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Characterization of Bee Bread Produced with Defined Starter Cultures Mimicking the Natural Fermentation Process

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Abstract: Bee bread is a product with unique properties for humans and bees that is produced through the fermentation of pollen in the honeycomb, mainly caused by lactic acid bacteria (LAB) and yeast strains present in the environment. It is a rich source of nutrients such as proteins, polyphenols and vitamins. Despite the potential nutritional value of bee bread, it is consumed at low levels, as harvesting bee bread from the hives is costly and difficult. This study aimed to produce a standard bee bread by using different strains of the fructophilic lactic acid bacteria (FLAB) *Lactobacillus kunkeei* and the yeasts *Starmeralla magnolia* MP-2 and *Zygosaccharomyces siamensis* MP-14, previously isolated from bee products. In this context, bee bread was produced from pollen by solid-state fermentation using selected FLAB and yeast species, which were then compared with spontaneously developed and commercially available bee bread in terms of microbial stability, physicochemical properties, total phenolic component amounts, in vitro digestibility and amino acid profiles. As a result, it was determined that bee bread made from bee pollen fermented with starter cultures showed improved characteristics than commercial bee bread and was more advantageous in terms of absorption as well as production processes.

Keywords: bee bread; bee pollen; lactic acid bacteria; solid-state fermentation (SSF)

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1. Introduction

Bee products, such as honey, propolis, pollen, royal jelly and bee venom, are among the most popular natural products used in traditional medicine due to the bioactive molecules they contain and their powerful healing properties [1]. Bee products have many components found in functional foods, such as prebiotics, probiotics, fibers, phytochemicals, bioactive peptides, minerals, vitamins and organic acids. Of these compounds, phenolics, flavonoids and carotenoids are important compounds suggested to be effective in terms of treating cancer, atherosclerosis, weak immune system, Parkinson's, Alzheimer's, cardiovascular diseases and arthritis patients [2].

Bee bread, one of the main functional bee products, is produced in honeycombs through the fermentation of the bee pollen by Lactic Acid Bacteria (LAB), yeasts and enzymes originating from the bees in the hive [3,4]. Pollen fermentation is a process that requires effective enzymatic activity due to pollen morphology and the exine wall until the wall is broken and the contents are released [5]. During the fermentation process of pollens, bacteria belonging to the genus *Lactobacillus* species, which comprise an important part of probiotics, play a major role [6]. Fructophilic lactic acid bacteria are lactic acid bacteria usually isolated from natural bee bread [7–10]. In addition to LAB strains, yeasts are also important for pollen fermentation to obtain valuable nutritional components [11]. There are yeasts belonging to *Starmerella* and *Zygosaccharomyces* species at different stages of natural bee bread formation [12]. Bee bread is a health-oriented product characterized by its rich chemical composition, nutritional values, digestible properties and biological

activities [4,13]. For this reason, in recent years, many attempts have been made to develop bee bread with pollen fermentation by simulating the natural biological process in laboratory conditions in order to develop a product suitable for human consumption [7,11,14,15]. Several reasons, including the ineffective production conditions, cost, small amount of final product availability as well as unstandardized final products, have motivated researchers to produce bee bread with starter cultures under in vitro conditions. The solid-state fermentation method is the most frequently used fermentation method used to convert bee pollen into bee bread in the literature [16–18]. This process includes the formation of a product with increased digestibility and accessibility from bee pollen, with high microbial stability, depending on various factors such as microorganism density, humidity, water activity, pH, temperature, substrate and oxygen content [6,7,16].

From this perspective, this study aimed to develop a starter culture for bee bread production and bee bread was produced through the fermentation of bee pollen using FLAB strains; *Lactobacillus kunkeei* AP13, *L. kunkeei* AP15, *L. kunkeei* AP16, *L. kunkeei* AP20, *L. kunkeei* AP24, *L. kunkeei* AP29, *L. kunkeei* AP31, *L. kunkeei* AP37, *L. kunkeei* AP42 and pectinolytic yeasts MP-2 *Starmeralla magnolia*, MP-14 *Zygosaccharomyces siamensis* isolated from bee bread and bee pollen obtained in our previous study. [8]. The characterization of the produced innovative bee bread was performed comparatively with bee bread obtained by spontaneous fermentation of bee pollen without using a starter culture and two different commercial bee bread.

2. Materials and Methods

2.1. Materials

The pollen samples used in the study were collected from Afyonkarahisar, Turkey. One of the commercial bee bread used in the study was obtained from a local market, and the other was obtained from a local producer from Istanbul, Turkey. Both the commercial bee bread obtained from the local market and the local producer were fermented spontaneously. Lactic acid bacteria and yeasts used in the study were obtained from Yildiz Technical University Food Engineering Department Culture Collection, isolated previously [8]. FLAB and yeast strains used in this study were tested in pollen extract for their growth as described previously and selected for the bee pollen fermentation process [7].

2.2. Methods

2.2.1. Fermentation of Bee Pollen

The method that forms the basis of the study, fermentation and drying of pollen samples under suitable conditions with solid-state fermentation, was carried out as follows: Lactic acid bacteria and yeasts to be used as starter cultures were incubated in FYP broth (10 g D-fructose (Merck), 10 g yeast extract (Merck), 5 g polypeptone (Merck), 2 g sodium acetate (Merck), 0.5 g Tween 80 (Merck), 0.2 g $MgSO_4 \cdot 7H_2O$ (Merck), 0.01 g $MnSO_4 \cdot 4H_2O$ (Merck), 0.01 g $FeSO_4 \cdot 7H_2O$ (Tekkim), 0.01 g NaCl (Merck), 0.05 g cycloheximide (Sigma-Aldrich), and 0.05 g sodium azide (Merck) (pH6.8)) and PDB broths (Merck, 24 g/L), respectively [8]. After incubation, the cultures were centrifuged at 1780× *g*, 24 °C for 10 min, and then the supernatant was discarded. Then, 10 mL of PBS (Phosphate-Buffered Saline, Sigma- Aldrich) was added to the pellet, vortexed for 10 s and centrifuged again at 11,780× *g*, 24 °C for 10 min. The concentration of the LAB strains was set to ~10^8 cfu ml^{-1}, and for yeasts, the concentration was 10^7 colony-forming units (cfu) ml^{-1}. Three starter culture mixes were prepared in this study: starter 1, containing the strains AP24, AP31 and AP42 of the fructophilic lactic acid bacteria (FLAB) *Lactobacillus kunkeei* and the yeasts *Starmeralla magnolia* MP-2 and *Zygosaccharomyces siamensis* MP-14; starter 2, containing the strains AP15, AP16 and AP29 of the FLAB *L. kunkeei* and the same yeasts used for starter 1; starter 3, containing *L. kunkeei* AP13, AP20 and AP37 strains and the same yeasts used for the two previous starters. The pollen samples fermented with starters 1, 2 and 3 were named SCFP1, SCFP2 and SCFP3, respectively. Multiflora bee pollen, brought to the laboratory under sterile conditions and kept at −18 °C, was weighed, and LAB and yeasts

were inoculated to the pollen samples at a final con. of 10^6 CFU g^{-1} and 10^5 CFU g^{-1}, respectively [7]. After preparation, fermentation was carried out at 30 °C for 14 days, followed by the drying process for 4 days in the incubator in contact with air. The obtained bee bread was stored at 4°C. The same procedures were performed for spontaneously fermented pollen without a starter, and sterile distilled water was used as a microorganism substitute [7].

2.2.2. Physicochemical Properties of Bee Bread

pH and Moisture Analysis

The pH determination of bee bread was determined using a pH meter (Mettler Toledo, Columbus, OH, USA). The moisture contents of the samples were determined in a Radwag moisture analyzer (MA.R series, Radom, Poland); 1 g of sample was used. The difference between the initial and final weight of the sample was weighed by the device, and the percent of moisture content was determined [19].

Carbohydrate and Organic Acid Levels

Then, 1 g of glass beads and 5 mL of 5% perchloric acid (Sigma-Aldrich) were added to 1 g of fermented pollen. The mixture was shaken at 4 °C, 500 rpm for 30 min on an orbital shaker, then sonicated in an ice bath for 1 min. The suspension was centrifuged at 10,000× *g* for 10 min, and the supernatant was passed through a 0.45 µm membrane filter for the analysis (Millipore Corporation) [13]. A CONCISE cARBoSep CHO 87C column was used for the monosaccharide composition analysis in HPLC with a RID-10A refractive index detector. The mobile phase was H_2O with a flow rate of 0.6 mL min^{-1}, and the column temperature was 85 °C. Glucose, galactose, maltose and fructose were used as standard sugars. For the organic acid analysis of the bee bread extracts, an Inertsil®ODS-3 C18 (250 × 4.6 mm 5 µm GL Sciences, Tokyo, Japan) column was used, and the detection was obtained using a PDA (Photodiode Array Detector) detector (SPD-M20A, Shimadzu, Kyoto, Japan) with a wavelength of 210 nm. Then, 10 mM $HClO_4$ was used as the mobile phase with a flow rate of 0.8 mL min^{-1}, and the column temperature was 40 °C. Calibration was performed using lactic, acetic, citric, propionic, oxalic, tartaric and formic acid standards to determine the amount of organic acids in the samples.

2.2.3. Amount of Free Phenolic Components in Bee Bread

The determination of the total phenolic substances was carried out according to the Folin–Ciocalteu method by Shehu (2016) [20]. For this purpose, the bee bread was extracted, as in the method of determining carbohydrate and organic acid levels. Gallic acid (Merck) was used as a reference standard. 0.5 mL of fermented pollen was mixed with 2.5 mL of foline citrate (Merck) and kept in the dark for 3 min. Then, 2 mL of Na_2CO_3 was added and incubated in the dark for 30 min. The same procedures were performed with distilled water as a blind. After incubation, absorbance was measured with a spectrophotometer (Optizen Pop UV spectrophotometer) at a wavelength of 760 nm. The results are expressed as mg gallic acid equivalent (mg GAE/g) [21].

2.2.4. In Vitro Protein Digestibility

Then, 1 g of dried fermented pollen samples were mixed with 0.002% pepsin (Merck) in 100 mL of 0.0075 N HCl (Merck) solution. The samples were incubated at 45 °C for 16 h with shaking. After filtration through Whatman paper, the protein content of the clear solution was analyzed using the Bradford method. For the Bradford method, 300 µL of Bradford reagent (ThermoFisher, Waltham, MA, USA) and 600 µL of distilled water were added to 10 µL of the sample and left for 10 min. Then, absorbance measurement was taken with a spectrophotometer (Optizen Pop UV spectrophotometer) at 595 nm. Bovine Serum Albumin (BSA) was used as the protein standard. While the digested protein content of fermented pollen is expressed as (g)/100 g of bee bread (pollen) total protein,

in vitro protein digestibility is expressed as % digested pollen dissolved after enzyme hydrolysis [22,23].

2.2.5. Amino Acid Profile

For amino acid profile analysis, the bee bread was extracted as follows: 1 g of glass beads and 5 mL of 5% perchloric acid were mixed together with 1 g of fermented pollen. The mixture was shaken at 4 °C, 500 rpm for 30 min on an orbital shaker, then sonicated in an ice bath for 1 min. The suspension was centrifuged at 10,000× *g* for 10 min, and the supernatant was filtered through a 0.45µm membrane filter (Millipore Corporation, Burlington, MA, USA). The obtained extract was analyzed for amino acids using an LC-MS/MS instrument, as described previously [7].

2.2.6. Detection of Microbial Consortium during Bee Bread Fermentation

FYP agar, MRS agar (Merck 68.2 g/L), PCA (Merck, 22.5 g/L) and PDA (Merck, 29 g/L) media were prepared for microbiological analysis of starter and spontaneously fermented pollen to count FLAB, LAB, total mesophilic aerobic bacteria, mold and yeast numbers, respectively. On fermentation days 0, 3, 5, 9, 12 and 15, 100 mg of each sample was taken and dissolved in 1 mL of PBS solution, followed by the preparation of serial dilutions. From the corresponding dilutions, the plating was applied to the corresponding agar plates. The FYP agar, MRS agar and PCA media to be used in the analysis were mixed with 0.05 g L^{-1} cyclohexemide, whereas the PDA medium was prepared with 0.1 g L^{-1} chloramphenicol to inhibit fungal and bacterial growth, respectively. The plates were incubated at 30°C, the colonies were counted, and the number obtained for each sample (fermented pollen or bee bread) was expressed as log cfu g^{-1} [7].

2.2.7. Statistical Analysis

The significant differences between the data obtained as a result of the characterization studies of bee bread were evaluated using Microsoft Office Excel Professional plus (2016 version) using one-way analysis of variance (ANOVA). The significant differences between the means were determined through the interpretation of the *p*-value.

3. Results and Discussion

3.1. Fermentation of Bee Pollen

During the fermentation, drying and storage processes, the images of the pollen fermented spontaneously and with three different starters for 18 days were macroscopically examined. As a result of the examination, it was observed that the starter-fermented pollen swelled on fermentation day 5 as a result of the metabolic activities of the microorganisms. The swelling was observed in the spontaneously fermented pollen on fermentation day 7. This shows that starter-fermented pollen can start fermentation earlier than spontaneously fermented pollen. It was observed that the starter-fermented pollen turned into fragmented granules, similar to bee bread in the hive, while the spontaneously fermented pollen was still moist and fluid as observed on fermentation day 18. As a result, it has been seen that starter-fermented pollen is more advantageous than spontaneously fermented pollen in terms of fermentation time, appearance and consistency, similar to natural bee bread on a macroscopic scale. Pollen fermentation on days 1, 7 and 18 is shown in Figure 1.

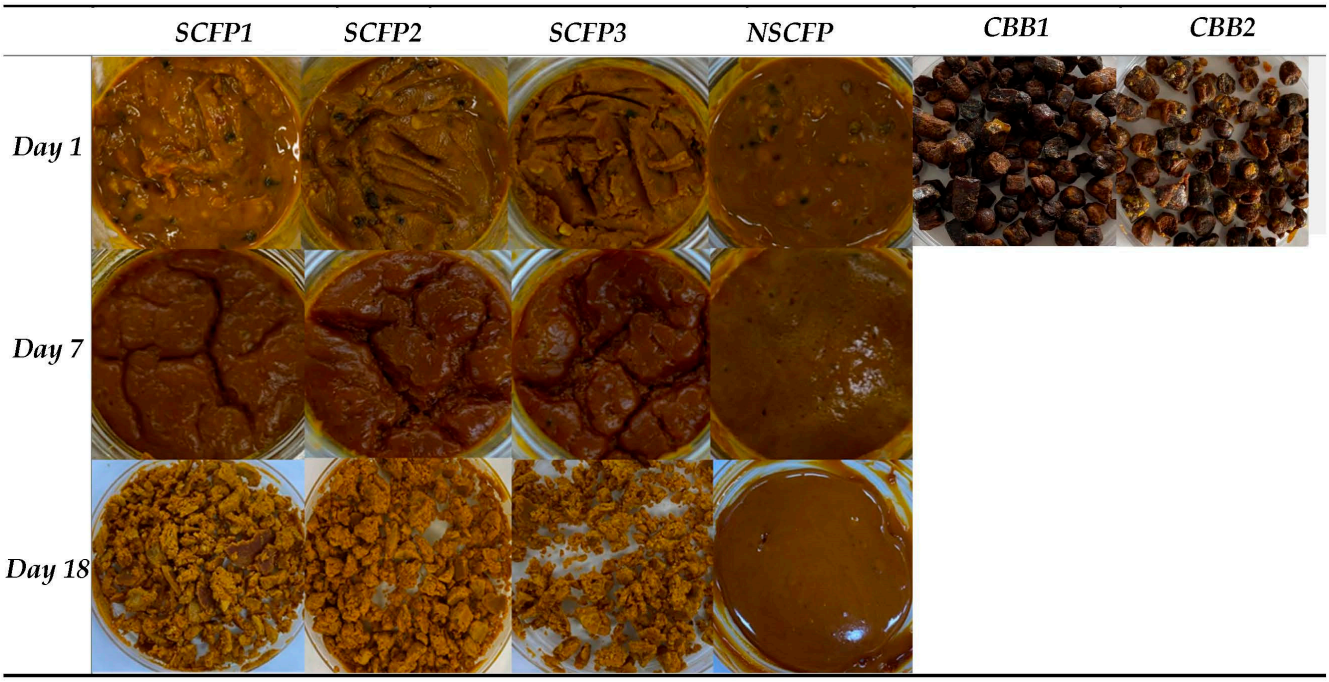

Figure 1. Visualization of pollen fermentation on days 1, 7 and 18. SCFP1: Starter-culture-fermented pollen-1; SCFP2: Starter-culture-fermented pollen-2; SCFP3: Starter-culture-fermented pollen-3; NSCFP: Spontaneously fermented pollen; CBB1: Commercial bee bread (bee bread derived from local market); CBB2: Commercial bee bread-2 (bee bread derived from local producers).

3.2. Physicochemical Properties of Bee Bread

The physicochemical properties of the bee bread samples are shown in Table 1. Looking at the data from days 1, 21 and 50, the pH values of the samples with the added starter culture were ~5.1, the commercial ones were ~4.9, and the initial pH values were similar to each other. After the first 21 days of storage, the pH was observed in the range of 4.5 ± 0.003~4.7 ±0.44 in starter culture samples, while no significant change was detected in spontaneously fermented and commercial samples. On day 50, the pH was fixed around ~4.2 for the ones to which the starter culture was added, while this value was determined as ~4.8 in the other samples. The initial moisture content of the samples was between 16.93% and 27.49%, and at the end of day 50, this value decreased for all samples.

Table 1. Physicochemical properties of bee bread.

		SCFP1 *	SCFP2	SCFP3	NSCF	CBB1	CBB2
pH	Day 1	5.13 ± 0.002 ^{a}	5.13 ± 0.001 ^{a}	5.13 ± 0.002 ^{a}	5.13 ± 0.002 ^{a}	4.91 ± 0.002 ^{b}	4.97 ± 0.004 ^{c}
	Day 21	4.51 ± 0.003 ^{a}	4.69 ± 0.001 ^{b}	4.76 ± 0.44 ^{c}	5.02 ± 0.009 ^{d}	4.90 ± 0.005 ^{d}	4.97 ± 0.004 ^{d}
	Day 50	4.15 ± 0.001 ^{a}	4.22 ± 0.006 ^{b}	4.24 ± 0.012 ^{c}	4.85 ± 0.004 ^{d}	4.80 ± 0.003 ^{de}	4.87 ± 0.014 ^{df}
% Moisture	Day 1	27.49 ± 0.002 ^{a}	26.3 ± 0.000 ^{ab}	27.02 ± 0.000 ^{a}	25.64 ± 0.003 ^{b}	12.69 ± 0.000 ^{c}	16.93 ± 0.000 ^{d}
	Day 21	22.28 ± 0.00 ^{a}	20.63 ± 0.004 ^{bfg}	21.42 ± 0.001 ^{af}	19.59 ± 0.001 ^{cg}	12.57 ± 0.000 ^{d}	16.36 ± 0.000 ^{e}
	Day 50	21.79 ± 0.000 ^{a}	19.62 ± 0.000 ^{b}	20.55 ± 0.000 ^{c}	18.93 ± 0.000 ^{d}	11.24 ± 0.001 ^{e}	15.19 ± 0.000 ^{f}

* SCFP1: Starter-culture-fermented pollen-1; SCFP2: Starter-culture-fermented pollen-2; SCFP3: Starter-culture-fermented pollen-3; NSCFP: Spontaneously fermented pollen; CBB1: Commercial bee bread; CBB2: Commercial bee bread-2. Those with statistically significant differences are listed with different letters in the same column.

The free sugar composition of the bee bread was examined, and the main sugar group in the bee bread was found to be fructose (Table 2). The samples contained fructose in the range of 7.54 g 100 g^{-1} to 14.36 g 100 g^{-1} and glucose in the range of 3.414 100 g^{-1} and 11,748 100 g^{-1}. In contrast, CBB1 had the highest fructose level, with 75.79%, and SCFP3 had the lowest value, with 55.10%. Bakour et al. (2019) found fructose (11.8 g 100 g^{-1}) and

glucose (5.7 g 100 g^{-1}) to be the main two sugars in the bee bread sample [24]. Dranca et al. (2020) [25] reported that Romanian bee bread contained these two sugars at 19.73 g 100 g^{-1} and 8.82 g 100 g^{-1}, respectively [24]. In another study, the fructose and glucose contents of the bee bread were found to be at 46% and 37% levels, respectively [26]. Contrary to these values, in another study, when four different Malaysian multi-floral bee breads were analyzed, fructose was around 10.270 ± 0.140 g 100 g^{-1} level, whereas glucose was at the 12.397 ± 0.980 g 100 g^{-1} level [27].

Table 2. Sugar and organic acid profile of bee bread.

		SCFP1 *	SCFP2	SCFP3	NSCFP	CBB1	CBB2
SUGAR COMPOSITION (g 100 g^{-1})	Maltose	ND	ND	ND	ND	ND	ND
	Glucose	9.64	8.91	10.08	11.74	3.41	4.26
	Fructose	12.93	11.55	12.37	14.36	10.68	7.54
	%Glucose	42.71	43.56	44.89	44.98	24.21	36.12
	%Fructose	57.29	56.44	55.11	55.02	75.79	63.88
ORGANIC ACID (mg g^{-1})	Oxalic acid	0.75	0.72	0.79	0.08	0.65	0.61
	Tartaric acid	0.92	ND [a]	0.96	0.28	ND	0.89
	Formic acid	1.45	1.29	1.45	0.88	0.25	1.40
	Lactic acid	3.30	3.42	4.40	4.15	0.45	1.21
	Acetic acid	3.51	2.96	3.57	ND	ND	2.08
	Citric acid	0.14	0.14	0.19	0.14	0.19	0.20
	Propionic acid	0.33	0.34	0.38	0.38	1.73	1.08

* SCFP1: Starter-culture-fermented pollen-1; SCFP2: Starter-culture-fermented pollen-2; SCFP3: Starter-culture-fermented pollen-3; NSCFP: Spontaneously fermented pollen; CBB1: Commercial bee bread; CBB2: Commercial bee bread-2, [a] not determined.

In terms of the organic acid content, the sample with the lowest organic acid content was non-starter-fermented pollen, while the highest was in the SCFPs (Table 2). The most abundant organic acid in the bee bread was lactic acid, and it was in the range of 0.45–4.40 mg g^{-1}, although other organic acids, such as acetic acid, might reach 2.08 mg g^{-1} in the bee bread samples. The high amount of lactic acid, especially in the samples with an added starter culture, may be associated with the fact that the selected cultures triggered the fermentation process. Tartaric acid was not detected in the SCFP2 and CBB1 samples, and acetic acid could not be detected in NSCFP and CBB1. Dranca et al. (2020) determined the values of 6.75 mg g^{-1} formic acid, 10.4 mg g^{-1} acetic acid and 1.30 mg g^{-1} propionic acid in the bee bread sample, but in that study, no lactic acid was detected [25]. The oxalic acid value was determined as 0.72–0.79 mg g^{-1} in our study in starter-used bee bread samples, and this range showed similar results to the previous study with Moroccan bee bread [24].

3.3. Amount of Free Phenolic Components in Bee Bread

The amount of the free phenolic contents of the bee bread was determined, and the results are given in Table 3. Accordingly, the phenolic content of the samples took values between 12.87 ± 0.2 mg GAE (Gallic acid equivalent) g^{-1} and 15.09 ± 0.077 mg GAE g^{-1}. Suleiman et al. (2021) reported the amount of free phenolic compounds to be 9.55 ± 1.00 and 17.44 ± 0.93 mg GAE g^{-1} in their study, in which they determined the amount of free phenolic components with the different extraction methods they applied to bee bread [27]. Sawicki et al. (2022) [28], in their study comparing bee products such as bee bread, bee pollen honey and beeswax, stated the phenolic content of bee bread to be 8.23 ±0.24 mg GAE g^{-1}. Studies have shown that the phenolic content of bee bread samples collected in different regions ranged between 2.5 and 37.15 mg GAE g^{-1} [21,28]. Othman et al. (2019) found that the total phenolic content of the bee bread extracts ranged from 14.19 to 15.38 mg GAE g^{-1} in their investigations [29]. When compared to Romanian BBA extract [30] with a total phenolic content of 8.32 mg GAE g^{-1}, these outcomes were superior. In another study, the phenolic content in the bee bread samples was observed

to be between 14 mg and 84 mg GAE g^{-1} [13]. These values, in general, were higher than our results. This may be explained by the higher availability of compounds resulting from the degradation of pollen structure during the fermentation of bee pollen [31]. Most studies have found that the total amount of phenolic compounds varies in different regions using different extraction techniques or depending on the source of the flowers [31,32]. Baltruaityte et al. (2007) [33] also revealed that the origin of the pollen, which clearly depends on the flora and the area in which it is located, also might have an impact on the pollen phenolic content.

Table 3. Free phenolic components and in vitro digestibility of bee bread.

	SCFP1 *	SCFP2	SCFP3	NSCFP	CBB1	CBB2
F.P.C. (mg GAE/g)	14.68 ± 0.09 [a]	14.16 ± 0.04 [b]	15.09 ± 0.07 [a]	13.95 ± 0.05 [b]	12.87 ± 0.02 [c]	13.33 ± 0.07 [c]
% I.v.D.	50.35 ± 0.03 [a]	48.28 ± 0.01 [b]	61.64 ± 0.01 [c]	45.32 ± 0.01 [d]	36.23 ± 0.01 [e]	35.74 ± 0.007 [f]

* SCFP1: Starter-culture-fermented pollen-1; SCFP2: Starter-culture-fermented pollen-2; SCFP3: Starter-culture-fermented pollen-3; NSCFP: Spontaneously fermented pollen; CBB1: Commercial bee bread; CBB2: Commercial bee bread-2; F.P.C: Free Phenolic Components; I.v.D: In vitro digestibility. Those with statistically significant differences are listed with different letters in the same row.

3.4. In vitro Digestibility of Bee Bread

The in vitro digestibility of bee bread was determined before and after the digestion process of the bee bread with pepsin. The % protein values of fermented bee bread after the digestion process are given in Table 3. After the fermentation process, the digestibility of the samples increased significantly. While the highest % digestibility value was observed in SCFP3 (61.64 ± 0.018) sample, the lowest digestibility was observed in CBB2 (35%, 74 ± 0.007). In previous studies, bee bread was shown to have greater bioavailability than bee pollen since the outer layer of pollen, known as the exine, is made of sporopollenin, which blocks the availability of nutrients [34]. There is a limit to the number of nutrients and bioactive substances that can be absorbed from pollen grain because of the exine, but in bee bread, the out layer has partly been destroyed by fermentation, so pollen's functional and energetically rich content is easier to assimilate and use [30]. It has been shown that the outer layer of pollen is only partially digested by humans through different in vitro simulations of human digestion, to a value between 48% and 59%, which has been used by researchers to prove that the exine is resistant to acid, even from stomachs [35]. A different investigation on the protein digestibility of bee bread and bee pollen revealed variations in the values for bee bread (79.1 g protein digested 100 g^{-1} total protein) and bee pollen (63.9 g protein digested 100 g^{-1} total protein) [34,35]. Similar to our findings, Di Cagno et al. (2019) [7] evaluated the digestibility of bee pollen before and after fermentation and reported that while the digestibility increased significantly after fermentation with selected species, this value changed less during spontaneous fermentation.

3.5. Amino Acid Profile of Bee Bread

The data in Table 4 show the amino acid contents of bee bread. As can be seen in Table 4, six different bee bread samples contain six essential amino acids, eight conditionally essential amino acids and eighteen non-essential amino acid groups. The most abundant essential amino acid in all samples were phenylalanine, alanine and leucine. The sample containing the highest percentage of these amino acids was SCFP3, with 14,447 g/100 g, 2.6086 g/100 g and 3.7982 g/100 g, respectively. The highest amount of conditionally essential amino acid contained in the samples was serine. In this group, SCFP3 (219,848 g/100 g) contained more of this amino acid than the others. Sarcosine, aspartic acid, gamma-aminobutyric acid and lysine were determined as the most common non-essential amino acids in bee bread samples. Commercial bee bread showed the lowest values for all amino acid values. Mohammad et al. (2020) showed the presence of 2.769 g/100 g phenylalanine and 1.036 g/100 g alanine in their study with Malaysian bee bread [36]. In other studies, with bee bread, aspartate, glutamate, asparagine, serine, glutamine, histidine,

glycine, threonine, arginine, alanine, gamma-aminobutyric acid, tyrosine, cysteine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, lysine, proline, valine, methionine, threonine and hydroxyproline have been reported as mostly determined amino acids [37,38]. Bayram et al. (2021) [39], in their study of five different Turkish bee bread, determined taurine, 1-2-aminobutyric acid, ethanolamine, L-ornithine, L-carnosine amino acids that are different from the amino acids listed above but similar to the amino acids in our study.

Table 4. Amino acid contents of bee bread.

E.a.a. (g/100 g)	**SCFP1 ***	**SCFP2**	**SCFP3**	**NSCFP**	**CBB1**	**CBB2**
L-Tryptophan	0.20	0.27	0.29	0.36	0.03	0.03
L-L-Phenylalanine	1.36	1.34	1.44	1.49	0.15	0.44
L-Leucine	3.49	3.34	3.79	3.66	0.20	0.98
L-isoleucine	0.86	0.88	0.96	1.00	0.01	0.27
L-Valine	1.67	1.62	1.78	0.02	0.19	0.05
L-Threonine	1.34	1.11	1.42	0.13	0.08	0.37
L-Histidine	1.98	1.28	1.70	2.14	0.18	0.66
1-Methyl-L-Histidine	0.03	0.06	0.03	0.06	0.06	0.03
3-Methyl-L-Histidine	0.06	0.03	0.01	0.01	0.02	0.01
L-Alanine	2.57	2.46	2.60	2.54	0.74	1.34
C.e.a.a. (g/100 g)	SCFP1	SCFP2	SCFP3	NSCFP	CBB1	CBB2
L-Tyrosine	1.54	1.49	1.60	1.67	0.14	0.54
L-Glutamine	ND	ND	ND	0.32	ND	ND
L-Glycine	0.68	0.61	0.67	0.57	0.16	0.31
L-Proline	3.07×10^{-4}	3.58×10^{-4}	3.53×10^{-4}	3.4×10^{-4}	1.86×10^{-4}	2.27×10^{-4}
Trans-4-hydroxy L-proline	0.36	1.17	1.16	1.27	0.28	0.40
L-Serine	1.91	2.07	2.19	1.97	0.26	0.68
O-Phospho-L-Serine	0.0254	0.023	0.025	0.006	0.027	0.005
L-Arginine	1.616	0.001	0.005	1.681	ND	0.008
L-Cystine	ND	0.01	0.36	0.02	0.11	0.16
L-ornithine	0.089	0.030	0.018	0.028	0.021	0.032
N.a.a. (g/100 g)	SCFP1	SCFP2	SCFP3	NSCFP	CBB1	CBB2
Taurine	0.146	0.129	0.112	0.102	0.119	0.112
3-Amino isobutyric acid	1.206	0.124	1.378	1.371	0.209	0.423
Gamma-aminobutyric acid	2.92	2.94	3.12	2.91	0.48	0.98
L-Norvaline	1.03	0.95	1.09	1.76	0.04	0.56
Ethanolamine	1.13	0.02	0.26	0.10	0.02	0.16
L-2-Aminoadipic acid	0.03	2.57	2.78	3.37	0.29	0.72
L-Aspartic acid	3.62	2.51	3.16	2.59	0.91	2.26
Sarcosine	5.76	5.85	6.10	5.99	1.60	2.90
Trans-4-hydroxy L-proline	0.36	1.17	1.16	1.27	0.28	0.40
L-Homocitrulline	0.505	0.518	0.514	0.510	ND	ND
L-Citrulline	ND	ND	ND	ND	0.4621	0.462
O-Phosphoryl Ethanolamine	0.020	0.021	0.003	0.013	0.011	0.018
DL-Homocystine	0.006	0.013	0.022	0.038	0.004	0.039
Argininosuccinic acid	0.009	4.175	4.152	ND	0.186	0.564
L-Carnosine	0.315	0.336	0.004	0.334	ND	0.011
L-Cystine	ND	0.013	0.368	0.028	0.112	0.167
DL-5-Hydroxylysine	0.018	ND	0.106	0.057	0.061	0.0535
L-Lysine	2.628	0.017	3.302	0.001	0.034	ND

* SCFP1: Starter-culture-fermented pollen-1; SCFP2: Starter-culture-fermented pollen-2; SCFP3: Starter-culture-fermented pollen-3; NSCFP: Spontaneously fermented pollen; CBB1: Commercial bee bread; CBB2: Commercial bee bread-2; E.a.a: Essential Amino acids; C.e.a.a: Conditionally essential amino acids; N.a.a: Nonessential amino acids; ND: not determined.

3.6. Microbial Profile of Bee Bread

Recent studies have demonstrated the importance of FLAB as well as yeasts for the fermentation of honey products, and using defined starter cultures can be the method of choice for the production of bee bread and other bee products under standardized conditions [7,12]. From this perspective, this study aimed to develop a starter culture for bee bread fermentation, and the microbiological consortium in the bee bread was detected using culture-dependent techniques. For this, the bee bread produced in this study were stored for 50 days, and FLAB, LAB, total mesophilic aerobic bacteria and total yeast counts were performed on day 1, 3, 5, 7, 14, 21, 30, 40 and 50. The samples of bee bread with starting culture added showed more microbial counts than the other samples (Table 5). This is assumed to be connected to the fact that the species we isolated from bee pollen in our earlier work [7] may have adapted better to the pollen environment. The FLAB, LAB and TMAB microbial numbers in the bee bread samples with added starter culture increased until the 14th day, after which a decrease was observed until the end of storage. While yeast development increased for these samples in the first 5 days, a decrease was observed towards the end of storage. For non-starter cultures, microbial numbers increased in the first 7 days but decreased thereafter. Yeast growth for these samples similarly increased until day 5 and then decreased. The first-day and last-day counts were evaluated for commercial bee bread samples. Accordingly, the initial microbial load of CBB1 and CBB2 were 5.36 ± 0.098–5.57 ± 0.084 for FLAB; 6.57 ± 0.141–6.40 ± 0.091 for LAB; 4.57 ± 0.141–4.515 ± 0.247 for TMAB and 3.36 ± 0.091–3.42 ± 0.169 log cfu/gr for yeast numbers, respectively. After 50 days of storage, these values were decreased for CBB1 and CBB2 to 2.1 ± 0.031–4.25 ± 0.162 for FLAB; 4.81 ± 0.084–4.47 ± 0.240 for LAB; 4.38 ± 0.113–2.26 ± 0.37 for TMAB 0.00–3.47 ± 0.240 log cfu/gr for yeast numbers. The initial values of the commercial species were almost equal to the microbial stability of the other species at the end of the 50th day, and it was determined that these values decreased significantly at the end of storage (Table 5). Di Cagno et al. (2019) similarly reported in their study that the LAB density increased over time and then decreased during the 50-day storage period [7]. Detry et al. (2020) observed the bee bread fermentation for a short period but detected similar observations [40]. In terms of commercial bee bread samples, FLAB, LAB, TMAB and TY numbers were altered between 2.1 ± 0.031–4.25 ± 0.162, 4.47 ± 0.240–4.81 ± 0.084, 2.26 ± 0.374–4.38 ± 0.113 and 3.01 ±0.140 3.47 ± 0.240 log cfu/gr suggesting the decrement of the microbial counts during the storage period.

Table 5. FLAB, LAB, TMAB and TY values of bee bread during storage (log cfu g^{-1}).

DAY		SCFP1	SCFP2	SCFP3	NSCFP
1.	FLAB *	7.04 ± 0.014	7.98 ± 0.035	7.38 ± 0.205	5.11 ± 0.035
	LAB	6.17 ± 0.007	8.17 ± 0.056	6.62 ± 0.219	5.13 ± 0.056
	TMAB	5.88 ± 0.021	6.79 ± 0.028	6.33 ± 0.084	4.5 ± 0.042
	TY	5.07 ± 0.042	4.97 ± 0.028	4.98 ± 0.049	4.82 ± 0.077
	FLAB	9.40 ± 0.091	8.43 ± 0.120	8.30 ± 0.106	7.17 ± 0.077
3.	LAB	9.44 ± 0.148	8.23 ± 0.304	8.37 ± 0.077	7.22 ± 0.134
	TMAB	8.20 ± 0.056	7.24 ± 0.141	7.17 ± 0.042	7.28 ± 0.205
	TY	5.67 ± 0.339	5.32 ± 0.176	5.38 ± 0.212	5.37 ± 0.077
	FLAB	10.50 ± 0.268	11.17 ± 0.205	11.19 ± 0.183	8.69 ± 0.374
	LAB	10.63 ± 0.438	10.2 ± 0.014	11.28 ± 0.212	8.45 ± 0.028
5.	TMAB	9.28 ± 0.212	9.22 ± 0.028	9.40 ± 0.028	8.62 ± 0.028
	TY	5.17 ± 0.084	5.03 ± 0.007	5.27 ± 0.056	4.93 ± 0.049
	FLAB	10.47 ± 0.374	11.58 ± 0.183	10.83 ± 0.134	9.72 ± 0.360
	LAB	10.57 ± 0.480	11.41 ± 0.162	8.83 ± 0.205	10.81 ± 0.233
	TMAB	9.38 ± 0.084	9.65 ± 0.021	9.33 ± 0.134	10.42 ± 0.473

Table 5. *Cont.*

DAY		SCFP1	SCFP2	SCFP3	NSCFP
7.	TY	3.85 ± 0.134	3.72 ± 0.049	3.21 ± 0.304	3.67 ± 0.282
	FLAB	11.26 ± 0.049	10.56 ± 0.346	10.51 ± 0.176	9.34 ± 0.282
	LAB	9.36 ± 0.077	8.52 ± 0.183	9.57 ± 0.261	8.52 ± 0.268
	TMAB	10.64 ± 0.466	10.78 ± 0.042	9.56 ± 0.049	10.36 ± 0.233
14.	TY	3.35 ± 0.169	3.67 ± 0.106	3.36 ± 0.091	3.32 ± 0.452
	FLAB	6.33 ± 0.134	8.77 ± 0.028	9.46 ± 0.106	7.21 ± 0.586
	LAB	8.42 ± 0.155	9.48 ± 0.530	8.65 ± 0.169	7.26 ± 0.219
	TMAB	8.75 ± 0.311	8.63 ± 0.183	7.47 ± 0.183	8.65 ± 0.353
	TY	3.51 ± 0.063	3.62 ± 0.028	3.47 ± 0.247	3.32 ± 0.459
21.	FLAB	7.13 ± 0.311	7.66 ± 0.148	7.92 ± 0.080	7.32 ± 0.296
	LAB	7.52 ± 0.325	8.50 ± 0.636	7.91 ± 0.240	7.03 ± 0.007
	TMAB	7.94 ± 0.021	8.58 ± 0.371	6.61 ± 0.106	8.78 ± 0.070
	TY	3.48 ± 0.162	3.42 ± 0.169	2.42 ± 0.169	2.17 ± 0.247
30.	FLAB	7.50 ± 0.671	6.56 ± 0.183	3.40 ± 0.021	5.79 ± 0.212
	LAB	6.73 ± 0.268	6.71 ± 0.261	4.55 ± 0.190	5.72 ± 0.120
	TMAB	6.63 ± 0.141	6.23 ± 0.268	5.48 ± 0.091	5.67 ± 0.056
	TY	3.36 ± 0.014	2.21 ± 0.296	2.47 ± 0.240	2.26 ± 0.374
40.	FLAB	6.29 ± 0.339	5.54 ± 0.035	4.59 ± 0.247	5.73 ± 0.296
	LAB	5.75 ± 0.325	5.64 ± 0.014	5.31 ± 0.438	5.77 ± 0.049
	TMAB	5.60 ± 0.120	4.65 ± 0.169	5.28 ± 0.339	5.40 ± 0.473
	TY	2.26 ± 0.374	ND	ND	2.25 ± 0.353
50.	FLAB	6.29 ± 0.339	5.54 ± 0.035	4.59 ± 0.247	5.73 ± 0.296
	LAB	5.75 ± 0.325	5.64 ± 0.014	5.31 ± 0.438	5.77 ± 0.049
	TMAB	5.60 ± 0.120	4.65 ± 0.169	5.28 ± 0.339	5.40 ± 0.473
	TY	2.26 ± 0.374	ND	ND	2.25 ± 0.353

* FLAB: Fructophilic lactic acid bacteria; LAB: Lactic acid bacteria; TM: Total mesophilic aerobic bacteria; TY: total yeast; ND: not detected.

4. Conclusions

Bee products are always valuable because of the benefits they provide for humans. In recent times, bee bread, one of these products, has attracted a lot of attention. Especially as a result of fermentation, the increase in the diversity and bioaccessibility of many valuable components, such as phenolic compounds, proteins and organic acids of bee pollen in bee bread, makes this product remarkable. In this study, bee bread/pollen isolate distinct FLAB and yeast strains were tested for their starter culture potential to produce bee bread under in vitro conditions. The result of this study demonstrated the importance of the selection of different starter cultures as well as the usage of starter cultures during bee bread production. The physicochemical properties of bee bread altered with the starter culture usage, and importantly, a significant increment in the digestibility of bee bread was observed following the starter culture application. The bee bread samples where a starter culture was used showed the highest phenylalanine, alanine and leucine contents as essential amino acids in comparison to the spontaneously fermented bee bread samples. These findings demonstrated that bee bread production could be conducted similarly to the natural fermentation process with selected starter cultures. With this developed fermentation protocol, in this study, it has been shown that bee bread with beneficial properties for humans can be produced in standardized and high quantities.

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References

1. Brown, R. Hive products: Pollen, propolis and royal jelly. *Bee World* **1989**, *3*, 109–117. [CrossRef]
2. Komosinska-Vassev, K.; Olczyk, P.; Kaźmierczak, J.; Mencner, L.; Olczyk, K. Bee pollen: Chemical composition and therapeutic application. *Evid.-Based Complement. Altern. Med.* **2015**, *2015*, 297425. [CrossRef]
3. Urcan, A.; Mărghitaș, L.A.; Dezmirean, D.S.; Bobiș, O.; Bonta, V.; Mureșan, C.I.; Mărgăoan, R. Chemical Composition and Biological Activities of Beebread-Review. *Bull. Univ. Agric. Sci. Vet. Med. Cluj-Napoca. Anim. Sci. Biotechnol.* **2017**, *74*, 6. [CrossRef]
4. Kieliszek, M.; Piwowarek, K.; Kot, A.M.; Błażejak, S.; Chlebowska-Śmigiel, A.; Wolska, I. Pollen and bee bread as new health-oriented products: A review. *Trends Food Sci. Technol.* **2018**, *3*, 170–180. [CrossRef]
5. Zhang, Z.; Cao, H.; Chen, C.; Chen, X.; Wei, Q.; Zhao, F. Effects of fermentation by Ganoderma lucidum and Saccharomyces cerevisiae on rape pollen morphology and its wall. *J. Food Sci. Technol.* **2017**, *3*, 4026–4034. [CrossRef]
6. Salazar-González, C.; Díaz-Moreno, C. The nutritional and bioactive aptitude of bee pollen for a solid-state fermentation process. *J. Apic. Res.* **2016**, *3*, 161–175. [CrossRef]
7. Di Cagno, R.; Filannino, P.; Cantatore, V.; Gobbetti, M. Novel solid-state fermentation of bee-collected pollen emulating the natural fermentation process of bee bread. *Food Microbiol.* **2019**, *3*, 218–230. [CrossRef]
8. Ispirli, H.; Dertli, E. Detection of fructophilic lactic acid bacteria (FLAB) in bee bread and bee pollen samples and determination of their functional roles. *J. Food Process. Preserv.* **2021**, *3*, e15414. [CrossRef]
9. Janashia, I.; Carminati, D.; Rossetti, L.; Zago, M.; Fornasari, M.E.; Haertlé, T.; Chanishvili, N.; Giraffa, G. Characterization of fructophilic lactic microbiota of Apis mellifera from the Caucasus Mountains. *Ann. Microbiol.* **2016**, *3*, 1387–1395. [CrossRef]
10. Acín Albiac, M.; Di Cagno, R.; Filannino, P.; Cantatore, V.; Gobbetti, M. How fructophilic lactic acid bacteria may reduce the FODMAPs content in wheat-derived baked goods: A proof of concept. *Microb. Cell Factories* **2020**, *3*, 182. [CrossRef] [PubMed]
11. Yan, S.; Li, Q.; Xue, X.; Wang, K.; Zhao, L.; Wu, L. Analysis of improved nutritional composition of bee pollen (Brassica campestris L.) after different fermentation treatments. *Int. J. Food Sci. Technol.* **2019**, *3*, 2169–2181. [CrossRef]
12. Detry, R.; Simon-Delso, N.; Bruneau, E.; Daniel, H.-M. Specialisation of yeast genera in different phases of bee bread maturation. *Microorganisms* **2020**, *3*, 1789. [CrossRef] [PubMed]
13. Tomás, A.; Falcão, S.I.; Russo-Almeida, P.; Vilas-Boas, M. Potentialities of beebread as a food supplement and source of nutraceuticals: Botanical origin, nutritional composition and antioxidant activity. *J. Apic. Res.* **2017**, *3*, 219–230. [CrossRef]
14. Adaškevičiūtė, V.; Kaškonienė, V.; Barčauskaitė, K.; Kaškonas, P.; Maruška, A. The Impact of Fermentation on Bee Pollen Polyphenolic Compounds Composition. *Antioxidants* **2022**, *3*, 645. [CrossRef] [PubMed]
15. Fuenmayor, C.A.; Quicazán, M.C.; Figueroa, J. Desarrollo de un suplemento nutricional mediante la fermentación en fase sólida de polen de abejas empleando bacterias ácido lácticas probióticas. *Aliment. Hoy* **2011**, *3*, 17–39.
16. Krishna, C. Solid-state fermentation systems—An overview. *Crit. Rev. Biotechnol.* **2005**, *3*, 1–30. [CrossRef]
17. Singhania, R.R.; Patel, A.K.; Soccol, C.R.; Pandey, A. Recent advances in solid-state fermentation. *Biochem. Eng. J.* **2009**, *3*, 13–18. [CrossRef]
18. Kostić, A.Ž.; Milinčić, D.D.; Barać, M.B.; Ali Shariati, M.; Tešić, Ž.L.; Pešić, M.B. The application of pollen as a functional food and feed ingredient—The present and perspectives. *Biomolecules* **2020**, *3*, 84. [CrossRef]
19. Urcan, A.C.; Criste, A.D.; Dezmirean, D.S.; Bobiș, O.; Bonta, V.; Dulf, F.V.; Mărgăoan, R.; Cornea-Cipcigan, M.; Campos, M.G. Botanical origin approach for a better understanding of chemical and nutritional composition of beebread as an important value-added food supplement. *LWT* **2021**, *3*, 111068. [CrossRef]
20. Shehu, A.; Ismail, S.; Rohin MA, K.; Harun, A.; Abd Aziz, A.; Haque, M. Antifungal properties of Malaysian Tualang honey and stingless bee propolis against Candida albicans and Cryptococcus neoformans. *J. Appl. Pharm. Sci.* **2016**, *3*, 044–050. [CrossRef]
21. Mayda, N.; Özkök, A.; Ecem Bayram, N.; Gerçek, Y.C.; Sorkun, K. Bee bread and bee pollen of different plant sources: Determination of phenolic content, antioxidant activity, fatty acid and element profiles. *J. Food Meas. Charact.* **2020**, *3*, 1795–1809. [CrossRef]
22. Sommano, S.R.; Bhat, F.M.; Wongkeaw, M.; Sriwichai, T.; Sunanta, P.; Chuttong, B.; Burgett, M. Amino acid profiling and chemometric relations of black dwarf honey and bee pollen. *Front. Nutr.* **2020**, *7*, 558579. [CrossRef]
23. Benavides-Guevara, R.M.; Quicazan, M.C.; Ramírez-Toro, C. Digestibility and availability of nutrients in bee pollen applying different pretreatments. *Ing. Y Compet.* **2017**, *3*, 119–128.
24. Bakour, M.; Fernandes, A.; Barros, L.; Sokovic, M.; Ferreira, I.C.; Lyoussi, B. Bee bread as a functional product: Chemical composition and bioactive properties. *LWT* **2019**, *109*, 276–282. [CrossRef]
25. Dranca, F.; Ursachi, F.; Oroian, M. Bee bread: Physicochemical characterization and phenolic content extraction optimization. *Foods* **2020**, *3*, 1358. [CrossRef]
26. Szczesna, T. Study on the sugar composition of honeybee-collected pollen. *J. Apic. Sci.* **2007**, *51*, 15–22.

27. Suleiman, J.B.; Mohamed, M.; Abu Bakar, A.B.; Nna, V.U.; Zakaria, Z.; Othman, Z.A.; Aroyehun, A.B. Chemical Profile, Antioxidant Properties and Antimicrobial Activities of Malaysian Heterotrigona itama Bee Bread. *Molecules* **2021**, *3*, 4943. [CrossRef]
28. Sawicki, T.; Starowicz, M.; Kłębukowska, L.; Hanus, P. The profile of polyphenolic compounds, contents of total phenolics and flavonoids, and antioxidant and antimicrobial properties of bee products. *Molecules* **2022**, *3*, 1301. [CrossRef]
29. Othman, Z.; Noordin, L.; Ghazali WS, W.; Omar, N.; Mohamed, M. Nutritional, phytochemical and antioxidant analysis of bee bread from different regions of Malaysia. *Indian J. Pharm. Sci.* **2019**, *3*, 955–960. [CrossRef]
30. Eva, I.; Miroslava, K.; Helena, F.; Jana, P.; Jana, H.; Valerii, B.; Serhii, V.; Leonora, A.; Zuzana, S.; Janette, M. Bee bread-perspective source of bioactive compounds for future. *Potravinarstvo* **2015**, *9*, 592–598. [CrossRef]
31. Carpes, S.T.; Begnini, R.; Alencar SM, d.e.; Masson, M.L. Study of preparations of bee pollen extracts, antioxidant and antibacterial activity. *Ciência E Agrotecnologia* **2007**, *3*, 1818–1825. [CrossRef]
32. Urcan, A.C.; Criste, A.D.; Dezmirean, D.S.; Mărgăoan, R.; Caeiro, A.; Graça Campos, M. Similarity of data from bee bread with the same taxa collected in India and Romania. *Molecules* **2018**, *3*, 2491. [CrossRef] [PubMed]
33. Baltrušaitytė, V.; Venskutonis, P.R.; Čeksterytė, V. Radical scavenging activity of different floral origin honey and beebread phenolic extracts. *Food Chem.* **2007**, *3*, 502–514. [CrossRef]
34. Atkin, S.L.; Barrier, S.; Cui, Z.; Fletcher, P.D.; Mackenzie, G.; Panel, V.; Sol, V.; Zhang, X. UV and visible light screening by individual sporopollenin exines derived from Lycopodium clavatum (club moss) and Ambrosia trifida (giant ragweed). *J. Photochem. Photobiol. B: Biol.* **2011**, *3*, 209–217. [CrossRef] [PubMed]
35. Campos, M.; Frigerio, C.; Lopes, J.; Bogdanov, S. What is the future of Bee-Pollen? *J. ApiProduct ApiMedical Sci.* **2010**, *2*, 131–144. [CrossRef]
36. Mohammad, S.M.; Mahmud-Ab-Rashid, N.-K.; Zawawi, N. Probiotic properties of bacteria isolated from bee bread of stingless bee Heterotrigona itama. *J. Apic. Res.* **2020**, *3*, 172–187. [CrossRef]
37. Donkersley, P.; Rhodes, G.; Pickup, R.W.; Jones, K.C.; Power, E.F.; Wright, G.A.; Wilson, K. Nutritional composition of honey bee food stores vary with floral composition. *Oecologia* **2017**, *3*, 749–761. [CrossRef]
38. Othman, Z.A.; Wan Ghazali, W.S.; Noordin, L.; Mohd Yusof, N.A.; Mohamed, M. Phenolic compounds and the anti-atherogenic effect of bee bread in high-fat diet-induced obese rats. *Antioxidants* **2019**, *3*, 33. [CrossRef]
39. Bayram, N.E.; Gercek, Y.C.; Çelik, S.; Mayda, N.; Kostić, A.Ž.; Dramićanin, A.M.; Özkök, A. Phenolic and free amino acid profiles of bee bread and bee pollen with the same botanical origin–similarities and differences. *Arab. J. Chem.* **2021**, *3*, 103004. [CrossRef]
40. Barry, J.P.; Metz, M.S.; Hughey, J.; Quirk, A.; Bochman, M.L. Two novel strains of Torulaspora delbrueckii isolated from the honey bee microbiome and their use in honey fermentation. *Fermentation* **2018**, *3*, 22. [CrossRef]