https://doi.org/10.31436/iiumej.v24i2.2709

EFFECTS OF HIVE SIZES AND MESH MATERIALS ON THE PRODUCTIVITY OF PROPOLIS AND HONEY PRODUCED BY *TETRAGONULA LAEVICEPS*

MUHAMMAD YUSUF ABDUH[1,2*], ANNISA SHABRINA[1], ANDREAS RADEN CAMAN[1], ARSY ELIA PRATIWI[1] AND MUHAMMAD IHSANU[1]

[1]*School of Life Sciences and Technology, Institut Teknologi Bandung, Bandung, Indonesia*
[2]*University Center of Excellence for Nutraceuticals, Bioscience and Biotechnology Research Center, Institut Teknologi Bandung, Bandung, Indonesia*

[*]*Corresponding author: yusuf@sith.itb.ac.id*

(Received: 4 January 2023; Accepted: 2 June 2023; Published on-line: 4 July 2023)

ABSTRACT: Propolis and honey are bioproducts produced by *Tetragonula laeviceps*, a stingless bee species. Both products have anti-inflammatory, antimicrobial, and antibacterial properties. Modular Tetragonula hives of different sizes were used in the cultivation of *T. laeviceps* to increase the productivity of propolis while maintaining the sustainability of the bee colonies. This study was carried out in Jatinangor, West Java, Indonesia, with three size variations: small (21 × 18 × 14 cm), medium (26 × 22 ×17 cm), and large (30 × 26 × 17 cm). Each hive was equipped with a mesh of a different material. The results obtained in this study showed that increasing the hive's size increases the productivity of propolis. The highest productivity of propolis (2.53 ± 0.37 g/colony/week) was obtained when *T. laeviceps* was cultivated in large hives equipped with a nylon-based mesh. The highest productivity of honey (0.78 ± 0.18 g/colony/week) was obtained when *T. laeviceps* was cultivated in medium hives equipped with an aluminum-based mesh. The harvested propolis was extracted using a maceration method. Total flavonoid and phenolic content of the propolis solution lies in the range of 1.77 ± 0.86 to 3.18 ± 1.43 mg QE/g propolis and 32.23 ± 14.09 to 112.13 ± 47.64 mg GAE/g propolis, respectively. The harvested honey had a water content of 21.86%, with 72.86% reducing sugar, 192.86 μg/mL vitamin C, and 2613.41 μg/mL of antioxidant content.

ABSTRAK: Propolis dan madu adalah produk bio yang dihasilkan oleh *Tetragonula laeviceps*, spesies lebah kelulut. Kedua-dua produk mempunyai ciri anti-radang, anti-mikrob dan antibakteria. Sarang Tetragonula modular dengan saiz yang berbeza digunakan dalam mengusahakan *T. laeviceps* bagi meningkatkan produktiviti propolis sambil mengekalkan kemampanan koloni lebah. Kajian ini dijalankan di Jatinangor, Jawa Barat, Indonesia, dengan tiga variasi saiz: kecil (21 × 18 × 14 cm), sederhana (26 × 22 × 17 cm), dan besar (30 × 26 ×17 cm). Setiap sarang dilengkapi dengan jaringan bahan yang berbeza. Dapatan kajian yang diperolehi melalui kajian ini menunjukkan bahawa penambahan saiz sarang dapat meningkatkan produktiviti propolis. Produktiviti tertinggi propolis (2.53 ± 0.37 g/koloni/minggu) diperoleh apabila *T. laeviceps* dibela dalam sarang besar yang dilengkapi dengan jaring berasaskan nilon. Produktiviti madu tertinggi (0.78 ± 0.18 g/koloni/minggu) diperoleh apabila *T. laeviceps* diusahakan dalam sarang sederhana yang dilengkapi dengan jaring berasaskan aluminium. Propolis yang dituai telah diekstrak menggunakan kaedah maserasi. Jumlah kandungan flavonoid dan kandungan fenolik larutan propolis berada dalam julat 1.77 ± 0.86 hingga 3.18 ± 1.43 mg QE/g propolis dan 32.23 ± 14.09 hingga 112.13 ± 47.64 mg GAE/g propolis, masing-masing. Madu yang dituai mempunyai kandungan air 21.86%, dengan 72.86% penurunan gula, 192.86 μg/mL vitamin C, dan 2613.41 μg/mL kandungan antioksidan.

KEYWORDS: *hive; honey; mesh, propolis; Tetragonula laeviceps*

1. INTRODUCTION

Bees produce various beneficial products such as honey, pollen, propolis, beeswax, and bee bread. Bees make propolis by mixing plant resin, which contains flavonoids, polyphenols, saponins, amino acids, and minerals, with wax and their saliva secretion [1]. Propolis consumption can reduce inflammation, inhibit scar tissue contraction, promote wound healing, and enhance stamina and overall health. Antibacterial and antifungal properties of propolis allow for its extensive use in the health and cosmetic industries [2]. Moreover, bees also produce honey from flower nectars, which comprise flavonoids and other phenolic compounds, contributing to antimicrobial, antioxidant, anti-inflammation, and anticancer activities [3].

Tetragonula laeviceps is a stingless bee species that produces propolis to seal cracks in their hive, to protect their hive from pests and diseases, and to build honey and pollen pots. *T. laeviceps* can produce propolis more abundantly compared to honeybees of genus *Apis* with productivities of 3 kg/colony/year and 20–30 g/colony/year, respectively [4]. Total flavonoid content in stingless bee propolis with quercetin equivalent (QE) reaches 4% (mg QE/g propolis extract), higher than *Apis* bee with only 1.5% [5].

However, honey productivity of *T. laeviceps* is relatively small compared with its propolis only, around 6.5 kg/year/colony [6]. The physicochemical properties in *T. laeviceps* honey include a pH of 3.15–4.66, acidity of 5.9–109 meq/kg, anti-microbial activity of 0.9–23 DN, 58–75.7 g/100 g of reducing sugar, 1.1–4.8 g/100g of sucrose, and water content (19.9–41.9 g/100 g) [7]. Therefore, *T. laeviceps* is widely cultivated by stingless beekeepers for its propolis.

There are several stingless beekeepers still beekeeping with bamboo, the bees' natural hive. Beekeeping with bamboo hives yields propolis at a rate of 2.36 g/colony/week [8]. However, the application of bamboo hives requires beekeepers to damage the hive during the harvesting process, which harms the colony and results in high contamination of pollen and honey in the propolis [9]. The hive design that is currently under development is a wooden box equipped with a mesh as a propolis trap.

A Modular *Tetragonula* Hive (MOTIVE) is a stingless beehive invention that combines a main wooden box and a perforated mesh to optimize the productivity of propolis [8,10,11]. The mesh in the hive contributes to an increase in propolis production compared to a simple wooden box without a mesh [8,10]. A MOTIVE can also sustain the colony and improve the quality of propolis harvested because they do not mix with non-propolis products [10]. MOTIVE beekeeping has been accomplished with MOTIVEs the size of 21×14×18 cm which resulted in propolis productivity of 2–2.8 g/colony/week [8,10]. A MOTIVE is constructed of three main parts: the wooden box for the colony, a mesh holder comprised of two wooden frames above the wooden box, and the top cover that is legged to create a ventilation space above the mesh [8,10].

The mesh in the MOTIVE is a perforated material that serves as a place for the bees to attach propolis. The mesh requires properties such as rust resistance, acid resistance, odorless, and processability [12]. Aluminum wire, nylon, and polyethylene are mesh materials that are widely used. Nylon membranes have high pH, high-temperature resistance, and a small pore size distribution [13]. Polyethylene membranes have a melting point of 115 °C and are flexible, whereas aluminum materials have high-temperature- and corrosion-resistant properties [14].

https://doi.org/10.31436/iiumej.v24i2.2709

In this study, *T. laeviceps* cultivation was carried out using MOTIVEs with various hive sizes and mesh materials. According to Abd-Elmawgood et al. [15], the smaller hive results in a higher temperature inside the hive. Internal conditions, such as temperature, will affect the condition and activity of the colony in the foraging process. In addition, the size of the hive, which is proportional to the mesh size, will affect the propolis production by the bees. Therefore, this study was conducted to determine the size of MOTIVE and the mesh material that generates optimum productivity of propolis and honey.

2. MATERIALS AND METHODS

2.1 Preparations of Colony

Twenty *Tetragonula laeviceps* colonies were obtained from a breeder in Kebumen, Central Java, Indonesia, in September–November 2018. The *T. laeviceps* colonies were in bamboo hives and acclimatized at the cultivation site for one to two months. The MOTIVE was sterilized with ultraviolet (UV) light for 30 minutes. Eighteen colonies were moved to the MOTIVE, and two colonies remained in the bamboo hive as control treatments. The colony inside the MOTIVE was re-acclimatized for two weeks.

2.2 Variation of Hive Size and Mesh Type

The MOTIVE used had three size variations: small (21 × 18 ×14 cm), medium (26 × 22 × 17 cm), and large (30 × 26 × 17 cm). Each hive size had three variations of mesh materials: nylon, polyethylene, and aluminum wire placed between the wooden box and the cover. The pore size of the nylon mesh was 1 × 1 mm, while the pore size of the polyethylene and aluminum wire mesh was 2 × 2 mm.

2.3 Harvesting of Propolis and Honey

The beekeeping process of *T. laeviceps* was carried out for eight weeks at the Wood Laboratory, Institut Teknologi Bandung, Jatinangor Campus, Sumedang, West Java. Propolis was harvested every two weeks, while honey was harvested at the end of the cultivation period. The honey harvesting process was accomplished using a syringe and glass pipette.

The mesh that was filled with propolis was weighed to determine the propolis weight. Propolis productivity of *T. laeviceps* per week can then be determined by dividing the propolis weight by two (for the two-weeks period). The harvested honey was then measured for its weight and volume. Honey productivity per week was determined by dividing the weight of the honey by eight (for the eight-weeks period).

2.4 Extraction of Propolis

The propolis was extracted according to the method of Machado et al. [16] with a slight modification. Harvested propolis was separated from the mesh using liquid nitrogen. The ratio of propolis to ethanol was 1:15 (w/v). The extraction was carried out on a shaker incubator (IKA KS 4000i Control) with a rotational speed of 500 rpm and a temperature of 40 °C for one hour in a dark room. The extract was filtered with a glass funnel and filter paper Whatman no. 2. The filtrate obtained was stored in a dark vial bottle. Then, 0.5 mL of the propolis filtrate sample was transferred to evaporating dishes to evaporate the solvent using a hot plate at 40 °C. During this process, the evaporating dish was covered with aluminum foil to reduce air and light exposure.

2.5 Determination of Total Flavonoid Content

Total flavonoid content (TFC) was determined using a quercetin calibration curve, as described by Machado et al. [16]. Standard quercetin (0.05 g) was dissolved in 50 mL ethanol to obtain 1000 µg/mL of quercetin stock solution. The stock solution was then diluted to obtain quercetin solutions with concentrations of 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, and 0 µg/mL, for the blank solution. 2 mL of standard solution was mixed with 2 mL of 2% $AlCl_3$ solution and incubated for 30 minutes. Next, the absorbance was measured at a wavelength of 415 nm with an ultraviolet-visible (UV-VIS) spectrophotometer (Shimadzu UV-1800). Before analyzing the sample, propolis extract from the evaporation process was dissolved in 10 mL 80% methanol. The propolis-methanol solution was added to 2% $AlCl_3$, and the absorbance was measured. Quercetin contents in the sample solution were considered as TFC in units of mg of quercetin equivalent per mL (mg QE/mL), determined by the following equation:

$$TFC_{sample\ solution} = \left(\frac{A-0.0296}{0.035}\right) \times N, \quad (1)$$

where A is the absorbance value of the sample, N is the dilution factor of the sample solution, and 0.0296 and 0.035 are the constant value and the coefficient of the quercetin calibration curve, respectively. Meanwhile, the TFC in propolis extract was determined by the following equation:

$$TFC_{propolis\ extract}\ (\text{mg QE/g}) = \frac{TFC_{\text{sample}}\left(\frac{mg}{mL}\right) \times V_{solution}\ (mL)}{m_{extract}\ (g)}, \quad (2)$$

where $V_{solution}$ (10 mL) is the total volume of propolis methanol extract solution, and $m_{extract}$ is the mass (g) of propolis extract (mass of remaining solids from the evaporation of propolis filtrate).

2.6 Determination of Total Phenolic Content

The total phenolic content (TPC) of propolis was determined according to the Folin-Ciocalteu method, as explained by Machado et al. [16]. A calibration curve was constructed by first dissolving 0.1 g of standard gallic acid in 10 mL ethanol and then diluting with 100 mL distilled water to obtain a stock solution with a concentration of 1000 µg/mL. The stock solution was diluted with distilled water to obtain standard solutions with concentrations of 750 µg/mL, 500 µg/mL, 250 µg/mL, 100 µg/mL, 50 µg/mL, and 0 µg/mL, for the blank solution. 0.5 mL of each standard solution was added to 2 mL of Folin-Ciocalteu reagent and 2.5 mL of 7.5% Na_2CO_3 solution. The mixture was then incubated for five minutes in a water bath at 50 °C. The absorbance of each standard solution was measured with a UV-VIS spectrophotometer at a wavelength of 765 nm. Sample analysis was performed by dissolving evaporated propolis extract in 5 mL ethanol 80%. Gallic acid contents in the sample solution were considered as TPC with a unit of mg of gallic acid equivalent per mL (mg GAE/mL). TPC in the sample was determined with the following equation:

$$TPC_{sample\ solutione}\ (\text{mg/mL}) = \left(\frac{A-0{,}1351}{0{,}0111}\right) \times N \quad (3)$$

where A is the absorbance value of the sample, N is the dilution factor of the sample solution, and 0.1351 and 0.0111 are the constant value and the coefficient of the gallic acid calibration curve, respectively. Meanwhile, the TPC in the propolis extract was determined with Eq. (2), with a $V_{solution}$ of 5 mL.

2.7 Characterization of Honey

Honey characterization was carried out at Sibaweh Laboratories, Bandung. The moisture content of the honey was measured according to Indonesian National Standard 01-3545-2004 (19- SNI, 2004) using a refractometer. Reducing sugar content was determined with the Luff-Schoorl method according to the Indonesian National Standard 01-2892-1994 (20-SNI, 1994). Antioxidant activity was determined with the DPPH (2,2-diphenyl-1-picrylhydrazyl) activity method as suggested by Sadeli et al. [17]. Vitamin C content was measured using a titration method according to Silva et al. [18].

3. RESULTS AND DISCUSSIONS

3.1 Productivity of Propolis Produced by *T. laeviceps*

T. laeviceps colonies that were cultivated in bamboo hives produced propolis at a rate of 0.67 ± 0.01 g/colony/week. The propolis productivity of the bees in small, medium, and large MOTIVEs with nylon, polyethylene, and aluminum meshes ranged from 1.12–2.52 g/colony/week (Table 1). *T. laeviceps* bees produce propolis to cover the holes in the hive for self-protection [19]. A MOTIVE is equipped with a perforated mesh to stimulate more propolis production by the bees. The productivity of propolis of the colonies in bamboo hives was lower than the colonies in the MOTIVEs. This phenomenon is related to the difference in cavity size in the hives. Bamboo hives have smaller cavity gaps, which extend vertically, resulting in less propolis produced and difficulty in harvesting.

Table 1: Productivity of propolis and honey (g/colony/week) produced by *T. laeviceps* in various hive sizes and mesh materials

Hive sizes	Mesh materials	Propolis productivity [g/colony/week]	Honey productivity [g/colony/week]
Small	Nylon	1.24 ± 0.21^{a}	0.09 ± 0.02^{c}
	Polyethylene	1.12 ± 0.58^{a}	0.08 ± 0.01^{c}
	Aluminum	1.12 ± 0.54^{a}	0.02 ± 0.01^{c}
Medium	Nylon	$1.51 \pm 0.63^{a,b}$	0.09 ± 0.07^{d}
	Polyethylene	$1.60 \pm 0.27^{a,b}$	0.12 ± 0.17^{d}
	Aluminum	$1.81 \pm 0.80^{a,b}$	0.78 ± 0.18^{d}
Large	Nylon	2.52 ± 0.37^{b}	$0.12 \pm 0.02^{c,d}$
	Polyethylene	2.32 ± 0.39^{b}	$0.35 \pm 0.35^{c,d}$
	Aluminum	1.31 ± 0.50^{b}	$0.03 \pm 0.01^{c,d}$

*Different letters indicate statistical differences (P-value < 0.05, Duncan's test).

The mesh sizes in this study were proportional to the MOTIVE size, such that the large MOTIVE has a larger mesh surface area than the small MOTIVE. Thus, larger MOTIVE results in higher propolis productivity as the bees need to cover more areas. This phenomenon was shown through the propolis productivity in the large MOTIVEs with nylon and polyethylene meshes, which were higher than that of the small and medium MOTIVE. However, the MOTIVE with an aluminum mesh did not show the same pattern. Propolis productivity in the large MOTIVE with an aluminum mesh was lower than that of the medium MOTIVE.

Propolis production by the bees was influenced by several factors, such as the colony condition during the beekeeping process [20]. In this study, the number of bees per colony varied with bee colonies. The large MOTIVEs with nylon, polyethylene, and aluminum meshes had 859, 1120, and 937 bees, respectively. These population numbers were enough for the bees to cover the mesh with propolis, as shown by the highest productivity of propolis in the large MOTIVEs. Similarly, the medium MOTIVE with nylon and aluminum meshes also had enough populations with 774 and 1788 bees, respectively. Interestingly, the medium MOTIVE with a polyethylene mesh only had 189 bees, but the colony could still meet the needs of propolis production to cover the mesh. This phenomenon is related to the task divisions of worker bees, which are determined based on anatomical and physical conditions, environmental stimuli, and age [21]. The bee colony was considered young, signified by the abundant brood cells and bee breads in the hive. Thus, the high needs of the colony affected the activity of the forager bees in collecting resin [22]. In this study, the age of colony and number of bees per colony were not pre-determined prior to the study but were calculated after the harvesting process, which resulted in different numbers of bees in the colonies in each treatment.

In the research conducted by Agussalim et al. [23], the highest productivity of propolis was produced by the bees in a wooden box hive sized 35 × 20 × 17.5 cm with a value of 3.85 ± 3.66 g/colony/week. This value was higher than the colony from a wooden box hive sized 40 × 20 × 20 cm with a productivity rate of 2.30 ± 1.82 g/colony/week. These results were similar to this study, where the productivity of propolis in the MOTIVEs with aluminum meshes was not linearly proportional to the size of the hive. This could be due to factors such as the number of worker bees, flight activities of the forager bees, and sources and availability of resins.

Table 1 shows that propolis productivity was more affected by hive size because propolis productivity differences were not significant in mesh material variation. The highest productivity of propolis was from the colonies in the large MOTIVE with a nylon mesh at 2.52 ± 0.37 g/colony/week. According to Tsakgarakis et al. [24], propolis production is higher with smaller mesh pore size, which has a larger total surface area. Pore sizes of the aluminum, nylon, and polyethylene meshes were 2 × 2 mm, 1 × 1 mm, and 2 × 2 mm, respectively. The small pore size of the nylon mesh results in more holes to cover but allows for easier propolis placement by the bees.

3.2 Productivity of Honey Produced by *T. laeviceps*

In addition to propolis, *T. laeviceps* bees produce honey at a rate of 0.02–0.78 g/colony/week. The honey tends to have a dark brown color with a distinctly sour taste. The results show that honey productivity is directly proportional to the size of MOTIVE with a polyethylene mesh, but not for the MOTIVE with nylon and aluminum meshes. According to Bushal et al. [25], honey productivity is not affected by the size of the hive but is affected by the size of the colony, the number of populations in the colony, and the bees' flight activity. Honey production tends to increase when the population increases owing to the growing need for bees to forage, which results in more forager bees. Based on the research conducted by Salatnaya [26], honey productivity using a 25 × 15 × 15 cm wooden box hive was 0.02–0.19 g/colony/week, less than the honey productivity of small MOTIVEs.

In addition to the colony population, honey productivity is influenced by nectar flow, or the availability of nectar in the environment [27]. The bees will collect nectar in warm weather for supply during the rainy season. However, honey production for stingless bees, in general, is not as high as the *Apis* honeybees owing to their smaller body sizes, which results in shorter flight distances [28]. The maximum flying distance of *Tetragonula*

stingless bees is approximately 712 m [29]. However, its small body prevents the stingless bee from collecting nectar and pollen on small flowers [30]. Several factors that can cause low honey production are the focus of worker bees on hive construction, waste disposal activity from the hive, and low productivity of the queen bee to produce worker bees [31].

In this study, honey productivity was directly proportional to propolis productivity in MOTIVEs with polyethylene and aluminum meshes. This was because large amounts of honey can provide energy for the bees to forage other resources, such as pollen and resin. Stingless bees, which are eusocial organisms, are interdependent to fulfil their needs and maintain hive conditions. These activities improved the sustainability of the colony, and the colony remained healthy and protected from pests and diseases.

3.3 Total Flavonoid and Total Phenolic Content of Propolis Extract

The harvested raw propolis was extracted. The resulting propolis extracts had a wide range of TFC and TPC. Propolis from the bamboo hive had a TFC and TPC of 3.42 ± 1.15 mg QE/g and TPC 284.62 ± 39.46 mg of GAE/g, respectively. The complete TFC and TPC of each MOTIVE size and mesh material are shown in Table 2. The propolis extraction process will remove the inert materials and keep the polyphenol fraction [32]. The results of this study indicate that there is no correlation between hive size and propolis quality. The difference in propolis quality is caused by numerous factors, such as bee genetics (species), different sources of plant resins, and microclimates [26]. This is supported by the research of Hakim & Abduh [8] and Abduh et al. [10], in which the colony of *T. laeviceps* in a MOTIVE sized 21 × 14 × 18 cm that was cultivated in the Cileunyi Wetan Village had a TFC of 6.64–11.42 mg QE/g extract, whereas a MOTIVE in Cibodas Village had a TFC amounting to 14.8 mg QE/g extract.

The extraction process of propolis also affects the dissolved flavonoids and phenolics content. The antioxidant content and activity of the propolis extract depend on the extraction method and the type of solvents used [33]. Propolis extracted by the maceration method for three days resulted in TFC and TPC of 52.17 mg QE/g extract and 26.13 mg GAE/g extract, respectively, whereas five days of maceration resulted in TFC and TPC of 68.04 mg QE/g extract and 46.68 mg QE/g extract, respectively [34]. These results indicate that the duration of the maceration process can affect the solubility and the yield of flavonoid and phenolic compounds associated with the mass transfer process of the compounds into the solvent. The solid-to-solvent ratio, temperature, and the maceration speed can also affect the TFC and TPC.

TFC and TPC in the propolis from the bamboo hive were also measured with the result of 3.42 ± 1.15 mg QE/g extract and 284.62 ± 39.46 mg GAE/g extract. TPCs in the propolis from MOTIVEs were lower than TPC in the propolis from the bamboo hive. The position of propolis in the hive may play a role in the chemical composition of propolis. According to Pratami et al. [35], propolis located inside the hive had a higher TFC and TPC compared with propolis located outside the hive, which was exposed to the external environment. Propolis in the MOTIVEs was more exposed to the external environment because of the ventilation space between the mesh and the top cover compared with propolis in the bamboo hive. Therefore, the difference in propolis position in the hive may be a factor that caused differences in the TPC of the propolis. Exposure to light, air, and high temperatures can change the structure of phenolic compounds affecting their degradation process [35].

Moreover, the chemical composition of propolis is also influenced by the source of the plant resins. During the beekeeping process, the bees produce propolis from plant resins obtained near the beekeeping sites, such as mahogany (*Swietenia macrophylla*), velvet apple

(*Diospyros blancoi*), gmelina (*Gmelina arborea*), guanacaste (*Enterolobium cyclocarpum*), and kassod tree (*Senna siamea*). The colors of propolis produced varied, light to dark brown, red, as well as greenish yellow. Color differences in the propolis indicate that the chemical composition and the active compounds in each propolis might also be different [10].

Table 2: Total flavonoid and total phenolic content of propolis produced by *T. laeviceps* in various hive sizes and mesh materials

Hive sizes	Mesh materials	Total Flavonoid Content (TFC) [mg QE/g extract]	Total Phenolic Content (TPC) [mg GAE/g extract]
Small	Nylon	3.18 ± 1.43^{a}	112.13 ± 47.64^{b}
	Polyethylene	2.60 ± 1.66^{a}	32.23 ± 14.09^{b}
	Aluminum	2.46 ± 1.21^{a}	80.45 ± 53.93^{b}
Medium	Nylon	1.95 ± 1.02^{a}	39.10 ± 15.91^{b}
	Polyethylene	1.78 ± 1.02^{a}	39.12 ± 23.35^{b}
	Aluminum	3.02 ± 1.50^{a}	54.58 ± 26.76^{b}
Large	Nylon	1.77 ± 0.86^{a}	77.61 ± 34.61^{b}
	Polyethylene	2.84 ± 2.01^{a}	52.44 ± 25.01^{b}
	Aluminum	2.84 ± 1.00^{a}	71.41 ± 27.30^{b}

*Different letters indicate statistical differences (P-value < 0.05, Duncan's test).

3.4 Characteristics of Honey Produced by *T. laeviceps*

The honey harvested from *T. laeviceps* was measured for its moisture, reducing sugars, antioxidants, and vitamin C contents. Table 3 shows that the moisture content, reducing sugars, and active antioxidants of *T. laeviceps* honey from MOTIVEs were lower than in the honey from the bamboo hive. However, the vitamin C content of honey from the MOTIVEs was higher than in honey from the bamboo hive.

High moisture content can reduce the quality of honey because the presence of water can accelerate the rate of yeast growth so that fermentation occurs. The type of yeast that causes fermentation in honey is *Zygosaccharomyces* [36]. The higher moisture content in the honey from the bamboo hive could be caused by the differences in the bamboo hive and MOTIVE structures. Bamboo hives are narrower and have less ventilation space and, hence, higher humidity compared to the MOTIVEs. Honey is also hygroscopic, able to absorb water from the environment. Therefore, the moisture content in honey could be influenced by environmental humidity [37].

According to Souza et al. [7], the moisture content in *Apis* bee honey is only 17%, lower than in honey from *Tetragonula* bees, which is 24%. The difference is caused by the more open structure of honey storage pots in *Apis* bees, so the rate of water evaporation in *Apis* honey will be faster than in *Tetragonula* honey. Honey from the MOTIVEs met the maximum moisture content requirement of 22% outlined by the Indonesian National Standard SNI 01-3545-2004 [38]. Yeast in honey will degrade sugars, such as glucose and fructose, into alcohol and CO_2. This will increase the reducing sugar content, which is the total amount of fructose and glucose in honey [37]. Table 3 shows that the reducing sugars in honey from the bamboo hive was higher than in honey from the MOTIVEs. This shows the correlation between moisture content and reducing sugar content in honey. Bees from the Meliponini family, such as *T. laeviceps*, generally have a reducing sugar content of 66% (w/w) with a sucrose content of 2.3% [9]. Based on the standard quality of honey from the

Indonesian National Standard SNI 01-3545-2004 [38], the minimum of total reducing sugar is 65%, and thus, honey from the bamboo hive and MOTIVEs still meets this requirement.

Table 3: Characteristics of honey produced by *T. laeviceps*

Parameters	Bamboo hive	MOTIVE
Moisture content [%]	24.08	21.86
Reducing sugar [%]	74.70	72.84
Active antioxidant/IC_{50} [µg/mL]	3544.88	2613.41
Vitamin C [µg/mL]	184.50	192.86

Honey contains many antioxidant compounds such as catalase, glucose oxidase, peroxidase, and phenolic compounds. The active antioxidant of *T. laeviceps* honey from a bamboo hive is higher than in honey from a MOTIVE. However, both honeys have higher antioxidant content compared to honey researched by Abduh et al. [10]. The antioxidant content of honey cultivated from this study in Cibodas and Cileunyi Wetan, West Java, were 1188.2 µg/mL and 1,341.88 µg/mL, respectively. The lower active antioxidant content in honey from this study could be caused by the location of cultivation, sources of nectar, and the age of honey.

Vitamin C (ascorbic acid) is one of the numerous compounds present in honey. The vitamin C content in honey from MOTIVEs is higher than in honey from bamboo hives, which are 192.86 µg/mL and 184.5 µg/mL, respectively. Different sources of nectar could cause the difference in vitamin C content [7]. Therefore, both the content of vitamin C and antioxidants in honey is strongly influenced by the plant nectar sources around the cultivation site.

4. CONCLUSION

In brief, increasing the hive's size increases the productivity of propolis. The highest productivity of propolis (2.53 ± 0.37 g/colony/week) was obtained when *T. laeviceps* was cultivated in large hives equipped with a nylon-based mesh. The highest productivity of honey (0.78 ± 0.18 g/colony/week) was obtained when *T. laeviceps* was cultivated in medium hives equipped with an aluminum-based mesh. The harvested propolis was extracted using a maceration method to determine the total flavonoid and phenolic content of the propolis which lies in the range of 1.77 ± 0.86 to 3.18 ± 1.43 mg QE/g propolis and 32.23 ± 14.09 to 112.13 ± 47.64 mg GAE/g propolis, respectively. Relevant properties of the harvested honey were also characterized and shown to meet the standard quality of honey. The result of this study provides an insight for larger scale cultivation of *T. laeviceps* to produce higher productivity of propolis from stingless bees.

ACKNOWLEDGMENT

This work was financially supported by Institut Teknologi Bandung (PPMI SITH 2022).

REFERENCES

[1] Nakajima Y, Tsuruma K, Shimazawa M, Mishima S, Hara H. (2009) Comparison of bee products based on assays of antioxidant capacities. BMC Complementary and Alternative Medicine, 9: 1-9.
[2] Silva CR, Baltazar F, Almeida AC. (2015) Propolis: A complex natural product with a plethora

of biological activities that can be explored for drug development. Evidence-Based Complementary and Alternative Medicine, 1-29. https://doi.org/10.1155/2015/206439
[3] Rao P.V, Krishnan KT, Salleh N, Gan SH. (2016) Biological and therapeutic effects of honey produced by honey bees and stingless bees: a comparative review. Revista Brasileira de Farmacognosia, 26: 657-664.
[4] Borror DJ, Triplehorn LA, Johnson NF. (1989) An introduction to the study of insects. 6th edition. Saunders College Publishing, Philadelphia.
[5] Fadhilah R, Rizkika K. (2015) Laba Lebah Tanpa Sengat, My Trubus Potential Business. Trubus Swadaya, Jakarta.
[6] Singh S, Ketan V, Saurabh S, Jain N, Tawale P. (2018) Propolis market research report - forecast to 2023. Market Research Future.
[7] Souza BA, Roubik DW, Barth OM, Heard TA, Enríquez E, Carvalho CA, Villas-Bôas J, Marchini L, Locatelli J, Persano-Oddo L, Almeida-Muradian L, Bogdanov S, Vit P. (2006) Composition of Stingless Bee Honey: Setting Quality Standards. Interciencia, 31: 867-875.
[8] Hakim MN, Abduh MY. (2019) Produksi propolis dari lebah *Tetragonula laeviceps* menggunakan sarang MOTIVE yang dilengkapi dengan sistem instrumentasi. Jurnal Otomasi Kontrol dan Instrumentasi, 10: 133-148.
[9] Hilmi M, Bradbear N, Mejia D. (2011) Beekeeping and sustainable livelihood, Food and Agriculture Organization of the United Nations, Rome.
[10] Abduh MY, Adam A, Fadhlullah M, Putra RE, Manurung R. (2020) Production of propolis and honey from Tetragonula laeviceps cultivated in Modular Tetragonula Hives. Heliyon, 6: 1-8.
[11] Adam A, Hakim MN, Oktaviani L, Inderaja BM, Manurung, R, Putra RE, Abduh MY. (2020) Techno-economic evaluation for integrated cultivation of coffee and stingless bees in West Java, Indonesia. Biological And Natural Resources Engineering Journal, 3: 28-36.
[12] Budiaman, Rahman A. (2006) Uji efektivitas empat variasi propolis trap terhadap produksi propolis lebah madu *Apis mellifera* L. Jurnal Perennial, 2: 1-4.
[13] Huang L, Bui N, Meyering MT, Hamlin TJ, Mccutcheon J. (2013) Novel hydrophilic nylon 6,6 microfiltration membrane supported thin film composite membranes for engineered osmosis. J. Memb. Sci., 437: 141-149.
[14] Surdia T, Saito S. (1995) Pengetahuan bahan teknik. Pradnya Paramita, Jakarta, Indonesia.
[15] Abd-Elmawgood BH, Al-Rajhi MA, El-Ashhab AO. (2015) Effect of the internal size and thermal insulation of the hive on bee colonies strength and productivity. Egyptian Journal of Agricultural Research, 93: 185-195.
[16] Machado BA, Silva RP, Barreto GD, Costa SS. Silva DF, Brandao HN, Rocha JLC, Dellagostin OA, Henriques JAP, Umsza-Gues MA, Padilha FF. (2016) Chemical composition and biological activity of extracts obtained by supercritical extraction and ethanolic extraction of brown, green and red Propolis derived from different geographic regions in Brazil. PLoS ONE, 11: 1-26.
[17] Sadeli RA. (2016) Uji Aktivitas Antioksidan dengan Metode DPPH (1,1-diphenyl-2-picrylhydrazyl) Ekstrak Bromelain Buah Nanas (*Ananas comosus* (L.) Merr.). Faculty of Pharmacy, Universitas Sanata Dharma, Yogyakarta.
[18] Silva CR, Simoni JA, Collins CH, Volpe PL. (1999) Ascorbic acid as a standard for iodometric titrations: an analytical experiment for general chemistry. Journal of Chemical Education, 76: 1421-1422.
[19] Sanpa S, Popova M, Bankova V, Tunkasiri T, Eitssayeam S, Chantawannakul P. (2015) Antibacterial compounds from propolis of *Tetragonula laeviceps* and *Tetrigona melanoleuca* (Hymenoptera: Apidae) from Thailand. PLoS ONE, 10: 1-11.
[20] Bhusal S. Thapa R. (2006) Response of colony strength to honey production: Regression and correlation analysis. Journal of the Institute of Agriculture and Animal Science, 27: 133-137.
[21] Akratanakul, P. (1990) Beekeeping in Asia, Food and Agriculture Organisation of the United Nation, Rome.
[22] Leonhardt SD, Dworschak K, Eltz T, Bluthgen N. (2007) Foraging loads of stingless bees and utilisation of stored nectar for pollen harvesting. Apidologie, 38: 125-135.

[23] Agussalim AA, Umami N, Erwan E. (2015) Production of stingless bees (*Trigona* sp.) propolis in various bee hives design. The 6th International Seminar on Tropical Animal Production, 20-22 October 2015, Universitas Gadjah Mada.
[24] Tsagkarakis AE, Katsikogianni T, Gardikis K, Katsenios I, Spanidi E, Balotis GN. (2017) Comparison of traps collecting propolis by honey bees. Advances in Entomology, 5: 68-74.
[25] Bushal SJ, Kafle L, Thapa RB, Shih CJ. (2011) Effect of Colony Strength on the Performance of Honeybees (*Apis mellifera*) in Nepal (Hymenoptera:Apidae). Sociobiology, 58: 435-447.
[26] Salatnaya, H. (2012) Produktivitas lebah Trigona sp. sebagai penghasil propolis perkebunan pala monokultur dan polikultir di Jawa Barat, Institut Pertanian Bogor, Bogor.
[27] Jager AJ, Taylor GJ, Greeff P, Lishman, AW. (2001) The effect of commercial propolis production on hive profitability. Proceedings of The 37th International Apicultural Congress.
[28] Faheem M, Aslam M, Razaq M. (2004) Pollination Ecology with Special to Insects: A Review, J. Res. (Sci), 15: 359-409.
[29] Smith, J. P., Heard, T. A., Beekman, M., Gloag. R., 2016. Flight range of the Australian stingless bee *Tetragonula carbonaria* (Hymenoptera: Apidae): Flight range of the bee *Tetragonula carbonaria*. Austral Entomology, 56: 1-4.
[30] Jalil AM, Kasmuri AR, Hadi H. (2017) Stingless Bee Honey, The Natural Wound Healer: A Review. Skin Pharmacology and Physiology, 30: 66-75.
[31] Agussalim AA, Umami N, Budiasatra IG. (2017) The Effect of Daily Activity Stingless Bees of Trigona sp. on Honey Production. The 7th International Seminar on Tropical Animal Production, 12-14 September, Universitas Gadjah Mada; pp. 223-227.
[32] Paviani LC, Fiorito G, Sacoda P, Cabral FA. (2013) Different Solvents For Extraction of Brazilian Green Propolis: Composition and Extraction Yield of Phenolic Compounds, III Iberoamerican Conference on Supercritical Fluids. Colombia.
[33] Narimane S, Demircan E, Salah A, Ozcelik B, Salah R. (2017) Correlation between antioxidant activity and phenolic acids profile and content of Algerian propolis: Influence of solvent. Pakistan Journal Pharmaceutical Sciences, 30: 1417-1423.
[34] Zin NB, Azemin A, Rodi MM, Mohd KS. (2018) Chemical Composition and Antioxidant Activity of Stingless Bee Propolis from Different Extraction Methods. International Journal of Engineering & Technology, 7: 90-95.
[35] Pratami DK, Mun'im A, Sundowo A, Sahlan M. 2018. Phytochemical profile and antioxidant activity of propolis ethanolic extract from *Tetragonula* Bee. Pharmacognosy Journal, 10: 128-135.
[36] Salas, PG, Soto AM, Carretero AM, Gutierrez AF. (2010) Phenolic-Compound-Extraction Systems for Fruit and Vegetable Samples. Molecules, 15: 8813-8826.
[37] Nanda PB, Radiati LE, Rosyidi D. (2015) Perbedaan Kadar Air, Glukosa, dan Fruktosa pada Madu Karet dan Madu Sonokeling, Universitas Brawijaya, Malang.
[38] Standar Nasional Indonesia (SNI). (2004) Madu (SNI 01-3545-2004). Badan Standardisasi Nasional, Jakarta.