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Identification of bioactive compounds and encapsulation of bee bread from *Heterotrigona itama* using a spray dryer with its antioxidant activity

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Abstract. *Sari E, Wardhani DAS, Agustina V, Agussalim, Hakim L, Wulandari R, Wibowo NA, Chua LS. 2024. Identification of bioactive compounds and encapsulation of bee bread from* Heterotrigona itama *using a spray dryer with its antioxidant activity. Biodiversitas 25: 2857-2865.* Stingless bee produces honey, propolis, and bee bread. Bee bread is known to have a high antioxidant. This study uses a spray dryer and its antioxidant activity, phytochemical, and bioactive compounds; therefore, it aims to encapsulate bee bread from *Heterotrigona itama* Cockerell 1918. The encapsulation of bee bread used several encapsulants, such as starch, lactose, and maltodextrin, in several formulas. The physical characterization was performed by analysis of hygroscopic properties by Scanning Electron Microscopy (SEM), X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), and DPPH antioxidant. The results showed that bee bread contains bioactive compounds, such as saponins, alkaloids, carbohydrates, quinones, flavonoids, tannins, phenols, and ninhydrin. The best encapsulant was a combination of starch and lactose with the formula 50:50 (% w/w), which has low hygroscopic properties. SEM analysis shows that bee bread does not bond well with filler at a 10.817 to 20.244 µm diameter. XRD analysis obtained a product crystallinity level of 71.0819%. FTIR analysis shows that bee bread has the most complex functional group, which was characterized by the presence of a wave at the point of 1,115.68 cm^{-1}, which indicates the presence of a group (C−O−C) that shows the spectrum of flavonoid group compounds with antioxidant activity of 588.402 ppm.

Keywords: Bee pollen, filler, *Heterotrigona itama***,** stingless bee, spray dryer

INTRODUCTION

Indonesia is a developing country whose population of productive age is at high risk of degenerative diseases. Basic Health Research reported that degenerative diseases in Indonesia reached 65.7%, including, Parkinson's, cancer, diabetes Alzheimer's, and osteoporosis, which are caused by an unhealthy lifestyle, including lots of fast food consumption, lack of exercise and nutritional intake, and frequent exposure to environmental pollution (Indonesia Health Ministry 2018). Therefore, to prevent these diseases, people must exercise diligently, consume more fruit, vegetables, whole grains, and foods rich in nutrients and vitamins, and get enough rest. One natural food that fulfills nutrients is bee bread, superfood that prevents degenerative diseases.

Honeybees and stingless bees can produce bee bread; in stingless bees, bee bread is also known as pot pollen. Bee bread was made from pollen as the raw material, which is collected by the worker bees (foragers) from plant flowers as the primary source of protein in their hives (Agussalim et al. 2017, 2018; Agus et al. 2019b; Erwan et al. 2021a, 2022; Supeno et al. 2022) those bee bread the workers use to produce royal jelly, also used as a functional food to improve human health (Anugrah et al. 2024). Furthermore, the stingless bee can produce honey from nectar as the raw material, obtained from plant flowers (flora nectar) and extrafloral nectar. Honey is their food source in the colony, stored in the honey-pot. In addition, propolis is also produced by the stingless bee, which is made of resin from plants collected by foragers and used to construct their hive and entrance, balm their hive, and pots used to store pollen and honey (Agus et al. 2019a, 2021; Agussalim et al. 2019a, 2019b, 2020, 2021, 2022, 2023; Erwan et al. 2020, 2021b, 2023; Dewi et al. 2021; Sabir et al. 2021; Supeno et al. 2021, 2022; Agussalim and Agus 2022; Rachmawati et al. 2022; Pratama et al. 2023). The foragers visit each flower until their pollen basket (corbicula) is filled with

pollen when collecting pollen, which is then taken to the nest to be processed into bee bread by fermentation (Agussalim et al. 2017, 2018; Bogdanov 2017; Agus et al. 2019b).

Bee bread contains a variety nutrients such as carbohydrates, sugar (glucose, maltose, fructose), protein, amino acids (taurine, tryptophan, l-tyrosine, l-isoleucine, l-leucine, l-phenylalanine, 3-amino isobutyric acid, gamma-aminobutyric acid, l-methionine, beta-alanine, l-2-aminoadipic acid, l-aspartic acid, l-valine, l-glutamic acid, l-2-aminobutyric acid, l-alanine, ethanolamine, l-serine, l-threonine, l-asparagine, l-glycine, l-proline, l-glutamine, l-arginine, sarcosine, l-cystine, l-cystathionine, l-ornithine, l-lysine, histidine, l-anserine, and l-carnosine), fatty acids (long-chain saturated fatty acids: C_{20} to C_{24} and medium-chain saturated fatty acids: C_{10} to C_{18}), organic acids (gluconic acid, formic acid, acetic acid, lactic acid, propionic acid, tartaric acid, succinic acid, butyric acid, and citric acid), minerals (Ca, Mg, K, Fe, P, Mn, Se, Cu, and Zn). Furthermore, bee bread contains vitamins (vitamins A, B, C, D, E, B_1, B_2, and K), antioxidants, bioactive compounds such as flavonoids, apigenin, chrysin, naringenin, gallic, caffeic, and ferulic acids, flavonol, kaempferol, quercetin, and glutathione (Khalifa et al. 2021; Bakour et al. 2022; Barta et al. 2022). These bioactive compounds have the benefit of neutralizing free radicals, thus protecting the body from various degenerative diseases and cancer, increasing the immune system, maintaining heart and liver functions, and accelerating wound healing (Bakour et al. 2022; Barta et al. 2022; Khutami et al. 2022).

The chemical compositions of the bee bread extract from three bee species in Indonesia, namely *Apis mellifera* Linnaeus 1758, *Apis cerana* Fabricius 1793, and *Tetragonula* sp., have been studied by Jaya et al. (2020); the total polyphenol and total flavonoids in *A. cerana* were significantly higher than in *A. mellifera* and *Tetragonula* sp., but their DPPH IC_{50} was lower. However, information on bee bread post-harvest processing is rare, especially the encapsulation. In our study, bee bread was prepared as the bio-supplement with the encapsulation process using several encapsulant materials (starch, lactose, maltodextrin, and the combination) to obtain stable product properties and characterizations and their antioxidant properties.

MATERIALS AND METHODS

Study area

The bee bread used in our study was directly taken from the stingless bee of *Heterotrigona itama* Cockerell 1918 farm in the Ujung Kulon National Park, Banten, Indonesia (6º47'05"S 105º22'30"E). Bee bread was harvested by cutting the propolis as the pot's construction sticks to the box walls. Afterward, the pots were opened, and propolis, as the bee bread cover, was removed. Bee bread was put into plastic bottles and then powdered using a spray dryer. This study was divided into four steps: bee bread drying; phytochemical identification; encapsulation, and analysis of hygroscopic and morphology properties using Scanning Electron Microscopy (SEM) and its crystallinity properties by X-ray diffraction (XRD); and then bioactive identification by Liquid Chromatography-Mass Spectrometer (LC-MS/MS), functional groups of bee bread powder was determined by Fourier Transform Infrared Spectroscopy (FTIR), and antioxidant activity by DPPH method.

Procedures

Bee bread drying

A tray dryer did the drying process of bee bread. This stage began by lining the tray using a sieve of 60 mesh and then inserting the tray into the tray dryer. Furthermore, the tray dryer was set at 50°C for 1.5 hours, and the drying process was continued until their weight was constant to ensure that the bee bread was fully dried.

Phytochemical analysis

We conducted several analyses on ash and water contents (AOAC 2005; Erwan and Agussalim 2022; Agussalim et al. 2021, 2023). Phytochemical screening was done by a method that was explained by Bakour et al. (2019) and Syafrizal et al. (2020). The bee bread was extracted by ultrasonic-assisted extraction (UAE), with the ratio between bee bread and ethanol 96% was 1 g: 5 mL. The UAE conditions were set at 40ºC for 30 minutes, with the frequency ranging from 20 kHz to 10 MHz. The resulting phytochemical screening of bee bread was alkaloids, flavonoids, carbohydrates, tannins, saponins, glycosides, quinones, phenols, triterpenoids, and ninhydrin. The presence of saponin was determined by mixing distilled water with the bee bread sample and presenting permanent foam in the solution. Alkaloids were performed by mixing Mayer's reagent with the bee bread, and a white precipitate was obtained. The carbohydrates were mixed with Molisch's reagent by the bee bread, and the color changed to purple or reddish. The quinones, tannins, and phenols were mixed with a 5% $FeCl_3$ solution into the bee bread, and the color changed to red, blue, or greenish-black, and blue or green, respectively. The flavonoids were mixed with NaOH solution in the bee bread, and the color changed to yellow. The glycosides were mixed with 10% $CHCl_3$ and NH_4OH solutions to the bee bread, and a red-brown ring was present. The ninhydrin was mixed with the ninhydrin reagent in the bee bread, changing the color to blue.

Bioactive identification

Bioactive identification by LC-MS/MS was used based on the previous method by Sari et al. (2021). Bee bread was extracted three times using a 1:1 ratio of methanol to water (LC-MS grade). Furthermore, the samples were vortexed for 1 min, and the solvent was added, followed by 30 min of sonication. After extraction, the samples were centrifuged at 12,000 rpm for 10 min, and the supernatants were collected and diluted 20 times for LC-MS injection.

Bee bread encapsulation

Bee bread encapsulation was performed by a spray dryer consisting of 3 formulas with different encapsulants. The first formula (F1) was starch and lactose with a ratio of 50:50 (% w/w), the second formula (F2) was lactose and maltodextrin with a ratio of 50:50 (% w/w), and the third

formula (F3) was the combination of starch, lactose, and maltodextrin with a ratio of 33.3:33.3:33.3 (% w/w). The bee bread sample and filler ratio used was 25:75 (% w/w), then the ingredients mixtures were dissolved in aquadest to obtain a solution with concentration of 30%. Furthermore, the composition used in this study was 75 g of formula and 175 mL of aquadest, thus producing 250 mL of solution. After that, the mixture was dissolved using an agitator, which was then dried using a mini spray dryer type (Buchi 190) at an inlet at 160°C and an outlet at 60 to 70°C.

Data analysis

All data on the phytochemicals, bioactive compounds, and encapsulation parameters were analyzed by descriptive analysis.

RESULTS AND DISCUSSION

Phytochemical and bioactive compounds

Bee bread in the stingless bee is also called pot-pollen and is used by the stingless bee as the primary protein source in the hives which are stored in the pots (Belina-Aldemita et al. 2019; Agussalim and Agus 2022; Supeno et al. 2022). Figure 1 presents the fresh bee bread (Figure 1.A) and the processed results (Figure 1.B) from a stingless *H. itama* bee in our study.

Figure 1.B shows that the bee bread moisture contents decreased after drying, and the color changed from brownish yellow to golden yellow. The purpose of drying is to maintain the bee bread quality so it doesn't get damaged when stored for an extended period. The freshness and durability of food ingredients are influenced by moisture, where food ingredients with high moisture will facilitate the growth of bacteria, yeast, and mold, thereby accelerating spoilage. The most bacterial growth can be prevented when food's water activity (aw) is under 0.9, yeasts 8.8, and molds 0.7. However, stable conditions prevent microbial growth when a_w is under 0.6 (Belina-Aldemita et al. 2019). The study revealed that the moisture of bee bread from *H. itama* was 9%, lower than the previous study's reported. Belina-Aldemita et al. (2019) reported that the bee bread moisture from a stingless bee of *Tetragonula biroi* Friese 1898 from the Philippines ranged from 14.15 to 16.73%, and *Tetragonisca angustula* Latreille 1811 from Venezuela was 23.34 to 24.69% (Vit et al. 2018). Furthermore, Chuttong et al. (2018) reported the moisture of bee bread in several stingless bees in Thailand, such as 16.1% for *Tetragonula laeviceps* Smith 1857, 31.7% for *Tetragonula testaceitarsis* Cameron 1901, 25.3% for *Lepidotrigona terminata* Smith 1878, and 22.8% for *Lepidotrigona flavibasis* Cockerell 1929. The difference in bee bread moisture was influenced by stingless bee species involved in bee bread production, plant types as the pollen source, and geographical conditions (Agussalim and Agus 2022).

The ash content determines the mineral amount of residue from the burning. The mineral content of bee bread *T. biroi* Friese from the Philippines are B, Mg, Ca, Mn, Fe, Cu, Zn, Rb, and Sr (Belina-Aldemita et al. 2019). The ash content or minerals content depends on the ingredients and burning method. In addition, the ash contents are also used to determine the parameters of the nutritional ingredient values. The result showed that the ash content of bee bread from *H. itama* was 2.16%. The ash contents of bee bread in our study were similar to those previously studied, such as Belina-Aldemita et al. (2019) *T. biroi* (Philippines) ranged from 3.23 to 3.97%. Furthermore, Chuttong et al. (2018) reported that the bee bread ash contents from *L. terminata* at 1.8%, *T. laeviceps* at 2.3%, *L. flavibasis* at 2.2%, and *T. testaceitarsis* at 2.2% from Thailand, *Melipona seminigra* Friese 1903 at 4.03% and *Melipona interrupta* Latreille 1811 of 2.74% from Brazil (Rebelo et al. 2016); and *T. angustula* of 2.06% from Venezuela (Vit et al. 2018). The difference in ash content is influenced by plant type, which produces pollen, botanical origin, and soil nutrients (Kalaycıoğlu et al. 2017; Belina-Aldemita et al. 2019; Liolios et al. 2019; Sabir et al. 2021).

A **B**

Figure 1. A. Fresh bee bread; B. After processing

The phytochemical analysis showed that the bee bread from a stingless bee of *H. itama* from Ujung Kulon National Park, Indonesia, contained eight compounds: saponins, alkaloids, carbohydrates, quinones, flavonoids, tannins, phenols, and ninhydrin (Table 1).

Saponin is a steroid or triterpene glycoside compound found in various plants. This compound has pharmacological effects, namely anti-bacterial and anti-viral, reducing blood glucose and blood clotting (Fiana and Oktaria 2016). Alkaloid compounds represent the primary secondary metabolites discovered in plant and animal tissues. Several alkaloid compounds have anti-diarrheal, anti-diabetic, anti-malarial, and anti-microbial properties. In addition, alkaloids have detoxification abilities that can remove toxins from the body (Ningrum et al. 2016). The function of carbohydrates is to produce energy for humans, sweet taste in food, regulate fat metabolism, and help excrete feces (Siregar 2014).

Quinones have pharmacological effects, such as antibiotics for both pain and new cell growth (Zhang et al. 2021), and flavonoids as anti-inflammatory and antioxidant (Agussalim et al. 2022), antiviral, cardioprotective, anti-diabetic, anti-cancer, and anti-aging (Zakaria et al. 2018). Tannin and phenolic are anti-inflammatory, antioxidant, anti-carcinogenic, and anti-microbial (Wink 2015). In addition, the ninhydrin compound is characterized by a color change to blue, indicating free amino acids that have metabolic functions in the body (Jeong et al. 2023).

The bioactive compounds of bee bread from *H. itama*, originating from Ujung Kulon in our study, are presented in Table 2. The bioactive compounds detected by LC-MS/MS of bee bread from *H. itama* were found to be 21 compounds (Table 2), where the high content of ajmalicine, including the main indole alkaloid which has strong antiarrhythmic and antihypertensive effects (Du et al. 2014).

The most commonly detected bioactive compounds in bee bread from stingless bees were kaempferol, quercetin, and isorhamnetin. Bee bread from stingless bees has health benefits such as antioxidant, anti-inflammatory, antibacterial anti-fungicidal, hepatoprotective, and anti-atherosclerotic (Mohammad et al. 2021). The bioactive compounds in bee bread are affected by bee species and plant types as the pollen source, which is used as the raw material to produce bee bread.

Table 1. The phytochemical of bee bread from stingless bee of *H. itama* from Ujung Kulon National Park, Indonesia

Phytochemical results		Descriptions
Saponins	+	Permanent foam for 0.4 cm
Alkaloids	+	White precipitate
Carbohydrates	+++	Forming a purple or reddish color
Quinones	++++	Forming a red color
Flavonoids	++++	Forming a yellow color
Tannins	++++	Forming a blue or greenish black
Glycosides	-	There is a red-brown ring
Phenols	++++	Forming a blue or green color
Triterpenoids	-	A red-brown color forms on the interface
Ninhydrin	++++	Forming a blue color

Note: + and ++ is low level; +++ is moderate level; ++++ is high level; - is not detected

Table 2. Bioactive compounds that LC-MS/MS detects in bee bread of *H. itama* from Ujung Kulon

Molecular formula	Estimation of cand structures
$C_{21}H_{24}N_2O$	(Ajmalicine)
$C_{14}H_{30}N_4S_3$	(1-butyl-3-[2-[2-(butylcarbamothioylamino)ethylsulfanyl]ethyl]thiourea)
$C_{23}H_{29}N_3O_3$	Ethyl [5-(N,N-diethylglycyl)-10,11-dihydro-5H-dibenzo[b,f]azepin-3-yl]carbamate
$C_{50}H_{103}N_3O_8$	(Cyclohexane;1-(cyclohexylamino)-3-ethoxypropan-2-ol;1-[1-(cyclohexylamino)-3-ethoxypropan-2-yl]oxy-3-ethoxypropan-2-ol)
$C_{22}H_{26}N_8O_6$	(1,4:3,6-Dianhydro-2-[(4-carboxy-3,3-dimethylbutanoyl)amino]-2,5-dideoxy-5-{5-[4-(1H-1,2,4-triazol-1-yl)phenoxy]-1H-tetrazol-1-yl}-L-iditol)
$C_4H_{10}ClN_3$	(2-Amino-1,4,5,6-tetrahydropyrimidine Hydrochloride)
$C_{24}H_{40}N_6O_4$	(N-(6-Amino-1-butyl-2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl)-2-[4-(1-azepanylcarbonyl)-1-piperidinyl]-N-ethylacetamide)
$C_{15}H_{35}N_3O_3$	(3-{2,2-Bis[(3-aminopropoxy)methyl]butoxy}-1-propanamine)
$C_{18}H_{37}NO_3$	((E)-2-aminooctadec-8-ene-1,3,4-triol)
C_8H_9N	(2-Methyl-5-vinylpyridine)
$C_{10}H_8O_3$	(Hymecromone)
$C_{16}H_{12}O_7$	(Isorhamnetin)
$C_{20}H_{35}NO_2$	(α-Linolenoyl Ethanolamide)
$C_{26}H_{30}N_2O_8$	(strictosamide)
$C_{11}H_{20}N_2O_3$	(Leucylproline)
$C_{17}H_{37}NOS$	(2-{[(2-Methoxyethyl)(3-pentanyl)amino]methyl}-2-propyl-1-pentanethiol)
$C_{12}H_8N_6O_5$	(2-(4-Nitro-1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)-N-(4H-1,2,4-triazol-4-yl)acetamide)
$C_{20}H_{22}N_6O_4$	(A2AR-agonist-1)
$C_{18}H_{35}NO$	((9Z)-9-Octadecenamide)
$C_{21}H_{36}O$	(Furan Fatty Acid F5)
$C_6H_2N_2O_4$	((Dicyanomethylidene)propanedioic acid)

Mohammad et al. (2021) reported that the bioactive compounds were found in bee bread from several stingless bee species collected from various origins consisting of 7-isorhamnetin, 6-naringenin, 2-tricetin, 4-8-methoxyherbacetin, 3-selagin, kaempferol, ferulic acid, ellagic acid, caffeic acid, isorhamnetin, luteolin, apigenin, quercetin, luteolin, 8 methoxykaempferol Quercetin, 8 methoxykaempferol Genkwanin, dihydroquercetin, p-hydroxycinnamic acid, isorhamnetin, isorhamnetin-3-O-(6"-O-E-p-coumaroyl)- β-D-glucopyranoside, digalloylshikimic acid, apigenin-6-C-glucoside, kaempferol 3,7-di-O-rhamnoside, quercetin-3,4-diglucoside, procyanidin dimmer digallate (a-type), isoorientin-2”-O-rhamnoside, gluconic acid, gluconic acid derivate, monogalloylglucose, protocatechuic acid 3-glucoside, ellagic acid dimer, kaempferol derivative, 6-hydroxykaempferol 3,6-diglucoside 7-glucuronide, quercetin 3,4'-diglucoside, ellagic acid, linoleic acid, protocatechuic acid 3-glucoside, quercetin-3-O-β-D-glucoside, linolenic acid, and rutin.

Bee bread encapsulation

The bee bread processed by a spray dryer in this study has a brownish-yellow color (Figure 1), and the moisture lost (Figure 2) from the F1, F2, and F3 were 62.6%, 63.9%, and 60.7%, respectively. Furthermore, the hygroscopic test of bee bread encapsulated in our study is presented in Figure 2. Hygroscopicity is the ability of a substance to absorb vapor from the air, and it is performed four times each 15 minutes for 1 h and 24 h.

The increase in weight of encapsulated bee bread after 1 h and 24 h hygroscopic test in this study was lower in F1 compared to F2 and F3 (Figure 2). Moreover, the F1 treatment showed that they experienced a slight color change to become more brownish compared to the initial condition, namely brownish yellow. Additionally, the treatment of F2 and F3 showed that the use of maltodextrin has a hygroscopic percentage value that is relatively greater compared to F1 due to maltodextrin filler can absorb more moisture from the air (Masrukan and Mindhayani 2019). Tan et al. (2015) explained that food material is stable when moisture is under 6%. In addition, the moisture of encapsulated bee bread in this study is lower according to the standard regulated by the Indonesian Food and Drug Authority (number 12 of 2024), stating that Simplicia's moisture was under 10%. However, the moisture in our study is higher when compared to propolis filler powder, which has a moisture of 3.33% (Pant et al. 2022).

The percentage solubility of encapsulated bee bread in this study was 30.52% due to the lactose being more soluble than starch. However, in the previous study, the solubility of skim milk was 79.29%. The low solubility from encapsulated bee bread in this study due to the higher temperature during the drying process using a spray dryer, which impacted particle hardening and promoted the development of an impenetrable layer over the particles that inhibit the diffusion rate of water molecules into the particles. This phenomenon decreased the particles' ability, further impacted by decreased solubility and dispersibility (Chegini and Ghobadian 2005). The SEM analysis was conducted to characterize the morphological surface of bee bread, which is covered by starch and lactose fillers (Figure 3). The morphology condition influences the characteristics of bee bread, like retention and release rate of active compounds.

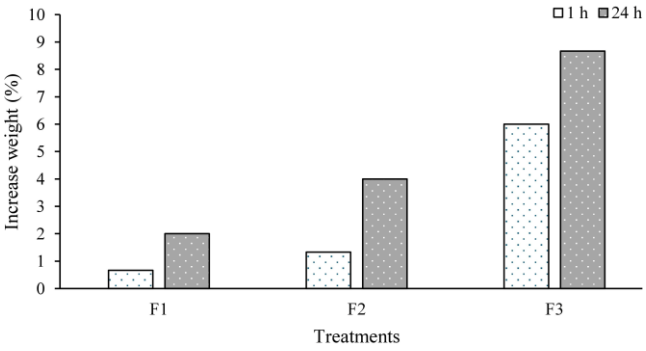
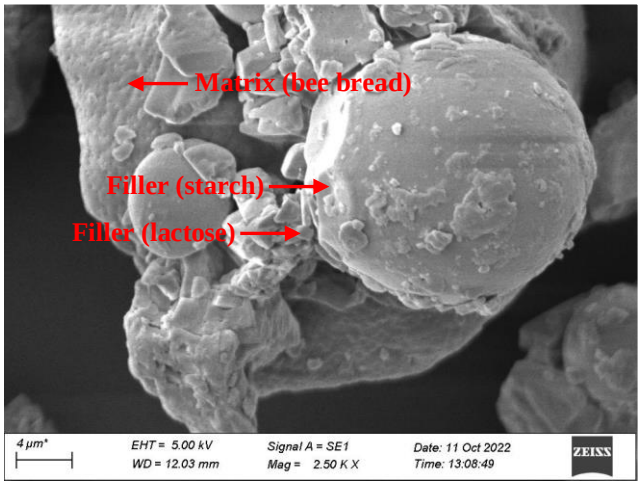
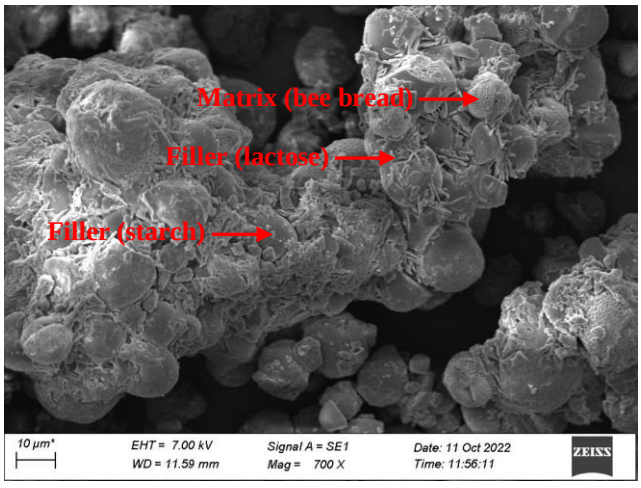


Figure 2. The increase in weight of encapsulated bee bread (F1: starch and lactose, ratio 50:50; F2: lactose and maltodextrin, ratio 50:50: F3: starch, lactose, and maltodextrin, ratio 33.3:33.3:33.3)



A



B

Figure 3. Bee bread morphology by SEM analysis A. Magnification of 700×; B. Magnification of 2,500×

The results of SEM morphology showed that the encapsulated bee bread from the F1 does not bond well between the core material with filler, which is impacted on bee bread and is not covered by the filler due to evaporation during the drying process using a spray dryer. Moisture evaporation can occur due to high temperatures during drying (inlet temperature of 160°C). At the magnification of 700× (Figure 3.A), the powder particle has a diameter ranging from 8 to 25 µm, while at 1000× has a diameter of 10.817 to 20.244 µm. Our finding follows a previous study reported by Schuck and Ouest (2011) that the bee bread filler produces particles with a diameter ranging from 10 to 250 µm.

The XRD analysis in this study shows the crystallinity structure of bee bread with a distance of 2θ (Figure 4). The diffractogram of F1 encapsulated bee bread powder shows that the peaks with the highest intensity were found at angles 2θ: 12.768º, 16.097º, 18.711º, 19.981º, 19.427º, 21.02º and 23.066º; the highest phase detected was polyphenol. This study diffractogram of the encapsulated bee bread powder has a similar pattern to the polyphenol-rich milk powder (Thakur and Nanda 2019). Our study's crystallinity of encapsulated bee bread powder was 71.0819%.

The result of the FTIR spectrum (Figure 5) from F1 encapsulated bee bread with filler of starch and lactose was found in several absorption bands (Table 3). Based on the test results showed that in encapsulated bee bread, there are O−H bonds (stretching) at the wave peak of 3,197.52 cm^{-1} (Castiglioni et al. 2019), stretching vibration C−H from carboxylic acid (lipid) and cellulose (Thakur and Nanda 2019). CH group is also present in the starch spectrum at the wave peak of 2,929.21 cm^{-1}, C−H deformation single bond at 1422.76 cm^{-1} (Castiglioni et al. 2019).

The bee bread powder F1 encapsulated was tested by DPPH (2,2-diphenyl-1-picrylhydrazyl) with ascorbic acid (vitamin C) and quercetin as the positive control. The absorbance was assayed using a UV-Vis spectrophotometer at the maximum wavelength (Agus et al. 2019a; Agussalim et al. 2022). The radical-scavenging activity was calculated as the percentage reduction of DPPH color intensity, namely percentage inhibition. The inhibition percentage reflects how effectively the antioxidant compound in the sample captures free radicals at the concentration of the test solution, and their inhibition was obtained in a linear equation (Figure 6).

The O−H group in the amino acid serine or symmetric bending which is included in the group of amino acid of leucine at the peak of 1,337.01 cm^{-1} (Maqsoudlou et al. 2020), the O−H group (alcohol), where this functional group is also found in lactose (Okoye et al. 2014) at the peak wave of 1,260.27 cm^{-1}, there are C−C and C−O vibration groups, which are compounds of phenolic and flavonoids at 1,141.11 cm^{-1} (Subari et al. 2012), the (C−O−C) group, which shows the spectrum of flavonoid compounds at the peak wave of 1,115.68 cm^{-1} (Pitriyana et al. 2017), the presence of C−C, C−O groups at 1,081.91 cm^{-1} (Castiglioni et al. 2019) and in the range 700-900 $cm^{-1,}$ there are C−O−C ring groups (C−O−C ring vibration) from carbohydrates in starch (Subari et al. 2012; Petrakis and Polissiou 2017).

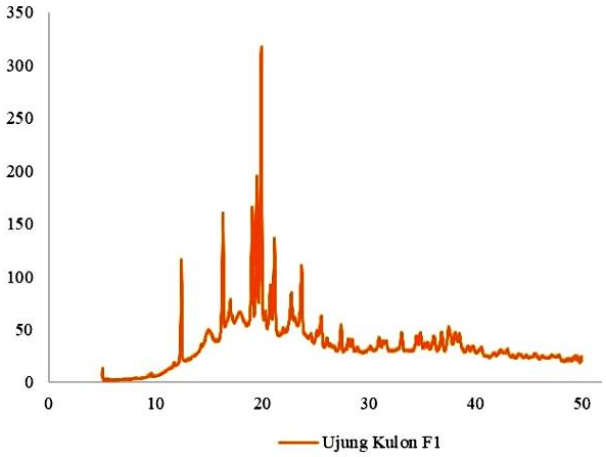


Figure 4. XRD result of encapsulated bee bread from F1 (starch and lactose, ratio 50:50)

Table 3. Infrared absorption of F1 encapsulated bee bread

Wavenumber	Grouping	References
3,400-3,000 cm^{-1}	O−H stretching N−H stretching (hydroxyls and aminos)	(Castiglioni et al. 2019)
2,800-2,932 cm^{-1}	C−H stretching from carboxylic acid (lipid) and cellulose	(Thakur and Nanda 2019)
1,700-1,600 cm^{-1}	C=O stretching (protein amide I, fatty acid) C−O stretching (amides, ketones, quinines)	(Fan et al. 2013) (Isopescu et al. 2020)
1,418.18-1,422.76 cm^{-1}	C−H stretching (lipid and cellulose)	(Castiglioni et al. 2019)
1,400-1,450 cm^{-1}	asymmetrical CH_3 bending, symmetrical CO_3 - stretching, and ring vibrations (free amino acids of valine, glutamic acid, and phenyalanine)	(Pavia et al. 2001)
1,450 cm^{-1}	C−H of CH_2 and CH_3, Aromatics (flavonoids and aromatic rings)	(Ibrahim et al. 2018)
1,375-1,345 cm^{-1}	CH3 (Aliphatic groups)	(Maqsoudlou et al. 2020)
1,337.01-1,338.83 cm^{-1}	O−H in serine amino acid or symmetric bending (amino acid leucine)	(Maqsoudlou et al. 2020)
1,260 cm^{-1}	O−H (alcohol)	(Okoye et al. 2014)
1,200.88-1,201.85 cm^{-1}	N−H deformation, C−N stretching (amide III)	(Castiglioni et al. 2019)
800-1,500 cm^{-1}	C−C and C−O vibration (phenolics and flavonoids)	(Subari et al. 2012)
1,000-1,050 cm^{-1}	NH_3 free amino acid of lysine	(Pérez-Masiá et al. 2015)
1,175-900 cm^{-1}	C−O stretching (saccharides)	(Petrakis and Polissiou 2017)
1,115.68 cm^{-1} and 1,115.34 cm^{-1}	C−O−C (flavonoid)	(Pitriyana et al. 2017)
1,076.46 cm^{-1} and 1,001.48 cm^{-1}	C−C, C−O, C−N, and C−C stretching vibrations from sugar and protein	(Castiglioni et al. 2019)
900-750 cm^{-1}	C−H bending (the carbohydrate) anomeric region of carbohydrate	(Subari et al. 2012; Petrakis and Polissiou 2017)

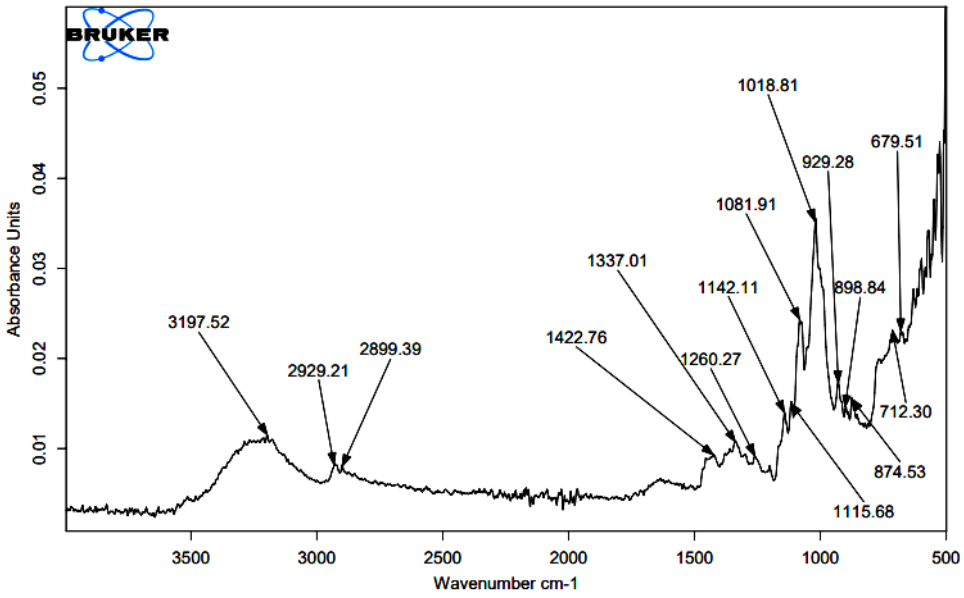


Figure 5. FTIR spectrum of F1 (starch and lactose, ratio 50:50) encapsulated bee bread

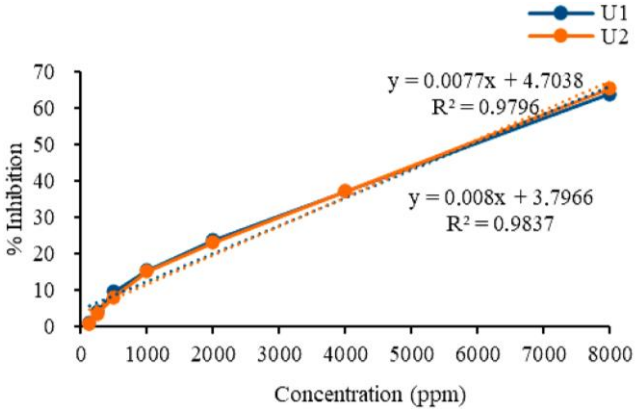


Figure 6. Linear equation of F1 (starch and lactose, ratio 50:50) encapsulated bee bread powder (U1) and ascorbic acid (U2)

Based on the linear equation, the phenolic compounds contribute 97.96% and 98.37% to the antioxidant for encapsulated bee bread powder (U1) and ascorbic acid (U2), respectively (Figure 6). Therefore, the IC_{50} was obtained at 5,884.02 ppm for U1 and 5.28 ppm for U2. The antioxidant IC_{50} are divided into several categories, including highest antioxidant when IC_{50} value is under 50 ppm, 100 to 150 ppm for moderate antioxidant, 150 to 200 ppm for weak antioxidant, and very weak antioxidant when IC_{50} is more than 200 ppm (Molyneux 2004). In this study, the antioxidant activity of bee bread encapsulated powder was categorized as very weak compared to the positive control from ascorbic acid. Jaya et al. (2020) reported that the DPPH IC_{50} of bee bread extract from Indonesian bees, such as *A. mellifera*, *A. cerana*, and *Tetragonula* sp., were 15,840 ppm, 5,070 ppm, and 14,440 ppm, respectively. The different DPPH of bee bread is affected by plant types as the pollen source, which impacts their chemical composition, bee species, and method used to process bee bread (for example, extraction and encapsulation) (Bakour et al. 2019). Bakour et al. (2022) reported that the antioxidant (DPPH, reducing power, and ABTS) of bee bread, which is extracted by three solvents (ethanol, methanol, and ethyl acetate), obtained the highest IC_{50} value recorded in ethyl acetate extract, followed by methanolic extract, and the lowest at ethanolic extract.

This study concluded that phytochemical screening of bee bread from *H. itama* contains saponin, alkaloids, carbohydrates, quinones, flavonoids, tannins, glycosides, phenols, and ninhydrin compounds. The best bee bread encapsulated formula in starch and lactose is 50:50 (% w/w), and the ratio of materials and filler is 25:75 (% w/w), characterized by low hygroscopic properties. In SEM analysis, encapsulated bee bread powder from Ujung Kulon has a diameter ranging from 10.817 to 20.244 µm, and crystallinity based on XRD analysis is obtained at 71.0819%. FTIR results show that in bee bread powder filled with starch and lactose, it was identified that there were groups contained in starch and lactose, shown in the alcohol OH functional group, which is the spectrum of lactose at a wave peak of 1,337.01 cm^{-1}. In addition, there are C–O and C–C functional groups at the wave peak of 1,142.11 cm^{-1}, CH_2–OH groups at the wave peak of 1,260.27 cm^{-1}, and CH groups at the peak of 2,929.21 cm^{-1}, which are also found in the starch spectrum. The antioxidant activity (DPPH) of bee bread powder encapsulated with starch and lactose had weak results, namely 5,884.02 ppm.

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