its quality, texture, consistency, taste, color, and prevent fermentation, acidification as well as its decomposition (Anand & Sati, 2013; Samal et al., 2017). Food preservatives can be classified into two classes, Class I and Class II. Class I is known as the natural preservatives (salt, sugar, vinegar, syrup, spices, honey and edible oil) while Class II is the artificial or chemical preservatives (benzoates, sorbates, nitrites and nitrates of sodium or potassium, sulfites, glutamates and glycerides) (Anand & Sati, 2013; Sharif et al., 2017).

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The benefits and safety of many artificial food preservatives have created concerns among the consumer as it has been shown to cause several health problems such as respiratory, cancer and kidney problems (Abdulmumeen et al., 2012). Thus, more research should be done to seek for natural and less harmful preservatives to meet the consumer health criteria. One of the popular natural food preservatives are honey which is commonly served as a natural sweetener. According to McKibben and Engeseth (2002), honey acts as a food preservative agent by minimizing the food oxidative degradation reactions as well as protecting the foods against microbial growth.

Natural food preservatives can be divided into two agents that are antimicrobial and antioxidant agents (Sharif et al., 2017). Nevertheless, this research is focusing on the antimicrobial agent of natural food preservatives which is the stingless bee honey. The antibacterial activity of honey can be classified into peroxide and non-peroxide components where hydrogen peroxide is the main contributor for anti-bacterial properties in honey (Amato et al., 2010; Ewnetu et al., 2013; Krushna et al., 2007). The non-peroxide factors are contributed by components such as -tocopherol, ascorbic acid, flavonoids, lysozymes, phenolic acids and aromatic acids where it is abundant with syringic acid and phenyllactic acid (McKibben & Engeseth, 2002; Krushna et al., 2007).

Honey is often used as a preservative in food products such as turkey slices, cheese, and milk (Antony et al., 2006; Belewu & Morakinyo, 2009; Krushna et al., 2007). In a recent study by Amechi & Hurdison (2019), the shelf life of kunun zaki, a non-alcoholic drink from Nigeria, was prolonged for up to five-days at room temperature (25°C) by adding honey. To date, there is no direct study investigating the effect of stingless bee honey on food preservation. Stingless bee honey has been increased in popularity due to its higher nutritional and medicinal value besides having greater sources of biologically active compounds compared to common bee honey (Al-Hatamleh et al., 2020). Rosli et al. (2020) reported that stingless bee honey possesses higher antimicrobial properties and less sugar content. For this reason, we aimed to investigate the effectiveness of stingless bee honey to preserve processed food and to compare its effectiveness with commercial honey.

Methods

Sample collection and preparation

In this study, honey from the Heterotrigona Itama species was harvested from Universiti Malaysia Pahang stingless bee farm located at Taman Pertanian Sultan Ahmad Shah Kuantan while one commercial honey was obtained from the Malaysian local market. The honey samples were stored at ambient temperature in the dark until the experiment. Sambal hitam (spicy black sauce mainly made from bilimbi, Averrhoa bilimbi) is obtained commercially from Zati's Sambal Hitam, Malaysia. Milk was purchased from local grocery stores and bread was ordered from a personal baker to ensure no preservative was added. All food samples were stored at 4°C until the analyses. The microbes used in this experiment, Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) were purchased from Central Laboratory, Universiti Malaysia Pahang

Determination of moisture content and sugar profile

Moisture content of the honey was determined using a handheld refractometer (RHB 90ATC, China). Sufficient amount of honey was thinly spread onto the glass of handheld refractometer and moisture content was recorded. The sugar profile (fructose, glucose, sucrose) of honey was evaluated by following the method by Malaysian Kelulut standard (MS 2683:2017) (Department of Standards Malaysia, 2017). High Performance Liquid Chromatography (HPLC) [model of 1260 Infinity II LC System, Agilent Technologies, US] was used along with the Zorbax column (Zorbax NH2 150 x 4.6 mm, Agilent Technologies, USA). The mobile phase used was acetonitrile: water (75:25, v/v) at a flow rate of 1 ml/min. Briefly, about 1g of honey was transferred into a 50 mL centrifuge tube containing 20 mL of distilled water and the solution was filtered through a 0.45 μm membrane filter. Then 2 μL of sample filtrate was injected into HPLC which was eluted through the Zorbax column and detected by refractive Index detector (RID) operated at 40°C. The retention times obtained from the standards were used to determine the retention time of honey samples.

Antibacterial analyses

Disc diffusion assay for inhibitory activity

The antibacterial analysis of two honey samples was studied following the method done by Berhanu (2014). The nutrient agar plate was inoculated with E. coli and S. aureus, separately. Wells at the size of 6 mm were punched into the surface of the agar using the bottom of sterile pipette tips. An aliquot (12 μL) of diluted honey samples was placed into the wells with honey concentration of 50%. The blank samples containing sterile distilled water were served as control. The plates were incubated at 37°C for 24 hours. After the incubation period the inhibition zones were measured. No antibacterial activity was considered if the inhibition zones of samples was less than 12 mm (in diameter) (Berhanu, 2014).

Evaluation of inhibitory effect of honey on the growth of foodborne pathogens

By adopting the method from Krushna et al. (2007), E. coli and S. aureus were inoculated into 10 mL of nutrient broth where each test tube was supplemented with 100 μL honey of different concentrations (10%, 25%, 50%, 75% and 100%). The cultures were incubated at 37oC for 24 hrs and the effect of honey on E.coli and S.aureus was assessed by monitoring the change in the absorbance at 550 nm using UV-Vis spectrophotometer (ThermoFisher Scientific, USA). Distilled water is used as the control.

Evaluation of honey as a preservative

A 100 μL of 10g/mL of honey solution was added to food samples (Sambal hitam, milk and bread) and stored at 4°C for 2 to 6 days. For sambal hitam, 1g of sambal was mixed with 10 mL of distilled water and blended using the Khind multifunctional food processor (BLC99). For milk, 1 mL of milk was serially diluted (10-1 to 10-6) in water. For bread, 1g bread was added into 10 mL distilled water and was blended using the Khind multifunctional food processor (BLC99). Then the turbidity of the mixtures (Sambal hitam, milk and bread) was recorded at 24 hour interval from 0 to 6 days using UV-Vis spectrophotometer (ThermoFisher Scientific, USA) at 550 nm absorbance. The results were compared with the food samples that were stored without honey (Krushna et al., 2007).

Findings

Moisture content and sugar profile

Table 1 shows the moisture content and sugar profile of harvested and commercial honey samples. Moisture content of harvested stingless bee honey recorded a value of 33.2 ± 0.06% while the commercial honey has slightly lower moisture content of 28.7 ± 0.02%. Nevertheless, the moisture content of both honey samples were within the limit range of Malaysian kelulut standard (<35%) (Department of Standards Malaysia, 2017). The higher moisture content in stingless bee honey was expected due to the humidity of their habitat (Julika et al., 2019). However, high moisture content was undesirable as it may affect the preservation ability of honey due to greater fermentation potential (Nordin et al., 2018; Prica et al., 2014) and can lead to microbial spoilage (Naila et al., 2022).

The values of glucose, fructose and sucrose in two honey samples were determined where the commercial honey showed higher value of these sugars (Naila et al., 2021) compared to stingless bee honey. The results of stingless bee honey were somewhat similar to the study done by Kek et al. (2017) and Se et al. (2018) where the sum of fructose and glucose were well below the standard requirement which was no more than 90.0 g/100g. According to Kek et al. (2017), the low fructose and glucose content may be due to the certain genera of stingless bee honey. Both honey samples had low sucrose levels demonstrating that no adulteration activity had occurred and honey were also harvested at the perfect maturation time (de Sousa et al., 2016). The sugar profile of these honey samples were evaluated because some authors had the opinion that sugar content in honey is responsible for their antibacterial effect. However, Krushna et al. (2007) had ruled out this opinion by proving that hydrogen peroxide is a more relevant factor contributing to the antibacterial effect of honey.

Table 2

The moisture content and sugar profile of harvested and commercial honey samples (n=3)

Honey samples	Moisture content (%)	Fructose (g/100g)	Glucose (g/100g)	Sucrose (g/100g)	F+G (g/100g)
Stingless bee	33.2 ± 0.06	20.8 ± 0.35	17.2 ± 0.73	0.03 ± 0.16	0.38 ± 38.0
Commercial	28.7 ± 0.02	37.4 ± 1.21	40.5 ± 0.26	0.19 ± 0.54	0.95 ± 77.9

Inhibitory activity of honey

The antibacterial activity for both honey samples is shown in Figure 1. Generally, stingless bee honey has a greater inhibition zone on both E. coli and S. aureus compared to commercial honey. The inhibition zone of stingless bee honey against E. coli varied from 17 to 20 mm while against S. aureus, the diameter ranged from 16 to 18 mm. Commercial honey had negligible antibacterial activity against E. coli with only 10.03 to 11.6 mm of inhibition zone. As for S. aureus, the diameter of the inhibition zone ranged between 12 to 15 mm in commercial honey. The lack of antibacterial properties shown by the commercial honey may be influenced by processing or storage conditions of this honey (Baltrušaitytė et al., 2007).

Figure 1. Antibacterial activity of stingless bee honey and commercial honey on pathogenic microorganisms by agar well diffusion method.

Honey inhibits growth of foodborne pathogen

The growth inhibition of both E. coli and S. aureus by honey samples was evaluated, and is illustrated in Figure 2. Stingless bee honey has greater inhibition growth in both pathogens compared to commercial honey. According to Krushna et al. (2017), honey with least concentration should inhibit at least 50% growth of pathogens. However, in our findings, the growth inhibition only reached 50% when the honey concentration was at 50%. Moreover, stingless bee honey was the only honey that was able to inhibit more than 50% growth (53.5%) of E. coli. This result indicated that the stingless bee honey was more effective against gram negative bacteria as agreed by Amato et al. (2010). As for commercial honey, the maximum inhibition growth was 36.97 to 40.83% for S.aureus at the maximum honey concentration (100%). Despite the lower inhibition growth, we still observed a similar trend as Krushna et al. (2017). According to Krushna et al. (2017) inhibition of bacterial growth enhanced with the increase of honey concentration. The increasing trend was possibly due to the role of hydrogen peroxide in honey.

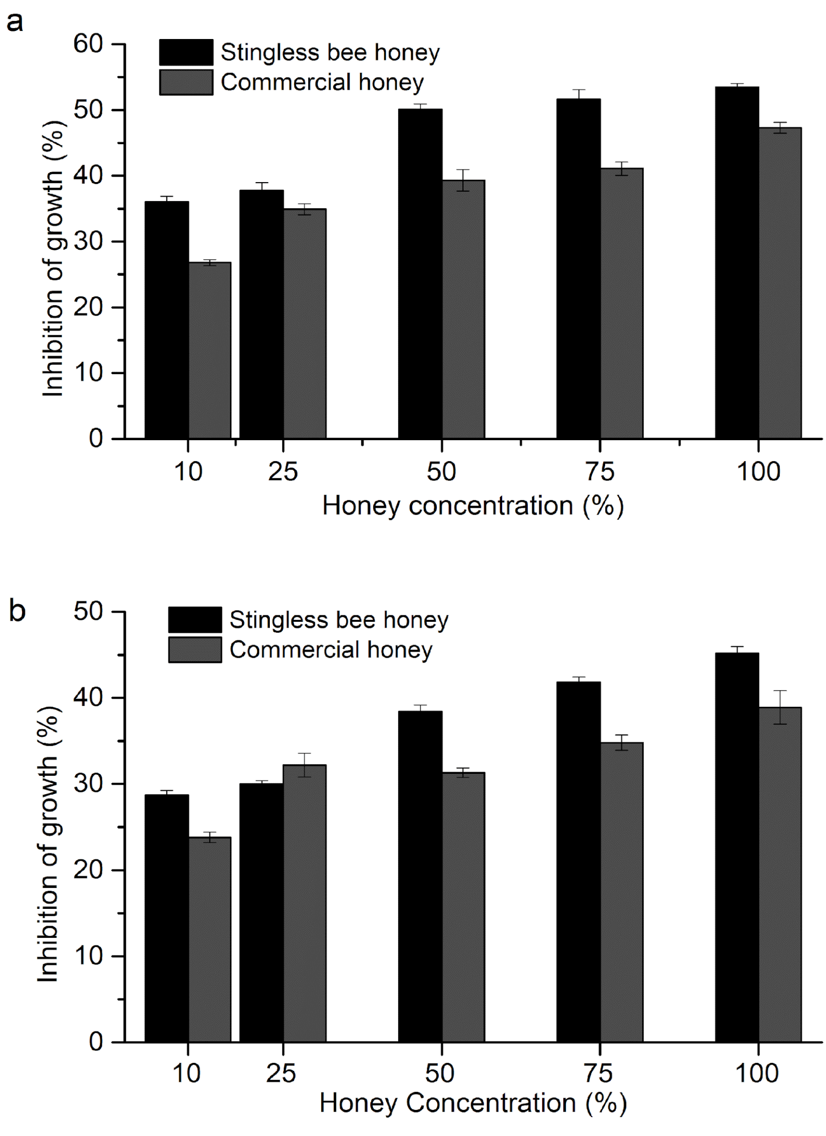

Figure 2. Inhibition of growth of stingless bee and commercial honey on a) E. coli and b) S.aureus

Honey as preservatives

The bacteria growth in three food samples (sambal hitam, milk and bread) were monitored. These results are illustrated in Figure 3. From the observation, foods that were preserved with stingless bee honey had the lowest turbidity compared to the control, commercial honey without added stingless bee honey. Besides that, food samples stored with both honey exhibited around 45% to 56% reduction in turbidity compared to food samples without addition of honey. These results are comparable with Krushna et al. (2017) where the authors reported around 50-55% decrease of turbidity in milk samples. Based on the preservative effect shown in this study, it can be deduced that honey, especially the stingless bee honey is suitable to be used as a natural preservative. This can be further proved with the study done by Amato et al. (2010) which stated that honey significantly increased the shelf life of packed fruit at 4°C. In addition, Antony et al. (2006) reported that packed sliced turkey showed negligible bacterial growth after 11 days storage with 15% honey addition.

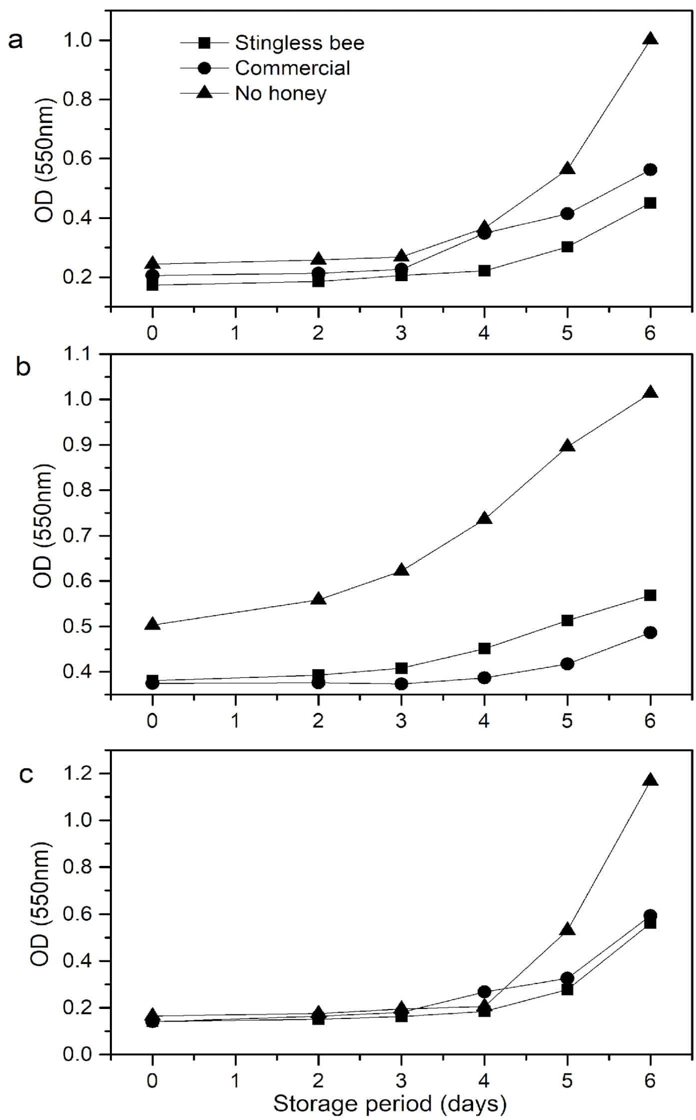

Figure 3. Turbidity assay of Sambal hitam (a) Milk (b) and Bread (c) at 550 nm

Conclusion

This paper reported on the ability of stingless bee honey from the Heterotrigona itama species to preserve some selected food samples which were the commercial sambal hitam, milk and bread. Stingless bee honey was better than commercial honey in terms of physicochemical and antibacterial properties. The stingless bee honey was more effective against E. coli and was able to inhibit more than 50% of bacterial growth in all food samples. The antibacterial properties of stingless bee honey may be influenced by the hydrogen peroxide content as referred to in the past studies. More analyses need to be carried out in the future to further verify the competence of stingless bee honey as a natural preservative. Analyses such as evaluating the role of hydrogen peroxide content in contributing to the antibacterial properties of honey as well as sensory and antioxidant properties assessment can be included in future study. This research is expected to contribute to the knowledge of stingless bee honey as a preservative agent against food pathogens. Additionally, the outcome of this research is to increase the values of stingless bee honey as a natural and low-cost preservative agent that may spark the interest of health concerned consumers

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References

Abdulmumeen, H. A., Risikat, A. N., & Sururah, A. R. (2012). Food: Its preservatives, additives and applications. *International Journal of Chemical and Biochemical Sciences,* 1(2012), 36-47.

Al-Hatamleh, M. A., Boer, J. C., Wilson, K. L., Plebanski, M., Mohamud, R., & Mustafa, M. Z. (2020). Antioxidant-based medicinal properties of stingless bee products: recent progress and future directions. *Biomolecules, 10*(6), 923.

Amechi, O. I., & Hurdison, D. C. (2019). Effectiveness of lemon juice and honey in shelf life enhancement of kunun zaki. *World Journal of Advanced Research and Reviews, 1*(1), 001-008.

Anand, S., & Sati, N. (2013). Artificial preservatives and their harmful effects: looking toward nature for safer alternatives. *International. Journal of Pharmaceutical Sciences and. Research, 4*(7), 2496-2501.

Antony, S., Rieck, J., Acton, J., Han, I., Halpin, E., & Dawson, P. (2006). Effect of dry honey on the shelf life of packaged turkey slices. *Poultry Science, 85*(10), 1811-1820.

Baltrušaitytė, V., Venskutonis, P. R., & Čeksterylė, V. (2007). Antibacterial activity of honey and beebread of different origin against S. aureus and S. epidermidis. *Food Technology & Biotechnology, 45*(2).

Belewu, M., & Morakinyo, O. (2009). Nutritional, microbiological and sensory evaluation of honey treated and untreated soft cheese. *European Journal of Scientific Research, 26*(1), 124-131.

Berhanu, A. (2014). Physico-chemical, microbiological and antibacterial properties of Apis mellipodae and Trigona spp. honey against bacterial pathogens. *World Journal of Agricultural Sciences, 10*(3), 112-120.

de Sousa, J. M. B., de Souza, E. L., Marques, G., de Toledo Benassi, M., Gullón, B., Pintado, M. M., & Magnani, M. (2016). Sugar profile, physicochemical and sensory aspects of monofloral honeys produced by different stingless bee species in Brazilian semi-arid region. *LWT-Food Science and Technology, 65*, 645-651.

D'Amato, D., Sinigaglia, M., & Corbo, M. R. (2010). Use of chitosan, honey and pineapple juice as filling liquids for increasing the microbiological shelf life of a fruit-based salad. *International Journal of Food Science & Technology, 45,* 1033-1041.

Department of Standards Malaysia. (2017). Malaysian Standard MS 2683/: 2017. *In Kelulut (Stingless bee) honey – Specification.* Department of Standard Malaysia (pp. 2683). Malaysia.

Ewnetu, Y., Lemma, W., & Birhane, N. (2013). Antibacterial effects of Apis mellifera and stingless bees honeys on susceptible and resistant strains of Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae in Gondar, Northwest Ethiopia. *BMC complementary and alternative medicine, 13*(1), 1-7.

Julika, W. N., Ajit, A., Sulaiman, A. Z., & Naila, A. (2019). Physicochemical and microbiological analysis of stingless bees honey collected from local market in Malaysia. *Indonesian Journal of Chemistry.* https://doi.org/https://doi.org/10.22146/ijc.40869

Kek, S. P., Chin, N. L., Tan, S. W., Yusof, Y. A., & Chua, L. S. (2017). Classification of honey from its bee origin via chemical profiles and mineral content. *Food Analytical Methods, 10,* 19-30.

Krushna, N. S., Kowsalya, A., Radha, S., & Narayanan, R. B. (2007). Honey as a natural preservative of milk. *Indian Journal of Experimental Biology, 45*(5), 459-464.

McKibben, J., & Engeseth, N. J. (2002). Honey as a protective agent against lipid oxidation in ground turkey. *Journal of Agricultural and Food Chemistry, 50*(3), 592-595.

Naila, A., Julika, W., Flint, S., Sulaiman, A. Z., Mohamed, A., & Ajit, A. (2021). Analysis of adulterants in international honey available in The Maldives. *ACS Food Science & Technology, 1*(8), 1373-1380.

Naila, A., WN, J., Abdul Hannan, N., Flint, S., Ziad Sulaiman, A., Mohamed, A., & Ajit, A. (2022). Microbiological and physiochemical quality of honey imported into the Maldives. *ACS Food Science & Technology, 2*(5), 836-843.

Nordin, A., Sainik, N. Q. A. V., Chowdhury, S. R., Saim, A. B., & Idrus, R. B. H. (2018). Physicochemical properties of stingless bee honey from around the globe: A comprehensive review. *Journal of Food Composition and Analysis, 73,* 91-102.

Prica, N., Baloš, M. Ž., Jakšić, S., Mihaljev, Ž., Kartalović, B., Babić, J., & Savić, S. (2014). Moisture and acidity as indicators of the quality of honey originating from Vojvodina region. *Archives of Veterinary Medicine, 7*(2), 99-109.

Rosli, F. N., Hazemi, M. H. F., Akbar, M. A., Basir, S., Kassim, H., & Bunawan, H. (2020). Stingless bee honey: Evaluating its antibacterial activity and bacterial diversity. *Insects, 11*(8), 500.

Samal, D., Gouda, S., & Patra, J. K. (2017). Food preservatives and their uses: a short report. *Asian Journal of Biology, 4*(1), 1-4.

Sharif, Z., Mustapha, F., Jai, J., Yusof, N. M., & Zaki, N. (2017). Review on methods for preservation and natural preservatives for extending the food longevity. *Chemical Engineering Research Bulletin,* 145-153.

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