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Thanks very much for the information and we hope our paper can be accepted and published in Biodiversitas [Kutipan teks disembunyikan]

Best Regards,

Dr. Ir. Erwan, M.Si. Faculty of Animal Science, University of Mataram, Indonesia

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The novelty of our study was the honey quality produced by the bee *Apis cerana* and the honey potency which are produced by sugar palm and coconut saps which have not studied by another researcher especially in Indonesia. Therefore, this manuscript is very informative for the beekeepers, researchers or scientist, and honey consumers.

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Sincerely yours,

(fill in your name, no need scanned autograph) Dr. Ir. Erwan, M.Si.

7

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Honey quality from the bee *Apis cerana*, honey potency produced by coconut and sugar palm saps

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8 Abstract. One of the big problems when keeping of honeybees is the limited of sustainable feed, especially in the rain season. The 9 objectives of this study were to evaluate the honey quality from the bee A. cerana based on the chemical composition, honey potency 10 produced by the coconut and sugar palm saps. This study using thirty colonies of the bee A. cerana were divided into six treatments 11 consists of sugar palm sap without sugar palm pollen; coconut sap without sugar palm pollen; coconut sap of 50% + sugar palm sap of 50% without sugar palm pollen; sugar palm sap was added by sugar palm pollen; coconut sap was added by sugar palm pollen; coconut 12 13 sap of 50% + sugar palm sap of 50% was added by sugar palm pollen. The chemical composition of honey from the A. cerana were 14 moisture (20.76 to 21.80%), reducing sugar (62.78 to 68.37%), sucrose (1.44 to 3.42%), diastase enzyme activity (5.17 to 9.04 DN), 15 hydroxymethylfurfural (2.24 to 5.81 mg/kg), and acidity (26.00 to 36.35 ml NaOH/kg). Honey potency produced by the coconut and 16 sugar palm saps in 100 hectares area produces honey was 1,542.857 tons/year and 1,150 tons/year, respectively. It can be concluded that 17 honey quality is produced by sugar palm and coconut saps, and potential as the bee feed.

18 Key words: Arenga pinnata, beekeeping, Cocos nucifera L., extrafloral nectar, multifloral nectar

19 **Running title:** Honey quality of *Apis cerana* produced by sugar palm and coconut saps

INTRODUCTION

21 Honeybee of A. cerana is one of the bees from the Apis genus which is include the local bee which is spread in some 22 regions in Indonesia are Kalimantan, Sumatera, Java, Bali, Lombok, Sumbawa, Sulawesi, Papua, and Seram (Radloff et al. 23 2011; Hepburn and Radloff 2011). In Indonesia, beekeeping of the bee A. cerana has been practiced by the beekeepers 24 using a traditional hives (for example using a coconut log hive) and semi modern hive (box hive without nest frame) to produce honey. Furthermore, several regions have been practices the beekeeping of the bee A. cerana has been reported by 25 Schouten et al. (2019) are Riau, North Sumatera, Lampung, Banten, Java, Yogyakarta region, Bali, and Lombok. 26 27 However, the beekeeping of A. cerana is mostly using traditional hives although using a box hives because is not 28 completed by the honey frame like a beekeeping of A. mellifera. The bee A. cerana can produce honey, bee bread, royal 29 jelly, and propolis, however their production is lower compared to the bee A. mellifera.

30 One of the problems faced by the beekeepers in Indonesia is the limited of feed sustainability as the raw material to 31 produce honey, bee bread, and royal jelly. The limitation feed is the very serious problem have been faced by the beekeepers because they have not area which is used to planted several plants which are used the feed source to produce 32 the honeybees products. Honeybees feeds is divided into two types namely nectar and pollen, where nectar is obtained by 33 34 the foragers from the plant flowers (nectar floral) and nectar extrafloral which is obtained by the foragers from stalk and 35 leaf of plants (Agussalim et al. 2018, 2017). Pollen is obtained by the foragers from plant flowers which is collected by using all body part and then deposited in the corbicula (Agussalim et al. 2018, 2017; Erwan et al. 2021a). When collecting 36 37 nectar and pollen from the plant flowers, the foragers is role as the pollinator agent by transporting pollen from the anther 38 to pistil so that the pollination process occurs. This process is continuously done by the foragers until their honey stomach 39 is full by a nectar and their corbicula has been deposited by the pollen. This pollination which is impacts on the increasing 40 the plants productivity (Pohorecka et al., 2014; Supeno et al., 2021).

One of the strategies to produce the sustainability honey from the bee *A. cerana* by using a sap from the plants such as sugar palm and coconut. Several studies have been conducted by using a sugar palm and coconut saps as the *A. cerana* feed. Erwan et al. (2021b) reported that the feed combination of coconut sap and sugar palm pollen as the *A. cerana* feed can enhancing the production of honey cells and bee bread cells. However, the use each of sap from coconut and sugar palm can increasing the honey and bee bread cells compared to control group without sap as the feed (multifloral nectar). Furthermore, Erwan et al. (2022) was also reported that the use of sugar palm and coconut saps which each added by sugar 47 palm pollen can improving the bee A. cerana productivity such as increase the honey production, brood cells number, and colony weight. In addition, in other study showed that the use of extrafloral nectar namely sugar palm (Arenga pinnata) 48 and coconut (Cocos nucifera L.) saps as the A. mellifera bee feed which is resulting the honey chemical composition 49 (reducing sugar, sucrose, acidity, moisture, and diastase enzyme activity) which are acceptable by Indonesian national 50 standard and international standard has been regulated by Codex Alimentarius (Erwan et al. 2020). However, the studied 51 about the chemical composition of honey from the bee A. cerana which are produced from the sugar palm sap, coconut sap 52 53 and their honey potency production from both sap sugar palm and coconut have not been studied. Therefore, the objectives 54 of this study were to evaluate the honey quality based on the chemical composition from the bee A. cerana, honey potency produced by the coconut and sugar palm saps. 55

56

MATERIALS AND METHODS

57 Study area

58 This research has been conducted in the North Duman Village (8°32'10"S 116°09'32"E), Lingsar Sub-district, West 59 Lombok (West Nusa Tenggara Province, Indonesia). In this research, we used thirty of A. cerana colonies were divided into six treatments and each five colonies per treatment as the replication. The saps were used in our study were obtained 60 61 from coconut (Cocos nucifera L.) and sugar palm (Arenga pinnata). The treatments in our study were sugar palm sap 62 without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0); coconut sap of 50% + 63 sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by 64 65 sugar palm pollen (SCP1).

The technique was used to given sugar palm and coconut saps and sugar palm pollen was according to previously method has been reported by Erwan et al. (2022, 2021b) briefly as follows: fresh coconut and sugar palm saps were given to the bee *A. cerana* by using a plastic plate and split bamboo were completed by 4 to 5 twigs for foragers perch. The plastic plate and split bamboo were placed one meter of the box hives, while the sugar palm pollen was hung besides and above of the box hives. The distance of 600 meters to place colony to avoid the foragers to collect pollen and sap from the other treatments.

72 Proceduress

73 Honey quality

74 Honey from the A. cerana was harvested after beekeeping for three months by using a coconut and sugar palm saps. Honey from the five hives in one treatment group was composited into one honey sample and then used to analysis of their 75 chemical composition. Honey quality from the A. cerana were evaluated based on the chemical composition consists of 76 77 moisture, reducing sugar, sucrose, diastase enzyme activity, hydroxymethylfurfural (HMF), and acidity. The moisture content was analyzed by using a proximate analysis based on the method from Association of Official Agricultural 78 79 Chemists (AOAC) (AOAC 2005). Reducing sugar was analyzed by using a Layne-Enyon method and sucrose content was 80 analyzed by a Luff Schoorl method were described by AOAC (2005). Diastase enzyme activity, hydroxymethylfurfural 81 (HMF), and free acidity were analyzed based on the harmonised methods of the international honey commission (Machado 82 et al. 2022).

83 Honey production from sugar palm and coconut saps

Sugar palm and coconut saps each ten liters were used to measuring the honey production from the bee *A. cerana* for three months of beekeeping. The sugar palm and coconut saps were placed in the plastic plate in front of the box hives at the distance of one meter. In addition, the honey production without using of sugar palm and coconut saps were measured for one year of the beekeeping which is used to calculate the contribution of sugar palm and coconut saps in honey production.

89 *Production of saps from coconut and sugar palm*

The production of sap from coconut was measured for a year, while the sugar palm sap based on the previously studied was used to calculate the production per hectare area and was also obtained from the deep interview with the farmers. The production of coconut and sugar palm saps per hectare which was calculated from the sap production per hectare multiplied by the tress number in one hectare area. After three months of beekeeping, honey from both treatments sugar palm and coconut saps were harvested to measure the honey production from the use of ten litters sap and then honey production was measured by cylinder glass

96 Data analysis

The data of honey quality, production potency of honey from sugar palm and coconut saps, honey production, and production of saps were analyzed by using a descriptive analysis (Steel et al. 1997).

RESULTS AND DISCUSSION

100 Moisture content of honey

101 Honey is composed by water as the second largest of honey constituent and its ranging from 15 to 21 g/100 g, 102 depending on the plant types as the nectar source which is affected by the botanical origin. Furthermore, honey moisture is 103 also affected by honey maturity level, processing postharvest, and storage condition (Da Silva et al. 2016). The honey 104 moisture is affecting the physical properties such as crystallization, viscosity, flavor, color, taste, solubility, specific gravity, and conservation (Da Silva et al. 2016; Escuredo et al. 2013). In addition, honey moisture is also affected by the 105 106 temperature and humidity or depending on the season (rain and dry seasons) and honey moisture can increase during the 107 postharvest processing such as storage condition because honey is hygroscopic that can absorbs the moisture in the air (Da 108 Silva et al. 2016; Karabagias et al. 2014).

109 The recent study showed that the honey moisture from the bee A. cerana which was produced by sugar palm and coconut saps and their combination was ranging from 20.76 to 21.80% (Table 1). This honey moisture content is accepted 110 by Indonesian national standard (SNI) where the moisture for beekeeping honey including the bee A. cerana and A. 111 112 mellifera is not exceed 22% (National Standardization Agency of Indonesia 2018) and higher compared to international 113 standard which is regulated by Codex Alimentarius is not exceed 20% (Thrasyvoulou et al. 2018). The variation of honey moisture of the bee A. cerana in our study may be caused by the different moisture content of both saps from sugar palm 114 and coconut, however in our study has not measured. The higher moisture content is requiring the long time to ripening of 115 honey and process decreasing of honey moisture have been started by the bees when they are taken a nectar from plant 116 flowers or saps as the raw material to produce honey. Furthermore, small portion of moisture content has been evaporated 117 in the honey sack before transferred to the other bee which is working in the hive. This transfer is rapid depending on the 118 temperature, colony strength, and nectar availability (Da Silva et al. 2016). 119

120 121

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Table 1. The moisture, reducing sugar, and sucrose contents of honey from the bee A. cerana

Treatments	Moisture (%)	Reducing sugar (%)	Sucrose (%)
SP0	21.60	65.24	2.86
CP0	20.76	68.37	1.96
SCP0	21.40	64.55	2.51
SP1	21.80	62.78	3.42
CP1	21.58	65.37	1.72
SCP1	20.98	67.33	1.44

Abbreviations: sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0); coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen (SCP1).

127 Honey production process is started from the foragers collecting a nectar from the plant flowers or extrafloral nectar 128 and then stored in honey stomach. After that, the foragers will be transferring a nectar has been collected to the other bees 129 whom working to processing a nectar into honey in their mouth, then put in honey stomach and then is transferred to other bees for several times until honey is ripening. A considerable of water amount will be evaporated in this process and this 130 continues with the help of wing fans that can regulate the air humidity for about 15 to 20 minutes (Balasubramanyam 131 2021; Zhang et al. 2021). The honey moisture content in our study was differed to reported by Wang et al. (2021) that 132 honey moisture from the bee A. cerana which is collected from 42 different honeycombs from China is ranging from 17.03 133 134 to 18.44%, 18.65% for A. cerana cerana from Hainan province, China (Wu et al. 2020), and 16.99% for A. cerana from 135 Borneo (Malaysian honey) (Moniruzzaman et al. 2013). Furthermore, Erwan et al. (2020) was also reported that the honey moisture was produced by the A. mellifera bee by using a sugar palm and coconut saps is ranging from 19.34 to 20.94%. 136 The different honey moisture content has been reported are affected by the different geographical origins which is impact 137 138 on the different plant types can be growth each region, different environmental condition (temperature and humidity), and 139 also different bee species which is impact on the different ability to evaporate water in the honey.

140 **Reducing sugar and sucrose contents of honey**

Sugars in honey are composed by monosaccharides for about 75%, disaccharides are 10 to 15%, and other sugars in small amounts. Honey sugars are responsible as the energy source, hygroscopic, viscosity, and granulation. Several sugars in honey have been reported such as glucose, fructose, sucrose, trehalose, rhamnose, isomaltose, nigerobiose, maltotriose, maltotetraose, melezitose, melibiose, maltulose, nigerose, raffinose, palatinose, erlose and others (Da Silva et al. 2016).

The recent study showed that the honey s reducing sugar from the bee *A. cerana* were beekeeping by using a sugar palm and coconut saps and their combination as the nectar source to produce honey is ranging from 62.78 to 68.37 % (Table 1). This honey reducing sugar is acceptable by the SNI for treatments SP0, CP0, CP1, and SCP1, but not acceptable for treatments SCP0, SP1 (Table 1), where the minimum reducing sugar is 65% (National Standardization Agency of Indonesia 2018). This sugar is produced by the mechanism of invertase enzyme activity that change the sap sucrose into simple sugars. It is known that this enzyme is responsible for the conversion of sucrose into glucose and fructose. These 151 sugars are included in reducing sugar group and as the main component present in honey. In honey maturity process, the 152 sucrose is break down by the invertase enzyme into simple sugars simultaneously and water will be evaporated so that it will be increasing the reducing sugar content. In addition, enzymes secreted by the worker bees are also can break down 153 the carbohydrate into simple sugars. Furthermore, other enzyme present in honey is diastase enzyme that role to break 154 down starch into simple sugars (Da Silva et al. 2016). The honey reducing sugar in our study (Table 1) was differed to 155 156 reported by Erwan et al. (2020) that honey reducing sugar from the bee A. mellifera which was produced by extrafloral 157 nectar (sugar palm and coconut saps) is ranging from 60.15 to 73.69%. The different reducing sugar may be affected by 158 the different bee species which is impact on the different their ability to evaporate water present in honey especially when they are convert the complex sugars into simple sugars and different season when done the study which are related to 159 160 temperature and humidity environmental.

161 The honey sucrose content from the bee A. cerana in our study is ranging from 1.44 to 3.42% (Table 1) and acceptable by SNI is not exceed 5% for the beekeeping honey including A. cerana and A. mellifera (National Standardization Agency 162 of Indonesia 2018) and also accepted by the International standard has been regulated by Codex Alimentarius is not exceed 163 5% for blossom and honeydew honeys (Thrasyvoulou et al. 2018). Naturally, sucrose present in honey in our study is 164 originated from sugar palm and coconut saps. The low of honey sucrose content in our study is caused the honey which is 165 166 harvested in mature condition that characterized by honey cells have been covered by the wax. Furthermore, the invertase enzyme which is produced by the worker bees is actively break down of sucrose from saps into simple sugars. There are 167 two types of invertase enzymes which are produced by the worker bees, namely glucoinvertase which is converts sucrose 168 into glucose and fructoinvertase which is converts sucrose into fructose. These enzymes are mostly derived from the bee's 169 170 secretion and only a small portion from the nectar, while the honeydew from the insect's secretion is mostly contain 171 invertase enzyme (Da Silva et al. 2016). The honey sucrose content in our study (Table 1) was differed to reported by 172 Erwan et al. (2020) that honey sucrose content from the bee A. mellifera was produced by extrafloral nectar (sugar palm and coconut saps) is ranging from 4.21 to 4.40%%. 173

174 The honey sucrose content is a very important parameter to evaluate the maturity of honey to identifying manipulation, 175 where the high levels may be indicated adulterations by adding the several sweeteners such cane sugar or refined beet 176 sugar. In addition, also indicating the early of harvest, where sucrose is not completed transformed into fructose and 177 glucose, the bees feeding artificial in prolonged time by using a sucrose syrup (Da Silva et al. 2016; Puscas et al. 2013; 178 Escuredo et al. 2013; Tornuk et al. 2013). Honey is sugar solution that is supersaturated and unstable so it's easy to crystallize. The honey crystallization is affected by concentration of glucose, fructose, and water. Fructose is the dominant 179 180 sugar present in honey from A. mellifera was produced by several plants as the nectar source which is used by workers to 181 produce honey such as eucalyptus, acacia, bramble, lime, chestnut, sunflower, and from honeydew, except in rape honey was produced by Brassica napus. Rape honey is higher in glucose and lower in fructose which is impact on the rapidly 182 crystallization (Escuredo et al. 2014). The sugars content present in honey is dependent on the geographical origins which 183 is impact on the different plant types can growth in each region and impact on the different sugars content from the nectar 184 which is produced by the nectary gland of plant flowers (Agus et al. 2021; Agussalim et al. 2019; Da Silva et al. 2016; 185 Escuredo et al. 2014; Tornuk et al. 2013). Furthermore, sugars content in honey is influenced by climate (season, 186 187 temperature, and humidity), processing (heating process), and storage time (Da Silva et al. 2016; Escuredo et al. 2014; 188 Tornuk et al. 2013).

189 Diastase enzyme activity and hydroxymethylfurfural of honey

190 The recent study showed that the diastase enzyme activity from the bee A. cerana honey was produced by the sugar palm and coconut saps was ranging from 5.17 to 9.04 DN (Table 2). This enzyme activity is acceptable by SNI with the 191 minimum of 3 DN for the beekeeping honey including the bee A. cerana and A. mellifera (National Standardization 192 193 Agency of Indonesia 2018) and also acceptable by international standard has been regulated by Codex Alimentarius with 194 the minimum 3 DN (Thrasyvoulou et al. 2018). One of the honey characteristics is contain enzymes which is originate 195 from the bees, pollen, and nectar from plant flowers, but the mostly enzymes are added by the bees when they are convert 196 nectar into honey (Da Silva et al. 2016; Thrasyvoulou et al. 2018). The honey diastase enzyme activity in our study (Table 197 2) was differed to reported by Erwan et al. (2020) that the diastase enzyme activity of honey from the bee A. mellifera was 198 produced by extrafloral nectar (sugar palm and coconut saps) is ranging from 16.48 to 17.12 Schade unit.

Diastases is divided into α- and β-amylases which are the natural enzymes present in honey. The α-amylase is separate the starch chain randomly in the central to produce dextrin, while the β-amylase to separate the maltose in the end chain. Diastase enzyme content in honey is influenced by nectar source (floral and extrafloral nectars) to produce honey and honey geographical origins which are impact on the different chemical composition of the nectar can be produced by the plants which is impact on the honey chemical composition especially diastase enzyme activity. In addition, the bee species is also influencing the activity diastase because it's related to the distance and the flowers plant numbers can be visited by the foragers when they are collecting nectar and pollen were using to produce honey and bee bread (Da Silva et al. 2016).

Generally, diastase enzyme is role to break down the complex sugars into simple sugars. This enzyme is role to digest of starch into maltose (disaccharide) and maltotriose (trisaccharide) which are sensitive to heat or thermolabile. Thus, this condition can be used to evaluate of overheating and preservation degree of honey (Da Silva et al. 2016). Furthermore, the diastase activity is also used to evaluate honey age which is related to storage time and the temperature because the diastase activity may be reducing when heating above 60°C and longtime storage (Da Silva et al. 2016; Yücel and Sultanoğlu 2013). The honey diastase activity from the bee *A. cerana* in our study (Table 2) was differed to reported by Wu et al. (2020) for multifloral honey produced by the *A. cerana cerana* from Hainan province (China) was 6.70 Göthe. Furthermore, also was differed to reported by Wang et al. (2021) that the diastase activity of *A. cerana* honey from Qinling Mountains (China) is ranging from 22.05 to 35.67 Göthe. The different diastase activity of honey from *A. cerana* were reported by previously researchers are influenced by the different plant types as the nectar source to produce honey, different sugars content, and different geographical origin.

217 Furthermore, the HMF of A. cerana honey was produced by the sugar palm and coconut saps in our study was ranging from 2.24 to 5.81 mg/kg (Table 2). This HMF indicate that honey from our study in fresh condition and acceptable by SNI 218 219 for the beekeeping honey including from A. cerana and A. mellifera is not exceed 40 mg/kg (National Standardization 220 Agency of Indonesia 2018) and also acceptable by the international standard has been regulated by Codex Alimentarius is 221 not exceed 40 mg/kg for blossom and honeydew honeys (Thrasyvoulou et al. 2018). The fresh honey after harvested is 222 generally contain the low of HMF is ranging from 0 to 4.12 mg/kg honey. Hydroxymethylfurfural is resulted from the 223 degradation of honey monosaccharide especially fructose and glucose under acid condition and accelerated by the heating. 224 This reaction is producing levulinic and formic acids (Da Silva et al. 2016).

225 226

Table 2. The diastase enzyme activity, hydroxymethylfurfural, and acidity of honey from the bee A. cerana

Treatments	Diastase enzyme	activity Hydroxymethylfurfural	Acidity (ml NaOH/kg)
Treatments	(DN)	(mg/kg)	
SP0	7.57	5.78	36.33
CP0	5.17	5.04	26.00
SCP0	9.04	4.75	28.60
SP1	6.86	4.77	29.68
CP1	8.51	5.81	28.26
SCP1	6.85	2.24	30.61

Abbreviations: sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0);
coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm
pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen (SCP1).

Hydroxymethyfurfural is formed after honey removed from the comb or when the wax covers was opened and the 232 advanced processing like heating process. The increasing of the HMF content is occur in honey with the high acidity and 233 accelerated by the heating process. However, the HMF content also influenced by several factors such as sugars content, 234 235 organic acids presence, pH, moisture content, water activity, and the plant types as the nectar source (floral source). In addition, HMF is also can be formed at the low temperatures, acidic condition, and sugars dehydration reactions. 236 Therefore, the higher of HMF content is impact on the honey color is darker (Da Silva et al. 2016; Tornuk et al. 2013). The 237 238 HMF of honey from the A. cerana in our study (Table 2) was differed to previously reported by Wu et al. (2020) for 239 multifloral honey of A. cerana cerana from China is 3.80 mg/kg and 1.69 mg/kg for A. cerana honey from Qinling 240 Mountains, China is 1.69 mg/kg. The different HMF content of honey from A. cerana were reported by previously 241 researchers are influenced by the different plant types as the nectar source to produce honey, different sugars content, and different geographical origin. 242

243 Acidity of honey

Free acidity is one of an important parameter to evaluate the honey deterioration which is characterized by the organic acids presence in equilibrium with internal esters, lactone and several inorganic ions such as sulfates, chlorides, and phosphates (Da Silva et al. 2016). The recent study showed that the honey acidity from *A. cerana* was produced by the sugar palm and coconut saps was ranging from 26.00 to 36.33 ml NaOH/kg (Table 2). The acidity of *A. cerana* honey in our study is acceptable by SNI is not exceed 50 ml NaOH/kg for the beekeeping honey including *A. cerana* and *A. mellifera*. Furthermore, is also acceptable of the international standard has been regulated by the Codex Alimentarius is not exceed 50 meq/kg for blossom and honeydew honeys (Thrasyvoulou et al. 2018).

The sour taste of honey originated from the several of organic and inorganic acids, where the dominant of organic acid present in honey is gluconic acid. This organic acid is produced by the enzyme activity of glucose-oxidase which is added by the bees when they are convert a nectar into honey, so can protecting a nectar until honey maturity. This protecting mechanism is occurred by the inhibit of microorganisms activity present in honey (Da Silva et al. 2016). This inhibit mechanism includes the combination several factors such as low moisture and presence hydrogen peroxide which is produced by the enzyme glucose-oxidase can inhibit the metabolism activity in the microbe cell through the destruction of cell wall resulting in change in cytoplasmic membrane permeability (Nainu et al. 2021; Pasias et al. 2018).

The acidity total content in honey is small quantity, but the present in honey is very important because can influencing the honey stability on the microorganisms, taste or flavor, and aroma of honey. The high acidity is indicating the fermentation process had been occurred when some reducing sugar is break down into acetic acid. Honey acidity content is related to the yeast number where them is break down some reducing sugar into ethanol and if it's reaction with the oxygen is formed the acetic acid which is increasing the honey acidity. The values higher of acidity may be indicating the 263 fermentation process of sugars into organic acids. The honey acidity is affected by several factors such as different content 264 of organic acids, different geographical origin and the seasonal when honey harvested (Da Silva et al. 2016; Tornuk et al. 2013). The honey acidity from the bee A. cerana in our study (Table 2) was differed to previously studied by Wu et al. 265 (2020) for A. cerana cerana honey is 0.80 mol/kg and Guerzou et al. (2021) is ranging 11 to 47 meq/kg for Algerian 266 honey. Furthermore, is differed to reported by Erwan et al. (2020) that honey acidity from the bee A. mellifera were 267 268 produced by extrafloral nectar (sugar palm and coconut saps) is ranging from 22.00 to 43.00 ml NaOH/kg. The different 269 acidity has been reported previously with our studied is affected by the different plant types as the nectar source to produce 270 honey, honey pH, geographical origin, and organic acids compound, however in our study has not measured the organic acid compound and honey pH. 271

272 Honey production potency from the sugar palm and coconut saps

273 Coconut and sugar palm plants have a good prospect to be developed because almost part of the plants can be utilized 274 which can contributing for communities' income. Generally, the main production from the coconut (Cocos nucifera L.) 275 was harvested is coconut fruit to advanced process into coconut oil and copra. Theses commodities have a high price, but 276 if just to producing coconut oil and copra are high risk for the farmers because they are just preparing in the raw material. 277 Therefore, the utilizing of the sap can be produced by the coconut and sugar palm were also potency feed for the bees was 278 used as the nectar source to produce honey. Sugar palm and coconut saps are the feed potential which is studied by Erwan 279 et al. (2021b) that the coconut and sugar palm saps can increasing the number of honey cell and bee bread cell of the bee 280 A. cerana. Furthermore, is also reported that sugar palm and coconut are improving the productivity of the bee A. cerana such as increase the brood cells number, colony weight, and the honey production (Erwan et al. 2022). In addition, the saps 281 282 from coconut and sugar palm are usually used by the farmers to produce sugar by using a traditional process.

The coconut plants can produce of 12 stalks in a year and in one of stalk can produce sap of 90 liters, thus, in one 283 284 coconut plant can produce of 1,080 liters of sap. Furthermore, if the farmers have one hectare of the land which are planted 285 by 100 coconut plants (distance 10 m \times 10 m), so can be produced for about 108,000 liters of coconut sap. To produce 1 286 kg of honey is required coconut sap for about 7 liters and in a year is required 84 liters to produce 12 kg of honey. Thus, 287 honey potency in a year from 100 hectares of the land can be calculated as follows: 10.800,000 liters of sap divided by 84 liters of sap and multiplied by 12 kg of honey and obtained 1,542,857,14 kg/year (1,542,857 tons/year) or equivalent with 288 128.571 tons/month in 100 hectares of the land. Based on the sap production showing that the coconut plants have a big 289 290 potency to produce honey. This potency was also supported by the harvest area of coconut in West Lombok (Nusa 291 Tenggara Province, Indonesia) was 10,629.36 hectares (Department of Agricultural and Plantations 2021).

Sugar palm plant can be tapped to collect sap for about 5 to 6 months in one stalk, but generally can be tapping not exceed of 4 months. Wahyuni et al. (2021) reported that the production of sugar palm sap per plant is ranging from 8 to 22 liters/plant or 300 to 400 liters/season (3 to 4 months) or 800 to 1,500 liters/plant/year (average is 1,150 liters/plant/year). Furthermore, if in one hectare of plantation we have 100 sugar palm plants with the distance for planted is $10 \text{ m} \times 10 \text{ m}$, so can be obtained of sap for 115,000 liters.

297 Based on the field investigation showed that to produce 1 kg of honey from the sugar palm sap is required for about 10 298 liters and in a year is required for about 120 liters to produce 12 kg of honey. Thus, the honey potency from the sugar palm 299 sap in a year from the 100 hectares of the sugar palm field is 11,500,000 liters which was divided by 120 liters and 300 multiplied by 12 kg, so is obtained 1,150,000 kg of honey per year (1,150 tons of honey) or equivalent with 95.833 301 tons/month in 100 hectares area. This potency indicate that the sugar palm sap has a big potency to produce honey which is 302 supported by the report data from the Department of Agricultural and Plantations (2021) that the sap production, sap productivity, and harvest area for sugar palm plants in West Lombok (West Nusa Tenggara Province, Indonesia) are 57.46 303 tones, 304.80 quintals/hectare, and 188.52, respectively, in the year of 2021. It can be concluded that Honey is produced 304 305 by the bee A. cerana from sugar palm and coconut saps as the feed have the quality which is acceptable by Indonesian 306 national standard and international standard has been regulated by the Codex Alimentarius. Honey potency production 307 from the coconut sap in 100 hectares area can produce honey of 1.542.857 tons/year or equivalent with 128.571 308 tons/month, while in sugar palm can produce honey of 1,150 tons/year or equivalent with 95.833 tons/month.

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[biodiv] Editor Decision

Smujo Editors <smujo.id@gmail.com>27 September 2022 pukul 10.02Kepada: Erwan <apiserwan@gmail.com>, Agussalim <agussalim@mail.ugm.ac.id>27 September 2022 pukul 10.02

Erwan, Agussalim:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Honey quality from the bee Apis cerana, honey potency produced by coconut and sugar palm saps".

Our decision is: Revisions Required

Reviewer A: Recommendation: Revisions Required

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erwan apis <apiserwan@gmail.com> Kepada: Smujo Editors <smujo.id@gmail.com> 29 September 2022 pukul 22.10

Dear Editor in Chief Biodiversitas

Thanks very much for the information and we will revise according to reviewer comments and submit to the system as soon as possible

[Kutipan teks disembunyikan]

Best Regards,

Dr. Ir. Erwan, M.Si. Faculty of Animal Science, University of Mataram, Indonesia ARTIKEL HASIL KOMENTAR DARI REVIEWER A (27 SEPTEMBER 2022)

Honey quality from the bee *Apis cerana*, honey potency produced by coconut and sugar palm saps

8 9 10 Abstract. One of the big problems when keeping of honeybees is the limited of sustainable feed, especially in the rain season. The objectives of this study were to evaluate the honey quality from the bee A. cerana based on the chemical composition, honey potency produced by the coconut and sugar palm saps. This study using thirty colonies of the bee A. cerana were divided into six treatments 11 consists of sugar palm sap without sugar palm pollen; coconut sap without sugar palm pollen; coconut sap of 50% + sugar palm sap of 12 13 50% without sugar palm pollen; sugar palm sap was added by sugar palm pollen; coconut sap was added by sugar palm pollen; coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen. The chemical composition of honey from the A. cerana were 14 moisture (20.76 to 21.80%), reducing sugar (62.78 to 68.37%), sucrose (1.44 to 3.42%), diastase enzyme activity (5.17 to 9.04 DN), 15 hydroxymethylfurfural (2.24 to 5.81 mg/kg), and acidity (26.00 to 36.35 ml NaOH/kg). Honey potency produced by the coconut and 16 17 sugar palm saps in 100 hectares area produces honey was 1,542.857 tons/year and 1,150 tons/year, respectively. It can be concluded that honey quality is produced by sugar palm and coconut saps, and potential as the bee feed.

18 Keywords: Arenga pinnata, beekeeping, Cocos nucifera L., extrafloral nectar, multifloral nectar

19 Running title: Honey quality of Apis cerana produced by sugar palm and coconut saps

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INTRODUCTION

21 Honeybee of A. cerana is one of the bees from the Apis genus which is include the local bee which is spread in some 22 regions in Indonesia are Kalimantan, Sumatera, Java, Bali, Lombok, Sumbawa, Sulawesi, Papua, and Seram (Radloff et al. 23 2011; Hepburn and Radloff 2011). In Indonesia, beekeeping of the bee A. cerana has been practiced by the beekeepers 24 using a traditional hives (for example using a coconut log hive) and semi modern hive (box hive without nest frame) to 25 produce honey. Furthermore, several regions have been practices the beekeeping of the bee A. cerana has been reported by 26 Schouten et al. (2019) are Riau, North Sumatera, Lampung, Banten, Java, Yogyakarta region, Bali, and Lombok. 27 However, the beekeeping of A. cerana is mostly using traditional hives although using a box hives because is not 28 completed by the honey frame like a beekeeping of A. mellifera. The bee A. cerana can produce honey, bee bread, royal 29 jelly, and propolis, however their production is lower compared to the bee A. mellifera.

30 One of the problems faced by the beekeepers in Indonesia is the limited of feed sustainability as the raw material to 31 produce honey, bee bread, and royal jelly. The limitation feed is the very serious problem have been faced by the 32 beekeepers because they have not area which is used to planted several plants which are used the feed source to produce 33 the honeybees products. Honeybees feeds is divided into two types namely nectar and pollen, where nectar is obtained by the foragers from the plant flowers (nectar floral) and nectar extrafloral which is obtained by the foragers from stalk and 34 35 leaf of plants (Agussalim et al. 2018, 2017). Pollen is obtained by the foragers from plant flowers which is collected by 36 using all body part and then deposited in the corbicula (Agussalim et al. 2018, 2017; Erwan et al. 2021a). When collecting 37 nectar and pollen from the plant flowers, the foragers is role as the pollinator agent by transporting pollen from the anther 38 to pistil so that the pollination process occurs. This process is continuously done by the foragers until their honey stomach 39 is full by a nectar and their corbicula has been deposited by the pollen. This pollination which is impacts on the increasing 40 the plants productivity (Pohorecka et al., 2014; Supeno et al., 2021).

One of the strategies to produce the sustainability honey from the bee *A. cerana* by using a sap from the plants such as sugar palm and coconut. Several studies have been conducted by using a sugar palm and coconut saps as the *A. cerana* feed. Erwan et al. (2021b) reported that the feed combination of coconut sap and sugar palm pollen as the *A. cerana* feed can enhancing the production of honey cells and bee bread cells. However, the use each of sap from coconut and sugar palm can increasing the honey and bee bread cells compared to control group without sap as the feed (multifloral nectar). Furthermore, Erwan et al. (2022) was also reported that the use of sugar palm and coconut saps which each added by sugar palm pollen can improving the bee *A. cerana* productivity such as increase the honey production, brood cells number, and **Commented** [11]: Maybe better the conclusion can be replaced by:

It can be concluded that the quality of *A. cerana* honey which are produced by the sugar palm and coconut saps are acceptable by the Indonesia national standard and international standard. The sugar palm and coconut saps have a big potential as the bee feed especially for the bee *A. cerana*.

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48 colony weight. In addition, in other study showed that the use of extrafloral nectar namely sugar palm (Arenga pinnata) 49 and coconut (Cocos nucifera L.) saps as the A. mellifera bee feed which is resulting the honey chemical composition 50 (reducing sugar, sucrose, acidity, moisture, and diastase enzyme activity) which are acceptable by Indonesian national 51 standard and international standard has been regulated by Codex Alimentarius (Erwan et al. 2020). However, the studied 52 about the chemical composition of honey from the bee A. cerana which are produced from the sugar palm sap, coconut sap 53 and their honey potency production from both sap sugar palm and coconut have not been studied. Therefore, the objectives 54 of this study were to evaluate the honey quality based on the chemical composition from the bee A. cerana, honey potency

55 produced by the coconut and sugar palm saps.

56

MATERIALS AND METHODS

57 Study area

This research has been conducted in the North Duman Village (8°32'10"S 116°09'32"E), Lingsar Sub-district, West 58 59 Lombok (West Nusa Tenggara Province, Indonesia). In this research, we used thirty of A. cerana colonies were divided 60 into six treatments and each five colonies per treatment as the replication. The saps were used in our study were obtained 61 from coconut (Cocos nucifera L.) and sugar palm (Arenga pinnata). The treatments in our study were sugar palm sap 62 without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0); coconut sap of 50% + 63 sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm pollen 64 (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by 65 sugar palm pollen (SCP1).

The technique was used to given sugar palm and coconut saps and sugar palm pollen was according to previously 66 method has been reported by Erwan et al. (2022, 2021b) briefly as follows: fresh coconut and sugar palm saps were given 67 to the bee A. cerana by using a plastic plate and split bamboo were completed by 4 to 5 twigs for foragers perch. The 68 plastic plate and split bamboo were placed one meter of the box hives, while the sugar palm pollen was hung besides and 69 70 above of the box hives. The distance of 600 meters to place colony to avoid the foragers to collect pollen and sap from the 71 other treatments.

72 Proceduress

73 Honey quality

74 Honey from the A. cerana was harvested after beekeeping for three months by using a coconut and sugar palm saps. 75 Honey from the five hives in one treatment group was composited into one honey sample and then used to analysis of their 76 chemical composition. Honey quality from the A. cerana were evaluated based on the chemical composition consists of 77 moisture, reducing sugar, sucrose, diastase enzyme activity, hydroxymethylfurfural (HMF), and acidity. The moisture 78 content was analyzed by using a proximate analysis based on the method from Association of Official Agricultural 79 Chemists (AOAC) (AOAC 2005). Reducing sugar was analyzed by using a Layne-Enyon method and sucrose content was 80 analyzed by a Luff Schoorl method were described by AOAC (2005). Diastase enzyme activity, hydroxymethylfurfural 81 (HMF), and free acidity were analyzed based on the harmonised methods of the international honey commission (Machado 82 et al. 2022).

83 Honey production from sugar palm and coconut saps

Sugar palm and coconut saps each ten liters were used to measuring the honey production from the bee A. cerana for 84 85 three months of beekeeping. The sugar palm and coconut saps were placed in the plastic plate in front of the box hives at the distance of one meter. In addition, the honey production without using of sugar palm and coconut saps were measured 86 87 for one year of the beekeeping which is used to calculate the contribution of sugar palm and coconut saps in honey 88 production.

89 Production of saps from coconut and sugar palm

90 The production of sap from coconut was measured for a year, while the sugar palm sap based on the previously studied 91 was used to calculate the production per hectare area and was also obtained from the deep interview with the farmers. The 92 production of coconut and sugar palm saps per hectare which was calculated from the sap production per hectare 93 multiplied by the tress number in one bectare area. After three months of beekeeping, honey from both treatments sugar 94 palm and coconut saps were harvested to measure the honey production from the use of ten litters sap and then honey 95 production was measured by cylinder glass

96 Data analysis

97 The data of honey quality, production potency of honey from sugar palm and coconut saps, honey production, and 98 production of saps were analyzed by using a descriptive analysis (Steel et al. 1997).

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Please add briefly the method used to harvest and obtained the sugar palm and coconut saps

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RESULTS AND DISCUSSION

100 Moisture content of honey

Honey is composed by water as the second largest of honey constituent and its ranging from 15 to 21 g/100 g, 101 102 depending on the plant types as the nectar source which is affected by the botanical origin. Furthermore, honey moisture is also affected by honey maturity level, processing postharvest, and storage condition (Da Silva et al. 2016). The honey 103 moisture is affecting the physical properties such as crystallization, viscosity, flavor, color, taste, solubility, specific 104 gravity, and conservation (Da Silva et al. 2016; Escuredo et al. 2013). In addition, honey moisture is also affected by the 105 106 temperature and humidity or depending on the season (rain and dry seasons) and honey moisture can increase during the 107 postharvest processing such as storage condition because honey is hygroscopic that can absorbs the moisture in the air (Da 108 Silva et al. 2016; Karabagias et al. 2014).

The recent study showed that the honey moisture from the bee A. cerana which was produced by sugar palm and 109 110 coconut saps and their combination was ranging from 20.76 to 21.80% (Table 1). This honey moisture content is accepted by Indonesian national standard (SNI) where the moisture for beekeeping honey including the bee A. cerana and A. 111 mellifera is not exceed 22% (National Standardization Agency of Indonesia 2018) and higher compared to international 112 113 standard which is regulated by Codex Alimentarius is not exceed 20% (Thrasyvoulou et al. 2018). The variation of honey 114 moisture of the bee A. cerana in our study may be caused by the different moisture content of both saps from sugar palm 115 and coconut, however in our study has not measured. The higher moisture content is requiring the long time to ripening of 116 honey and process decreasing of honey moisture have been started by the bees when they are taken a nectar from plant 117 flowers or saps as the raw material to produce honey. Furthermore, small portion of moisture content has been evaporated 118 in the honey sack before transferred to the other bee which is working in the hive. This transfer is rapid depending on the 119 temperature, colony strength, and nectar availability (Da Silva et al. 2016). 120

121 **Table 1.** The moisture, reducing sugar, and sucrose contents of honey from the bee *A. cerana*

Treatments	Moisture (%)	Reducing sugar (%)	Sucrose (%)
SP0	21.60	65.24	2.86
CP0	20.76	68.37	1.96
SCP0	21.40	64.55	2.51
SP1	21.80	62.78	3.42
CP1	21.58	65.37	1.72
SCP1	20.98	67.33	1.44

Abbreviations: sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0); coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen (SCP1).

Honey production process is started from the foragers collecting a nectar from the plant flowers or extrafloral nectar 127 128 and then stored in honey stomach. After that, the foragers will be transferring a nectar has been collected to the other bees 129 whom working to processing a nectar into honey in their mouth, then put in honey stomach and then is transferred to other bees for several times until honey is ripening. A considerable of water amount will be evaporated in this process and this 130 131 continues with the help of wing fans that can regulate the air humidity for about 15 to 20 minutes (Balasubramanyam 132 2021: Zhang et al. 2021). The honey moisture content in our study was differed to reported by Wang et al. (2021) that 133 honey moisture from the bee A. cerana which is collected from 42 different honeycombs from China is ranging from 17.03 to 18.44%, 18.65% for A. cerana cerana from Hainan province, China (Wu et al. 2020), and 16.99% for A. cerana from 134 Borneo (Malaysian honey) (Moniruzzaman et al. 2013). Furthermore, Erwan et al. (2020) was also reported that the honey 135 136 moisture was produced by the A. mellifera bee by using a sugar palm and coconut saps is ranging from 19.34 to 20.94%. The different honey moisture content has been reported are affected by the different geographical origins which is impact 137 138 on the different plant types can be growth each region, different environmental condition (temperature and humidity), and 139 also different bee species which is impact on the different ability to evaporate water in the honey.

140 Reducing sugar and sucrose contents of honey

Sugars in honey are composed by monosaccharides for about 75%, disaccharides are 10 to 15%, and other sugars in small amounts. Honey sugars are responsible as the energy source, hygroscopic, viscosity, and granulation. Several sugars in honey have been reported such as glucose, fructose, sucrose, trehalose, rhamnose, isomaltose, nigerobiose, maltotriose, maltotetraose, melezitose, melibiose, maltulose, nigerose, raffinose, palatinose, erlose and others (Da Silva et al. 2016).

The recent study showed that the honey s reducing sugar from the bee *A. cerana* were beekeeping by using a sugar palm and coconut saps and their combination as the nectar source to produce honey is ranging from 62.78 to 68.37 % (Table 1). This honey reducing sugar is acceptable by the SNI for treatments SP0, CP0, CP1, and SCP1, but not acceptable

for treatments SCP0, SP1 (Table 1), where the minimum reducing sugar is 65% (National Standardization Agency of Indonesia 2018). This sugar is produced by the mechanism of invertase enzyme activity that change the sap sucrose into simple sugars. It is known that this enzyme is responsible for the conversion of sucrose into glucose and fructose. These **Commented [126]:** The results and discussion is very great and comprehensive, but any minor corrections

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151 sugars are included in reducing sugar group and as the main component present in honey. In honey maturity process, the 152 sucrose is break down by the invertase enzyme into simple sugars simultaneously and water will be evaporated so that it 153 will be increasing the reducing sugar content. In addition, enzymes secreted by the worker bees are also can break down 154 the carbohydrate into simple sugars. Furthermore, other enzyme present in honey is diastase enzyme that role to break 155 down starch into simple sugars (Da Silva et al. 2016). The honey reducing sugar in our study (Table 1) was differed to 156 reported by Erwan et al. (2020) that honey reducing sugar from the bee A. mellifera which was produced by extrafloral 157 nectar (sugar palm and coconut saps) is ranging from 60.15 to 73.69%. The different reducing sugar may be affected by 158 the different bee species which is impact on the different their ability to evaporate water present in honey especially when 159 they are convert the complex sugars into simple sugars and different season when done the study which are related to 160 temperature and humidity environmental.

161 The honey sucrose content from the bee A. cerana in our study is ranging from 1.44 to 3.42% (Table 1) and acceptable by SNI is not exceed 5% for the beekeeping honey including A. cerana and A. mellifera (National Standardization Agency 162 of Indonesia 2018) and also accepted by the International standard has been regulated by Codex Alimentarius is not exceed 163 5% for blossom and honeydew honeys (Thrasyvoulou et al. 2018). Naturally, sucrose present in honey in our study is 164 165 originated from sugar palm and coconut saps. The low of honey sucrose content in our study is caused the honey which is harvested in mature condition that characterized by honey cells have been covered by the wax. Furthermore, the invertase 166 167 enzyme which is produced by the worker bees is actively break down of sucrose from saps into simple sugars. There are two types of invertase enzymes which are produced by the worker bees, namely glucoinvertase which is converts sucrose 168 into glucose and fructoinvertase which is converts sucrose into fructose. These enzymes are mostly derived from the bee's 169 secretion and only a small portion from the nectar, while the honeydew from the insect's secretion is mostly contain 170 171 invertase enzyme (Da Silva et al. 2016). The honey sucrose content in our study (Table 1) was differed to reported by Erwan et al. (2020) that honey sucrose content from the bee A. mellifera was produced by extrafloral nectar (sugar palm 172 and coconut saps) is ranging from 4.21 to 4.40%%. 173

The honey sucrose content is a very important parameter to evaluate the maturity of honey to identifying manipulation, 174 175 where the high levels may be indicated adulterations by adding the several sweeteners such cane sugar or refined beet 176 sugar. In addition, also indicating the early of harvest, where sucrose is not completed transformed into fructose and 177 glucose, the bees feeding artificial in prolonged time by using a sucrose syrup (Da Silva et al. 2016; Puscas et al. 2013; 178 Escuredo et al. 2013; Tornuk et al. 2013). Honey is sugar solution that is supersaturated and unstable so it's easy to 179 crystallize. The honey crystallization is affected by concentration of glucose, fructose, and water. Fructose is the dominant 180 sugar present in honey from A. mellifera was produced by several plants as the nectar source which is used by workers to 181 produce honey such as eucalyptus, acacia, bramble, lime, chestnut, sunflower, and from honeydew, except in rape honey 182 was produced by Brassica napus. Rape honey is higher in glucose and lower in fructose which is impact on the rapidly 183 crystallization (Escuredo et al. 2014). The sugars content present in honey is dependent on the geographical origins which 184 is impact on the different plant types can growth in each region and impact on the different sugars content from the nectar 185 which is produced by the nectary gland of plant flowers (Agus et al. 2021; Agussalim et al. 2019; Da Silva et al. 2016; Escuredo et al. 2014; Tornuk et al. 2013). Furthermore, sugars content in honey is influenced by climate (season, 186 temperature, and humidity), processing (heating process), and storage time (Da Silva et al. 2016; Escuredo et al. 2014; 187 Tornuk et al. 2013). 188

189 Diastase enzyme activity and hydroxymethylfurfural of honey

190 The recent study showed that the diastase enzyme activity from the bee A. cerana honey was produced by the sugar palm and coconut saps was ranging from 5.17 to 9.04 DN (Table 2). This enzyme activity is acceptable by SNI with the 191 minimum of 3 DN for the beekeeping honey including the bee A. cerana and A. mellifera (National Standardization 192 193 Agency of Indonesia 2018) and also acceptable by international standard has been regulated by Codex Alimentarius with 194 the minimum 3 DN (Thrasyvoulou et al. 2018). One of the honey characteristics is contain enzymes which is originate 195 from the bees, pollen, and nectar from plant flowers, but the mostly enzymes are added by the bees when they are convert nectar into honey (Da Silva et al. 2016; Thrasyvoulou et al. 2018). The honey diastase enzyme activity in our study (Table 196 197 2) was differed to reported by Erwan et al. (2020) that the diastase enzyme activity of honey from the bee A. mellifera was 198 produced by extrafloral nectar (sugar palm and coconut saps) is ranging from 16.48 to 17.12 Schade unit.

199 Diastases is divided into α - and β -amylases which are the natural enzymes present in honey. The α -amylase is separate the starch chain randomly in the central to produce dextrin, while the β -amylase to separate the maltose in the end chain. Diastase enzyme content in honey is influenced by nectar source (floral and extrafloral nectars) to produce honey and honey geographical origins which are impact on the different chemical composition of the nectar can be produced by the plants which is impact on the honey chemical composition especially diastase enzyme activity. In addition, the bee species is also influencing the activity diastase because it's related to the distance and the flowers plant numbers can be visited by the foragers when they are collecting nectar and pollen were using to produce honey and bee bread (Da Silva et al. 2016).

Generally, diastase enzyme is role to break down the complex sugars into simple sugars. This enzyme is role to digest of starch into maltose (disaccharide) and maltotriose (trisaccharide) which are sensitive to heat or thermolabile. Thus, this condition can be used to evaluate of overheating and preservation degree of honey (Da Silva et al. 2016). Furthermore, the diastase activity is also used to evaluate honey age which is related to storage time and the temperature because the

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diastase activity may be reducing when heating above 60°C and longtime storage (Da Silva et al. 2016; Yücel and Sultanoğlu 2013). The honey diastase activity from the bee *A. cerana* in our study (Table 2) was differed to reported by Wu et al. (2020) for multifloral honey produced by the *A. cerana cerana* from Hainan province (China) was 6.70 Göthe. Furthermore, also was differed to reported by Wang et al. (2021) that the diastase activity of *A. cerana* honey from Qinling Mountains (China) is ranging from 22.05 to 35.67 Göthe. The different diastase activity of honey from *A. cerana* were reported by previously researchers are influenced by the different plant types as the nectar source to produce honey, different sugars content, and different geographical origin.

Furthermore, the HMF of A. cerana honey was produced by the sugar palm and coconut saps in our study was ranging 217 218 from 2.24 to 5.81 mg/kg (Table 2). This HMF indicate that honey from our study in fresh condition and acceptable by SNI 219 for the beekeeping honey including from A. cerana and A. mellifera is not exceed 40 mg/kg (National Standardization 220 Agency of Indonesia 2018) and also acceptable by the international standard has been regulated by Codex Alimentarius is 221 not exceed 40 mg/kg for blossom and honeydew honeys (Thrasyvoulou et al. 2018). The fresh honey after harvested is 222 generally contain the low of HMF is ranging from 0 to 4.12 mg/kg honey. Hydroxymethylfurfural is resulted from the 223 degradation of honey monosaccharide especially fructose and glucose under acid condition and accelerated by the heating. 224 This reaction is producing levulinic and formic acids (Da Silva et al. 2016).

Table 2. The diastase enzyme activity, hydroxymethylfurfural, and acidity of honey from the bee A. cerana

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Treatments	Diastase enzyme activity (DN)	Hydroxymethylfurfural (mg/kg)	Acidity (ml NaOH/kg)
SP0	7.57	5.78	36.33
CP0	5.17	5.04	26.00
SCP0	9.04	4.75	28.60
SP1	6.86	4.77	29.68
CP1	8.51	5.81	28.26
SCP1	6.85	2.24	30.61

Abbreviations: sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0);
 coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm
 pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar
 palm pollen (SCP1).

232 Hydroxymethyfurfural is formed after honey removed from the comb or when the wax covers was opened and the 233 advanced processing like heating process. The increasing of the HMF content is occur sin honey with the high acidity and 234 accelerated by the heating process. However, the HMF content also sinfluenced by several factors such as sugars content, 235 organic acids presence, pH, moisture content, water activity, and the plant types as the nectar source (floral source). In 236 addition, HMF is also can be formed at the low temperatures, acidic condition, and sugars dehydration reactions. 237 Therefore, the higher of HMF content is impact on the honey color is darker (Da Silva et al. 2016; Tornuk et al. 2013). The 238 HMF of honey from the A. cerana in our study (Table 2) was differed to previously reported by Wu et al. (2020) for 239 multifloral honey of A. cerana cerana from China is 3.80 mg/kg and 1.69 mg/kg for A. cerana honey from Qinling 240 Mountains, China is 1.69 mg/kg. The different HMF content of honey from A. cerana were reported by previously researchers are influenced by the different plant types as the nectar source to produce honey, different sugars content, and 241 242 different geographical origin.

243 Acidity of honey

Free acidity is one of an important parameter to evaluate the honey deterioration which is characterized by the organic acids presence in equilibrium with internal esters, lactone and several inorganic ions such as sulfates, chlorides, and phosphates (Da Silva et al. 2016). The recent study showed that the honey acidity from *A. cerana* was produced by the sugar palm and coconut saps was ranging from 26.00 to 36.33 ml NaOH/kg (Table 2). The acidity of *A. cerana* honey in our study is acceptable by SNI is not exceed 50 ml NaOH/kg for the beekeeping honey including *A. cerana* and *A. mellifera*. Furthermore, is also acceptable of the international standard has been regulated by the Codex Alimentarius is not exceed 50 meq/kg for blossom and honeydew honeys (Thrasyvoulou et al. 2018).

The sour taste of honey originated from the several of organic and inorganic acids, where the dominant of organic acid present in honey is gluconic acid. This organic acid is produced by the enzyme activity of glucose-oxidase which is added by the bees when they are convert a nectar into honey, so can protecting a nectar until honey maturity. This protecting mechanism is occurred, by the inhibit of microorganisms activity present in honey (Da Silva et al. 2016). This inhibit mechanism includes the combination several factors such as low moisture and presence hydrogen peroxide which is produced by the enzyme glucose-oxidase can inhibit the metabolism activity in the microbe cell through the destruction of cell wall resulting in change in cytoplasmic membrane permeability (Nainu et al. 2021; Pasias et al. 2018).

The acidity total content in honey is small quantity, but the present in honey is very important because can influencing the honey stability on the microorganisms, taste or flavor, and aroma of honey. The high acidity is indicating the fermentation process had been occurred when some reducing sugar is break down into acetic acid. Honey acidity content is related to the yeast number where them is break down some reducing sugar into ethanol and if it's reaction with the oxygen is formed the acetic acid which is increasing the honey acidity. The values higher of acidity may be indicating the Commented [I61]: Should be it was also

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263 fermentation process of sugars into organic acids. The honey acidity is affected by several factors such as different content 264 of organic acids, different geographical origin and the seasonal when honey harvested (Da Silva et al. 2016; Tornuk et al. 265 2013). The honey acidity from the bee A. cerana in our study (Table 2) was differed to previously studied by Wu et al. 266 (2020) for A. cerana cerana honey is 0.80 mol/kg and Guerzou et al. (2021) is ranging 11 to 47 meq/kg for Algerian 267 honey. Furthermore, is differed to reported by Erwan et al. (2020) that honey acidity from the bee A. mellifera were 268 produced by extrafloral nectar (sugar palm and coconut saps) is ranging from 22.00 to 43.00 ml NaOH/kg. The different 269 acidity has been reported previously with our studied is affected by the different plant types as the nectar source to produce 270 honey, honey pH, geographical origin, and organic acids compound, however in our study has not measured the organic 271 acid compound and honey pH.

272 Honey production potency from the sugar palm and coconut saps

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Coconut and sugar palm plants have a good prospect to be developed because almost part of the plants can be utilized 273 274 which can contributing for communities' income. Generally, the main production from the coconut (Cocos nucifera L.) 275 was harvested is coconut fruit to advanced process into coconut oil and copra. Theses commodities have a high price, but 276 if just to producing coconut oil and copra are high risk for the farmers because they are just preparing in the raw material. 277 Therefore, the utilizing of the sap can be produced by the coconut and sugar palm were also potency feed for the bees was 278 used as the nectar source to produce honey. Sugar palm and coconut saps are the feed potential which is studied by Erwan 279 et al. (2021b) that the coconut and sugar palm saps can increasing the number of honey cell and bee bread cell of the bee 280 A. cerana. Furthermore, is also reported that sugar palm and coconut are improving the productivity of the bee A. cerana 281 such as increase the brood cells number, colony weight, and the honey production (Erwan et al. 2022). In addition, the saps 282 from coconut and sugar palm are usually used by the farmers to produce sugar by using a traditional process.

283 The coconut plants can produce of 12 stalks in a year and in one of stalk can produce sap of 90 liters, thus, in one 284 coconut plant can produce of 1,080 liters of sap. Furthermore, if the farmers have one hectare of the land which are planted 285 by 100 coconut plants (distance $10 \text{ m} \times 10 \text{ m}$), so can be produced for about 108,000 liters of coconut sap. To produce 1 286 kg of honey is required coconut sap for about 7 liters and in a year is required 84 liters to produce 12 kg of honey. Thus, 287 honey potency in a year from 100 hectares of the land can be calculated as follows: 10,800,000 liters of sap divided by 84 288 liters of sap and multiplied by 12 kg of honey and obtained 1,542,857,14 kg/year (1,542.857 tons/year) or equivalent with 289 128.571 tons/month in 100 hectares of the land. Based on the sap production showing that the coconut plants have a big 290 potency to produce honey. This potency was also supported by the harvest area of coconut in West Lombok (Nusa Tenggara Province, Indonesia) was 10,629.36 hectares (Department of Agricultural and Plantations 2021). 291

292 Sugar palm plant can be tapped to collect sap for about 5 to 6 months in one stalk, but generally can be tapping not 293 exceed of 4 months. Wahyuni et al. (2021) reported that the production of sugar palm sap per plant is ranging from 8 to 22 294 liters/plant or 300 to 400 liters/season (3 to 4 months) or 800 to 1,500 liters/plant/year (average is 1,150 liters/plant/year). 295 Furthermore, if in one hectare of plantation we have 100 sugar palm plants with the distance for planted is $10 \text{ m} \times 10 \text{ m}$, so 296 can be obtained of sap for 115,000 liters.

297 Based on the field investigation showed that to produce 1 kg of honey from the sugar palm sap is required for about 10 liters and in a year is required for about 120 liters to produce 12 kg of honey. Thus, the honey potency from the sugar palm 298 sap in a year from the 100 hectares of the sugar palm field is 11,500,000 liters which was divided by 120 liters and 299 300 multiplied by 12 kg, so is obtained 1,150,000 kg of honey per year (1,150 tons of honey) or equivalent with 95.833 301 tons/month in 100 hectares area. This potency indicate that the sugar palm sap has a big potency to produce honey which is 302 supported by the report data from the Department of Agricultural and Plantations (2021) that the sap production, sap productivity, and harvest area for sugar palm plants in West Lombok (West Nusa Tenggara Province, Indonesia) are 57.46 303 tones, 304.80 quintals/hectare, and 188.52, respectively, in the year of 2021. It can be concluded that Honey is produced 304 305 by the bee A. cerana from sugar palm and coconut saps as the feed have the quality which is acceptable by Indonesian 306 national standard and international standard has been regulated by the Codex Alimentarius. Honey potency production from the coconut sap in 100 hectares area can produce honey of 1,542.857 tons/year or equivalent with 128.571 307 308 tons/month, while in sugar palm can produce honey of 1,150 tons/year or equivalent with 95.833 tons/month.

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Honey quality from the bee *Apis cerana*, honey potency produced by coconut and sugar palm saps

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8 Abstract. One of the big problems when keeping of honeybees is the limited of sustainable feed, especially in the rain season. The 9 objectives of this study were to evaluate the honey quality from the bee A. cerana based on the chemical composition, honey potency 10 produced by the coconut and sugar palm saps. This study using thirty colonies of the bee A. cerana were divided into six treatments 11 consists of sugar palm sap without sugar palm pollen; coconut sap without sugar palm pollen; coconut sap of 50% + sugar palm sap of 12 50% without sugar palm pollen; sugar palm sap was added by sugar palm pollen; coconut sap was added by sugar palm pollen; coconut 13 sap of 50% + sugar palm sap of 50% was added by sugar palm pollen. The chemical composition of honey from the A. cerana were 14 moisture (20.76 to 21.80%), reducing sugar (62.78 to 68.37%), sucrose (1.44 to 3.42%), diastase enzyme activity (5.17 to 9.04 DN), 15 hydroxymethylfurfural (2.24 to 5.81 mg/kg), and acidity (26.00 to 36.35 ml NaOH/kg). Honey potency produced by the coconut and sugar palm saps in 100 hectares area produces honey was 1,542.857 tons/year and 1,150 tons/year, respectively. It can be concluded that 16 17 the quality of A. cerana honey which are produced by the sugar palm and coconut saps are acceptable by the Indonesia national standard 18 and international standard. The sugar palm and coconut saps have a big potential as the bee feed especially for the bee A. cerana.

19 Key words: Arenga pinnata, beekeeping, Cocos nucifera L., extrafloral nectar, multifloral nectar

20 Running title: Honey quality of *Apis cerana* produced by sugar palm and coconut saps

INTRODUCTION

22 Honeybee of A. cerana is one of the bees from the Apis genus which is include the local bee which is spread in some 23 regions in Indonesia are Kalimantan, Sumatera, Java, Bali, Lombok, Sumbawa, Sulawesi, Papua, and Seram (Radloff et al. 24 2011; Hepburn and Radloff 2011). In Indonesia, beekeeping of the bee A. cerana has been practiced by the beekeepers 25 using traditional hives (for example using a coconut log hive) and semi modern hive (box hive without nest frame) to produce honey. Furthermore, several regions have been practicing the beekeeping of the bee A. cerana has been reported 26 by Schouten et al. (2019) are Riau, North Sumatera, Lampung, Banten, Java, Yogyakarta region, Bali, and Lombok. 27 28 However, the beekeeping of A. cerana is mostly using traditional hives or use box hives, but is not completed by the honey 29 frame like a beekeeping of A. mellifera. The bee A. cerana can produce honey, bee bread, royal jelly, and propolis, 30 however their production is lower compared to the bee A. mellifera.

One of the problems faced by the beekeepers in Indonesia is the limited feed sustainability as the raw material to 31 32 produce honey, bee bread, and royal jelly. The limitation feed is the very serious problem have been faced by the 33 beekeepers because they have no area which is used to plant several plants which are used as the feed source to produce the honeybees products. Honeybees feeds is divided into two types namely nectar and pollen, where nectar is obtained by 34 35 the foragers from the plant flowers (nectar floral) and nectar extrafloral which is obtained by the foragers from stalk and 36 leaf of plants (Agussalim et al. 2018, 2017). Pollen is obtained by the foragers from plant flowers which is collected by 37 using all body parts and then deposited in the corbicula (Agussalim et al. 2018, 2017; Erwan et al. 2021a). When collecting 38 nectar and pollen from the plant flowers, the foragers role as the pollinator agent by transporting pollen from the anther to 39 pistil so that the pollination process occurs. This process is continuously done by the foragers until their honey stomach is 40 full of nectar and their corbicula has been deposited by the pollen. This pollination impacts on the increasing the plants productivity (Pohorecka et al., 2014; Supeno et al., 2021). 41

One of the strategies to produce the sustainability honey from the bee *A. cerana* by using a sap from the plants such as sugar palm and coconut. Several studies have been conducted by using a sugar palm and coconut saps as the *A. cerana* feed. Erwan et al. (2021b) reported that the feed combination of coconut sap and sugar palm pollen as the *A. cerana* feed can enhance the production of honey cells and bee bread cells. However, the use of sap from coconut and sugar palm can increasing the honey and bee bread cells compared to control group without sap as the feed (multifloral nectar). Furthermore, Erwan et al. (2022) was also reported that the use of sugar palm and coconut saps which are each added by sugar palm pollen can improving the bee A. cerana productivity such as increase the honey production, brood cells number, and colony weight. In addition, in other study showed that the use of extrafloral nectar namely sugar palm (Arenga pinnata) and coconut (Cocos nucifera L.) saps as the A. mellifera bee feed which is resulting the honey chemical composition (reducing sugar, sucrose, acidity, moisture, and diastase enzyme activity) which are acceptable by Indonesian national standard and international standard has been regulated by Codex Alimentarius (Erwan et al. 2020). However, the studies about the chemical composition of honey from the bee A. cerana which are produced from the sugar palm sap, coconut sap and their honey potency production from both sap sugar palm and coconut have not been studied. Therefore, the objectives of this study were to evaluate the honey quality based on the chemical composition from the bee A. cerana, honey potency produced by the coconut and sugar palm saps.

MATERIALS AND METHODS

58 Study area

This research has been conducted in the North Duman Village (8°32'10"S 116°09'32"E), Lingsar Sub-district, West Lombok (West Nusa Tenggara Province, Indonesia). In this research, we used thirty of *A. cerana* colonies were divided into six treatments and each five colonies per treatment as the replication. The saps were used in our study were obtained from the stalk of coconut (*Cocos nucifera* L.) and sugar palm (*Arenga pinnata*) and the pollen source from the sugar palm were shown in Figure 1. The stalks of coconut and sugar palm were cut and then put in the plastic bottle which was used to storage the sap which was secreted by their stalks. The treatments in our study were sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0); coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen (SCP1).



Figure 1. Coconut sap (left), sugar palm sap (center), and sugar palm pollen (right)

The technique was used to given sugar palm and coconut saps and sugar palm pollen (shown in Figure 2) was according to previously method has been reported by Erwan et al. (2022, 2021b) briefly as follows: fresh coconut and sugar palm saps were given to the bee *A. cerana* by using a plastic plate and split bamboo were completed by 4 to 5 twigs for foragers perch. The plastic plate and split bamboo were placed one meter of the box hives, while the sugar palm pollen was hung besides and above of the box hives. The distance of 600 meters to place colony to avoid the foragers to collect pollen and sap from the other treatments.

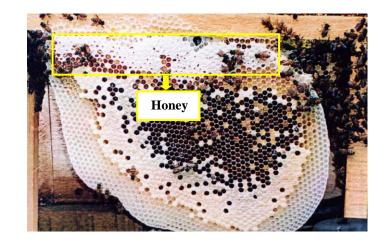


Figure 2. Technique to given the sugar palm and coconut saps (left) and sugar palm pollen (right) (Erwan et al. 2022, 2021b)

107 **Procedures**

108 Honey quality

109 Honey from the A. cerana (shown in Figure 2) was harvested after beekeeping for three months by using a coconut and 110 sugar palm saps. Honey from the five hives in one treatment group was composited into one honey sample and then used to analysis of their chemical composition. Honey quality from the A. cerana were evaluated based on the chemical 111 composition consists of moisture, reducing sugar, sucrose, diastase enzyme activity, hydroxymethylfurfural (HMF), and 112 acidity. The moisture content was analyzed by using a proximate analysis based on the method from Association of 113 Official Agricultural Chemists (AOAC) (AOAC 2005). Reducing sugar was analyzed by using a Layne-Enyon method and 114 115 sucrose content was analyzed by a Luff Schoorl method were described by AOAC (2005). Diastase enzyme activity, hydroxymethylfurfural (HMF), and free acidity were analyzed based on the harmonised methods of the international honey 116 117 commission (Machado et al. 2022). 118



134 **Figure 2.** Honey from *A. cerana* was produced from the sugar palm and coconut saps

135 Honey production from sugar palm and coconut saps

Sugar palm and coconut saps each ten liters were used to measuring the honey production from the bee *A. cerana* for three months of beekeeping. The sugar palm and coconut saps were placed in the plastic plate in front of the box hives at the distance of one meter. In addition, the honey production without using of sugar palm and coconut saps were measured for one year of the beekeeping which is used to calculate the contribution of sugar palm and coconut saps in honey production. Honey from *A. cerana* was harvested with cut the honey cells (Figure 2) and squezed to separate wax and honey. Afterward, honey was measured their production by using a digital scale and stored in the regrigerator.

142 Production of saps from coconut and sugar palm

The production of sap from coconut was measured for a year, while the sugar palm sap based on the previously studied was used to calculate the production per hectare area and was also obtained from the deep interview with the farmers. The production of coconut and sugar palm saps per hectare which was calculated from the sap production per tree multiplied by the trees number in one hectare area. After three months of beekeeping, honey from both treatments sugar palm and coconut saps were harvested to measure the honey production from the use of ten litters sap and then honey production was measured by cylinder glass

149 Data analysis

The data of honey quality, production potency of honey from sugar palm and coconut saps, honey production, and production of saps were analyzed by using a descriptive analysis (Steel et al. 1997).

152

RESULTS AND DISCUSSION

153 Moisture content of honey

Honey is composed by water as the second largest of honey constituents and its ranging from 15 to 21 g/100 g, depending on the plant types as the nectar source which is affected by the botanical origin. Furthermore, honey moisture is also affected by honey maturity level, processing postharvest, and storage condition (Da Silva et al. 2016). The honey moisture is affecting the physical properties such as crystallization, viscosity, flavor, color, taste, solubility, specific gravity, and conservation (Da Silva et al. 2016; Escuredo et al. 2013). In addition, honey moisture is also affected by the temperature and humidity or depending on the season (rain and dry seasons) and honey moisture can increase during the 160 postharvest processing such as storage condition because honey is hygroscopic that can absorbs the moisture in the air (Da 161 Silva et al. 2016; Karabagias et al. 2014).

The recent study showed that the honey moisture from the bee A. cerana which was produced by sugar palm and 162 163 coconut saps and their combination was ranging from 20.76 to 21.80% (Table 1). This honey moisture content is accepted by Indonesian national standard (SNI) where the moisture for beekeeping honey including the bee A. cerana and A. 164 mellifera is not exceed 22% (National Standardization Agency of Indonesia 2018) and higher compared to international 165 166 standard which is regulated by Codex Alimentarius is not exceed 20% (Thrasyvoulou et al. 2018). The variation of honey 167 moisture of the bee A. cerana in our study may be caused by the different moisture content of both saps from sugar palm and coconut, however in our study has not been measured. The higher moisture content is requiring the long time to 168 ripening of honey and process of decreasing honey moisture has been started by the bees when they are taking nectar from 169 170 plant flowers or saps as the raw material to produce honey. Furthermore, a small portion of moisture content has been 171 evaporated in the honey sack before transferred to the other bee which is working in the hive. This transfer is rapid 172 depending on the temperature, colony strength, and nectar availability (Da Silva et al. 2016).

¹⁷³

174	Table 1. The moisture, reducing sugar, and sucrose contents of honey from the bee A. ceran	na
1/7	Table 1. The moisture, reducing sugar, and sucrose contents of noney from the beer A. certa	nu

Treatments	Moisture (%)	Reducing sugar (%)	Sucrose (%)
SP0	21.60	65.24	2.86
CP0	20.76	68.37	1.96
SCP0	21.40	64.55	2.51
SP1	21.80	62.78	3.42
CP1	21.58	65.37	1.72
SCP1	20.98	67.33	1.44

175 Abbreviations: sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0); 176 coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm 177 pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar 178 palm pollen (SCP1). 179

180 Honey production process is started from the foragers collecting a nectar from the plant flowers or extrafloral nectar and then stored in the honey stomach. After that, the foragers will be transferring a nectar that has been collected to the 181 182 other bees who are working to process a nectar into honey in their mouth, then put in honey stomach and then is transferred to other bees for several times until honey is ripening. A considerable amount of water will be evaporated in 183 this process and this continues with the help of wing fans that can regulate the air humidity for about 15 to 20 minutes 184 185 (Balasubramanyam 2021; Zhang et al. 2021). The honey moisture content in our study was differed to reported by Wang et 186 al. (2021) that honey moisture from the bee A. cerana which is collected from 42 different honeycombs from China is 187 ranging from 17.03 to 18.44%, 18.65% for A. cerana cerana from Hainan province, China (Wu et al. 2020), and 16.99% 188 for A. cerana from Borneo (Malaysian honey) (Moniruzzaman et al. 2013). Furthermore, Erwan et al. (2020) was also 189 reported that the honey moisture produced by the A. mellifera bee by using sugar palm and coconut saps is ranging from 19.34 to 20.94%. The different honey moisture content has been reported are affected by the different geographical origins 190 191 which is impact on the different plant types can be growth each region, different environmental condition (temperature and 192 humidity), and also different bee species which is impact on the different ability to evaporate water in the honey.

193 Reducing sugar and sucrose contents of honey

194 Sugars in honey are composed by monosaccharides for about 75%, disaccharides are 10 to 15%, and other sugars in 195 small amounts. Honey sugars are responsible as the energy source, hygroscopic, viscosity, and granulation. Several sugars 196 in honey have been reported such as glucose, fructose, sucrose, trehalose, rhamnose, isomaltose, nigerobiose, maltotriose, 197 maltotetraose, melezitose, melibiose, maltulose, nigerose, raffinose, palatinose, erlose and others (Da Silva et al. 2016).

198 The recent study showed that the honey reducing sugar from the bee A. cerana were beekeeping by using a sugar palm 199 and coconut saps and their combination as the nectar source to produce honey is ranging from 62.78 to 68.37 % (Table 1). 200 This honey reducing sugar is acceptable by the SNI for treatments SP0, CP0, CP1, and SCP1, but not acceptable for 201 treatments SCP0, SP1 (Table 1), where the minimum reducing sugar is 65% (National Standardization Agency of 202 Indonesia 2018). This sugar is produced by the mechanism of invertase enzyme activity that change the sap sucrose into simple sugars. It is known that this enzyme is responsible for the conversion of sucrose into glucose and fructose. These 203 204 sugars are included in reducing sugar group and as the main component present in honey. In honey maturity process, the 205 sucrose is break down by the invertase enzyme into simple sugars simultaneously and water will be evaporated so that it 206 will be increasing the reducing sugar content. In addition, enzymes secreted by the worker bees are also can break down 207 the carbohydrate into simple sugars. Furthermore, other enzyme present in honey is diastase enzyme that role to break down starch into simple sugars (Da Silva et al. 2016). The honey reducing sugar in our study (Table 1) was differed to 208 209 reported by Erwan et al. (2020) that honey reducing sugar from the bee A. mellifera which was produced by extrafloral nectar (sugar palm and coconut saps) is ranging from 60.15 to 73.69%. The different reducing sugar may be affected by 210 the different bee species which is impact on the different their ability to evaporate water present in honey especially when 211 212 they are convert the complex sugars into simple sugars and different season when done the study which are related to

213 temperature and humidity environmental. 214 The honey sucrose content from the bee A. cerana in our study is ranging from 1.44 to 3.42% (Table 1) and acceptable 215 by SNI is not exceed 5% for the beekeeping honey including A. cerana and A. mellifera (National Standardization Agency of Indonesia 2018) and also accepted by the International standard has been regulated by Codex Alimentarius is not exceed 216 5% for blossom and honeydew honeys (Thrasyvoulou et al. 2018). Naturally, sucrose present in honey in our study 217 originated from sugar palm and coconut saps. The low honey sucrose content in our study is caused by the honey which is 218 219 harvested in mature condition that is characterized by honey cells that have been covered by wax. Furthermore, the 220 invertase enzyme which is produced by the worker bees actively break down sucrose from saps into simple sugars. There 221 are two types of invertase enzymes which are produced by the worker bees, namely glucoinvertase which converts sucrose into glucose and fructoinvertase which converts sucrose into fructose. These enzymes are mostly derived from the bee's 222 223 secretion and only a small portion from the nectar, while the honeydew from the insect's secretion mostly contains 224 invertase enzymes (Da Silva et al. 2016). The honey sucrose content in our study (Table 1) was differed to reported by 225 Erwan et al. (2020) that honey sucrose content from the bee A. mellifera was produced by extrafloral nectar (sugar palm 226 and coconut saps) is ranging from 4.21 to 4.40%%.

227 The honey sucrose content is a very important parameter to evaluate the maturity of honey to identify manipulation, 228 where the high levels may be indicate adulterations by adding the several sweeteners such cane sugar or refined beet sugar. 229 In addition, also indicating the early of harvest, where sucrose is not completed transformed into fructose and glucose, the 230 bees feeding artificial in prolonged time by using a sucrose syrup (Da Silva et al. 2016; Puscas et al. 2013; Escuredo et al. 231 2013; Tornuk et al. 2013). Honey is sugar solution that is supersaturated and unstable so it's easy to crystallize. The honey crystallization is affected by concentration of glucose, fructose, and water. Fructose is the dominant sugar present in honey 232 233 from A. mellifera was produced by several plants as the nectar source which is used by workers to produce honey such as 234 eucalyptus, acacia, bramble, lime, chestnut, sunflower, and from honeydew, except in rape honey was produced by 235 Brassica napus. Rape honey is higher in glucose and lower in fructose which is impact on the rapidly crystallization (Escuredo et al. 2014). The sugars content present in honey is dependent on the geographical origins which is impact on 236 the different plant types can growth in each region and impact on the different sugars content from the nectar which is 237 238 produced by the nectary gland of plant flowers (Agus et al. 2021; Agussalim et al. 2019; Da Silva et al. 2016; Escuredo et 239 al. 2014; Tornuk et al. 2013). Furthermore, sugars content in honey is influenced by climate (season, temperature, and 240 humidity), processing (heating process), and storage time (Da Silva et al. 2016; Escuredo et al. 2014; Tornuk et al. 2013).

241 Diastase enzyme activity and hydroxymethylfurfural of honey

242 The recent study showed that the diastase enzyme activity from the bee A. cerana honey was produced by the sugar palm and coconut saps was ranging from 5.17 to 9.04 DN (Table 2). This enzyme activity is acceptable by SNI with the 243 244 minimum of 3 DN for the beekeeping honey including the bee A. cerana and A. mellifera (National Standardization Agency of Indonesia 2018) and also acceptable by international standard has been regulated by Codex Alimentarius with 245 the minimum 3 DN (Thrasyvoulou et al. 2018). One of the honey characteristics is contain enzymes which is originate 246 247 from the bees, pollen, and nectar from plant flowers, but the mostly enzymes are added by the bees when they are convert 248 nectar into honey (Da Silva et al. 2016; Thrasyvoulou et al. 2018). The honey diastase enzyme activity in our study (Table 249 2) was differed to reported by Erwan et al. (2020) that the diastase enzyme activity of honey from the bee A. mellifera was 250 produced by extrafloral nectar (sugar palm and coconut saps) is ranging from 16.48 to 17.12 Schade unit.

Diastases are divided into α - and β -amylases which are the natural enzymes present in honey. The α -amylase separates the starch chain randomly in the central to produce dextrin, while the β -amylase separates the maltose in the end chain. Diastase enzyme content in honey is influenced by nectar source (floral and extrafloral nectars) to produce honey and honey geographical origins which are impact on the different chemical composition of the nectar can be produced by the plants which is impact on the honey chemical composition especially diastase enzyme activity. In addition, the bee species is also influencing the activity diastase because it's related to the distance and the flowers plant numbers can be visited by the foragers when they are collecting nectar and pollen were used to produce honey and bee bread (Da Silva et al. 2016).

258 Generally, diastase enzyme is role to break down complex sugars into simple sugars. This enzyme is role to digesting 259 starch into maltose (disaccharide) and maltotriose (trisaccharide) which are sensitive to heat or thermolabile. Thus, this condition can be used to evaluate the overheating and preservation degree of honey (Da Silva et al. 2016). Furthermore, 260 261 the diastase activity is also used to evaluate honey age which is related to storage time and the temperature because the diastase activity may be reducing when heating above 60°C and longtime storage (Da Silva et al. 2016; Yücel and 262 263 Sultanoğlu 2013). The honey diastase activity from the bee A. cerana in our study (Table 2) was differed to reported by 264 Wu et al. (2020) for multifloral honey produced by the A. cerana cerana from Hainan province (China) was 6.70 Göthe. 265 Furthermore, it was also differed to reported by Wang et al. (2021) that the diastase activity of A. cerana honey from Qinling Mountains (China) is ranging from 22.05 to 35.67 Göthe. The different diastase activity of honey from A. cerana 266 267 were reported by previously researchers are influenced by the different plant types as the nectar source to produce honey, 268 different sugars content, and different geographical origin.

Furthermore, the HMF of *A. cerana* honey was produced by the sugar palm and coconut saps in our study was ranging from 2.24 to 5.81 mg/kg (Table 2). This HMF indicate that honey from our study in fresh condition and acceptable by SNI for the beekeeping honey including from *A. cerana* and *A. mellifera* is not exceed 40 mg/kg (National Standardization Agency of Indonesia 2018) and also acceptable by the international standard has been regulated by Codex Alimentarius is 273 not to exceed 40 mg/kg for blossom and honeydew honeys (Thrasyvoulou et al. 2018). The fresh honey after harvested is generally contains the low of HMF is ranging from 0 to 4.12 mg/kg honey. Hydroxymethylfurfural is the result of the 274 degradation of honey monosaccharide, especially fructose and glucose, under acid conditions and accelerated by heating. 275 This reaction is producing levulinic and formic acids (Da Silva et al. 2016). 276

Table 2. The diastase enzyme activity, hydroxymethylfurfural, and acidity of honey from the bee <i>A. cerana</i>					
Treatments	Diastase enzyme (DN)	activity Hydroxymethylfurfura (mg/kg)	l Acidity (ml NaOH/kg)		
SP0	7.57	5.78	36.33		
CP0	5.17	5.04	26.00		
SCP0	9.04	4.75	28.60		
SP1	6.86	4.77	29.68		
CP1	8.51	5.81	28.26		
SCP1	6.85	2.24	30.61		

279 Abbreviations: sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0); 280 coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm 281 pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar 282 palm pollen (SCP1). 283

284 Hydroxymethyfurfural is formed after honey removed from the comb or when the wax cover was opened and the 285 advanced processing like heating process. The increasing of the HMF content occurs in honey with the high acidity and accelerated by the heating process. However, the HMF content is also influenced by several factors such as sugars content, 286 287 organic acids presence, pH, moisture content, water activity, and the plant types as the nectar source (floral source). In 288 addition, HMF can also be formed at low temperatures, acidic condition, and sugars dehydration reactions. Therefore, the 289 higher of HMF content's impact on the honey color is darker (Da Silva et al. 2016; Tornuk et al. 2013). The HMF of 290 honey from the A. cerana in our study (Table 2) was differed to previously reported by Wu et al. (2020) for multifloral honey of A. cerana cerana from China is 3.80 mg/kg and 1.69 mg/kg for A. cerana honey from Qinling Mountains, China 291 292 is 1.69 mg/kg. The different HMF content of honey from A. cerana were reported by previously researchers are influenced 293 by the different plant types as the nectar source to produce honey, different sugars content, and different geographical 294 origin.

295 Acidity of honey

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296 Free acidity is one of an important parameter to evaluate the honey deterioration which is characterized by the organic 297 acids presence in equilibrium with internal esters, lactone and several inorganic ions such as sulfates, chlorides, and 298 phosphates (Da Silva et al. 2016). The recent study showed that the honey acidity from A. cerana was produced by the 299 sugar palm and coconut saps was ranging from 26.00 to 36.33 ml NaOH/kg (Table 2). The acidity of A. cerana honey in 300 our study is acceptable by SNI is not to exceed 50 ml NaOH/kg for the beekeeping honey including A. cerana and A. 301 *mellifera*. Furthermore, it is also acceptable by the international standard has been regulated by the Codex Alimentarius is 302 not to exceed 50 meq/kg for blossom and honeydew honeys (Thrasyvoulou et al. 2018).

303 The sour taste of honey originated from the several organic and inorganic acids, where the dominant organic acid 304 present in honey is gluconic acid. This organic acid is produced by the enzyme activity of glucose-oxidase which is added 305 by the bees when they convert a nectar into honey, so it can protect a nectar until honey maturity. This protecting mechanism is caused by the inhibition of microorganisms activity in honey (Da Silva et al. 2016). This inhibit mechanism 306 307 includes the combination several factors such as low moisture and presence hydrogen peroxide which is produced by the enzyme glucose-oxidase can inhibit the metabolism activity in the microbe cell through the destruction of cell wall 308 309 resulting in change in cytoplasmic membrane permeability (Nainu et al. 2021; Pasias et al. 2018).

310 The acidity total content in honey is small quantity, but the present in honey is very important because can influencing 311 the honey stability on the microorganisms, taste or flavor, and aroma of honey. The high acidity is indicating the fermentation process had been occurred when some reducing sugar is break down into acetic acid. Honey acidity content is 312 related to the yeast number where them is break down some reducing sugar into ethanol and if it's reaction with the 313 oxygen is formed the acetic acid which is increasing the honey acidity. The values higher of acidity may be indicating the 314 315 fermentation process of sugars into organic acids. The honey acidity is affected by several factors such as different content 316 of organic acids, different geographical origin and the seasonal when honey harvested (Da Silva et al. 2016; Tornuk et al. 2013). The honey acidity from the bee A. cerana in our study (Table 2) was differed to previously studied by Wu et al. 317 (2020) for A. cerana cerana honey is 0.80 mol/kg and Guerzou et al. (2021) is ranging 11 to 47 meq/kg for Algerian 318 honey. Furthermore, is differed to reported by Erwan et al. (2020) that honey acidity from the bee A. mellifera were 319 produced by extrafloral nectar (sugar palm and coconut saps) is ranging from 22.00 to 43.00 ml NaOH/kg. The different 320 321 acidity has been reported previously with our studied is affected by the different plant types as the nectar source to produce 322 honey, honey pH, geographical origin, and organic acids compound, however in our study has not measured the organic 323 acid compound and honey pH.

324 Honey production potency from the sugar palm and coconut saps

325 Coconut and sugar palm plants have a good prospect to be developed because almost part of the plants can be utilized which can contribute to communities' income. Generally, the main production from the coconut (Cocos nucifera L.) was 326 harvested as coconut fruit to advance the process into coconut oil and copra. These commodities have a high price, but 327 producing coconut oil and copra are high risk for the farmers because they are just preparing raw material. Therefore, the 328 329 utilizing of the sap can be produced by the coconut and sugar palm were also potency feed for the bees was used as the 330 nectar source to produce honey. Sugar palm and coconut saps are the feed potential which is studied by Erwan et al. 331 (2021b) that the coconut and sugar palm saps can increase the number of honey cell and bee bread cells of the bee A. cerana. Furthermore, it is also reported that sugar palm and coconut are improving the productivity of the bee A. cerana 332 333 such as increasing the brood cells number, colony weight, and the honey production (Erwan et al. 2022). In addition, the 334 saps from coconut and sugar palm are usually used by the farmers to produce sugar by using a traditional process.

335 The coconut plants can produce 12 stalks in a year and in one stalk can produce sap of 90 liters, thus, in one coconut plant can produce 1,080 liters of sap. Furthermore, if the farmers have one hectare of the land which are planted by 100 336 coconut plants (distance 10 m \times 10 m), so can be produced for about 108,000 liters of coconut sap. To produce 1 kg of 337 honey requires coconut sap for about 7 liters and in a year 84 liters is required to produce 12 kg of honey. Thus, honey 338 potency in a year from 100 hectares of the land can be calculated as follows: 10,800,000 liters of sap divided by 84 liters 339 340 of sap and multiplied by 12 kg of honey and obtained 1,542,857,14 kg/year (1,542.857 tons/year) or equivalent with 341 128.571 tons/month in 100 hectares of the land. Based on the sap production showing that the coconut plants have a big potency to produce honey. This potency was also supported by the harvest area of coconut in West Lombok (Nusa 342 343 Tenggara Province, Indonesia) was 10,629.36 hectares (Department of Agricultural and Plantations 2021).

344 Sugar palm plants can be tapped to collect sap for about 5 to 6 months in one stalk, but generally can be tapped not to 345 exceed 4 months. Wahyuni et al. (2021) reported that the production of sugar palm sap per plant is ranging from 8 to 22 liters/plant or 300 to 400 liters/season (3 to 4 months) or 800 to 1,500 liters/plant/year (average is 1,150 liters/plant/year). 346 Furthermore, if in one hectare of plantation we have 100 sugar palm plants with the distance for planted is $10 \text{ m} \times 10 \text{ m}$, so 347 can be obtained of sap for 115,000 liters. 348

349 The field investigation showed that to produce 1 kg of honey from the sugar palm sap is required for about 10 liters and 350 in a year it is required for about 120 liters to produce 12 kg of honey. Thus, the honey potency from the sugar palm sap in 351 a year from the 100 hectares of the sugar palm field is 11,500,000 liters which was divided by 120 liters and multiplied by 12 kg, so is obtained 1,150,000 kg of honey per year (1,150 tons of honey) or equivalent with 95.833 tons/month in 100 352 353 hectares area. This potency indicate that the sugar palm sap has a big potency to produce honey which is supported by the 354 report data from the Department of Agricultural and Plantations (2021) that the sap production, sap productivity, and harvest area for sugar palm plants in West Lombok (West Nusa Tenggara Province, Indonesia) are 57.46 tones, 304.80 355 quintals/hectare, and 188.52, respectively, in the year of 2021. It can be concluded that honey is produced by the bee A. 356 cerana from sugar palm and coconut saps as the feed have the quality which are acceptable by Indonesian national 357 standard and international standard has been regulated by the Codex Alimentarius. Honey potency production from the 358 coconut sap in 100 hectares area can produce honey of 1,542.857 tons/year or equivalent with 128.571 tons/month, while 359 360 in sugar palm can produce honey of 1,150 tons/year or equivalent with 95.833 tons/month.

361

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EDITOR DECISION: KOMENTAR KEDUA DARI REVIEWER A TERHADAP ARTIKEL DAN ARTIKEL HASIL KOMENTAR KEDUA DARI REVIEWER A (1 NOVEMBER 2022)



[biodiv] Editor Decision

Smujo Editors <smujo.id@gmail.com> Kepada: Erwan <apiserwan@gmail.com>, Agussalim <agussalim@mail.ugm.ac.id>

1 November 2022 pukul 10.35

Erwan, Agussalim:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Honey quality from the bee Apis cerana, honey potency produced by coconut and sugar palm saps".

Our decision is: Revisions Required

Reviewer A:

This study aimed to evaluate the honey quality based on the chemical composition from the bee A. cerana and the honey potency produced by the coconut and sugar palm saps. The paper is clear objectives. The topic is an important subject. However, I have the following comments for revision consideration that I put in the text.

Recommendation: Revisions Required

Biodiversitas Journal of Biological Diversity

A-12166-Article Text-1062944-1-4-20221004.doc 2024K



[biodiv] Editor Decision

erwan apis <apiserwan@gmail.com> Kepada: Smujo Editors <smujo.id@gmail.com> 1 November 2022 pukul 13.26

Dear Editor in Chief Biodiversitas

Thanks very much for the information and we will revise according to reviewer comments [Kutipan teks disembunyikan]

Best Regards,

Dr. Ir. Erwan, M.Si. Faculty of Animal Science, University of Mataram, Indonesia ARTIKEL HASIL KOMENTAR KEDUA DARI REVIEWER A

Honey quality from the bee *Apis cerana*, honey potency produced by coconut and sugar palm saps

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Abstract. One of the big problems when keeping-of honeybees is the limited of sustainable feed, especially in the rainy season. The objectives of this study were to evaluate the honey quality from the bee A. cerana based on the chemical composition, and honey 10 11 potency produced by the coconut and sugar palm saps. This study using thirty colonies of the bee A. cerana wasere divided into six treatments consistings of sugar palm sap without sugar palm pollen; coconut sap without sugar palm pollen; coconut sap of 50% + sugar 12 13 14 palm sap of 50% without sugar palm pollen; sugar palm sap was added by sugar palm pollen; coconut sap was added by sugar palm pollen; coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen. The chemical composition of honey from the *A. cerana* were moisture (20.76 to 21.80%), reducing sugar (62.78 to 68.37%), sucrose (1.44 to 3.42%), diastase enzyme activity (5.17 to 15 9.04 DN), hydroxymethylfurfural (2.24 to 5.81 mg/kg), and acidity (26.00 to 36.35 ml NaOH/kg). Honey potency produced by the 16 17 coconut and sugar palm saps in 100 hectares area produces honey was 1,542.857 tons/year and 1,150 tons/year, respectively. It can be concluded that the quality of A. cerana honey, which are produced by the sugar palm and coconut saps, isare acceptable by the 18 Indonesian national standard-and international standards. The sugar palm and coconut saps have a big potential as the bee feed, 19 especially for the bee A. cerana.

20 Key words: Arenga pinnata, beekeeping, Cocos nucifera L., extrafloral nectar, multifloral nectar

21 **Running title:** Honey quality of *Apis cerana* produced by sugar palm and coconut saps

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INTRODUCTION

23 The hHoneybee of A. cerana is one of the bees from the Apis genus, which is includes the local bee which is spread in 24 some regions in Indonesia, including -are Kalimantan, Sumatera, Java, Bali, Lombok, Sumbawa, Sulawesi, Papua, and 25 Seram (Radloff et al. 2011; Hepburn and Radloff 2011). In Indonesia, beekeeping of the bee A. cerana has been practiced 26 27 by-the beekeepers using traditional hives (for example using a coconut log hive) and semi-modern hives (box hives without nest frames) to produce honey. Furthermore, several regions have been practicing the beekeeping of the bee A. 28 cerana has been reported by Schouten et al. (2019) are Riau, North Sumatera, Lampung, Banten, Java, Yogyakarta region, 29 30 Bali, and Lombok. However, the beekeeping of A. cerana is mostly using traditional hives or use box hives; but is not completed by the honey frame like thea beekeeping of A. mellifera. The bee A. cerana can produce honey, bee bread, royal 31 jelly, and propolis. H, however their production is lower compared to the bee A. mellifera.

32 One of the problems faced by the beekeepers in Indonesia is the limited of feed sustainability as the raw material to 33 produce honey, bee bread, and royal jelly. The limitation feed is athe very serious problem that has we been faced by the 34 beekeepers because they have no area which is-used to plant several plants which are used as the feed source to produce 35 the honeybees' products. Honeybees feeds areis divided into two types, namely nectar and pollen, where nectar is obtained 36 by the foragers from the plant flowers (nectar floral) and nectar extrafloral, which is obtained by the foragers from stalk 37 and leaf of plants (Agussalim et al. 2018, 2017). Pollen is obtained by the foragers from plant flowers which is collected 38 by using all body parts and then deposited in the corbicula (Agussalim et al. 2018, 2017; Erwan et al. 2021a). When 39 collecting nectar and pollen from the plant flowers, the foragers role as the pollinator agent by transporting pollen from the 40 anther to pistil so that the pollination process occurs. This process is continuously done by the foragers until their honey 41 stomach is full of nectar and their corbicula has been deposited by the pollen. This pollination impacts on the increasing 42 the plants productivity (Pohorecka et al., 2014; Supeno et al., 2021).

43 One of the strategies to produce the sustainableility honey from the bee *A. cerana* is by using a sap from the plants such 44 as sugar palm and coconut. Several studies have been conducted by using a-sugar palm and coconut saps as the *A. cerana* 45 feed. Erwan et al. (2021b) reported that the feed combination of coconut sap and sugar palm pollen as the *A. cerana* 46 couldar enhance the production of honey cells and bee bread cells. However, the use of sap from coconut and sugar palm 47 can increaseing the honey and bee bread cells compared to the control group without sap as the feed (multi-floral nectar). Commented [Rev1]: please add the citation.

Furthermore, Erwan et al. (2022) was-also reported that the usinge of sugar palm and coconut saps which are each added withby sugar palm pollen can improve ing the bee A. cerana productivity, such as increasing the honey production, brood cells number, and colony weight. In addition, in-another study showed that the use of extrafloral nectar, namely sugar palm (Arenga pinnata) and coconut (Cocos nucifera L.) saps as the A. mellifera bee feed, which is resulting the honey chemical composition (reducing sugar, sucrose, acidity, moisture, and diastase enzyme activity) which are acceptable by Indonesian national standard and the international standard has been regulated by Codex Alimentarius (Erwan et al. 2020). However, the studies about the chemical composition of honey from the bee A. cerana-which are produced from the sugar palm sap, coconut sap. and their honey potency production from both sap sugar palm and coconut have yet to be not been studied. Therefore, the objectives of this study were to evaluate the honey quality based on the chemical composition of rom the bee A. cerana and, the honey potency produced by the coconut and sugar palm saps.

MATERIALS AND METHODS

9 Study area

This research has been conducted in the North Duman Village ($8^{\circ}32'10''S_{\pm}$ 116'09'32''E), Lingsar Sub-district, West Lombok (West Nusa Tenggara Province, Indonesia). In this research, we used thirty-of *A. cerana* colonies were-divided into six treatments and everyach five colonies per treatment as the replication. The saps were-used in our study were obtained from the stalk of cocont (*Cocos nucifera* L.) and sugar palm (*Arenga pinnata*) and the pollen source from the sugar palm were shown in (Figure 1). The stalks of coconut and sugar palm were cut and then put in athe plastic bottle, which was used to storage the sap which was-secreted by their stalks. The treatments in our study were sugar palm pollen (SP0); coconut sap without added by-sugar palm pollen (CP0); coconut sap applies (SCP0); sugar palm sap was added by-sugar palm pollen (SP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen (SCP1).



Figure 1. Coconut sap (left), sugar palm sap (center), and sugar palm pollen (right)

The technique was used to given sugar palm and coconut saps and sugar palm pollen (shown in Fig.ure 2) was according to the previously method has been reported by Erwan et al. (2022, 2021b) briefly as follows: fresh coconut and sugar palm saps were given to the bee *A. cerana* by using a plastic plate and split bamboo wasere completed by 4 to 5 twigs for foragers perch. The plastic plate and split bamboo wasere placed one meter fromoft the box hives, while the sugar palm pollen was hung besides and above-off the box hives. The distance of 600 meters to place the colory to avoid the foragers to collecting pollen and sap from the other treatments.



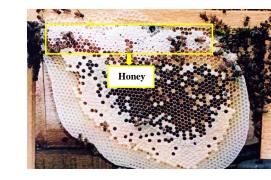
108 Figure 2. Technique to given the sugar palm and coconut saps (left) and sugar palm pollen (right) (Erwan et al. 2022, 2021b)

109 Procedures

107

110 Honey quality

111 Honey from the A. cerana (shown in Fig.ure 2) was harvested after beekeeping for three months by-using a-coconut and sugar palm saps. Honey from the five hives in one treatment group was composited into one honey sample and then 112 113 used to analysis of their chemical composition. Honey quality from the A. cerana wasere evaluated based on the chemical 114 115 composition consisisting to moisture, reducing sugar, sucrose, diastase enzyme activity, hydroxymethylfurfural (HMF), and acidity. The moisture content was analyzed by using a proximate analysis based on the method from the Association of Official Agricultural Chemists (AOAC) (AOAC 2005). Reducing sugar was analyzed by using a Layne-Enyon method, 116 117 and sucrose content was analyzed by a Luff Schoorl method, were described by AOAC (2005). Diastase enzyme activity, 118 hydroxymethylfurfural (HMF), and free acidity were analyzed based on the harmonizsed methods of the international 119 honey commission (Machado et al. 2022). 120



136 Figure 2. Honey from A. cerana was produced from the sugar palm and coconut saps

137 Honey production from sugar palm and coconut saps

Sugar palm and coconut saps everyach ten liters were used to measureing the honey production from the bee *A. cerana* for three months of beekeeping. The sugar palm and coconut saps were placed on the plastic plate in front of the box hives at <u>athe</u> distance of one meter. In addition, the honey production without using of sugar palm and coconut saps were-was measured for one year of the beekeeping, which is used to calculates the contribution of sugar palm and coconut saps in honey production. Honey from *A. cerana* was harvested with cut the honey cells (Figure 2) and squeezed to separate wax and honey. Afterward, honey was measured their-production by using a digital scale and stored in the refgrigerator.

144 Production of saps from coconut and sugar palm

The production of sap from coconut was measured for a year, while the sugar palm sap based on the previously studied was used to calculate the production per hectare area and was also obtained from the deep interview with the farmers. The production of coconut and sugar palm saps per hectare which was calculated from the sap production per tree multiplied by the trees-number of trees in a_one hectare area. After three months of beekeeping, honey from both treatments_ sugar palm and coconut saps were harvested to measure the honey production from the use of ten litters sap_ and then honey production was measured by cylinder glass

151 Data analysis

152 The data onf honey quality, production potency of honey from sugar palm and coconut saps, honey production, and production of saps were analyzed by using a descriptive analysis (Steel et al. 1997).

154

RESULTS AND DISCUSSION

155 Moisture content of honey

Honey is composed <u>of by</u> water as the second largest of honey constituents<u>, and its</u> ranging from 15 to 21 g/100 g, depending on the plant <u>speciestypes</u> as the nectar source, which is affected by the botanical origin. Furthermore, honey moisture is also affected by honey maturity level, processing postharvest, and storage conditions (Da Silva et al. 2016). The honey moisture is affect<u>sing</u> the physical properties such as crystallization, viscosity, flavor, color, taste, solubility, specific gravity, and conservation (Da Silva et al. 2016; Escuredo et al. 2013). In addition, honey moisture is also affected by the temperature and humidity or depending on the season (rainy and dry seasons) and <u>H</u>honey moisture can increase during the postharvest processing, such as storage conditions, because honey is hygroscopic that can absorbs the moisture in the air (Da Silva et al. 2016; Karabagias et al. 2014).

A The recent study showed that the honey moisture from the bee A. cerana, which was produced by sugar palm and 164 165 coconut saps, and their combination was ranging from 20.76 to 21.80% (Table 1). This honey moisture content is accepted 166 by the Indonesian national standard (SNI), where the moisture for beekeeping honey, including the bee A. cerana and A. 167 mellifera, does is not exceed 22% (National Standardization Agency of Indonesia 2018) and is higher compared to the 168 international standard which is regulated by Codex Alimentarius regulated is not exceed 20% (Thrasyvoulou et al. 2018). 169 The variation of honey moisture of the bee A. cerana in our study may be caused by the different moisture content of both 170 saps from sugar palm and coconut, however in-our study has not been measured. The higher moisture content is 171 requiresing the a long time to for ripening of honey, and process of decreasing honey moisture has been started by the bees 172 start the process of -decreasing honey moisture when they are takingtake nectar from plant flowers or saps as the raw 173 material to produce honey. Furthermore, a small portion of moisture content has been evaporated in the honey sack before 174 being transferred to the other bee, which is working in the hive. This transfer is rapid depending on the temperature, 175 colony strength, and nectar availability (Da Silva et al. 2016).

176

Treatments	Moisture (%)	Reducing sugar (%)	Sucrose (%)
SP0	21.60	65.24	2.86
CP0	20.76	68.37	1.96
SCP0	21.40	64.55	2.51
SP1	21.80	62.78	3.42
CP1	21.58	65.37	1.72
SCP1	20.98	67.33	1.44

Abbreviations: sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0); coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen (SCP1).

183 The Hhoney production process is started withfrom the foragers collecting-a nectar from the plant flowers or extrafloral 184 nectar and then stored in the honey stomach. After that, the foragers will be transferring athe nectar that has been collected 185 to the other bees who are working to process athe nectar into honey in their mouth, then put it in the honey stomach and 186 then is-transferred_it to other bees for-several times until honey is ripening. A considerable amount of water will be 187 evaporated in this process, which and this continues with the help of wing fans that can regulate the air humidity for about 188 15 to 20 minutes (Balasubramanyam 2021; Zhang et al. 2021). The honey moisture content in our study was-differed 189 from to reported by Wang et al. (2021) that honey moisture from the bee A. cerana, which is collected from 42 different 190 honeycombs from China rangesis ranging from 17.03 to 18.44%, 18.65% for A. cerana cerana from Hainan province, 191 China (Wu et al. 2020), and 16.99% for A. cerana from Borneo (Malaysian honey) (Moniruzzaman et al. 2013). 192 Furthermore, Erwan et al. (2020) was also reported that the honey moisture produced by the A. mellifera bee by using 193 sugar palm and coconut saps is rangesing from 19.34 to 20.94%. The different honey moisture content has been reported to 194 beare affected by the different geographical origins, which is impacts on the different plant types that can be grownth in 195 each region, different environmental conditions (temperature and humidity), and also different bee species, which is 196 impact-on the different ability to evaporate water in the honey.

197 Reducing sugar and sucrose contents of honey

Sugars in honey are composed of by monosaccharides for about 75%, disaccharides are 10 to 15%, and other sugars in small amounts. Honey sugars are responsible as the energy source, hygroscopic, viscosity, and granulation. Several sugars in honey have been reported such as glucose, fructose, sucrose, trehalose, rhamnose, isomaltose, nigerobiose, maltotriose, maltotetraose, melezitose, melibiose, maltulose, nigerose, raffinose, palatinose, erlose and others (Da Silva et al. 2016).

202 A The recent study showed that the honey_reducing sugar from the bee A. cerana wasere beekeeping by using a sugar 203 palm and coconut saps, and their combination as the nectar source to produce honey is rangesing from 62.78 to 68.37 % 204 (Table 1). This honey-reducing sugar is acceptable by the SNI for treatments SP0, CP0, CP1, and SCP1, but not 205 acceptable for treatments SCP0, and SP1 (Table 1), where the minimum reducing sugar is 65% (National Standardization 206 Agency of Indonesia 2018). This sugar is produced by the mechanism of invertase enzyme activity that changes the sap 207 sucrose into simple sugars. It is known that this enzyme is responsible for the convertingsion of sucrose into glucose and fructose. These sugars are included in the reducing sugar group and as the main component present in honey. In the honey 208 209 maturity process, the sucrose is break down by the invertase enzyme into simple sugars simultaneously, and water will be 210 211 evaporated tso that it will be increaseing the reduceding sugar content. In addition, enzymes secreted by the worker bees are also can also break down the carbohydrate into simple sugars. Furthermore, another enzyme present in honey is the 212 diastase enzyme that role to breaks down starch into simple sugars (Da Silva et al. 2016). The honey-reducing sugar in our

study (Table 1) was differed from what wasto reported by Erwan et al. (2020), that honey reducing sugar from the bee *A. mellifera* which was produced by extrafloral nectar (sugar palm and coconut saps) is rangesing from 60.15 to 73.69%. The different reducing sugar may be affected by the different bee species, which is impacts on the different their ability to evaporate water present in honey, especially when they are convert the complex sugars into simple sugars and different seasons when done the study which are is related to temperature and humidity environmental.

218 The honey sucrose content from the bee A. cerana in our study is-rangesing from 1.44 to 3.42% (Table 1) and 219 acceptable by SNI is not exceed 5% for the beekeeping honey including A. cerana and A. mellifera (National 220 Standardization Agency of Indonesia 2018) and also accepted by the International standard has been regulated by Codex 221 Alimentarius is not exceed 5% for blossom and honeydew honeys (Thrasyvoulou et al. 2018). Naturally, sucrose present in 222 honey in our study originated from sugar palm and coconut saps. The low honey sucrose content in our study is caused by 223 the honey which is harvested in mature condition that is characterized by honey cells that have been covered by wax. 224 Furthermore, the invertase enzyme which is produced by the worker bees actively breaks down sucrose from saps into 225 simple sugars. There are two types of invertase enzymes that which are produced by the worker bees, namely 226 glucoinvertase, which converts sucrose into glucose and fructoinvertase, which converts sucrose into fructose. These 227 enzymes are mostly derived from the bee's secretion and only a small portion from the nectar, while the honeydew from 228 the insect's secretion mostly contains invertase enzymes (Da Silva et al. 2016). The honey sucrose content in our study (Table 1) was differed from to reported by Erwan et al. (2020), that honey sucrose content from the bee A. mellifera was 229 230 produced by extrafloral nectar (sugar palm and coconut saps) is ranging from 4.21 to 4.40%%.

The honey sucrose content is a very important parameter to evaluate the maturity of honey to identify manipulation, 231 232 where the high levels may be-indicate adulterations by adding the several sweeteners such as cane sugar or refined beet 233 sugar. In addition, also indicating the early of harvest, where sucrose is not completelyd transformed into fructose and 234 glucose, the bees feeding artificially for ain prolonged time-by using a sucrose syrup (Da Silva et al. 2016; Puscas et al. 235 2013; Escuredo et al. 2013; Tornuk et al. 2013). Honey is a sugar solution that is supersaturated and unstable so it's easy to crystallize. The honey crystallization is affected by the concentration of glucose, fructose, and water. Fructose is the 236 237 dominant sugar present in honey from A. mellifera was produced by several plants as the nectar source that workers 238 usewhich is used by workers to produce honey such as eucalyptus, acacia, bramble, lime, chestnut, sunflower, and from 239 honeydew, except in rape honey was produced by Brassica napus. Rape honey is higher in glucose and lowers in fructose 240 which is impacts on theits rapidly crystallization (Escuredo et al. 2014). The sugars content present in honey is dependent 241 on the geographical origins which is-impacts on the different plant types that can growth in each region and impact-on the 242 different sugars content from the nectar, which is produced by the nectary gland of plant flowers (Agus et al. 2021; 243 Agussalim et al. 2019; Da Silva et al. 2016; Escuredo et al. 2014; Tornuk et al. 2013). Furthermore, the sugars content in honey is influenced by climate (season, temperature, and humidity), processing (heating process), and storage time (Da 244 245 Silva et al. 2016; Escuredo et al. 2014; Tornuk et al. 2013).

246 Diastase enzyme activity and hydroxymethylfurfural (HMF) of honey

247 TheA recent study showed that the diastase enzyme activity from the bee A. cerana honey was-produced by the sugar 248 palm and coconut saps was rangeding from 5.17 to 9.04 DN (Table 2). This enzyme activity is acceptable by SNI with the 249 a_minimum of 3 DN for the beekeeping honey, including the bee A. cerana and A. mellifera (National Standardization 250 Agency of Indonesia 2018), and also acceptable by the international standard has been regulated by Codex Alimentarius 251 with the minimum 3 DN (Thrasyvoulou et al. 2018). One of the honey characteristics is that it contains enzymes which is 252 originatinge from the bees, pollen, and nectar from plant flowers, but the mostly enzymes are added by the bees when they 253 are convert nectar into honey (Da Silva et al. 2016; Thrasyvoulou et al. 2018). The honey diastase enzyme activity in our 254 study (Table 2) was differed from what waste reported by Erwan et al. (2020), that the diastase enzyme activity of honey 255 from the bee A. mellifera was produced by extrafloral nectar (sugar palm and coconut saps) is ranging from 16.48 to 17.12 256 Schade unit.

257 Diastases are divided into α - and β -amylases, which are the natural enzymes present in honey. The α -amylase separates 258 the starch chain randomly in the centerral to produce dextrin, while the β -amylase separates the maltose in the end chain. 259 The nectar source Diastase enzyme content in honey is influenceds diatase enzyme content in honey by nectar source 260 (floral and extrafloral nectars) to produce honey and honey geographical origins, which are impacts on the different 261 chemical composition of the nectar can be produced by the plants which is-impacts on the honey chemical composition, especially diastase enzyme activity. In addition, the bee species are is also influencing the activity diastase because it's 262 263 related to the distance, and the flowers plant numbers that can be visited by the foragers when they are collecting nectar 264 and pollen were used to produce honey and bee bread (Da Silva et al. 2016).

265 Generally, the diastase enzyme has theis role of to breaking down complex sugars into simple sugars. In addition, tThis 266 enzyme is role to digesting starch into maltose (disaccharide) and maltotriose (trisaccharide), which are sensitive to heat or 267 thermolabile. Thus, this condition can be used to evaluate the overheating and preservation degree of honey (Da Silva et al. 2016). Furthermore, the diastase activity is also used to evaluate honey age_which is_related to storage time and the 268 temperature because the diastase activity may be reducing when heating above 60°C and longtime storage (Da Silva et al. 2016; Yücel and Sultanoğlu 2013). The honey diastase activity from the bee *A. cerana* in our study (Table 2) was differed 271 to reported byfrom Wu et al. (2020) for multifloral honey produced by the *A. cerana cerana* from the Hainan province 272 (China) was 6.70 Göthe, Furthermore, it-was also differed from to reported by Wang et al. (2021) that the diastase activity of A. cerana honey from Qinling Mountains (China) is ranging from 22.05 to 35.67 Göthe. The different diastase activitiesy of honey from A. cerana were reported by previously researchers are influenced by the different plant types as the nectar source to produce honey, different sugars content, and different geographical origin.

273 274 275 276 277 278 279 Furthermore, the HMF of A. cerana honey was produced by the sugar palm and coconut saps in our study was rangeding from 2.24 to 5.81 mg/kg (Table 2). This HMF indicates that honey from our study in fresh condition and acceptable by SNI for the beekeeping honey, including from A. cerana and A. mellifera, is not exceed 40 mg/kg (National Standardization Agency of Indonesia 2018) and also acceptable by the international standard has been regulated by Codex 280 Alimentarius is not to exceed 40 mg/kg for blossom and honeydew honeys (Thrasyvoulou et al. 2018). After harvesting, 281 The fresh honey after harvested is generally contains athe low-of-HMF is ranging from 0 to 4.12 mg/kg honey. Hydroxymethylfurfural is the result of the degradation of honey monosaccharides, especially fructose and glucose, under 282 acid conditions and accelerated by heating. This reaction is producesing levulinic and formic acids (Da Silva et al. 2016). 283 284

Table 2. The diastase enzyme activity, hydroxymethylfurfural, and acidity of honey from the bee A. cerana 285

Tarata	Diastase enzyme	activity	Hydroxymethylfurfural	Acidity (ml NaOH/kg)
Treatments	(DN)	-	(mg/kg)	
SP0	7.57		5.78	36.33
CP0	5.17		5.04	26.00
SCP0	9.04		4.75	28.60
SP1	6.86		4.77	29.68
CP1	8.51		5.81	28.26
SCP1	6.85		2.24	30.61
SNI	<u>>3</u>		<40	<u><50</u>
Codex Alimentarus	>3		<40	<50

286 Abbreviations: sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0); 287 288 coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar 289 palm pollen (SCP1).

289 290 291 292 293 Hydroxymethylfurfural is formed after the honey is removed from the comb or when the wax cover iwas opened and the advanced processing like heating process. The increaseing of the HMF content occurs in honey with the-high acidity and is accelerated by the heating process. However, the HMF content is also influenced by several factors such as sugars 294 content, organic acids presence, pH, moisture content, water activity, and the plant types as the nectar source (floral 295 source). In addition, HMF can also be formed at low temperatures, acidic conditions, and sugars dehydration reactions. 296 Therefore, the higher of-HMF content's impact on the honey color is darker (Da Silva et al. 2016; Tornuk et al. 2013). The 297 HMF of honey from the A. cerana in our study (Table 2) was differed to previously reported by Wu et al. (2020) for 298 multifloral honey of A. cerana cerana from China is 3.80 mg/kg and 1.69 mg/kg for A. cerana honey from Qinling 299 Mountains, China is 1.69 mg/kg. The different HMF content of honey from A. cerana were reported by previously 300 researchers are influenced by the different plant types as the nectar source to produce honey, different sugars content, and 301 different geographical origin.

302 Acidity of honey

303 Free acidity is one of the an-important parameters to evaluate the honey deterioration which is characterized by the 304 presence of the organic acids presence in equilibrium with internal esters, lactone, and several inorganic ions such as 305 sulfates, chlorides, and phosphates (Da Silva et al. 2016). This The recent study showed that the honey acidity from A. 306 cerana was produced by the sugar palm and coconut saps was rangeding from 26.00 to 36.33 ml NaOH/kg (Table 2). The 307 acidity of A. cerana honey in our study is acceptable by SNI is not to exceed 50 ml NaOH/kg for the beekeeping honey, including A. cerana and A. mellifera. Furthermore, it is also acceptable by the international standard has been regulated by 308 309 the Codex Alimentarius is not to exceed 50 meq/kg for blossom and honeydew honeys (Thrasyvoulou et al. 2018).

310 The sour taste of honey originated from the several organic and inorganic acids, where the dominant organic acid present in honey is gluconic acid. This organic acid is produced by the enzyme activity of glucose-oxidase, which is added 311 312 by the bees when they convert a-nectar into honey, so that it can protect thea nectar until honey maturity. This protectioning mechanism is caused by the inhibitiong of microorganisms' activity in honey (Da Silva et al. 2016). This 313 314 inhibit mechanism includes the combination of several factors, such as low moisture and the presence of hydrogen 315 316 peroxide, which is produced by the enzyme glucose-oxidase can inhibit the metabolism activity in the microbe cell through the destruction of the cell wall resulting in a change in cytoplasmic membrane permeability (Nainu et al. 2021; Pasias et al. 317 2018).

The total acidity total content in honey is a small quantity. Still, , but the presencet in honey is very important because 318 319 it can influenceing the honey stability on the microorganisms, taste or flavor, and aroma of honey. The high acidity is 320 indicatesing the fermentation process had been occurrsed when some reducing sugar is brokeneak down into acetic acid. 321 Honey acidity content is related to the yeast number where theym is break down some reducing sugar into ethanol, and if 322 theit's reaction with the oxygen is formed, the acetic acid which is increasing the honey acidity. Therefore, the higher

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323 acidity values higher of acidity may be-indicateing the sugars fermentation process of sugars-into organic acids. The 324 hHoney acidity is affected by several factors, such as different content of organic acids, different geographical origins, and 325 the seasonal when honey is harvested (Da Silva et al. 2016; Tornuk et al. 2013). The honey acidity from the bee A. cerana 326 in our study (Table 2) was-differed to from previously studied by Wu et al. (2020) for A. cerana cerana honey is 0.80 327 mol/kg, and Guerzou et al. (2021) is-ranging from 11 to 47 meq/kg for Algerian honey. Furthermore, it is differed from to 328 reported by Erwan et al. (2020) that honey acidity from the bee A. mellifera wasere produced by extrafloral nectar (sugar 329 palm and coconut saps) is ranging from 22.00 to 43.00 ml NaOH/kg. The different acidity has been reported previously 330 with our studyied is affected by the different plant types as the nectar source to produce honey, honey pH, geographical 331 origin, and organic acids compound ar however in our study has not measured the organic acid compound and honey pH.

332 Honey production potency from the sugar palm and coconut saps

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Coconut and sugar palm plants have a good prospect to be developed because almost part of the plants can be utilized 333 334 which can contributinge to communities' income. Generally, the main production from the coconut (Cocos nucifera L.) 335 was harvested as coconut fruit to advance the process into coconut oil and copra. These commodities have a high price, but 336 producing coconut oil and copra are high risk for the farmers because they are just preparing raw materials. Therefore, the 337 utilizing of the sap can be produced by the coconut and sugar palm were also potency feed for the bees was used as the 338 nectar source to produce honey. Sugar palm and coconut saps are the feed potential which is studied by Erwan et al. 339 (2021b) that the coconut and sugar palm saps can increase the number of honey cells and bee bread cells of the bee A. 340 cerana. Furthermore, it is also reported that sugar palm and coconut are improving the productivity of the bee A. cerana. 341 such as increasing the brood cells number, colony weight, and the honey production (Erwan et al. 2022). In addition, the 342 saps from coconut and sugar palms are usually used by the farmers to produce sugar by using a traditional process.

343 The coconut plants can produce 12 stalks in a year, and in-one stalk can produce sap of 90 liters, <u>T</u> thus, in-one coconut 344 plant can produce 1,080 liters of sap. Furthermore, if the farmers have one hectare of the land which are planted by 100 coconut plants (distance 10 m \times 10 m), so they can be produced for about 108,000 liters of coconut sap. To produce 1 kg 345 346 of honey requires coconut sap for about 7 liters, and in a year 84 liters areis required to produce 12 kg of honey. Thus, 347 honey potency in a year from 100 hectares of the land can be calculated as follows: 10,800,000 liters of sap divided by 84 348 liters of sap and multiplied by 12 kg of honey and obtained 1,542,857,14 kg/year (1,542.857 tons/year) or equivalent with 349 128.571 tons/month in 100 hectares of the land. Based on the sap production showing that the coconut plants have a big 350 potency to produce honey. This potency was also supported by the harvest area of coconut in West Lombok (Nusa Tenggara Province, Indonesia) was 10,629.36 hectares (Department of Agricultural and Plantations 2021). 351

352 Sugar palm plants can be tapped to collect sap for about 5 to 6 months in one stalk, but generally can be tapped not to 353 exceed 4 months. Wahyuni et al. (2021) reported that the production of sugar palm sap per plant is rangesing from 8 to 22 354 liters/plant or 300 to 400 liters/season (3 to 4 months) or 800 to 1,500 liters/plant/year (average is 1,150 liters/plant/year). 355 Furthermore, if in one hectare of the plantation, we have 100 sugar palm plants, with the distance for plantinged is 10 m × 356 10 m, so can be obtained of sap for 115,000 liters.

357 The field investigation showed that to producinge 1 kg of honey from the sugar palm sap is required for about 10 liters. 358 and in a year, it is required for about 120 liters to produce 12 kg of honey. Thus, the honey potency from the sugar palm sap in a year from the 100 hectares of the sugar palm field is 11,500,000 liters which was divided by 120 liters and 359 360 multiplied by 12 kg, so is obtained 1,150,000 kg of honey per year (1,150 tons of honey) or equivalent with 95.833 361 tons/month in 100 hectares area. This potency indicates that the sugar palm sap has a big potency to produce honey which is supported by the report data from the Department of Agricultural and Plantations (2021) that the sap production, sap 362 productivity, and harvest area for sugar palm plants in West Lombok (West Nusa Tenggara Province, Indonesia) are 57.46 363 364 tones, 304.80 quintals/hectare, and 188.52, respectively, in the year of 2021. Therefore, Hi can be concluded that honey is 365 produced by the bee A. cerana from sugar palm and coconut saps as the feed have athe quality which that isare acceptable 366 by Indonesian national standard, and the international standard has been regulated by the Codex Alimentarius. Honey potency production from the coconut sap in 100 hectares area can produce honey of 1,542.857 tons/year or equivalent with 367 368 128.571 tons/month, while in sugar palm can produce honey of 1,150 tons/year or equivalent with 95.833 tons/month.

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Honey quality from the bee *Apis cerana*, honey potency produced by coconut and sugar palm saps

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8 Abstract. One of the big problems when keeping honeybees is the limited of sustainable feed, especially in the rainy season. The 9 objectives of this study were to evaluate the honey quality from the bee A. cerana based on the chemical composition, and honey 10 potency produced by the coconut and sugar palm saps. This study using thirty colonies of the bee A. cerana was divided into six 11 treatments consistings of sugar palm sap without sugar palm pollen; coconut sap without sugar palm pollen; coconut sap of 50% + sugar 12 palm sap of 50% without sugar palm pollen; sugar palm sap was added by sugar palm pollen; coconut sap was added by sugar palm 13 pollen; coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen. The chemical composition of honey from the A. 14 cerana were moisture (20.76 to 21.80%), reducing sugar (62.78 to 68.37%), sucrose (1.44 to 3.42%), diastase enzyme activity (5.17 to 15 9.04 DN), hydroxymethylfurfural (2.24 to 5.81 mg/kg), and acidity (26.00 to 36.35 ml NaOH/kg). Honey potency produced by the coconut and sugar palm saps in 100 hectares area produces honey was 1,542.857 tons/year and 1,150 tons/year, respectively. It can be 16 17 concluded that the quality of A. cerana honey, produced by the sugar palm and coconut saps, is acceptable by the Indonesian national 18 and international standards. The sugar palm and coconut saps have a big potential as the bee feed especially for the bee A. cerana.

19 Key words: Arenga pinnata, beekeeping, Cocos nucifera L., extrafloral nectar, multifloral nectar

20 Running title: Honey quality of *Apis cerana* produced by sugar palm and coconut saps

INTRODUCTION

22 The honeybee of A. cerana is one of the bees from the Apis genus which includes the local bee which is spread in some 23 regions in Indonesia, including Kalimantan, Sumatera, Java, Bali, Lombok, Sumbawa, Sulawesi, Papua, and Seram 24 (Radloff et al. 2011; Hepburn and Radloff 2011). In Indonesia, beekeeping of the bee A. cerana has been practiced by 25 beekeepers using traditional hives (for example using a coconut log hive) and semi-modern hives (box hives without nest frames) to produce honey. Furthermore, several regions have been practicing the beekeeping of the bee A. cerana has been 26 reported by Schouten et al. (2019) are Riau, North Sumatera, Lampung, Banten, Java, Yogyakarta region, Bali, and 27 28 Lombok. However, the beekeeping of A. cerana is mostly using traditional hives or use box hives but is not completed by 29 the honey frame like the beekeeping of A. mellifera. The bee A. cerana can produce honey, bee bread, royal jelly, and propolis. However their production is lower compared to the bee A. mellifera (Agussalim and Agus 2022). 30

31 One of the problems faced by the beekeepers in Indonesia is the limited of feed sustainability as the raw material to 32 produce honey, bee bread, and royal jelly. The limitation feed is a very serious problem that has been faced by beekeepers 33 because they have no area used to plant several plants which are used as the feed source to produce the honeybees' products. Honeybee feeds are divided into two types, namely nectar and pollen, where nectar is obtained by the foragers 34 35 from the plant flowers (nectar floral) and nectar extrafloral, which is obtained by the foragers from stalk and leaf of plants (Agussalim et al. 2018, 2017). Pollen is obtained by the foragers from plant flowers which is collected by using all body 36 37 parts and then deposited in the corbicula (Agussalim et al. 2018, 2017; Erwan et al. 2021a). When collecting nectar and 38 pollen from the plant flowers, the foragers role as the pollinator agent by transporting pollen from the anther to pistil so 39 that the pollination process occurs. This process is continuously done by the foragers until their honey stomach is full of 40 nectar and their corbicula has been deposited by the pollen. This pollination impacts on the increasing the plants productivity (Pohorecka et al. 2014; Supeno et al. 2021). 41

One of the strategies to produce sustainable honey from the bee *A. cerana* is by using sap from the plants such as sugar palm and coconut. Several studies have been conducted by using sugar palm and coconut saps as the *A. cerana* feed. Erwan et al. (2021b) reported that the feed combination of coconut sap and sugar palm pollen as the *A. cerana* feed could enhance the production of honey cells and bee bread cells. However, the use of sap from coconut and sugar palm can increase the honey and bee bread cells compared to the control group without sap as the feed (mult-ifloral nectar). Furthermore, Erwan et al. (2022) also reported using sugar palm and coconut saps which are each added with sugar palm pollen can improve the bee A. cerana productivity, such as increasing honey production, brood cell number, and colony weight. In addition, another study showed that the use of extrafloral nectar namely sugar palm (Arenga pinnata) and coconut (Cocos nucifera L.) saps as the A. mellifera bee feed which is resulting the honey chemical composition (reducing sugar, sucrose, acidity, moisture, and diastase enzyme activity) which are acceptable by Indonesian national standard and the international standard has been regulated by Codex Alimentarius (Erwan et al. 2020). However, studies about the chemical composition of honey from the bee A. cerana produced from the sugar palm sap, coconut sap, and their honey potency production from both sap sugar palm and coconut have vet to be studied. Therefore, the objectives of this study were to evaluate the honey quality based on the chemical composition of the bee A. cerana, honey potency produced by the coconut and sugar palm saps.

MATERIALS AND METHODS

58 Study area

This research has been conducted in the North Duman Village (8°32'10"S 116°09'32"E), Lingsar Sub-district, West Lombok (West Nusa Tenggara Province, Indonesia). In this research, we used thirty *A. cerana* colonies were divided into six treatments and every five colonies per treatment as the replication. The saps used in our study were obtained from the stalk of coconut (*Cocos nucifera* L.) and sugar palm (*Arenga pinnata*) and the pollen source from the sugar palm (Figure 1). The stalks of coconut and sugar palm were cut and then put in a plastic bottle which was used to store the sap secreted by their stalks. The treatments in our study were sugar palm sap without added by sugar palm pollen (SPO); coconut sap without added sugar palm pollen (CPO); coconut sap of 50% + sugar palm sap of 50% without added sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added sugar palm pollen (SCP1).



Figure 1. Coconut sap (left), sugar palm sap (center), and sugar palm pollen (right)

The technique was used to give sugar palm and coconut saps and sugar palm pollen (Figure 2) was according to the previous method has been reported by Erwan et al. (2022, 2021b) briefly as follows: fresh coconut and sugar palm saps were given to the bee *A. cerana* by ussing a plastic plate and split bamboo was completed by 4 to 5 twigs for foragers perch. The plastic plate and split bamboo was placed one meter from the box hives, while the sugar palm pollen was hung besides and above of the box hives. The distance of 600 meters to place the colony to avoid the foragers collecting pollen and sap from the other treatments.

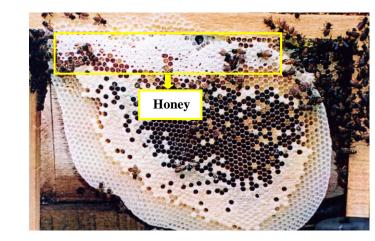


Figure 2. Technique to given the sugar palm and coconut saps (left) and sugar palm pollen (right) (Erwan et al. 2022, 2021b)

107 **Procedures**

108 Honey quality

109 Honey from the A. cerana (Figure 3) was harvested after beekeeping for three months using coconut and sugar palm 110 saps. Honey from the five hives in one treatment group was composited into one honey sample and then used to analysis of their chemical composition. Honey quality from the A. cerana was evaluated based on the chemical composition 111 consisting of moisture, reducing sugar, sucrose, diastase enzyme activity, hydroxymethylfurfural (HMF), and acidity. The 112 moisture content was analyzed by using a proximate analysis based on the method from the Association of Official 113 114 Agricultural Chemists (AOAC) (AOAC 2005). Reducing sugar was analyzed using a Layne-Enyon method and sucrose 115 content was analyzed by a Luff Schoorl method, described by AOAC (2005). Diastase enzyme activity, hydroxymethylfurfural (HMF), and free acidity were analyzed based on the harmonized methods of the international 116 117 honey commission (Machado et al. 2022).



134 **Figure 3.** Honey from *A. cerana* was produced from the sugar palm and coconut saps

135 Honey production from sugar palm and coconut saps

Sugar palm and coconut saps every ten liters were used to measure the honey production from the bee *A. cerana* for three months of beekeeping. The sugar palm and coconut saps were placed on the plastic plate in front of the box hives at a distance of one meter. In addition, the honey production without using sugar palm and coconut saps was measured for one year of the beekeeping, which calculates the contribution of sugar palm and coconut saps in honey production. Honey from *A. cerana* was harvested with cut the honey cells (Figure 3) and squeezed to separate wax and honey. Afterward, honey was measured production by using a digital scale and stored in the refrigerator.

142 Production of saps from coconut and sugar palm

The production of sap from coconut was measured for a year and also based on dept interview with farmers, while the sugar palm sap based on the previously studied was used to calculate the production per hectare area and was also obtained from the deep interview with the farmers. The production of coconut and sugar palm saps per hectare was calculated from the sap production per tree multiplied by the number of trees in a one hectare area. After three months of beekeeping, honey from both treatments sugar palm and coconut saps were harvested to measure the honey production from the use of ten litters sap, and then honey production was measured by cylinder glass

149 Data analysis

The data on honey quality, production potency of honey from sugar palm and coconut saps, honey production, and production of saps were analyzed by using descriptive analysis (Steel et al. 1997).

152

RESULTS AND DISCUSSION

153 Moisture content of honey

Honey is composed of water as the second largest of honey constituents, ranging from 15 to 21 g/100 g, depending on the plant species as the nectar source which is affected by the botanical origin. Furthermore, honey moisture is also affected by honey maturity level, processing postharvest, and storage conditions (Da Silva et al. 2016). The honey moisture affects the physical properties such as crystallization, viscosity, flavor, color, taste, solubility, specific gravity, and conservation (Da Silva et al. 2016; Escuredo et al. 2013). In addition, honey moisture is also affected by the temperature and humidity depending on the season (rainy and dry seasons). Honey moisture can increase during postharvest processing such as storage conditions, because honey is hygroscopic that can absorb the moisture in the air (Da
 Silva et al. 2016; Karabagias et al. 2014).

A recent study showed that the honey moisture from the bee A. cerana, produced by sugar palm and coconut saps and 162 163 their combination was ranging from 20.76 to 21.80% (Table 1). This honey moisture content is accepted by the Indonesian national standard (SNI), where the moisture for beekeeping honey, including the bee A. cerana and A. mellifera, does not 164 exceed 22% (National Standardization Agency of Indonesia 2018) and is higher compared to the international standard 165 166 which Codex Alimentarius regulated is not exceed 20% (Thrasyvoulou et al. 2018). The variation of honey moisture of the 167 bee A. cerana in our study may be caused by the different moisture content of both saps from sugar palm and coconut, however our study has not been measured. The higher moisture content requires a long time for ripening of honey, and the 168 169 bees start the process of decreasing honey moisture when they take nectar from plant flowers or saps as the raw material to 170 produce honey. Furthermore, a small portion of moisture content has been evaporated in the honey sack before being 171 transferred to the other bee which is working in the hive. This transfer is rapid depending on the temperature, colony 172 strength, and nectar availability (Da Silva et al. 2016).

173

174	Table 1. The moisture, reducing sugar, and sucrose contents of honey from the bee <i>A. cerana</i>

Treatments	Moisture (%)	Reducing sugar (%)	Sucrose (%)
SP0	21.60	65.24	2.86
CP0	20.76	68.37	1.96
SCP0	21.40	64.55	2.51
SP1	21.80	62.78	3.42
CP1	21.58	65.37	1.72
SCP1	20.98	67.33	1.44

Abbreviations: sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0);
coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm
pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen (SCP1).

180 The honey production process is started with the foragers collecting nectar from the plant flowers or extrafloral nectar 181 and then stored in the honey stomach. After that, the foragers will transfer the nectar that has been collected to the other 182 bees who are working to process the nectar into honey in their mouth, then put it in the honey stomach and then transfer it to other bees several times until honey is ripening. A considerable amount of water will be evaporated in this process, 183 which continues with the help of wing fans that can regulate the air humidity for about 15 to 20 minutes 184 185 (Balasubramanyam 2021; Zhang et al. 2021). The honey moisture content in our study differed from Wang et al. (2021) 186 that honey moisture from the bee A. cerana which is collected from 42 different honeycombs from China ranges from 187 17.03 to 18.44%, 18.65% for A. cerana cerana from Hainan province, China (Wu et al. 2020), and 16.99% for A. cerana 188 from Borneo (Malaysian honey) (Moniruzzaman et al. 2013). Furthermore, Erwan et al. (2020) also reported that the 189 honey moisture produced by the A. mellifera bee by using sugar palm and coconut saps ranges from 19.34 to 20.94%. The different honey moisture content has been reported to be affected by the different geographical origins, impacts the 190 191 different plant types that can be grown in each region, different environmental conditions (temperature and humidity), and 192 also different bee species, which impact the different ability to evaporate water in the honey.

193 Reducing sugar and sucrose contents of honey

Sugars in honey are composed of monosaccharides for about 75%, disaccharides are 10 to 15%, and other sugars in small amounts. Honey sugars are responsible as the energy source, hygroscopic, viscosity, and granulation. Several sugars in honey have been reported such as glucose, fructose, sucrose, trehalose, rhamnose, isomaltose, nigerobiose, maltotriose, maltotetraose, melezitose, melibiose, maltulose, nigerose, raffinose, palatinose, erlose and others (Da Silva et al. 2016).

198 A recent study showed that the honey reducing sugar from the bee A. cerana was beekeeping by using sugar palm and 199 coconut saps, and their combination as the nectar source to produce honey ranges from 62.78 to 68.37 % (Table 1). This 200 honey reducing sugar is acceptable by the SNI for treatments SP0, CP0, CP1, and SCP1 but not acceptable for treatments 201 SCP0, and SP1 (Table 1), where the minimum reducing sugar is 65% (National Standardization Agency of Indonesia 202 2018). This sugar is produced by the mechanism of invertase enzyme activity that change the sap sucrose into simple 203 sugars. It is known that this enzyme is responsible for converting of sucrose into glucose and fructose. These sugars are 204 included in the reducing sugar group and the main component in honey. In the honey maturity process, the sucrose is break 205 down by the invertase enzyme into simple sugars simultaneously, and water will be evaporated to increase the reduced 206 sugar content. In addition, enzymes secreted by the worker bees can also break down the carbohydrate into simple sugars. 207 Furthermore, another enzyme in honey is the diastase enzyme that breaks down starch into simple sugars (Da Silva et al. 208 2016). The honey reducing sugar in our study (Table 1) differed from what was reported by Erwan et al. (2020), that honey 209 reducing sugar from the bee A. mellifera which was produced by extrafloral nectar (sugar palm and coconut saps) ranges 210 from 60.15 to 73.69%. The different reducing sugar may be affected by the different bee species, which impacts their ability to evaporate water in honey, especially when they convert the complex sugars into simple sugars and different 211 212 seasons when the study is related to temperature and humidity environmental.

213 The honey sucrose content from the bee A. cerana in our study ranges from 1.44 to 3.42% (Table 1) and acceptable by 214 SNI is not exceed 5% for the beekeeping honey including A. cerana and A. mellifera (National Standardization Agency of Indonesia 2018) and also accepted by the International standard has been regulated by Codex Alimentarius is not exceed 215 5% for blossom and honeydew honey (Thrasyvoulou et al. 2018). Naturally, sucrose present in honey in our study 216 originated from sugar palm and coconut saps. The low honey sucrose content in our study is caused by the honey 217 218 harvested in mature condition characterized by honey cells that have been covered by wax. Furthermore, the invertase 219 enzyme which is produced by the worker bees actively breaks down sucrose from saps into simple sugars. There are two 220 types of invertase enzymes that are produced by the worker bees, namely glucoinvertase which converts sucrose into glucose and fructoinvertase, which converts sucrose into fructose. These enzymes are mostly derived from the bee's 221 222 secretion and only a small portion from the nectar, while the honeydew from the insect's secretion mostly contains 223 invertase enzymes (Da Silva et al. 2016). The honey sucrose content in our study (Table 1) differed from Erwan et al. 224 (2020), that honey sucrose content from the bee A. mellifera was produced by extrafloral nectar (sugar palm and coconut 225 saps) is ranging from 4.21 to 4.40%%.

226 The honey sucrose content is a very important parameter to evaluate the maturity of honey to identify manipulation, 227 where the high levels may indicate adulterations by adding several sweeteners such as cane sugar or refined beet sugar. In 228 addition, indicating the early harvest, where sucrose is not completely transformed into fructose and glucose, the bees feed 229 artificially for a prolonged time using a sucrose syrup (Da Silva et al. 2016; Puscas et al. 2013; Escuredo et al. 2013; 230 Tornuk et al. 2013). Honey is a sugar solution that is supersaturated and unstable so it's easy to crystallize. The honey crystallization is affected by the concentration of glucose, fructose, and water. Fructose is the dominant sugar present in 231 232 honey from A. mellifera was produced by several plants as the nectar source that workers use to produce honey such as 233 eucalyptus, acacia, bramble, lime, chestnut, sunflower, and from honeydew, except in rape honey was produced by 234 Brassica napus. Rape honey is higher in glucose and lower in fructose which impacts its rapid crystallization (Escuredo et al. 2014). The sugars content present in honey is dependent on the geographical origins which impacts on the different 235 plant types that can grow in each region and impacts the different sugars content from the nectar, which is produced by the 236 237 nectary gland of plant flowers (Agus et al. 2021; Agussalim et al. 2019; Da Silva et al. 2016; Escuredo et al. 2014; Tornuk 238 et al. 2013). Furthermore, the sugar content in honey is influenced by climate (season, temperature, and humidity), 239 processing (heating process), and storage time (Da Silva et al. 2016; Escuredo et al. 2014; Tornuk et al. 2013).

240 Diastase enzyme activity and hydroxymethylfurfural (HMF) of honey

241 A recent study showed that the diastase enzyme activity from the bee A. cerana honey produced by the sugar palm and 242 coconut saps ranges from 5.17 to 9.04 DN (Table 2). This enzyme activity is acceptable by SNI with a minimum of 3 DN for beekeeping honey including the bee A. cerana and A. mellifera (National Standardization Agency of Indonesia 2018), 243 244 and also acceptable by the international standard has been regulated by Codex Alimentarius with the minimum 3 DN (Thrasyvoulou et al. 2018). One of the honey characteristics is that it contains enzymes originating from the bees, pollen, 245 246 and nectar from plant flowers, but mostly enzymes are added by the bees when they are convert nectar into honey (Da 247 Silva et al. 2016; Thrasyvoulou et al. 2018). The honey diastase enzyme activity in our study (Table 2) differed from what 248 was reported by Erwan et al. (2020) that the diastase enzyme activity of honey from the bee A. mellifera was produced by 249 extrafloral nectar (sugar palm and coconut saps) is ranging from 16.48 to 17.12 Schade unit.

Diastases are divided into α - and β -amylases, the natural enzymes present in honey. The α -amylase separates the starch chain randomly in the center to produce dextrin, while the β -amylase separates the maltose in the end chain. The nectar source influences diastase enzyme content in honey (floral and extrafloral nectars) to produce honey and honey geographical origins, which impacts the different chemical composition of the nectar can be produced by the plants which is impact on the honey chemical composition especially diastase enzyme activity. In addition, the bee species are also influencing the activity diastase because it's related to the distance, and the flowers plant numbers that can be visited by the foragers when they are collecting nectar and pollen used to produce honey and bee bread (Da Silva et al. 2016).

257 Generally, the diastase enzyme has the role of breaking down complex sugars into simple sugars. In addition, this 258 enzyme is role to digesting starch into maltose (disaccharide) and maltotriose (trisaccharide) which are sensitive to heat or 259 thermolabile. Thus, this condition can be used to evaluate the overheating and preservation degree of honey (Da Silva et al. 260 2016). Furthermore, diastase activity is also used to evaluate honey age related to storage time and temperature because the 261 diastase activity may be reducing when heating above 60°C and longtime storage (Da Silva et al. 2016; Yücel and 262 Sultanoğlu 2013). The honey diastase activity from the bee A. cerana in our study (Table 2) was differed from Wu et al. 263 (2020) for multifloral honey produced by the A. cerana cerana from the Hainan province (China) was 6.70 Göthe. 264 Furthermore, it also differed from Wang et al. (2021) that the diastase activity of A. cerana honey from Qinling Mountains (China) is ranging from 22.05 to 35.67 Göthe. The different diastase activities of honey from A. cerana were reported by 265 266 previous researchers are influenced by the different plant types as the nectar source to produce honey, different sugar 267 content, and different geographical origin.

Furthermore, the HMF of *A. cerana* honey produced by the sugar palm and coconut saps in our study ranges from 2.24 to 5.81 mg/kg (Table 2). This HMF indicates that honey from our study in fresh condition and acceptable by SNI for beekeeping honey, including from *A. cerana* and *A. mellifera*, not exceed 40 mg/kg (National Standardization Agency of Indonesia 2018) and also acceptable by the international standard regulated by Codex Alimentarius is not to exceed 40 272 mg/kg for blossom and honeydew honey (Thrasyvoulou et al. 2018). After harvesting, fresh honey generally contains a low HMF ranges from 0 to 4.12 mg/kg honey. Hydroxymethylfurfural is the result of the degradation of honey 273 monosaccharides, especially fructose and glucose, under acid conditions and accelerated by heating. This reaction produce 274 levulinic and formic acids (Da Silva et al. 2016). 275

Table 2. The diastase enzyme activity, hydroxymethylfurfural, and acidity of honey from the bee A. cerana Hydroxymethylfurfural Acidity (ml NaOH/kg) Diastase enzyme activity Treatments (DN) (mg/kg) SP0 7.57 5.78 36.33 CP0 5.17 5.04 26.00 SCP0 9.04 4.75 28.60 SP1 6.86 4.77 29.68 CP1 8.51 5.81 28.26 SCP1 6.85 2.2430.61

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278 Abbreviations: sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0); 279 coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm 280 pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar 281 palm pollen (SCP1). 282

Hydroxymethyfurfural is formed after the honey is removed from the comb or when the wax cover is opened and the 283 284 advanced processing like heating process. The increase of the HMF content occurs in honey with the acidity and is accelerated by the heating process. However, the HMF content is also influenced by sugars content, organic acids 285 286 presence, pH, moisture content, water activity, and the plant types as the nectar source (floral source). In addition, HMF 287 can also be formed at low temperatures, acidic conditions, and sugar dehydration reactions. Therefore, the higher HMF content's impact on the honey color is darker (Da Silva et al. 2016; Tornuk et al. 2013). The HMF of honey from the A. 288 cerana in our study (Table 2) was differed to previously reported by Wu et al. (2020) for multifloral honey of A. cerana 289 290 cerana from China is 3.80 mg/kg and 1.69 mg/kg for A. cerana honey from Qinling Mountains, China is 1.69 mg/kg. The 291 different HMF content of honey from A. cerana reported by previous researchers are influenced by the different plant 292 types as the nectar source to produce honey, different sugars content, and different geographical origin.

293 Acidity of honey

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294 Free acidity is one of the important parameters to evaluate honey deterioration which is characterized by the presence 295 of the organic acids in equilibrium with internal esters, lactone and several inorganic ions such as sulfates, chlorides, and 296 phosphates (Da Silva et al. 2016). This study showed that the honey acidity from A. cerana produced by the sugar palm 297 and coconut saps ranges from 26.00 to 36.33 ml NaOH/kg (Table 2). The acidity of A. cerana honey in our study is 298 acceptable by SNI not to exceed 50 ml NaOH/kg for the beekeeping honey including A. cerana and A. mellifera. 299 Furthermore, it is also acceptable by the international standard has been regulated by the Codex Alimentarius is not to 300 exceed 50 meg/kg for blossom and honeydew honeys (Thrasyvoulou et al. 2018).

301 The sour taste of honey originated from several organic and inorganic acids, where the dominant organic acid present in honey is gluconic acid. This organic acid is produced by the enzyme activity of glucose-oxidase which is added by the 302 303 bees when they convert nectar into honey, so that it can protect the nectar until honey maturity. This protection mechanism 304 is caused by inhibiting of microorganisms activity in honey (Da Silva et al. 2016). This inhibit mechanism includes the 305 combination of several factors, such as low moisture and the presence of hydrogen peroxide which is produced by the enzyme glucose-oxidase can inhibit the metabolism activity in the microbe cell through the destruction of the cell wall 306 307 resulting in a change in cytoplasmic membrane permeability (Nainu et al. 2021; Pasias et al. 2018).

308 The total acidity content in honey is a small quantity. Still, the presence in honey is very important because it can 309 influence the honey stability on the microorganisms, taste or flavor, and aroma of honey. The high acidity indicates the 310 fermentation process occurrs when some reducing sugar is break down into acetic acid. Honey acidity content is related to the yeast number where they break down some reducing sugar into ethanol, and if the reaction with the oxygen is formed, 311 the acetic acid which is increasing the honey acidity. Therefore, the higher acidity values may indicate the sugars 312 313 fermentation process into organic acids. Honey acidity is affected by several factors such as different content of organic 314 acids, different geographical origins, and the season when honey is harvested (Da Silva et al. 2016; Tornuk et al. 2013). 315 The honey acidity from the bee A. cerana in our study (Table 2) differed from previous studied by Wu et al. (2020) for A. cerana cerana honey is 0.80 mol/kg and Guerzou et al. (2021) ranges from 11 to 47 meq/kg for Algerian honey. 316 Furthermore, it is differed from Erwan et al. (2020) that honey acidity from the bee A. mellifera was produced by 317 extrafloral nectar (sugar palm and coconut saps) ranges from 22.00 to 43.00 ml NaOH/kg. The different acidity reported 318 319 previously with our study is affected by the different plant types as the nectar source to produce honey, honey pH, 320 geographical origin, and organic acids compound; however our study has not measured the organic acid compound and 321 honey pH.

322 Honey production potency from the sugar palm and coconut saps

323 Coconut and sugar palm plants have a good prospect to be developed because almost part of the plants can be utilized contributing to communities' income. Generally, the main product from the coconut (Cocos nucifera L.) was harvested as 324 coconut fruit to advance the process into coconut oil and copra. These commodities have a high price, but producing 325 326 coconut oil and copra are high risk for the farmers because they are just preparing raw materials. Therefore, the utilizing of 327 the sap can be produced by the coconut and sugar palm were also potency feed for the bees was used as the nectar source 328 to produce honey. Sugar palm and coconut saps are the feed potential studied by Erwan et al. (2021b) that the coconut and 329 sugar palm saps can increase the number of honey and bee bread cells of the bee A. cerana. Furthermore, it is also reported 330 that sugar palm and coconut are improving the productivity of the bee A. cerana such as increasing the brood cells number, 331 colony weight, and honey production (Erwan et al. 2022). In addition, the saps from coconut and sugar palm are usually 332 used by farmers to produce sugar using a traditional process.

333 The coconut plants can produce 12 stalks in a year, and one stalk can produce sap of 90 liters. Thus, one coconut plant can produce 1,080 liters of sap. Furthermore, if the farmers have one hectare of land planted by 100 coconut plants 334 (distance 10 m \times 10 m), so they can produce about 108,000 liters of coconut sap. To produce 1 kg of honey requires 335 coconut sap for about 7 liters and in a year 84 liters are required to produce 12 kg of honey. Thus, honey potency in a year 336 from 100 hectares of land can be calculated as follows: 10,800,000 liters of sap divided by 84 liters of sap and multiplied 337 338 by 12 kg of honey and obtained 1,542,857,14 kg/year (1,542.857 tons/year) or equivalent with 128.571 tons/month in 100 339 hectares of the land. Based on the sap production showing that the coconut plants have a big potency to produce honey. 340 This potency was also supported by the harvest area of coconut in West Lombok (Nusa Tenggara Province, Indonesia) was 341 10,629.36 hectares (Department of Agricultural and Plantations 2021).

342 Sugar palm plants can be tapped to collect sap for about 5 to 6 months in one stalk, but generally can be tapped not to 343 exceed 4 months. Wahyuni et al. (2021) reported that the production of sugar palm sap per plant ranges from 8 to 22 liters/plant or 300 to 400 liters/season (3 to 4 months) or 800 to 1,500 liters/plant/year (average is 1,150 liters/plant/year). 344 345 Furthermore, if in one hectare of the plantation we have 100 sugar palm plants, the distance for planting is $10 \text{ m} \times 10 \text{ m}$, so 346 can be obtained of sap for 115,000 liters.

347 The field investigation showed that producing 1 kg of honey from the sugar palm sap required about 10 liters and in a 348 year, it is required about 120 liters to produce 12 kg of honey. Thus, the honey potency from the sugar palm sap in a year 349 from the 100 hectares of the sugar palm field is 11,500,000 liters which was divided by 120 liters and multiplied by 12 kg, 350 so is obtained 1,150,000 kg of honey per year (1,150 tons of honey) or equivalent with 95.833 tons/month in 100 hectares 351 area. This potency indicate that the sugar palm sap has a big potency to produce honey which is supported by the report 352 data from the Department of Agricultural and Plantations (2021) that the sap production, sap productivity, and harvest area for sugar palm plants in West Lombok (West Nusa Tenggara Province, Indonesia) are 57.46 tones, 304.80 353 354 quintals/hectare, and 188.52, respectively, in the year of 2021. Therefore, it can be concluded that honey is produced by 355 the bee A. cerana from sugar palm and coconut saps as the feed have at quality that is acceptable by Indonesian national standard, and the international standard has been regulated by the Codex Alimentarius. Honey potency production from the 356 coconut sap in 100 hectares area can produce honey of 1,542.857 tons/year or equivalent with 128.571 tons/month, while 357 358 sugar palm can produce honey of 1,150 tons/year or equivalent with 95.833 tons/month.

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Honey quality from the bee *Apis cerana*, honey potency produced by coconut and sugar palm saps

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Abstract. *Erwan, Agus Salim. 2022. Honey quality from the bee* Apis cerana, *honey potency produced by coconut and sugar palm saps. Biodiversitas 23: xxxx.* One of the big problems when keeping honeybees is the limited of sustainable feed, especially in the rainy season. The objectives of this study were to evaluate the honey quality from the bee *A. cerana* based on the chemical composition, and honey potency produced by the coconut and sugar palm saps. This study using thirty colonies of the bee *A. cerana* was divided into six treatments consisting of sugar palm sap without sugar palm pollen; coconut sap without sugar palm pollen; coconut sap of 50% + sugar palm sap of 50% without sugar palm pollen; sugar palm sap was added by sugar palm pollen. The chemical composition of honey from the *A. cerana* were moisture (20.76 to 21.80%), reducing sugar (62.78 to 68.37%), sucrose (1.44 to 3.42%), diastase enzyme activity (5.17 to 9.04 DN), hydroxymethylfurfural (2.24 to 5.81 mg/kg), and acidity (26.00 to 36.35 ml NaOH/kg). Honey potency produced by the coconut and sugar palm and coconut saps, is acceptable by the Indonesian national and international standards. The sugar palm and coconut saps have a big potential as the bee feed especially for the bee *A. cerana*.

Keywords: Arenga pinnata, beekeeping, Cocos nucifera L., extrafloral nectar, multifloral nectar

INTRODUCTION

The honeybee of A. cerana is one of the bees from the Apis genus which includes the local bee which is spread in some regions in Indonesia, including Kalimantan, Sumatera, Java, Bali, Lombok, Sumbawa, Sulawesi, Papua, and Seram (Radloff et al. 2011; Hepburn and Radloff 2011). In Indonesia, beekeeping of the bee A. cerana has been practiced by beekeepers using traditional hives (for example using a coconut log hive) and semi-modern hives (box hives without nest frames) to produce honey. Furthermore, several regions have been practicing the beekeeping of the bee A. cerana has been reported by Schouten et al. (2019) are Riau, North Sumatera, Lampung, Banten, Java, Yogyakarta region, Bali, and Lombok. However, the beekeeping of A. cerana is mostly using traditional hives or use box hives but is not completed by the honey frame like the beekeeping of A. mellifera. The bee A. cerana can produce honey, bee bread, royal jelly, and propolis. However, their production is lower compared to the bee A. mellifera (Agussalim and Agus 2022).

One of the problems faced by the beekeepers in Indonesia is the limited of feed sustainability as the raw material to produce honey, bee bread, and royal jelly. The limitation feed is a very serious problem that has been faced by beekeepers because they have no area used to plant several plants which are used as the feed source to produce the honeybees' products. Honeybee feeds are divided into two types, namely nectar and pollen, where nectar is obtained by the foragers from the plant flowers (nectar floral) and nectar extrafloral, which is obtained by the foragers from stalk and leaf of plants (Agussalim et al. 2018, 2017). Pollen is obtained by the foragers from plant flowers which is collected by using all body parts and then deposited in the corbicula (Agussalim et al. 2018, 2017; Erwan et al. 2021a). When collecting nectar and pollen from the plant flowers, the foragers role as the pollinator agent by transporting pollen from the anther to pistil so that the pollination process occurs. This process is continuously done by the foragers until their honey stomach is full of nectar and their corbicula has been deposited by the pollen. This pollination impacts on the increasing the plants productivity (Pohorecka et al. 2014; Supeno et al. 2021).

One of the strategies to produce sustainable honey from the bee *A. cerana* is by using sap from the plants such as sugar palm and coconut. Several studies have been conducted by using sugar palm and coconut saps as the *A. cerana* feed. Erwan et al. (2021b) reported that the feed combination of coconut sap and sugar palm pollen as the *A. cerana* feed could enhance the production of honey cells and bee bread cells. However, the use of sap from coconut and sugar palm can increase the honey and bee bread cells compared to the control group without sap as the feed (mult-ifloral nectar). Furthermore, Erwan et al. (2022) also reported using sugar palm and coconut saps which are each added with sugar palm pollen can improve the bee *A. cerana* productivity, such as increasing honey production, brood cell number, and colony weight. In addition, another study showed that the use of extrafloral nectar namely sugar palm (*Arenga pinnata*) and coconut (*Cocos nucifera* L.) saps as the *A. mellifera* bee feed which is resulting the honey chemical composition (reducing sugar, sucrose, acidity, moisture, and diastase enzyme activity) which are acceptable by Indonesian national standard and the international standard has been regulated by Codex Alimentarius (Erwan et al. 2020). However, studies about the chemical composition of honey from the bee *A. cerana* produced from the sugar palm sap, coconut sap, and their honey potency production from both sap sugar palm and coconut have yet to be studied. Therefore, the objectives of this study were to evaluate the honey quality based on the chemical composition of the bee *A. cerana*, honey potency produced by the coconut and sugar palm saps.

MATERIALS AND METHODS

Study area

This research has been conducted in the North Duman Village (8°32'10" S 116°09'32" E), Lingsar Sub-district, West Lombok (West Nusa Tenggara Province, Indonesia). In this research, we used thirty *A. cerana* colonies were divided into six treatments and every five colonies per treatment as the replication. The saps used in our study

were obtained from the stalk of coconut (*Cocos nucifera* L.) and sugar palm (*Arenga pinnata*) and the pollen source from the sugar palm (Figure 1). The stalks of coconut and sugar palm were cut and then put in a plastic bottle which was used to store the sap secreted by their stalks. The treatments in our study were sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added sugar palm pollen (CP0); coconut sap of 50% + sugar palm sap of 50% without added sugar palm pollen (SP1); coconut sap of 50% + sugar palm sap of 50% + sugar palm sap of 50% + sugar palm pollen (SP1); coconut sap of 50% + sugar palm sap of 50% + sugar palm sap of 50% was added sugar palm pollen (SP1); coconut sap of 50% + sugar palm sap of 50% was added sugar palm pollen (SCP1).

The technique was used to give sugar palm and coconut saps and sugar palm pollen (Figure 2) was according to the previous method has been reported by Erwan et al. (2022, 2021b) briefly as follows: fresh coconut and sugar palm saps were given to the bee *A. cerana* by using a plastic plate and split bamboo was completed by 4 to 5 twigs for foragers perch. The plastic plate and split bamboo was placed one meter from the box hives, while the sugar palm pollen was hung besides and above of the box hives. The distance of 600 meters to place the colony to avoid the foragers collecting pollen and sap from the other treatments.



Figure 1. Coconut sap (left), sugar palm sap (center), and sugar palm pollen (right)



Figure 2. Technique to given the sugar palm and coconut saps (left) and sugar palm pollen (right) (Erwan et al. 2022, 2021b)

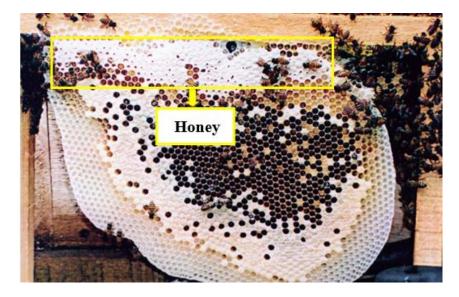


Figure 3. Honey from A. cerana was produced from the sugar palm and coconut saps

Procedures

Honey quality

Honey from the A. cerana (Figure 3) was harvested after beekeeping for three months using coconut and sugar palm saps. Honey from the five hives in one treatment group was composited into one honey sample and then used to analysis of their chemical composition. Honey quality from the A. cerana was evaluated based on the chemical composition consisting of moisture, reducing sugar, sucrose, diastase enzyme activity, hydroxymethylfurfural (HMF), and acidity. The moisture content was analyzed by using a proximate analysis based on the method from the Association of Official Agricultural Chemists (AOAC) (AOAC 2005). Reducing sugar was analyzed using a Layne-Enyon method and sucrose content was analyzed by a Luff Schoorl method, described by AOAC (2005). Diastase enzyme activity, HMF, and free acidity were analyzed based on the harmonized methods of the international honey commission (Machado et al. 2022).

Honey production from sugar palm and coconut saps

Sugar palm and coconut saps every ten liters were used to measure the honey production from the bee *A. cerana* for three months of beekeeping. The sugar palm and coconut saps were placed on the plastic plate in front of the box hives at a distance of one meter. In addition, the honey production without using sugar palm and coconut saps was measured for one year of the beekeeping, which calculates the contribution of sugar palm and coconut saps in honey production. Honey from *A. cerana* was harvested with cut the honey cells (Figure 3) and squeezed to separate wax and honey. Afterward, honey was measured production by using a digital scale and stored in the refrigerator.

Production of saps from coconut and sugar palm

The production of sap from coconut was measured for a year and also based on dept interview with farmers, while the sugar palm sap based on the previously studied was used to calculate the production per hectare area and was also obtained from the deep interview with the farmers. The production of coconut and sugar palm saps per hectare was calculated from the sap production per tree multiplied by the number of trees in a one hectare area. After three months of beekeeping, honey from both treatments sugar palm and coconut saps were harvested to measure the honey production from the use of ten litters sap, and then honey production was measured by cylinder glass

Data analysis

The data on honey quality, production potency of honey from sugar palm and coconut saps, honey production, and production of saps were analyzed by using descriptive analysis (Steel et al. 1997).

RESULTS AND DISCUSSION

Moisture content of honey

Honey is composed of water as the second largest of honey constituents, ranging from 15 to 21 g/100 g, depending on the plant species as the nectar source which is affected by the botanical origin. Furthermore, honey moisture is also affected by honey maturity level, processing postharvest, and storage conditions (Da Silva et al. 2016). The honey moisture affects the physical properties such as crystallization, viscosity, flavor, color, taste, solubility, specific gravity, and conservation (Da Silva et al. 2016; Escuredo et al. 2013). In addition, honey moisture is also affected by the temperature and humidity depending on the season (rainy and dry seasons). Honey moisture can increase during postharvest processing such as storage conditions, because honey is hygroscopic that can absorb the moisture in the air (Da Silva et al. 2016; Karabagias et al. 2014).

Treatments	Moisture (%)	Reducing sugar (%)	Sucrose (%)
SP0	21.60	65.24	2.86
CP0	20.76	68.37	1.96
SCP0	21.40	64.55	2.51
SP1	21.80	62.78	3.42
CP1	21.58	65.37	1.72
SCP1	20.98	67.33	1.44

Table 1. The moisture, reducing sugar, and sucrose contents of honey from the bee A. cerana

Abbreviations: sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0); coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen (SP1); coconut sap of 50% was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen (SP1).

A recent study showed that the honey moisture from the bee A. cerana, produced by sugar palm and coconut saps and their combination was ranging from 20.76 to 21.80% (Table 1). This honey moisture content is accepted by the Indonesian national standard (SNI), where the moisture for beekeeping honey, including the bee A. cerana and A. mellifera, does not exceed 22% (National Standardization Agency of Indonesia 2018) and is higher compared to the international standard which Codex Alimentarius regulated is not exceed 20% (Thrasyvoulou et al. 2018). The variation of honey moisture of the bee A. cerana in our study may be caused by the different moisture content of both saps from sugar palm and coconut, however our study has not been measured. The higher moisture content requires a long time for ripening of honey, and the bees start the process of decreasing honey moisture when they take nectar from plant flowers or saps as the raw material to produce honey. Furthermore, a small portion of moisture content has been evaporated in the honey sack before being transferred to the other bee which is working in the hive. This transfer is rapid depending on the temperature, colony strength, and nectar availability (Da Silva et al. 2016).

The honey production process is started with the foragers collecting nectar from the plant flowers or extrafloral nectar and then stored in the honey stomach. After that, the foragers will transfer the nectar that has been collected to the other bees who are working to process the nectar into honey in their mouth, then put it in the honey stomach and then transfer it to other bees several times until honey is ripening. A considerable amount of water will be evaporated in this process, which continues with the help of wing fans that can regulate the air humidity for about 15 to 20 minutes (Balasubramanyam 2021; Zhang et al. 2021). The honey moisture content in our study differed from Wang et al. (2021) that honey moisture from the bee A. cerana which is collected from 42 different honeycombs from China ranges from 17.03 to 18.44%, 18.65% for A. cerana cerana from Hainan province, China (Wu et al. 2020), and 16.99% for A. cerana from Borneo (Malaysian honey) (Moniruzzaman et al. 2013). Furthermore, Erwan et al. (2020) also reported that the honey moisture produced by the A. mellifera bee by using sugar palm and coconut saps ranges from 19.34 to 20.94%. The different honey moisture content has been reported to be affected by the different geographical origins, impacts the different plant types that can be grown in each region, different environmental conditions (temperature and humidity), and also different bee species, which impact the different ability to evaporate water in the honey.

Reducing sugar and sucrose contents of honey

Sugars in honey are composed of monosaccharides for about 75%, disaccharides are 10 to 15%, and other sugars in small amounts. Honey sugars are responsible as the energy source, hygroscopic, viscosity, and granulation. Several sugars in honey have been reported such as glucose, fructose, sucrose, trehalose, rhamnose, isomaltose, nigerobiose, maltotriose, maltotetraose, melezitose, melibiose, maltulose, nigerose, raffinose, palatinose, erlose and others (Da Silva et al. 2016).

A recent study showed that the honey reducing sugar from the bee A. cerana was beekeeping by using sugar palm and coconut saps, and their combination as the nectar source to produce honey ranges from 62.78 to 68.37 % (Table 1). This honey reducing sugar is acceptable by the SNI for treatments SP0, CP0, CP1, and SCP1 but not acceptable for treatments SCP0, and SP1 (Table 1), where the minimum reducing sugar is 65% (National Standardization Agency of Indonesia 2018). This sugar is produced by the mechanism of invertase enzyme activity that change the sap sucrose into simple sugars. It is known that this enzyme is responsible for converting of sucrose into glucose and fructose. These sugars are included in the reducing sugar group and the main component in honey. In the honey maturity process, the sucrose is break down by the invertase enzyme into simple sugars simultaneously, and water will be evaporated to increase the reduced sugar content. In addition, enzymes secreted by the worker bees can also break down the carbohydrate into simple sugars. Furthermore, another enzyme in honey is the diastase enzyme that breaks down starch into simple sugars (Da Silva et al. 2016). The honey reducing sugar in our study (Table 1) differed from what was reported by Erwan et al. (2020), that honey reducing sugar from the bee A. mellifera which was produced by extrafloral nectar (sugar palm and coconut saps) ranges from 60.15 to 73.69%. The different reducing sugar may be affected by the different bee species, which impacts their ability to evaporate water in honey, especially when they convert the complex sugars into simple sugars and different seasons when the study is related to temperature and humidity environmental.

The honey sucrose content from the bee *A. cerana* in our study ranges from 1.44 to 3.42% (Table 1) and acceptable by SNI is not exceed 5% for the beekeeping honey including *A. cerana* and *A. mellifera* (National

Standardization Agency of Indonesia 2018) and also accepted by the International standard has been regulated by Codex Alimentarius is not exceed 5% for blossom and honeydew honey (Thrasyvoulou et al. 2018). Naturally, sucrose present in honey in our study originated from sugar palm and coconut saps. The low honey sucrose content in our study is caused by the honey harvested in mature condition characterized by honey cells that have been covered by wax. Furthermore, the invertase enzyme which is produced by the worker bees actively breaks down sucrose from saps into simple sugars. There are two types of invertase enzymes that are produced by the worker bees, namely glucoinvertase which converts sucrose into glucose and fructoinvertase, which converts sucrose into fructose. These enzymes are mostly derived from the bee's secretion and only a small portion from the nectar, while the honeydew from the insect's secretion mostly contains invertase enzymes (Da Silva et al. 2016). The honey sucrose content in our study (Table 1) differed from Erwan et al. (2020), that honey sucrose content from the bee A. mellifera was produced by extrafloral nectar (sugar palm and coconut saps) is ranging from 4.21 to 4.40%%.

The honey sucrose content is a very important parameter to evaluate the maturity of honey to identify manipulation, where the high levels may indicate adulterations by adding several sweeteners such as cane sugar or refined beet sugar. In addition, indicating the early harvest, where sucrose is not completely transformed into fructose and glucose, the bees feed artificially for a prolonged time using a sucrose syrup (Da Silva et al. 2016; Puscas et al. 2013; Escuredo et al. 2013; Tornuk et al. 2013). Honey is a sugar solution that is supersaturated and unstable so it's easy to crystallize. The honey crystallization is affected by the concentration of glucose, fructose, and water. Fructose is the dominant sugar present in honey from A. mellifera was produced by several plants as the nectar source that workers use to produce honey such as eucalyptus, acacia, bramble, lime, chestnut, sunflower, and from honeydew, except in rape honey was produced by Brassica napus. Rape honey is higher in glucose and lower in fructose which impacts its rapid crystallization (Escuredo et al. 2014). The sugars content present in honey is dependent on the geographical origins which impacts on the different plant types that can grow in each region and impacts the different sugars content from the nectar, which is produced by the nectary gland of plant flowers (Agus et al. 2021; Agussalim et al. 2019; Da Silva et al. 2016; Escuredo et al. 2014; Tornuk et al. 2013). Furthermore, the sugar content in honey is influenced by climate (season, temperature, and humidity), processing (heating process), and storage time (Da Silva et al. 2016; Escuredo et al. 2014; Tornuk et al. 2013).

Diastase enzyme activity and hydroxymethylfurfural (HMF) of honey

A recent study showed that the diastase enzyme activity from the bee *A. cerana* honey produced by the sugar palm and coconut saps ranges from 5.17 to 9.04 DN (Table 2). This enzyme activity is acceptable by SNI with a minimum of 3 DN for beekeeping honey including the bee *A. cerana* and *A. mellifera* (National Standardization Agency of Indonesia 2018), and also acceptable by the international standard has been regulated by Codex Alimentarius with the minimum 3 DN (Thrasyvoulou et al. 2018). One of the honey characteristics is that it contains enzymes originating from the bees, pollen, and nectar from plant flowers, but mostly enzymes are added by the bees when they are convert nectar into honey (Da Silva et al. 2016; Thrasyvoulou et al. 2018). The honey diastase enzyme activity in our study (Table 2) differed from what was reported by Erwan et al. (2020) that the diastase enzyme activity of honey from the bee *A. mellifera* was produced by extrafloral nectar (sugar palm and coconut saps) is ranging from 16.48 to 17.12 Schade unit.

Diastases are divided into α - and β -amylases, the natural enzymes present in honey. The α -amylase separates the starch chain randomly in the center to produce dextrin, while the β -amylase separates the maltose in the end chain. The nectar source influences diastase enzyme content in honey (floral and extrafloral nectars) to produce honey and honey geographical origins, which impacts the different chemical composition of the nectar can be produced by the plants which is impact on the honey chemical composition especially diastase enzyme activity. In addition, the bee species are also influencing the activity diastase because it's related to the distance, and the flowers plant numbers that can be visited by the foragers when they are collecting nectar and pollen used to produce honey and bee bread (Da Silva et al. 2016).

Generally, the diastase enzyme has the role of breaking down complex sugars into simple sugars. In addition, this enzyme is role to digesting starch into maltose (disaccharide) and maltotriose (trisaccharide) which are sensitive to heat or thermolabile. Thus, this condition can be used to evaluate the overheating and preservation degree of honey (Da Silva et al. 2016). Furthermore, diastase activity is also used to evaluate honey age related to storage time and temperature because the diastase activity may be reducing when heating above 60°C and longtime storage (Da Silva et al. 2016; Yücel and Sultanoğlu 2013). The honey diastase activity from the bee A. cerana in our study (Table 2) was differed from Wu et al. (2020) for multifloral honey produced by the A. cerana cerana from the Hainan province (China) was 6.70 Göthe. Furthermore, it also differed from Wang et al. (2021) that the diastase activity of A. cerana honey from Qinling Mountains (China) is ranging from 22.05 to 35.67 Göthe. The different diastase activities of honey from A. cerana were reported by previous researchers are influenced by the different plant types as the nectar source to produce honey, different sugar content, and different geographical origin.

Furthermore, the HMF of *A. cerana* honey produced by the sugar palm and coconut saps in our study ranges from 2.24 to 5.81 mg/kg (Table 2). This HMF indicates that honey from our study in fresh condition and acceptable by SNI for beekeeping honey, including from *A. cerana* and *A. mellifera*, not exceed 40 mg/kg (National Standardization Agency of Indonesia 2018) and also acceptable by the international standard regulated by Codex Alimentarius is not to exceed 40 mg/kg for blossom and honeydew honey (Thrasyvoulou et al. 2018). After harvesting, fresh honey generally contains a low HMF ranges from 0 to 4.12 mg/kg honey. Hydroxymethylfurfural is the result of the degradation of honey monosaccharides, especially fructose and glucose, under acid conditions and accelerated by heating. This reaction produce levulinic and formic acids (Da Silva et al. 2016).

Hydroxymethyfurfural is formed after the honey is removed from the comb or when the wax cover is opened and the advanced processing like heating process. The increase of the HMF content occurs in honey with the acidity and is accelerated by the heating process. However, the HMF content is also influenced by sugars content, organic acids presence, pH, moisture content, water activity, and the plant types as the nectar source (floral source). In addition, HMF can also be formed at low temperatures, acidic conditions, and sugar dehydration reactions. Therefore, the higher HMF content's impact on the honey color is darker (Da Silva et al. 2016; Tornuk et al. 2013). The HMF of honey from the A. cerana in our study (Table 2) was differed to previously reported by Wu et al. (2020) for multifloral honey of A. cerana cerana from China is 3.80 mg/kg and 1.69 mg/kg for A. cerana honey from Qinling Mountains, China is 1.69 mg/kg. The different HMF content of honey from A. cerana reported by previous researchers are influenced by the different plant types as the nectar source to produce honey, different sugars content, and different geographical origin.

Acidity of honey

Free acidity is one of the important parameters to evaluate honey deterioration which is characterized by the presence of the organic acids in equilibrium with internal esters, lactone and several inorganic ions such as sulfates, chlorides, and phosphates (Da Silva et al. 2016). This study showed that the honey acidity from *A. cerana* produced by the sugar palm and coconut saps ranges from 26.00 to 36.33 ml NaOH/kg (Table 2). The acidity of *A. cerana* honey in our study is acceptable by SNI not to exceed 50 ml NaOH/kg for the beekeeping honey including *A. cerana* and *A. mellifera*. Furthermore, it is also acceptable by the international standard has been regulated by the Codex

Alimentarius is not to exceed 50 meq/kg for blossom and honeydew honeys (Thrasyvoulou et al. 2018).

The sour taste of honey originated from several organic and inorganic acids, where the dominant organic acid present in honey is gluconic acid. This organic acid is produced by the enzyme activity of glucose-oxidase which is added by the bees when they convert nectar into honey, so that it can protect the nectar until honey maturity. This protection mechanism is caused by inhibiting of microorganisms activity in honey (Da Silva et al. 2016). This inhibit mechanism includes the combination of several factors, such as low moisture and the presence of hydrogen peroxide which is produced by the enzyme glucose-oxidase can inhibit the metabolism activity in the microbe cell through the destruction of the cell wall resulting in a change in cytoplasmic membrane permeability (Nainu et al. 2021; Pasias et al. 2018).

The total acidity content in honey is a small quantity. Still, the presence in honey is very important because it can influence the honey stability on the microorganisms, taste or flavor, and aroma of honey. The high acidity indicates the fermentation process occurs when some reducing sugar is break down into acetic acid. Honey acidity content is related to the yeast number where they break down some reducing sugar into ethanol, and if the reaction with the oxygen is formed, the acetic acid which is increasing the honey acidity. Therefore, the higher acidity values may indicate the sugars fermentation process into organic acids. Honey acidity is affected by several factors such as different content of organic acids, different geographical origins, and the season when honey is harvested (Da Silva et al. 2016; Tornuk et al. 2013). The honey acidity from the bee A. cerana in our study (Table 2) differed from previous studied by Wu et al. (2020) for A. cerana cerana honey is 0.80 mol/kg and Guerzou et al. (2021) ranges from 11 to 47 meq/kg for Algerian honey. Furthermore, it is differed from Erwan et al. (2020) that honey acidity from the bee A. mellifera was produced by extrafloral nectar (sugar palm and coconut saps) ranges from 22.00 to 43.00 ml NaOH/kg. The different acidity reported previously with our study is affected by the different plant types as the nectar source to produce honey, honey pH, geographical origin, and organic acids compound; however, our study has not measured the organic acid compound and honey pH.

Table 2. The diastase enzyme activity, hydroxymethylfurfural, and acidity of honey from the bee A. cerana

Treatments	Diastase enzyme activity (DN)	Hydroxymethylfurfural (mg/kg)	Acidity (ml NaOH/kg)
SP0	7.57	5.78	36.33
CP0	5.17	5.04	26.00
SCP0	9.04	4.75	28.60
SP1	6.86	4.77	29.68
CP1	8.51	5.81	28.26
SCP1	6.85	2.24	30.61

Abbreviations: sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0); coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen (SP1); coconut sap of 50% was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen (SP1).

Honey production potency from the sugar palm and coconut saps

Coconut and sugar palm plants have a good prospect to be developed because almost part of the plants can be utilized contributing to communities' income. Generally, the main product from the coconut (Cocos nucifera L.) was harvested as coconut fruit to advance the process into coconut oil and copra. These commodities have a high price, but producing coconut oil and copra are high risk for the farmers because they are just preparing raw materials. Therefore, the utilizing of the sap can be produced by the coconut and sugar palm was also potency feed for the bees was used as the nectar source to produce honey. Sugar palm and coconut saps are the feed potential studied by Erwan et al. (2021b) that the coconut and sugar palm saps can increase the number of honey and bee bread cells of the bee A. cerana. Furthermore, it is also reported that sugar palm and coconut are improving the productivity of the bee A. cerana such as increasing the brood cells number, colony weight, and honey production (Erwan et al. 2022). In addition, the saps from coconut and sugar palm are usually used by farmers to produce sugar using a traditional process.

The coconut plants can produce 12 stalks in a year, and one stalk can produce sap of 90 liters. Thus, one coconut plant can produce 1,080 liters of sap. Furthermore, if the farmers have one hectare of land planted by 100 coconut plants (distance $10 \text{ m} \times 10 \text{ m}$), so they can produce about 108,000 liters of coconut sap. To produce 1 kg of honey requires coconut sap for about 7 liters and in a year 84 liters are required to produce 12 kg of honey. Thus, honey potency in a year from 100 hectares of land can be calculated as follows: 10,800,000 liters of sap divided by 84 liters of sap and multiplied by 12 kg of honey and obtained 1,542,857,14 kg/year (1,542.857 tons/year) or equivalent with 128.571 tons/month in 100 hectares of the land. Based on the sap production showing that the coconut plants have a big potency to produce honey. This potency was also supported by the harvest area of coconut in West Lombok (Nusa Tenggara Province, Indonesia) was 10,629.36 hectares (Department of Agricultural and Plantations 2021).

Sugar palm plants can be tapped to collect sap for about 5 to 6 months in one stalk, but generally can be tapped not to exceed 4 months. Wahyuni et al. (2021) reported that the production of sugar palm sap per plant ranges from 8 to 22 liters/plant or 300 to 400 liters/season (3 to 4 months) or 800 to 1,500 liters/plant/year (average is 1,150 liters/plant/year). Furthermore, if in one hectare of the plantation we have 100 sugar palm plants, the distance for planting is 10 m \times 10 m, so can be obtained of sap for 115,000 liters.

The field investigation showed that producing 1 kg of honey from the sugar palm sap required about 10 liters and in a year, it is required about 120 liters to produce 12 kg of honey. Thus, the honey potency from the sugar palm sap in a year from the 100 hectares of the sugar palm field is 11,500,000 liters which was divided by 120 liters and multiplied by 12 kg, so is obtained 1,150,000 kg of honey per year (1,150 tons of honey) or equivalent with 95.833 tons/month in 100 hectares area. This potency indicate that the sugar palm sap has a big potency to produce honey which is supported by the report data from the Department of Agricultural and Plantations (2021) that the sap production, sap productivity, and harvest area for sugar palm plants in West Lombok (West Nusa Tenggara 57.46 Province. Indonesia) are tones. 304.80 quintals/hectare, and 188.52, respectively, in the year of 2021. Therefore, it can be concluded that honey is produced by the bee A. cerana from sugar palm and coconut saps as the feed have at quality that is acceptable by Indonesian national standard, and the international standard has been regulated by the Codex Alimentarius. Honey potency production from the coconut sap in 100 hectares area can produce honey of 1,542.857 tons/year or equivalent with 128.571 tons/month, while sugar palm can produce honey of 1,150 tons/year or equivalent with 95.833 tons/month.

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Honey quality from the bee *Apis cerana*, honey potency produced by coconut and sugar palm saps

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Abstract. *Erwan, Agussalim.* 2022. Honey quality from the bee Apis cerana, honey potency produced by coconut and sugar palm saps. Biodiversitas 23: xxxx. One of the big problems when keeping honeybees is the limited of sustainable feed, especially in the rainy season. The objectives of this study were to evaluate the honey quality from the bee *A. cerana* based on the chemical composition, and honey potency produced by the coconut and sugar palm saps. This study using thirty colonies of the bee *A. cerana* was divided into six treatments consisting of sugar palm sap without sugar palm pollen; coconut sap without sugar palm pollen; coconut sap of 50% + sugar palm sap of 50% without sugar palm pollen; sugar palm sap was added by sugar palm pollen. The chemical composition of honey from the *A. cerana* were moisture (20.76 to 21.80%), reducing sugar (62.78 to 68.37%), sucrose (1.44 to 3.42%), diastase enzyme activity (5.17 to 9.04 DN), hydroxymethylfurfural (2.24 to 5.81 mg/kg), and acidity (26.00 to **36.33** ml NaOH/kg). Honey potency produced by the coconut and sugar palm and coconut saps, is acceptable by the Indonesian national and international standards. The sugar palm and coconut saps have a big potential as the bee feed especially for the bee *A. cerana*.

Keywords: Arenga pinnata, beekeeping, Cocos nucifera L., extrafloral nectar, multifloral nectar

INTRODUCTION

The honeybee of A. cerana is one of the bees from the Apis genus which includes the local bee which is spread in some regions in Indonesia, including Kalimantan, Sumatera, Java, Bali, Lombok, Sumbawa, Sulawesi, Papua, and Seram (Radloff et al. 2011; Hepburn and Radloff 2011). In Indonesia, beekeeping of the bee A. cerana has been practiced by beekeepers using traditional hives (for example using a coconut log hive) and semi-modern hives (box hives without nest frames) to produce honey. Furthermore, several regions have been practicing the beekeeping of the bee A. cerana has been reported by Schouten et al. (2019) are Riau, North Sumatera, Lampung, Banten, Java, Yogyakarta region, Bali, and Lombok. However, the beekeeping of A. cerana is mostly using traditional hives or use box hives but is not completed by the honey frame like the beekeeping of A. mellifera. The bee A. cerana can produce honey, bee bread, royal jelly, and propolis. However, their production is lower compared to the bee A. mellifera (Agussalim and Agus 2022; Schouten et al. 2019).

One of the problems faced by the beekeepers in Indonesia is the limited of feed sustainability as the raw material to produce honey, bee bread, and royal jelly. The limitation feed is a very serious problem that has been faced by beekeepers because they have no area used to plant several plants which are used as the feed source to produce the honeybees' products. Honeybee feeds are divided into two types, namely nectar and pollen, where nectar is obtained by the foragers from the plant flowers (nectar floral) and nectar extrafloral, which is obtained by the foragers from stalk and leaf of plants (Agussalim et al. 2018, 2017). Pollen is obtained by the foragers from plant flowers which is collected by using all body parts and then deposited in the corbicula (Agussalim et al. 2018, 2017; Erwan et al. 2021a). When collecting nectar and pollen from the plant flowers, the foragers role as the pollinator agent by transporting pollen from the anther to pistil so that the pollination process occurs. This process is continuously done by the foragers until their honey stomach is full of nectar and their corbicula has been deposited by the pollen. This pollination impacts on the increasing the plants productivity (Pohorecka et al. 2014; Supeno et al. 2021).

One of the strategies to produce sustainable honey from the bee *A. cerana* is by using sap from the plants such as sugar palm and coconut. Several studies have been conducted by using sugar palm and coconut saps as the *A. cerana* feed. Erwan et al. (2021b) reported that the feed combination of coconut sap and sugar palm pollen as the *A. cerana* feed could enhance the production of honey cells and bee bread cells. However, the use of sap from coconut and sugar palm can increase the honey and bee bread cells compared to the control group without sap as the feed (multi-floral nectar). Furthermore, Erwan et al. (2022) also reported using sugar palm and coconut saps which are each added with sugar palm pollen can improve the bee *A. cerana* productivity, such as increasing honey production, brood cell number, and colony weight. In addition, another study showed that the use of extrafloral nectar namely sugar palm (Arenga pinnata) and coconut (Cocos nucifera L.) saps as the A. mellifera bee feed which is resulting the honey chemical composition (reducing sugar, sucrose, acidity, moisture, and diastase enzyme activity) which are acceptable by Indonesian national standard and the international standard has been regulated by Codex Alimentarius (Erwan et al. 2020). However, studies about the chemical composition of honey from the bee A. cerana produced from the sugar palm sap, coconut sap, and their honey potency production from both sap sugar palm and coconut have yet to be studied. Therefore, the objectives of this study were to evaluate the honey quality based on the chemical composition of the bee A. cerana, honey potency produced by the coconut and sugar palm saps.

MATERIALS AND METHODS

Study area

This research has been conducted in the North Duman Village (8°32'10" S 116°09'32" E), Lingsar Sub-district, West Lombok (West Nusa Tenggara Province, Indonesia). In this research, we used thirty *A. cerana* colonies were divided into six treatments and every five colonies per treatment as the replication. The saps used in our study

were obtained from the stalk of coconut (*Cocos nucifera* L.) and sugar palm (*Arenga pinnata*) and the pollen source from the sugar palm (Figure 1). The stalks of coconut and sugar palm were cut and then put in a plastic bottle which was used to store the sap secreted by their stalks. The treatments in our study were sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added sugar palm pollen (CP0); coconut sap of 50% + sugar palm sap of 50% without added sugar palm pollen (SP1); coconut sap of 50% + sugar palm pollen (SP1); coconut sap of 50% + sugar palm sap of 50% was added sugar palm pollen (SP1); coconut sap of 50% + sugar palm sap of 50% was added sugar palm pollen (SP1).

The technique was used to give sugar palm and coconut saps and sugar palm pollen (Figure 2) was according to the previous method has been reported by Erwan et al. (2022, 2021b) briefly as follows: fresh coconut and sugar palm saps were given to the bee *A. cerana* by using a plastic plate and split bamboo was completed by 4 to 5 twigs for foragers perch. The plastic plate and split bamboo was placed one meter from the box hives, while the sugar palm pollen was hung besides and above of the box hives. The distance of 600 meters to place the colony to avoid the foragers collecting pollen and sap from the other treatments.



Figure 1. Coconut sap (left), sugar palm sap (center), and sugar palm pollen (right)



Figure 2. Technique to given the sugar palm and coconut saps (left) and sugar palm pollen (right) (Erwan et al. 2022, 2021b)

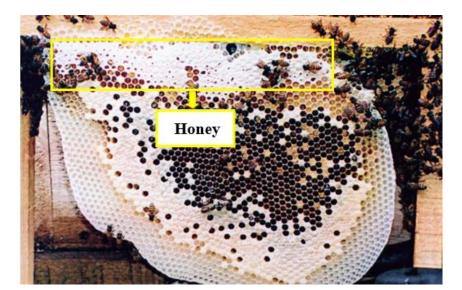


Figure 3. Honey from A. cerana was produced from the sugar palm and coconut saps

Procedures

Honey quality

Honey from the A. cerana (Figure 3) was harvested after beekeeping for three months using coconut and sugar palm saps. Honey from the five hives in one treatment group was composited into one honey sample and then used to analysis of their chemical composition. Honey quality from the A. cerana was evaluated based on the chemical composition consisting of moisture, reducing sugar, sucrose, diastase enzyme activity, hydroxymethylfurfural (HMF), and acidity. The moisture content was analyzed by using a proximate analysis based on the method from the Association of Official Agricultural Chemists (AOAC) (AOAC 2005). Reducing sugar was analyzed using a Layne-Enyon method and sucrose content was analyzed by a Luff Schoorl method, described by AOAC (2005). Diastase enzyme activity, HMF, and free acidity were analyzed based on the harmonized methods of the international honey commission (Machado et al. 2022).

Honey production from sugar palm and coconut saps

Sugar palm and coconut saps every ten liters were used to measure the honey production from the bee *A. cerana* for three months of beekeeping. The sugar palm and coconut saps were placed on the plastic plate in front of the box hives at a distance of one meter. In addition, the honey production without using sugar palm and coconut saps was measured for one year of the beekeeping, which calculates the contribution of sugar palm and coconut saps in honey production. Honey from *A. cerana* was harvested with cut the honey cells (Figure 3) and squeezed to separate wax and honey. Afterward, honey was measured production by using a digital scale and stored in the refrigerator.

Production of saps from coconut and sugar palm

The production of sap from coconut was measured for a year and also based on dept interview with farmers, while the sugar palm sap based on the previously studied was used to calculate the production per hectare area and was also obtained from the deep interview with the farmers. The production of coconut and sugar palm saps per hectare was calculated from the sap production per tree multiplied by the number of trees in a one hectare area. After three months of beekeeping, honey from both treatments sugar palm and coconut saps were harvested to measure the honey production from the use of ten litters sap, and then honey production was measured by cylinder glass

Data analysis

The data on honey quality, production potency of honey from sugar palm and coconut saps, honey production, and production of saps were analyzed by using descriptive analysis (Steel et al. 1997).

RESULTS AND DISCUSSION

Moisture content of honey

Honey is composed of water as the second largest of honey constituents, ranging from 15 to 21 g/100 g, depending on the plant species as the nectar source which is affected by the botanical origin. Furthermore, honey moisture is also affected by honey maturity level, processing postharvest, and storage conditions (Da Silva et al. 2016). The honey moisture affects the physical properties such as crystallization, viscosity, flavor, color, taste, solubility, specific gravity, and conservation (Da Silva et al. 2016; Escuredo et al. 2013). In addition, honey moisture is also affected by the temperature and humidity depending on the season (rainy and dry seasons). Honey moisture can increase during postharvest processing such as storage conditions, because honey is hygroscopic that can absorb the moisture in the air (Da Silva et al. 2016; Karabagias et al. 2014).

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Table 1. The moisture, reducing sugar, and sucrose contents of honey from the bee A. cerana

Treatments	Moisture (%)	Reducing sugar (%)	Sucrose (%)
SP0	21.60	65.24	2.86
CP0	20.76	68.37	1.96
SCP0	21.40	64.55	2.51
SP1	21.80	62.78	3.42
CP1	21.58	65.37	1.72
SCP1	20.98	67.33	1.44

Abbreviations: sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0); coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen (SCP1).

A recent study showed that the honey moisture from the bee A. cerana, produced by sugar palm and coconut saps and their combination was ranging from 20.76 to 21.80% (Table 1). This honey moisture content is accepted by the Indonesian national standard (SNI), where the moisture for beekeeping honey, including the bee A. cerana and A. mellifera, does not exceed 22% (National Standardization Agency of Indonesia 2018) and is higher compared to the international standard which Codex Alimentarius regulated is not exceed 20% (Thrasyvoulou et al. 2018). The variation of honey moisture of the bee A. cerana in our study may be caused by the different moisture content of both saps from sugar palm and coconut, however our study has not been measured. The higher moisture content requires a long time for ripening of honey, and the bees start the process of decreasing honey moisture when they take nectar from plant flowers or saps as the raw material to produce honey. Furthermore, a small portion of moisture content has been evaporated in the honey sack before being transferred to the other bee which is working in the hive. This transfer is rapid depending on the temperature, colony strength, and nectar availability (Da Silva et al. 2016).

The honey production process is started with the foragers collecting nectar from the plant flowers or extrafloral nectar and then stored in the honey stomach. After that, the foragers will transfer the nectar that has been collected to the other bees who are working to process the nectar into honey in their mouth, then put it in the honey stomach and then transfer it to other bees several times until honey is ripening. A considerable amount of water will be evaporated in this process, which continues with the help of wing fans that can regulate the air humidity for about 15 to 20 minutes (Balasubramanyam 2021; Zhang et al. 2021). The honey moisture content in our study differed from Wang et al. (2021) that honey moisture from the bee A. cerana which is collected from 42 different honeycombs from China ranges from 17.03 to 18.44%, 18.65% for A. cerana cerana from Hainan province, China (Wu et al. 2020), and 16.99% for A. cerana from Borneo (Malaysian honey) (Moniruzzaman et al. 2013). Furthermore, Erwan et al. (2020) also reported that the honey moisture produced by the A. mellifera bee by using sugar palm and coconut saps ranges from 19.34 to 20.94%. The different honey moisture content has been reported to be affected by the different geographical origins, impacts the different plant types that can be grown in each region, different

environmental conditions (temperature and humidity), and also different bee species, which impact the different ability to evaporate water in the honey.

Reducing sugar and sucrose contents of honey

Sugars in honey are composed of monosaccharides for about 75%, disaccharides are 10 to 15%, and other sugars in small amounts. Honey sugars are responsible as the energy source, hygroscopic, viscosity, and granulation. Several sugars in honey have been reported such as glucose, fructose, sucrose, trehalose, rhamnose, isomaltose, nigerobiose, maltotriose, maltotetraose, melezitose, melibiose, maltulose, nigerose, raffinose, palatinose, erlose and others (Da Silva et al. 2016).

A recent study showed that the honey reducing sugar from the bee A. cerana was beekeeping by using sugar palm and coconut saps, and their combination as the nectar source to produce honey ranges from 62.78 to 68.37% (Table 1). This honey reducing sugar is acceptable by the SNI for treatments SP0, CP0, CP1, and SCP1 but not acceptable for treatments SCP0, and SP1 (Table 1), where the minimum reducing sugar is 65% (National Standardization Agency of Indonesia 2018). This sugar is produced by the mechanism of invertase enzyme activity that change the sap sucrose into simple sugars. It is known that this enzyme is responsible for converting of sucrose into glucose and fructose. These sugars are included in the reducing sugar group and the main component in honey. In the honey maturity process, the sucrose is break down by the invertase enzyme into simple sugars simultaneously, and water will be evaporated to increase the reduced sugar content. In addition, enzymes secreted by the worker bees can also break down the carbohydrate into simple sugars. Furthermore, another enzyme in honey is the diastase enzyme that breaks down starch into simple sugars (Da Silva et al. 2016). The honey reducing sugar in our study (Table 1) differed from what was reported by Erwan et al. (2020), that honey reducing sugar from the bee A. mellifera which was produced by extrafloral nectar (sugar palm and coconut saps) ranges from 60.15 to 73.69%. The different reducing sugar may be affected by the different bee species, which impacts their ability to evaporate water in honey, especially when they convert the complex sugars into simple sugars and different seasons when the study is related to temperature and humidity environmental.

The honey sucrose content from the bee A. cerana in our study ranges from 1.44 to 3.42% (Table 1) and acceptable by SNI is not exceed 5% for the beekeeping honey including A. cerana and A. mellifera (National Standardization Agency of Indonesia 2018) and also accepted by the International standard has been regulated by Codex Alimentarius is not exceed 5% for blossom and honeydew honey (Thrasyvoulou et al. 2018). Naturally, sucrose present in honey in our study originated from sugar palm and coconut saps. The low honey sucrose content in our study is caused by the honey harvested in mature condition characterized by honey cells that have been covered by wax. Furthermore, the invertase enzyme which is produced by the worker bees actively breaks down sucrose from saps into simple sugars. There are two types of invertase enzymes that are produced by the worker bees, namely glucoinvertase which converts sucrose into glucose and fructoinvertase, which converts sucrose into fructose. These enzymes are mostly derived from the bee's secretion and only a small portion from the nectar, while the honeydew from the insect's secretion mostly contains invertase enzymes (Da Silva et al. 2016). The honey sucrose content in our study (Table 1) differed from Erwan et al. (2020), that honey sucrose content from the bee A. mellifera was produced by extrafloral nectar (sugar palm and coconut saps) is ranging from 4.21 to 4.40%%.

The honey sucrose content is a very important parameter to evaluate the maturity of honey to identify manipulation, where the high levels may indicate adulterations by adding several sweeteners such as cane sugar or refined beet sugar. In addition, indicating the early harvest, where sucrose is not completely transformed into fructose and glucose, the bees feed artificially for a prolonged time using a sucrose syrup (Da Silva et al. 2016; Puscas et al. 2013; Escuredo et al. 2013; Tornuk et al. 2013). Honey is a sugar solution that is supersaturated and unstable so it's easy to crystallize. The honey crystallization is affected by the concentration of glucose, fructose, and water. Fructose is the dominant sugar present in honey from A. mellifera was produced by several plants as the nectar source that workers use to produce honey such as eucalyptus, acacia, bramble, lime, chestnut, sunflower, and from honeydew, except in rape honey was produced by Brassica napus. Rape honey is higher in glucose and lower in fructose which impacts its rapid crystallization (Escuredo et al. 2014). The sugars content present in honey is dependent on the geographical origins which impacts on the different plant types that can grow in each region and impacts the different sugars content from the nectar, which is produced by the nectary gland of plant flowers (Agus et al. 2021; Agussalim et al. 2019; Da Silva et al. 2016; Escuredo et al. 2014; Tornuk et al. 2013). Furthermore, the sugar content in honey is influenced by climate (season, temperature, and humidity), processing (heating process), and storage time (Da Silva et al. 2016; Escuredo et al. 2014; Tornuk et al. 2013).

Diastase enzyme activity and hydroxymethylfurfural (HMF) of honey

A recent study showed that the diastase enzyme activity from the bee A. cerana honey produced by the sugar palm and coconut saps ranges from 5.17 to 9.04 DN (Table 2). This enzyme activity is acceptable by SNI with a minimum of 3 DN for beekeeping honey including the bee A. cerana and A. mellifera (National Standardization Agency of Indonesia 2018), and also acceptable by the international standard has been regulated by Codex Alimentarius with the minimum 3 DN (Thrasyvoulou et al. 2018). One of the honey characteristics is that it contains enzymes originating from the bees, pollen, and nectar from plant flowers, but mostly enzymes are added by the bees when they are convert nectar into honey (Da Silva et al. 2016; Thrasyvoulou et al. 2018). The honey diastase enzyme activity in our study (Table 2) differed from what was reported by Erwan et al. (2020) that the diastase enzyme activity of honey from the bee A. mellifera was produced by extrafloral nectar (sugar palm and coconut saps) is ranging from 16.48 to 17.12 Schade unit.

Diastases are divided into α - and β -amylases, the natural enzymes present in honey. The α -amylase separates the starch chain randomly in the center to produce dextrin, while the β -amylase separates the maltose in the end chain. The nectar source influences diastase enzyme content in honey (floral and extrafloral nectars) to produce honey and honey geographical origins, which impacts the different chemical composition of the nectar can be produced by the plants which is impact on the honey chemical composition especially diastase enzyme activity. In addition, the bee species are also influencing the activity diastase because it's related to the distance, and the flowers plant numbers that can be visited by the foragers when they are collecting nectar and pollen used to produce honey and bee bread (Da Silva et al. 2016).

Generally, the diastase enzyme has the role of breaking down complex sugars into simple sugars. In addition, this enzyme is role to digesting starch into maltose (disaccharide) and maltotriose (trisaccharide) which are sensitive to heat or thermolabile. Thus, this condition can be used to evaluate the overheating and preservation degree of honey (Da Silva et al. 2016). Furthermore, diastase activity is also used to evaluate honey age related to storage time and temperature because the diastase activity may be reducing when heating above 60°C and longtime storage (Da Silva et al. 2016; Yücel and Sultanoğlu 2013). The honey diastase activity from the bee A. cerana in our study (Table 2) was differed from Wu et al. (2020) for multifloral honey produced by the A. cerana cerana from the Hainan province (China) was 6.70 Göthe. Furthermore, it also differed from Wang et al. (2021) that the diastase activity of A. cerana honey from Qinling Mountains (China) is ranging from 22.05 to 35.67 Göthe. The different diastase activities of honey from A. cerana were reported by previous researchers are influenced by the different plant types as the nectar source to produce honey, different sugar content, and different geographical origin.

Furthermore, the HMF of *A. cerana* honey produced by the sugar palm and coconut saps in our study ranges from 2.24 to 5.81 mg/kg (Table 2). This HMF indicates that honey from our study in fresh condition and acceptable by SNI for beekeeping honey, including from *A. cerana* and *A. mellifera*, not exceed 40 mg/kg (National Standardization Agency of Indonesia 2018) and also acceptable by the international standard regulated by Codex Alimentarius is not to exceed 40 mg/kg for blossom and honeydew honey (Thrasyvoulou et al. 2018). After harvesting, fresh honey generally contains a low HMF ranges from 0 to 4.12 mg/kg honey. Hydroxymethylfurfural is the result of the degradation of honey monosaccharides, especially fructose and glucose, under acid conditions and accelerated by heating. This reaction produce levulinic and formic acids (Da Silva et al. 2016).

Hydroxymethylfurfural is formed after the honey is removed from the comb or when the wax cover is opened and the advanced processing like heating process. The increase of the HMF content occurs in honey with the acidity and is accelerated by the heating process. However, the HMF content is also influenced by sugars content, organic acids presence, pH, moisture content, water activity, and the plant types as the nectar source (floral source). In addition, HMF can also be formed at low temperatures, acidic conditions, and sugar dehydration reactions. Therefore, the higher HMF content's impact on the honey color is darker (Da Silva et al. 2016; Tornuk et al. 2013). The HMF of honey from the A. cerana in our study (Table 2) was differed to previously reported by Wu et al. (2020) for multifloral honey of A. cerana cerana from China is 3.80 mg/kg and 1.69 mg/kg for A. cerana honey from Qinling Mountains, China is 1.69 mg/kg. The different HMF content of honey from A. cerana reported by previous researchers are influenced by the different plant types as the nectar source to produce honey, different sugars content, and different geographical origin.

Acidity of honey

Free acidity is one of the important parameters to evaluate honey deterioration which is characterized by the presence of the organic acids in equilibrium with internal esters, lactone and several inorganic ions such as sulfates, chlorides, and phosphates (Da Silva et al. 2016). This study showed that the honey acidity from *A. cerana* produced by the sugar palm and coconut saps ranges from 26.00 to 36.33 ml NaOH/kg (Table 2). The acidity of *A. cerana* honey in our study is acceptable by SNI not to exceed 50 ml NaOH/kg for the beekeeping honey including *A. cerana* and *A. mellifera*. Furthermore, it is also acceptable by the international standard has been regulated by the Codex Alimentarius is not to exceed 50 meq/kg for blossom and honeydew honeys (Thrasyvoulou et al. 2018).

The sour taste of honey originated from several organic and inorganic acids, where the dominant organic acid present in honey is gluconic acid. This organic acid is produced by the enzyme activity of glucose-oxidase which is added by the bees when they convert nectar into honey, so that it can protect the nectar until honey maturity. This protection mechanism is caused by inhibiting of microorganisms activity in honey (Da Silva et al. 2016). This inhibit mechanism includes the combination of several factors, such as low moisture and the presence of hydrogen peroxide which is produced by the enzyme glucose-oxidase can inhibit the metabolism activity in the microbe cell through the destruction of the cell wall resulting in a change in cytoplasmic membrane permeability (Nainu et al. 2021; Pasias et al. 2018).

The total acidity content in honey is a small quantity. Still, the presence in honey is very important because it can influence the honey stability on the microorganisms, taste or flavor, and aroma of honey. The high acidity indicates the fermentation process occurs when some reducing sugar is break down into acetic acid. Honey acidity content is related to the yeast number where they break down some reducing sugar into ethanol, and if the reaction with the oxygen is formed, the acetic acid which is increasing the honey acidity. Therefore, the higher acidity values may indicate the sugars fermentation process into organic acids. Honey acidity is affected by several factors such as different content of organic acids, different geographical origins, and the season when honey is harvested (Da Silva et al. 2016; Tornuk et al. 2013). The honey acidity from the bee A. cerana in our study (Table 2) differed from previous studied by Wu et al. (2020) for A. cerana cerana honey is 0.80 mol/kg and Guerzou et al. (2021) ranges from 11 to 47 meq/kg for Algerian honey. Furthermore, it is differed from Erwan et al. (2020) that honey acidity from the bee A. mellifera was produced by extrafloral nectar (sugar palm and coconut saps) ranges from 22.00 to 43.00 ml NaOH/kg. The different acidity reported previously with our study is affected by the different plant types as the nectar source to produce honey, honey pH, geographical origin, and organic acids compound; however, our study has not measured the organic acid compound and honey pH.

Table 2. The diastase enzyme activity, hydroxymethylfurfural, and acidity of honey from the bee A. cerana

Treatments	Diastase enzyme activity (DN)	Hydroxymethylfurfural (mg/kg)	Acidity (ml NaOH/kg)
SP0	7.57	5.78	36.33
CP0	5.17	5.04	26.00
SCP0	9.04	4.75	28.60
SP1	6.86	4.77	29.68
CP1	8.51	5.81	28.26
SCP1	6.85	2.24	30.61

Abbreviations: sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0); coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen (SCP1).

Honey production potency from the sugar palm and coconut saps

Coconut and sugar palm plants have a good prospect to be developed because almost part of the plants can be utilized contributing to communities' income. Generally, the main product from the coconut (Cocos nucifera L.) was harvested as coconut fruit to advance the process into coconut oil and copra. These commodities have a high price, but producing coconut oil and copra are high risk for the farmers because they are just preparing raw materials. Therefore, the utilizing of the sap can be produced by the coconut and sugar palm was also potency feed for the bees was used as the nectar source to produce honey. Sugar palm and coconut saps are the feed potential studied by Erwan et al. (2021b) that the coconut and sugar palm saps can increase the number of honey and bee bread cells of the bee A. cerana. Furthermore, it is also reported that sugar palm and coconut are improving the productivity of the bee A. cerana such as increasing the brood cells number, colony weight, and honey production (Erwan et al. 2022). In addition, the saps from coconut and sugar palm are usually used by farmers to produce sugar using a traditional process.

The coconut plants can produce 12 stalks in a year, and one stalk can produce sap of 90 liters. Thus, one coconut plant can produce 1,080 liters of sap. Furthermore, if the farmers have one hectare of land planted by 100 coconut plants (distance 10 m \times 10 m), so they can produce about 108,000 liters of coconut sap. To produce 1 kg of honey requires coconut sap for about 7 liters and in a year 84 liters are required to produce 12 kg of honey. Thus, honey potency in a year from 100 hectares of land can be calculated as follows: 10,800,000 liters of sap divided by 84 liters of sap and multiplied by 12 kg of honey and obtained 1,542,857,14 kg/year (1,542.857 tons/year) or equivalent with 128.571 tons/month in 100 hectares of the land. Based on the sap production showing that the coconut plants have a big potency to produce honey. This potency was also supported by the harvest area of coconut in West Lombok (Nusa Tenggara Province, Indonesia) was 10,629.36 hectares (Department of Agricultural and Plantations 2021).

Sugar palm plants can be tapped to collect sap for about 5 to 6 months in one stalk, but generally can be tapped not to exceed 4 months. Wahyuni et al. (2021) reported that the production of sugar palm sap per plant ranges from 8 to 22 liters/plant or 300 to 400 liters/season (3 to 4 months) or 800 to 1,500 liters/plant/year (average is 1,150 liters/plant/year). Furthermore, if in one hectare of the plantation we have 100 sugar palm plants, the distance for planting is 10 m \times 10 m, so can be obtained of sap for 115,000 liters.

The field investigation showed that producing 1 kg of honey from the sugar palm sap required about 10 liters and in a year, it is required about 120 liters to produce 12 kg of honey. Thus, the honey potency from the sugar palm sap in a year from the 100 hectares of the sugar palm field is 11,500,000 liters which was divided by 120 liters and multiplied by 12 kg, so is obtained 1,150,000 kg of honey per year (1,150 tons of honey) or equivalent with 95.833 tons/month in 100 hectares area. This potency indicate that the sugar palm sap has a big potency to produce honey which is supported by the report data from the Department of Agricultural and Plantations (2021) that the sap production, sap productivity, and harvest area for sugar palm plants in West Lombok (West Nusa Tenggara 57.46 Province, Indonesia) are tones. 304.80 quintals/hectare, and 188.52, respectively, in the year of 2021. Therefore, it can be concluded that honey is produced by the bee A. cerana from sugar palm and coconut saps as the feed have at quality that is acceptable by Indonesian national standard, and the international standard has been regulated by the Codex Alimentarius. Honey potency production from the coconut sap in 100 hectares area can produce honey of 1,542.857 tons/year or equivalent with 128.571 tons/month, while sugar palm can produce honey of 1,150 tons/year or equivalent with 95.833 tons/month.

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EDITOR DECISION: ACCEPT SUMBISSION (12 NOVEMBER 2022)



[biodiv] Editor Decision

Ayu Astuti <smujo.id@gmail.com> 12 November 2022 pukul 07.14 Kepada: ERWAN <apiserwan@gmail.com>, AGUSSALIM <agussalim@mail.ugm.ac.id>

ERWAN, AGUSSALIM:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Honey quality from the bee Apis cerana, honey potency produced by coconut and sugar palm saps".

Our decision is to: Accept Submission

Biodiversitas Journal of Biological Diversity



[biodiv] Editor Decision

erwan apis <apiserwan@gmail.com> Kepada: Ayu Astuti <smujo.id@gmail.com> 12 November 2022 pukul 19.44

Dear Editor in Chief Biodiversitas

Thanks very much for the information [Kutipan teks disembunyikan] --Best Regards,

Dr. Ir. Erwan, M.Si. Faculty of Animal Science, University of Mataram, Indonesia BUKTI SUBMIT CORRECTED PROOF/PROOFREAD KEDUA DAN ARTIKEL HASIL PROOFREAD KEDUA (12 NOVEMBER 2022)



[biodiv] Editor Decision

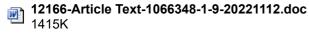
erwan apis <apiserwan@gmail.com> Kepada: Ayu Astuti <smujo.id@gmail.com> 12 November 2022 pukul 19.57

Dear Editor in Chief Biodiversitas

We have been check the copyedited in the system and we found some mistake for example in Universitas Mataram should be University of Mataram, the references not have DOI, so DOI we have been deleted and the references are Agussalim, Agus A. 2022. Production of honey, pot-pollen and propolis production from Indonesian stingless bee *Tetragonula laeviceps* and the physicochemical properties of honey: A review. Livest Res Rural Dev 34 (8): 66.

Agussalim, Agus A, Nurliyani, Umami N. 2019. The sugar content profile of honey produced by the Indonesian Stingless bee, *Tetragonula laeviceps*, from different regions. Livest Res Rural Dev 31 (6): 91.

Please find the correction in attached file [Kutipan teks disembunyikan]



FILE ARTIKEL HASIL PROOFREAD KEDUA

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Honey quality from the bee *Apis cerana*, honey potency produced by coconut and sugar palm saps

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Abstract. *Erwan, Agussalim. 2022. Honey quality from the bee* Apis cerana, *honey potency produced by coconut and sugar palm saps. Biodiversitas 23: 5854-5861.* One of the big problems when keeping honeybees is the limited sustainable feed, especially in the rainy season. The objectives of this study were to evaluate the honey quality from the bee *Apis cerana* based on the chemical composition and honey potency produced by the coconut and sugar palm saps. This study using thirty colonies of the bee *A. cerana* was divided into six treatments consisting of sugar palm sap without sugar palm pollen, coconut sap without sugar palm pollen, coconut sap of 50% + sugar palm sap of 50% without sugar palm pollen, sugar palm sap was added by sugar palm pollen; coconut sap was added by sugar palm pollen; coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen. The chemical composition of honey from the *A. cerana* was moisture (20.76 to 21.80%), reducing sugar (62.78 to 68.37%), sucrose (1.44 to 3.42%), diastase enzyme activity (5.17 to 9.04 DN), hydroxymethylfurfural (2.24 to 5.81 mg/kg), and acidity (26.00 to 36.33 mL NaOH/kg). Honey potency produced by the coconut and sugar palm saps in 100 hectares area produces honey was 1542.857 tons/year and 1150 tons/year, respectively. It can be concluded that the quality of *A. cerana* honey, produced by the sugar palm and coconut saps, is acceptable by the Indonesian national and international standards. The sugar palm and coconut saps have big potential as bee feed, especially for the bee *A. cerana*.

Keywords: Apis cerana, coconut, honey, sugar palm pollen

INTRODUCTION

The honeybee of Apis cerana is one of the bees from the Apis genus, which includes the local bee which is spread in some regions in Indonesia, including Kalimantan, Sumatera, Java, Bali, Lombok, Sumbawa, Sulawesi, Papua, and Seram (Hepburn and Radloff 2011; Radloff et al. 2011). In Indonesia, beekeeping of the bee A. cerana has been practiced by beekeepers using traditional hives (for example, using a coconut log hive) and semi-modern hives (box hives without nest frames) to produce honey. Furthermore, several regions have been practicing the beekeeping of the bee A. cerana, has been reported by Schouten et al. (2019) are Riau, North Sumatera, Lampung, Banten, Java, Yogyakarta region, Bali, and Lombok. However, the beekeeping of A. cerana is mostly using traditional hives or use box hives but is not completed by the honey frame like the beekeeping of A. mellifera. The bee A. cerana can produce honey, bee bread, royal jelly, and propolis. However, their production is lower compared to the bee A. mellifera (Schouten et al. 2019; Agussalim and Agus 2022).

One of the problems faced by the beekeepers in Indonesia is the limited of feed sustainability as the raw material to produce honey, bee bread, and royal jelly. The limitation of feed is a very serious problem that has been faced by beekeepers because they have no area used to plant several plants which are used as the feed source to produce the honeybees' products. Honeybee feeds are divided into two types, namely nectar and pollen, where nectar is obtained by the foragers from the plant flowers (nectar floral) and nectar extrafloral, which is obtained by the foragers from stalk and leaf of plants (Agussalim et al. 2018, 2017). Pollen is obtained by the foragers from plant flowers which are collected by using all body parts and then deposited in the corbicula (Agussalim et al. 2017, 2018; Erwan et al. 2021a). When collecting nectar and pollen from the plant flowers, the forager's role as the pollinator agent by transporting pollen from the anther to the pistil so that the pollination process occurs, this process is continuously done by the foragers until their honey stomach is full of nectar and their corbicula has been deposited by the pollen. This pollination impacts the increase of the plant's productivity (Pohorecka et al. 2014; Supeno et al. 2021).

One of the strategies to produce sustainable honey from the bee *A. cerana* is by using sap from the plants such as sugar palm and coconut. Several studies have been conducted by using sugar palm and coconut saps as the *A. cerana* feed. Erwan et al. (2021b) reported that the feed combination of coconut sap and sugar palm pollen as the *A. cerana* feed could enhance the production of honey cells and bee bread cells. However, using sap from coconut and sugar palms can increase the honey and bee bread cells compared to the control group without sap as the feed (multi-floral nectar). Furthermore, Erwan et al. (2022) also reported using sugar palm and coconut saps which are each added with sugar palm pollen, can improve the bee *A*.

cerana productivity, such as increasing honey production, brood cell number, and colony weight. In addition, another study showed that the use of extrafloral nectar namely sugar palm (Arenga pinnata) and coconut (Cocos nucifera) saps as the A. mellifera bee feed which is resulting the honey chemical composition (reducing sugar, sucrose, acidity, moisture, and diastase enzyme activity) which are acceptable by Indonesian national standard and the international standard has been regulated by Codex Alimentarius (Erwan et al. 2020). However, studies about the chemical composition of honey from the bee A. cerana produced from the sugar palm sap, coconut sap, and their honey potency production from both sap sugar palm and coconut have yet to be studied. Therefore, the objectives of this study were to evaluate the honey quality based on the chemical composition of the bee A. cerana honey potency produced by the coconut and sugar palm saps.

MATERIALS AND METHODS

Study area

This research has been conducted in the North Duman Village (8°32'10" S 116°09'32" E), Lingsar Sub-district, West Lombok, West Nusa Tenggara Province, Indonesia. In this research, we used thirty *A. cerana* colonies divided

into six treatments and every five colonies per treatment as the replication. The saps used in our study were obtained from the stalk of coconut (*Cocos nucifera*) and sugar palm (*Arenga pinnata*) and the pollen source from the sugar palm (Figure 1). The stalks of coconut and sugar palm were cut and then put in a plastic bottle which was used to store the sap secreted by their stalks. The treatments in our study were sugar palm sap without added by sugar palm pollen (SP0), coconut sap without added sugar palm pollen (CP0), coconut sap of 50% + sugar palm sap of 50% without added sugar palm pollen (SCP0); sugar palm sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added sugar palm pollen (SCP1).

The technique used to give sugar palm and coconut saps and sugar palm pollen (Figure 2) was according to the previous method has been reported by Erwan et al. (2021b, 2022) briefly as follows: fresh coconut and sugar palm saps were given to the bee *A. cerana* by using a plastic plate and split bamboo was completed by 4 to 5 twigs for foragers perch. The plastic plate and split bamboo was placed one meter from the box hives, while the sugar palm pollen was hung beside and above the box hives. The distance of 600 meters to place the colony to avoid the foragers collecting pollen and sap from the other treatments.



Figure 1. Coconut sap (left), sugar palm sap (center), and sugar palm pollen (right)



Figure 2. Technique to given the sugar palm and coconut saps (*left*) and sugar palm pollen (*right*) (Erwan et al. 2021b, 2022)

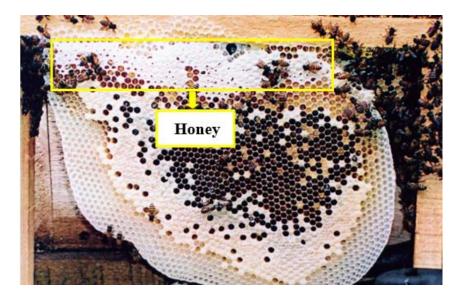


Figure 3. Honey from Apis cerana was produced from the sugar palm and coconut saps

Procedures

Honey quality

Honey from the A. cerana (Figure 3) was harvested after beekeeping for three months using coconut and sugar palm saps. Honey from the five hives in one treatment group was composited into one honey sample and then used to analysis of their chemical composition. Honey quality from the A. cerana was evaluated based on the chemical composition consisting of moisture, reducing sugar. sucrose, diastase enzyme activity, hydroxymethylfurfural (HMF), and acidity. The moisture content was analyzed by using a proximate analysis based on the method from the Association of Official Agricultural Chemists (AOAC) (AOAC 2005). Reducing sugar was analyzed using a Layne-Enyon method and sucrose content was analyzed by a Luff Schoorl method, described by AOAC (2005). Diastase enzyme activity, HMF, and free acidity were analyzed based on the harmonized methods of the international honey commission (Machado et al. 2022).

Honey production from sugar palm and coconut saps

Sugar palm and coconut saps every ten liters were used to measure the honey production from the bee *A. cerana* for three months of beekeeping. The sugar palm and coconut saps were placed on the plastic plate in front of the box hives at a distance of one meter. In addition, the honey production without using sugar palm and coconut saps was measured for one year of beekeeping, which calculates the contribution of sugar palm and coconut saps in honey production. Honey from *A. cerana* was harvested with cut the honey cells (Figure 3) and squeezed to separate wax and honey. Afterward, honey was measured production by using a digital scale and stored in the refrigerator.

Production of saps from coconut and sugar palm

The production of sap from coconut was measured for a year and also based on dept interviews with farmers, while the sugar palm sap based on the previously studied was used to calculate the production per hectare area and was also obtained from the deep interview with the farmers. The production of coconut and sugar palm saps per hectare was calculated from the sap production per tree multiplied by the number of trees in a one-hectare area. After three months of beekeeping, honey from both treatments, sugar palm and coconut saps, were harvested to measure the honey production from the use of ten litters sap, and then honey production was measured by cylinder glass

Data analysis

The data on honey quality, production potency of honey from sugar palm and coconut saps, honey production, and production of saps were analyzed by using descriptive analysis (Steel et al. 1997).

RESULTS AND DISCUSSION

Moisture content of honey

Honey is composed of water as the second largest of honey constituents, ranging from 15 to 21g/100 g, depending on the plant species as the nectar source, which is affected by the botanical origin. Furthermore, honey moisture is also affected by honey maturity level, processing postharvest, and storage conditions (Da Silva et al. 2016). The honey moisture affects the physical properties such as crystallization, viscosity, flavor, color, taste, solubility, specific gravity, and conservation (Escuredo et al. 2013; Da Silva et al. 2016). In addition, honey moisture is also affected by the temperature and humidity depending on the season (rainy and dry seasons). Honey moisture can increase during postharvest processing, such as storage conditions because honey is hygroscopic that can absorb the moisture in the air (Karabagias et al. 2014; Da Silva et al. 2016).

Treatments	Moisture (%)	Reducing sugar (%)	Sucrose (%)
SP0	21.60	65.24	2.86
CP0	20.76	68.37	1.96
SCP0	21.40	64.55	2.51
SP1	21.80	62.78	3.42
CP1	21.58	65.37	1.72
SCP1	20.98	67.33	1.44

Table 1. The moisture, reducing sugar, and sucrose contents of honey from the bee Apis cerana

Notes: sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0); coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen (SCP1)

A recent study showed that the honey moisture from the bee A. cerana, produced by sugar palm and coconut saps and their combination ranged from 20.76 to 21.80% (Table 1). This honey moisture content is accepted by the Indonesian national standard (SNI), where the moisture for beekeeping honey, including the bee A. cerana and A. mellifera, does not exceed 22% (National Standardization Agency of Indonesia 2018) and is higher compared to the international standard which Codex Alimentarius regulated is not exceeded 20% (Thrasvvoulou et al. 2018). The variation of honey moisture of the bee A. cerana in our study may be caused by the different moisture content of both saps from sugar palm and coconut, however, our study has not been measured. The higher moisture content requires a long time for the ripening of honey, and the bees start the process of decreasing honey moisture when they take nectar from plant flowers or saps as the raw material to produce honey. Furthermore, a small portion of moisture content has been evaporated in the honey sack before being transferred to the other bee, which is working in the hive. This transfer is rapid depending on the temperature, colony strength, and nectar availability (Da Silva et al. 2016).

The honey production process is started with the foragers collecting nectar from the plant flowers or extrafloral nectar and then stored in the honey stomach. After that, the foragers will transfer the nectar that has been collected to the other bees who are working to process the nectar into honey in their mouth, then put it in the honey stomach and then transfer it to other bees several times until honey is ripening. A considerable amount of water will be evaporated in this process, which continues with the help of wing fans that can regulate the air humidity for about 15 to 20 minutes (Balasubramanyam 2021; Zhang et al. 2021). The honey moisture content in our study differed from Wang et al. (2021), that honey moisture from the bee A. cerana, which is collected from 42 different honeycombs from China, ranges from 17.03 to 18.44%, 18.65% for A. cerana cerana from Hainan province, China (Wu et al. 2020), and 16.99% for A. cerana from Borneo (Malaysian honey) (Moniruzzaman et al. 2013). Furthermore, Erwan et al. (2020) also reported that the honey moisture produced by the A. mellifera bee by using sugar palm and coconut saps ranges from 19.34 to 20.94%. The different honey moisture content has been reported to be affected by the different geographical origins, impacts the different plant types that can be grown in each region, different environmental conditions (temperature and humidity), and also different bee species, which impact the different ability to evaporate water in the honey.

Reducing sugar and sucrose contents of honey

Sugars in honey are composed of monosaccharides for about 75%, disaccharides are 10 to 15%, and other sugars in small amounts. Honey sugars are responsible as the energy source, hygroscopic, viscosity, and granulation. Several sugars in honey have been reported such as glucose, fructose, sucrose, trehalose, rhamnose, isomaltose, nigerobiose, maltotriose, maltotetraose, melezitose, melibiose, maltulose, nigerose, raffinose, palatinose, erlose and others (Da Silva et al. 2016).

A recent study showed that the honey-reducing sugar from the bee A. cerana was beekeeping by using sugar palm and coconut saps, and their combination as the nectar source to produce honey ranges from 62.78 to 68.37% (Table 1). This honey-reducing sugar is acceptable by the SNI for treatments SP0, CP0, CP1, and SCP1 but not acceptable for treatments SCP0, and SP1 (Table 1), where the minimum reducing sugar is 65% (National Standardization Agency of Indonesia 2018). This sugar is produced by the mechanism of invertase enzyme activity that changes the sap sucrose into simple sugars. It is known that this enzyme is responsible for converting sucrose into glucose and fructose. These sugars are included in the reducing sugar group and the main component in honey. In the honey maturity process, the sucrose is broken down by the invertase enzyme into simple sugars simultaneously, and water will be evaporated to increase the reduced sugar content. In addition, enzymes secreted by the worker bees can also break down the carbohydrate into simple sugars. Furthermore, another enzyme in honey is the diastase enzyme that breaks down starch into simple sugars (Da Silva et al. 2016). The honey-reducing sugar in our study (Table 1) differed from what was reported by Erwan et al. (2020), that honey-reducing sugar from the bee A. mellifera which was produced by extrafloral nectar (sugar palm and coconut saps) ranges from 60.15 to 73.69%. The different reducing sugar may be affected by the different bee species, which impacts their ability to evaporate water in honey, especially when they convert the complex sugars

into simple sugars and different seasons when the study is related to temperature and humidity environmental.

The honey sucrose content from the bee A. cerana in our study ranges from 1.44 to 3.42% (Table 1) and acceptable by SNI is not exceed 5% for the beekeeping honey including A. cerana and A. mellifera (National Standardization Agency of Indonesia 2018) and also accepted by the international standard has been regulated by Codex Alimentarius is not exceed 5% for blossom and honeydew honey (Thrasyvoulou et al. 2018). Naturally, sucrose present in honey in our study originated from sugar palm and coconut saps. The low honey sucrose content in our study is caused by the honey harvested in a mature condition characterized by honey cells that have been covered by wax. Furthermore, the invertase enzyme which is produced by the worker bees actively breaks down sucrose from saps into simple sugars. There are two types of invertase enzymes that are produced by the worker bees, namely glucoinvertase, which converts sucrose into glucose and fructoinvertase, which converts sucrose into fructose. These enzymes are mostly derived from the bee's secretion and only a small portion from the nectar, while the honeydew from the insect's secretion mostly contains invertase enzymes (Da Silva et al. 2016). The honey sucrose content in our study (Table 1) differed from Erwan et al. (2020), that honey sucrose content from the bee A. mellifera was produced by extrafloral nectar (sugar palm and coconut saps) ranging from 4.21 to 4.40%.

The honey sucrose content is a very important parameter to evaluate the maturity of honey to identify manipulation, where the high levels may indicate adulterations by adding several sweeteners such as cane sugar or refined beet sugar. In addition, indicating the early harvest, where sucrose is not completely transformed into fructose and glucose, the bees feed artificially for a prolonged time using a sucrose syrup (Escuredo et al. 2013; Puscas et al. 2013; Tornuk et al. 2013; Da Silva et al. 2016). Honey is a sugar solution that is supersaturated and unstable, so it's easy to crystallize. The honey crystallization is affected by the concentration of glucose, fructose, and water. Fructose is the dominant sugar present in honey from A. mellifera was produced by several plants as the nectar source that workers use to produce honey, such as eucalyptus, acacia, bramble, lime, chestnut, sunflower, and from honeydew, except in rape honey was produced by Brassica napus. Rape honey is higher in glucose and lowers in fructose which impacts its rapid crystallization (Escuredo et al. 2014). The sugars content present in honey is dependent on the geographical origins, which impacts the different plant types that can grow in each region and impacts the different sugars content from the nectar, which is produced by the nectary gland of plant flowers (Tornuk et al. 2013; Escuredo et al. 2014; Da Silva et al. 2016; Agussalim et al. 2019; Agus et al. 2021). Furthermore, the sugar content in honey is influenced by climate (season, temperature, and humidity), processing (heating process), and storage time (Tornuk et al. 2013; Escuredo et al. 2014; Da Silva et al. 2016).

Diastase enzyme activity and hydroxymethylfurfural (HMF) of honey

A recent study showed that the diastase enzyme activity from the bee A. cerana honey produced by the sugar palm and coconut saps ranges from 5.17 to 9.04 DN (Table 2). This enzyme activity is acceptable by SNI with a minimum of 3 DN for beekeeping honey, including the bee A. cerana and A. mellifera (National Standardization Agency of Indonesia 2018), and also acceptable by the international standard has been regulated by Codex Alimentarius with the minimum 3 DN (Thrasyvoulou et al. 2018). One of the honey characteristics is that it contains enzymes originating from the bees, pollen, and nectar from plant flowers, but mostly enzymes are added by the bees when they convert nectar into honey (Da Silva et al. 2016; Thrasyvoulou et al. 2018). The honey diastase enzyme activity in our study (Table 2) differed from what was reported by Erwan et al. (2020) that the diastase enzyme activity of honey from the bee A. mellifera was produced by extrafloral nectar (sugar palm and coconut saps) is ranging from 16.48 to 17.12 Schade unit.

Diastases are divided into α - and β -amylases, the natural enzymes present in honey. The α -amylase separates the starch chain randomly in the center to produce dextrin, while the β -amylase separates the maltose in the end chain. The nectar source influences diastase enzyme content in honey (floral and extrafloral nectars) to produce honey and honey geographical origins, which impacts the different chemical compositions of the nectar that can be produced by the plants, which impacts the honey chemical composition, especially diastase enzyme activity. In addition, the bee species are also influencing the activity diastase because it's related to the distance and the flowers plant numbers that can be visited by the foragers when they are collecting nectar and pollen used to produce honey and bee bread (Da Silva et al. 2016).

Generally, the diastase enzyme has the role of breaking down complex sugars into simple sugars. In addition, this enzyme is a role in digesting starch into maltose (disaccharide) and maltotriose (trisaccharide), which are sensitive to heat or thermolabile. Thus, this condition can be used to evaluate the overheating and preservation degree of honey (Da Silva et al. 2016). Furthermore, diastase activity is also used to evaluate honey age-related to storage time and temperature because the diastase activity may be reduced when heating above 60°C and longtime storage (Yücel and Sultanoğlu 2013; Da Silva et al. 2016). The honey diastase activity from the bee A. cerana in our study (Table 2) differed from Wu et al. (2020) for multifloral honey produced by the A. cerana cerana from the Hainan province (China) was 6.70 Göthe. Furthermore, it also differed from Wang et al. (2021) that the diastase activity of A. cerana honey from Qinling Mountains (China) ranged from 22.05 to 35.67 Göthe. The different diastase activities of honey from A. cerana were reported by previous researchers and are influenced by the different plant types as the nectar source to produce honey, different sugar content, and different geographical origin.

Furthermore, the HMF of *A. cerana* honey produced by the sugar palm and coconut saps in our study ranges from

2.24 to 5.81 mg/kg (Table 2). This HMF indicates that honey from our study in fresh condition and acceptable by SNI for beekeeping honey, including from A. cerana and A. mellifera, does not exceed 40 mg/kg (National Standardization Agency of Indonesia 2018) and is also acceptable by the international standard regulated by Codex Alimentarius is not to exceed 40 mg/kg for blossom and honeydew honey (Thrasyvoulou et al. 2018). After harvesting, fresh honey generally contains a low HMF ranging from 4.12 mg/kg honev. 0 to Hydroxymethylfurfural is the result of the degradation of honey monosaccharides, especially fructose and glucose, under acid conditions and accelerated by heating. This reaction produces levulinic and formic acids (Da Silva et al. 2016).

Hydroxymethylfurfural is formed after the honey is removed from the comb or when the wax cover is opened and the advanced processing like heating process. The increase of the HMF content occurs in honey with acidity and is accelerated by the heating process. However, the HMF content is also influenced by sugar content, organic acids presence, pH, moisture content, water activity, and the plant types as the nectar source (floral source). In addition, HMF can also be formed at low temperatures, acidic conditions, and sugar dehydration reactions. Therefore, the higher HMF content's impact on the honey color is darker (Tornuk et al. 2013; Da Silva et al. 2016). The HMF of honey from the A. cerana in our study (Table 2) was differed to previously reported by Wu et al. (2020) for multifloral honey of A. cerana cerana from China is 3.80 mg/kg and 1.69 mg/kg for A. cerana honey from Qinling Mountains, China is 1.69 mg/kg. The different HMF content of honey from A. cerana reported by previous researchers are influenced by the different plant types as the nectar source to produce honey, different sugar content, and different geographical origin.

Acidity of honey

Free acidity is one of the important parameters to evaluate honey deterioration which is characterized by the presence of the organic acids in equilibrium with internal esters, lactone, and several inorganic ions such as sulfates, chlorides, and phosphates (Da Silva et al. 2016). This study showed that the honey acidity from *A. cerana* produced by the sugar palm and coconut saps ranges from 26.00 to 36.33 mL NaOH/kg (Table 2). The acidity of *A. cerana* honey in our study is acceptable by SNI not to exceed 50 mL NaOH/kg for the beekeeping honey, including *A. cerana* and *A. mellifera*. Furthermore, it is also acceptable by the international standard has been regulated by the Codex Alimentarius is not to exceed 50 meq/kg for blossom and honeydew honey (Thrasyvoulou et al. 2018).

The sour taste of honey originated from several organic and inorganic acids, where the dominant organic acid present in honey is gluconic acid. This organic acid is produced by the enzyme activity of glucose-oxidase, which is added by the bees when they convert nectar into honey so that it can protect the nectar until honey maturity. This protection mechanism is caused by inhibiting of microorganisms' activity in honey (Da Silva et al. 2016). This inhibit mechanism includes the combination of several factors, such as low moisture and the presence of hydrogen peroxide, which is produced by the enzyme glucoseoxidase can inhibit the metabolism activity in the microbe cell through the destruction of the cell wall resulting in a change in cytoplasmic membrane permeability (Pasias et al. 2018; Nainu et al. 2021).

The total acidity content in honey is a small quantity. Still, the presence of honey is very important because it can influence the honey stability on the microorganisms, taste or flavor, and aroma of honey. The high acidity indicates the fermentation process occurs when some reducing sugar is broken down into acetic acid. Honey acidity content is related to the yeast number where they break down some reducing sugar into ethanol, and if the reaction with the oxygen is formed, the acetic acid which is increasing the honey acidity. Therefore, the higher acidity values may indicate the sugars fermentation process into organic acids. Honey acidity is affected by several factors, such as different content of organic acids, different geographical origins, and the season when honey is harvested (Tornuk et al. 2013; Da Silva et al. 2016). The honey acidity from the bee A. cerana in our study (Table 2) differed from previous studied by Wu et al. (2020) for A. cerana cerana honey is 0.80 mol/kg, and Guerzou et al. (2021) ranges from 11 to 47 meq/kg for Algerian honey. Furthermore, it differed from Erwan et al. (2020) that honey acidity from the bee A. mellifera was produced by extrafloral nectar (sugar palm and coconut saps) ranging from 22.00 to 43.00 mL NaOH/kg. The different acidity reported previously with our study is affected by the different plant types as the nectar source to produce honey, honey pH, geographical origin, and organic acids compound; however, our study has not measured the organic acid compound and honey pH.

Table 2. The diastase enzyme activity, hydroxymethylfurfural, and acidity of honey from the bee Apis cerana

Treatments	Diastase enzyme activity (DN)	Hydroxymethylfurfural (mg/kg)	Acidity (mL NaOH/kg)
SP0	7.57	5.78	36.33
CP0	5.17	5.04	26.00
SCP0	9.04	4.75	28.60
SP1	6.86	4.77	29.68
CP1	8.51	5.81	28.26
SCP1	6.85	2.24	30.61

Notes: sugar palm sap without added by sugar palm pollen (SP0); coconut sap without added by sugar palm pollen (CP0); coconut sap of 50% + sugar palm sap of 50% without added by sugar palm pollen (SCP0); sugar palm sap was added by sugar palm pollen (SP1); coconut sap was added by sugar palm pollen (CP1); coconut sap of 50% + sugar palm sap of 50% was added by sugar palm pollen (SCP1)

Honey production potency from the sugar palm and coconut saps

Coconut and sugar palm plants have a good prospect to be developed because almost part of the plants can be utilized, contributing to communities' income. Generally, the main product from the coconut (Cocos nucifera) was harvested as coconut fruit to advance the process into coconut oil and copra. These commodities have a high price but producing coconut oil and copra are high risk for the farmers because they are just preparing raw materials. Therefore, the utilizing of the sap can be produced by the coconut and sugar palm was also potency feed for the bees was used as the nectar source to produce honey. Sugar palm and coconut saps are the feed potential studied by Erwan et al. (2021b) that the coconut and sugar palm saps can increase the number of honey and bee bread cells of the bee A. cerana. Furthermore, it is also reported that sugar palm and coconut are improving the productivity of the bee A. cerana, such as increasing the number of brood cells number, colony weight, and honey production (Erwan et al. 2022). In addition, the saps from coconut and sugar palms are usually used by farmers to produce sugar using a traditional process.

The coconut plants can produce 12 stalks in a year, and one stalk can produce sap of 90 liters. Thus, one coconut plant can produce 1080 liters of sap. Furthermore, if the farmers have one hectare of land planted by 100 coconut plants (distance 10×10 m), so they can produce about 108,000 liters of coconut sap. To produce 1 kg of honey requires coconut sap for about 7 liters and in a year, 84 liters are required to produce 12 kg of honey. Thus, honey potency in a year from 100 hectares of land can be calculated as follows: 10,800,000 liters of sap divided by 84 liters of sap and multiplied by 12 kg of honey and obtained 1,542,857,14 kg/year (1542.857 tons/year) or equivalent with 128.571 tons/month in 100 hectares of the land. Based on the sap production showing that the coconut plants have a big potency to produce honey. This potency was also supported by the harvest area of coconut in West Lombok (Nusa Tenggara Province, Indonesia) was 10,629.36 hectares (Department of Agricultural and Plantations 2021).

Sugar palm plants can be tapped to collect sap for about 5 to 6 months in one stalk but generally can be tapped not to exceed 4 months. Wahyuni et al. (2021) reported that the production of sugar palm sap per plant ranges from 8 to 22 liters/plant or 300 to 400 liters/season (3 to 4 months) or 800 to 1500 liters/plant/year (average is 1150 liters/plant/year). Furthermore, if in one hectare of the plantation we have 100 sugar palm plants, the distance for planting is 10×10 m, so can be obtained of sap for 115,000 liters.

The field investigation showed that producing 1 kg of honey from the sugar palm sap required about 10 liters and, in a year, it required about 120 liters to produce 12 kg of honey. Thus, the honey potency from the sugar palm sap in a year from the 100 hectares of the sugar palm field is 11,500,000 liters which was divided by 120 liters and multiplied by 12 kg, so obtained 1,150,000 kg of honey per year (1150 tons of honey) or equivalent with 95.833 tons/month in 100 hectares area. This potency indicates that the sugar palm sap has a big potency to produce honey which is supported by the report data from the Department of Agricultural and Plantations (2021) that the sap production, sap productivity, and harvest area for sugar palm plants in West Lombok (West Nusa Tenggara 57.46 Province. Indonesia) are tones. 304.80 quintals/hectare, and 188.52, respectively, in the year of 2021. Therefore, it can be concluded that honey is produced by the bee A. cerana from sugar palm and coconut saps as the feed have at a quality that is acceptable by Indonesian national standards, and the international standard has been regulated by the Codex Alimentarius. Honey potency production from the coconut sap in 100 hectares area can produce honey of 1542.857 tons/year or equivalent with 128.571 tons/month, while sugar palm can produce honey of 1150 tons/year or equivalent with 95.833 tons/month.

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BUKTI KONFIRMASI TELAH DILAKUKAN PEMBAYARAN TAGIHAN BIODIVERSITAS (16 NOVEMBER 2022)



[biodiv] New notification from Biodiversitas Journal of Biological Diversity

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EDITOR DECISION: EDITING COMPLETE AND SENDING IT TO PRODUCTION (18 NOVEMBER 2022)



[biodiv] Editor Decision

2 pesan

Smujo Editors <support@mail.smujo.id> 18 November 2022 pukul 16.34 Kepada: ERWAN <apiserwan@gmail.com>, AGUSSALIM <agussalim@mail.ugm.ac.id>

ERWAN, AGUSSALIM:

The editing of your submission, "Honey quality from the bee Apis cerana, honey potency produced by coconut and sugar palm saps," is complete. We are now sending it to production.

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erwan apis <apiserwan@gmail.com> Kepada: Smujo Editors <support@mail.smujo.id>

Dear Editor in Chief Biodiversitas

Thanks very much for the good information [Kutipan teks disembunyikan]

Best Regards,

Dr. Ir. Erwan, M.Si. Faculty of Animal Science, University of Mataram, Indonesia 18 November 2022 pukul 20.07