Bee Bread Boosts Probiotic Yoghurt: Unveiling the Impact on Physiochemical, Microbiological, and Sensory Attributes

Nilay KEYVAN[1,a,✉], Özen YURDAKUL[2,b]

[1]Burdur Mehmet Akif University, Institute of Health Science, Department of Food Science and Technology, Burdur, Türkiye; [2]Burdur Mehmet Akif University, Faculty of Veterinary Medicine, Department of Food Science and Technology, Burdur, Türkiye

[a]ORCID: 0000-0002-6717-2793; [b]ORCID: 0000-0001-7680-015X

ARTICLE INFO

Article History
Received : 13.07.2023
Accepted : 08.03.2024
DOI: 10.33988/auvfd.1326701

Keywords
Bee bread
Yoghurt
Probiotic

✉Corresponding author
nilaykeyvan@gmail.com

How to cite this article: Keyvan N, Yurdakul Ö (XXXX): Bee Bread Boosts Probiotic Yoghurt: Unveiling the Impact on Physiochemical, Microbiological, and Sensory Attributes. Ankara Univ Vet Fak Derg, XX (X), 000-000. DOI: 10.33988/auvfd.1326701.

ABSTRACT

This study aimed to investigate the effects of bee bread ratios of 0.5%, 1%, and 2%, respectively on some parameters in probiotic yoghurt production. The bee bread composition contained the elements B, Ca, Fe, K, Mg, Na, P, and Zn. The analysis of sugar composition revealed the presence of fructose, glucose, and sucrose. The organic acid and phenolic substance content were assessed. The following values were obtained: oxalic acid (1.26 mg/g), malic acid (7.79 mg/g), ascorbic acid (0.91 mg/g), citric acid (2.73 mg/g), *p*-coumaric acid (15.3 µg/g) and kaempferol (5.562.4 µg/g). The study determined the tocopherol content, specifically alpha (7.09 µg/g), beta (0.4 µg/g), gamma (0.77 µg/g), and delta (0.31 µg/g). A total of 55 distinct components were identified while analyzing the volatile and aroma profiles. This study found that the IC_{50} value of bee bread was 1.414 mg/mL. Bee bread did not affect physicochemical parameters such as pH, acidity, dry matter, ash, milk fat, and water holding capacity ($P>0.05$) but affected protein and syneresis ($P<0.05$). The addition of bee bread positively affected *Streptococcus thermophilus* and *Lactobacillus bulgaricus,* and *Lactobacillus acidophilus* LA-5 activity was preserved at around 10^7 kob/g during storage ($P<0.05$). Adding bee bread affected the color parameters L*, a*, and b* values ($P<0.05$). Consumers preferred the group to which 0.5% bee bread was offered following sensory analytical evaluation. The study has demonstrated that adding bee bread during yoghurt production can effectively maintain probiotic activity.

Introduction

Bee bread is primarily composed of pollen, honey, and secretions from the salivary glands of honey bees (43). Bees utilize nectar as their primary carbohydrate source, whereas pollen is a crucial source of proteins, lipids, vitamins, and minerals for bee bread production (42). The substance provides food for worker bees and developing larvae (27). Bee bread is considered a more easily digestible form of pollen because the bee's enzymes digest the pollen's outer shell during fermentation (18). The fermentation process carried out by lactic acid bacteria in the honey stomach of bees contributes to the transformation and preservation of the stored pollen, resulting in the formation of bee bread (39). Several research studies into the chemical composition of bee bread have revealed that it typically consists of water, protein, free amino acids, carbohydrates, fatty acids, minerals, vitamins, and numerous types of other bioactive compounds, including kaempferol, rutin, quercetin, luteolin, and rosmarinic acid (4, 5, 11, 23). Bee bread has many biological properties, including antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, and anticancer properties (5). Bee bread has been the subject of extensive research due to its unique nutritional qualities and possible benefits for health (31). Several studies have indicated that bee bread could enhance the immune system, promote digestion, and provide anti-inflammatory properties (30). Bee bread is considered a nutritional supplement due to its biological effects (34).

Probiotic yoghurt contains live microorganisms, known as probiotics, which confer health benefits on the host when consumed in adequate amounts (17). These

probiotics can improve the composition of the colonic microflora and exert health benefits independent of gastrointestinal colonization (22). The use of probiotic bacteria in yoghurt production has been explored to enhance its prophylactic properties (38). Probiotic yoghurt has also been found to inhibit pathogenic microorganisms such as *Staphylococcus aureus*, which may be attributed to the probiotic bacteria or the antibacterial substances they secrete (40). In addition to its cardiovascular and antimicrobial effects, probiotic yoghurt has been studied for its potential benefits in various health conditions. For example, daily probiotic yoghurt consumption has improved the albumin-to-creatinine ratio, estimated glomerular filtration rate, and metabolic parameters in patients with type 2 diabetes with nephropathy (16). Probiotic yoghurt has also been found to have potential anticarcinogenic effects, hypocholesterolemic effects, and the ability to alleviate lactose malabsorption and allergies (38). The study's objective was to investigate the effect of bee bread on physiochemical, microbiological, and sensory properties during the production of probiotic yoghurt.

Materials and Methods

The milk used for probiotic yoghurt production was obtained from the Official Milk Production Store of Burdur Mehmet Akif Ersoy University. Nu-trish LA5 (*Lactobacillus acidophilus* LA-5) and yoghurt culture YF-L903 (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) were purchased from Christen Hansen Laboratories in Copenhagen, Denmark. The bee bread used for the experimental group studies was obtained from hives in Karaman Province, Turkey (37°08'50.7"N 33°31'45.2" E). The chemicals used for the analyses were purchased from Sigma-Aldrich Co. (St. Louis, USA).

Study Design: *L. acidophilus* LA5 and bee bread were not included in the study's control groups, which were designated as group A. The only starters that were used were yoghurt starters. A combination of yoghurt starter cultures and *L. acidophilus* LA5 probiotic bacteria was included in the control test group's composition, which was Group B. A study strategy was created wherein the experimental groups, designated as C, D, and E, were assigned bee bread ratios of 0.5%, 1%, and 2% respectively. The purpose of these groups was to assess the physicochemical, microbiological, and sensory impacts of adding bee bread to yoghurts.

Characterization of Bee Bread Samples: The mineral composition of bee bread was analyzed by using inductively coupled plasma-optic emission spectroscopy (ICP-OES, Perkin Elmer OPTIMA 5300 DV, USA), with a focus on macroelements and microelements (33). High-performance liquid chromatography (HPLC, Shimadzu HPLC 10A VP, Shimadzu, Japan) was used to analyze samples for *p*-coumaric acid, quercetin, kaempferol, and free sugar using the method provided by Veberic et al. (44). Barros et al. (6) assessed the tocopherol content using HPLC, following the methodology previously described. In addition, the content of oxalic acid, one of the organic acid components of bee bread, was determined by HPLC (28). The volatile and aroma profile was determined by gas-chromatography–mass spectrometry (GC/MS) (Shimadzu GC-2010 Plus, Japan; Shimadzu GCMS-QP2010 SE (Detector)) solid-phase micro-extraction (SPME) (6). The 1,1-diphenyl-2-picrylhydrazyl (DPPH) method was used to evaluate the antioxidant activity of bee bread samples (32).

Production of Probiotic Yoghurt with Bee Bread: In the yoghurt production process, the milk was supplemented with 3% skimmed milk powder (Bagdat Baharat, Turkey). After applying heat treatment at a temperature of 90°C for 10 minutes, the homogenized milk received a subsequent cooling process to reach a temperature of 43°C. Subsequently, the milk was divided into five equal portions by introducing 2% starter and probiotic cultures. The experimental groups were administered bee bread dissolved in water at concentrations of 0.5%, 1%, and 2%. Subsequently, 100 grams of polystyrene containers were filled and subjected to incubation at a temperature of 42°C for a duration of 3.5 hours. Following the incubation period, the yoghurt samples were subjected to a cooling process, reducing their temperature to 4°C. Subsequently, these samples were stored at this specific temperature for a duration of 28 days, as reported by Tamime and Robinson (41) and Ozcan et al. (36). The study was designed to include three replications, and data analyses were conducted at intervals of 0, 7, 14, 21, and 28 days.

Microbiological Analysis: The samples of yoghurt that were examined for *S. thermophilus* were cultivated using M-17 agar (Oxoid CM785) (9). The current study used MRS 5.4 Agar (De Man Ragosa Sharpe, Difco 288210) as the medium for the examination of *L. bulgaricus* (10). The method ISO 20128/IDF192 reported was used to detect probiotic *L. acidophilus* LA-5 (20).

Physicochemical Analysis: The physicochemical parameters, including pH, acidity, dry matter, ash, milk fat, and water holding capacity, were assessed for yoghurt products using the Official Methods of Analyses (2). The

Kjeldahl method was used to conduct a crude protein analysis of yoghurt samples (45). Syneresis was analyzed using the methodology described by Wu et al. (46). The color analysis was performed using a colorimeter (Konika Minolta, CR 400, Osaka, Japan). The analysis involved the assessment of the L* (lightness), a* (red/green), and b* (yellow/blueness) parameters according to the Hunter scale.

Sensory Analysis: A study was conducted to evaluate the sensory characteristics of yoghurt samples over a period of 28 days under cold storage conditions. The evaluation was carried out by a panel of 10 individuals who had received comprehensive training in dairy product assessment, following the methodology proposed by Canbulat and Özcan (8).

Statistical Analysis: The study was replicated three times, and triplicate measurements were conducted for each parameter on the 1st, 7th, 14th, 21st and 28th day of storage. The statistical analysis of the data was conducted using SPSS 25.0 software (SPSS Inc., USA). The physicochemical composition data, including pH, acidity, dry matter, ash, milk fat, syneresis, and water holding capacity, were assessed using the generalized linear mixed model (GLMM) procedure. Additionally, microbiological analysis and sensory evaluation were also conducted and included in the evaluation. In the statistical design, fixed effects were assigned to groups and storage duration, whereas a random effect was assigned to replications. The Tukey multiple comparison test was employed to assess significant disparities among the average means. Statistical significance was determined when the p-value was less than 0.05 for differences observed among mean values. The chemical composition data, including protein, fat, and ash concentrations, as well as color attributes, were subjected to examination using a one-way analysis of variance (ANOVA). The findings were presented as mean values accompanied by standard errors (SE) of the mean.

Results

Content Analysis of Bee Bread: The data presented in Table 1 were obtained by analyzing the macroelement and microelement composition of the bee bread sample. The evaluation involved examining the presence of various elements, including B, Ca, Cr, Fe, K, Mg, Mn, Mo, Na, P, and Zn. In this study, an investigation was conducted on the sugar content of bee bread, resulting in the determination of fructose (149.4 mg/g), glucose (92 mg/g), and sucrose (21.1 mg/g). The assessment of the organic acid content in bee bread revealed the presence of oxalic acid (1.26 mg/g), malic acid (7.79 mg/g), ascorbic acid (0.91 mg/g), and citric acid (2.73 mg/g). In the scope of this study, the evaluation of the phenolic compound content of bee bread sample revealed the presence of *p*-coumaric acid (15.3 µg/g) and kaempferol (5562.4 µg/g). The concentrations of tocopherols, including alpha (7.09 µg/g), beta (0.4 µg/g), gamma (0.77 µg/g), and delta (0.31 µg/g), was determined by the study. In this study's parameters, 55 different components were identified by analyzing volatile/aroma profiles using GC/MS SPME. The components that were detected in the highest proportions are as follows: acetic acid (42.89%), octane (6.64%), 6-Methyl-5-hepten-2-one (5.62%), 3,5,5-Trimethyl-2-cyclohexanone (3.92%), 9-Nonadecane (3.78%), dimethyl sulfide (3.53%), nonanal (2.71%), methyl acetate (2.10%), and penten-3-one (2%). Other components were detected in proportions below 2% (Table 2). In the current study, the antioxidant activity of bee bread was determined using DPPH, and the IC_{50} value of bee bread was found to be 1.414 mg/mL (Figure 1).

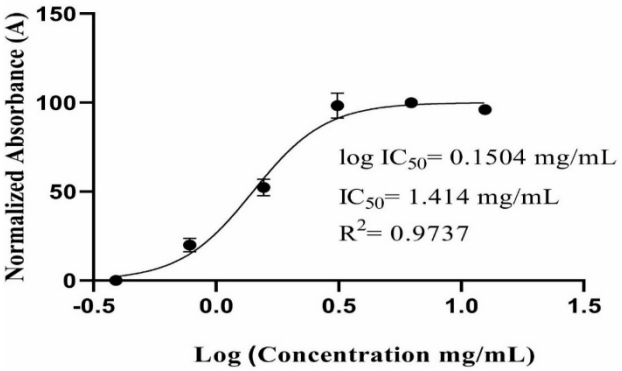

Figure 1. Bee bread IC_{50} value.

Table 1. The mineral content of bee bread.

Elements	Wavelenght (nm)	Content (mg/g)
B	249.677	0.013 ± 0.002
Ca	317.933	1.585 ± 0.162
Cr	267.716	< 0.005 ppm
Fe	238.204	0.111 ± 0.017
K	766.490	3.422 ± 0.043
Mg	285.213	0.564 ± 0.017
Mn	257.610	< 0.005 ppm
Mo	202.031	< 0.010 ppm
Na	589.592	0.056 ± 0.009
P	213.617	2.893 ± 0.076
Zn	206.200	0.013 ± 0.001

Table 2. Bee bread volatile compounds using GC/MS SPME.

Peak	R. Time	Name	Area	Area%
1	1.372	Ethyl alcohol	122132	0.68
2	1.443	Isopropenyl alcohol	264215	1.48
3	1.512	Dimethyl sulfide	632605	3.53
4	1.530	Methyl acetate	375229	2.10
5	1978	Acetic acid	7676538	42.89
6	2.217	2-Butenal	76342	0.73
7	2.250	3-Hydroxybutanal	46932	0.26
8	2.337	2-Pentanone	68819	0.38
9	2.554	Penten-3-one	358612	2.00
10	2.715	Heptanal	287448	1.61
11	2.815	2.5-Dimethylfuran	10899	0.06
12	3.428	Dimethyl disulfide	235749	1.32
13	3.665	(E)- 2-Pentenal	148120	0.83
14	3.818	3-Methyl-3-butenenitrile	119998	0.67
15	3.868	Toluene	101126	0.57
16	4.5627	Octane	1187763	6.64
17	5.531	Furfural 2-Furaldehyde	208555	1.17
18	6.6160	2-Hexenal	111234	0.62
19	6.611	o-Xylene	59561	0.33
20	6.660	p-Xylene	27292	0.15
21	7.322	Styrene	324620	1.81
22	7.668	Nonane	280952	1.57
23	7.731	Heptanal	121700	0.68
24	8.005	2-Acetylfuran	16516	0.09
25	8.030	Butyrolactone 2(3H)-Furanone. dihydro-	44587	0.25
26	8.107	2,6-Dimethylpyrazine	260670	1.46
27	8.549	Methyl caproate	40122	0.22
28	8.793	alpha- Pinene	95342	0.53
29	9.549	gamma- Valerolactone	21618	0.12
30	9.889	Benzaldehyde	41721	0.23
31	10.054	Dimethyl trisulfide	60981	0.34
32	10.284	Sabinene	25470	0.14
33	10.808	6-Methyl-5-hepten-2-one	1005430	5.62
34	11.285	3-Ethyl-1,4-hexadiene	78005	0.44
35	11.400	Decane	145345	0.81
36	11.512	Octanal	171343	0.96
37	11.656	cis- Ocimene	41193	0.23
38	11.865	(E,E)-2,4-Heptadienal	89802	0.50
39	12.276	Para Cymene	17543	0.10
40	12.466	Limonene	351807	1.97
41	13.050	Benzeneacetaldehyde	56476	0.32
42	13.206	beta-trans-Ocimene	21532	0.12
43	13.623	gamma- Terpinene	36112	0.20
44	14.155	(3E,5E)-3,5-Octadien-2-one	48730	0.27
45	14.954	2-Nonanone	13912	0.08
46	15.315	2,3,3-Trimethyloctane	160251	0.90
47	15.473	Nonanal	484166	2.71
48	16.047	3,5,5-Trimethyl-2-cyclohexenone	701488	3.92
49	16.964	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	33128	0.19
50	17.920	3,5,5-Trimethyl-1,4-cyclohexanedione	17958	0.10
51	19.151	Dodecane	47638	0.27
52	19.354	Decanal	152864	0.85
53	21.877	2,3,6,7-Tetramethyl octane	31218	0.17
54	28.250	Tetradecane	61462	0.34
55	29.281	9-Nonadecene	677095	3.78

DOI: 10.33988/auvfd.1326701

Table 3. The effect on protein values during storage in yoghurt experimental groups.

	Days				
Group	**1st day**	**7th day**	**14th day**	**21st day**	**28th day**
A	3.73 ± 0.01^{ze}	$3.81\pm0,04^{kd}$	$3.98\pm0,01^{yc}$	$4.23\pm0,02^{xa}$	4.11 ± 0.01^{xb}
B	3.74 ± 0.05^{zc}	3.91 ± 0.06^{zb}	3.92 ± 0.04^{yb}	4.18 ± 0.06^{xa}	4.28 ± 0.03^{xa}
C	3.86 ± 0.01^{yc}	4.00 ± 0.01^{yzbc}	3.89 ± 0.06^{yc}	4.14 ± 0.03^{xab}	4.18 ± 0.10^{xa}
D	3.83 ± 0.02^{yb}	4.04 ± 0.02^{yb}	3.49 ± 0.14^{zc}	4.14 ± 0.01^{xa}	4.17 ± 0.09^{xa}
E	4.02 ± 0.03^{xb}	4.31 ± 0.01^{xb}	4.31 ± 0.02^{xa}	4.27 ± 0.20^{xab}	4.15 ± 0.09^{xab}

a, b, c, d (→) Significant differences exist between the days indicated by different letters on the same line (P<0.05). x, y, z, k (↓) Significant differences exist between groups that are indicated by different letters in the same column (P<0.05). A: Control *L. acidophilus* LA5 (-), B: Control *L. acidophilus* LA5 (+), C: %0.5 bee bread, D: %1 bee bread, E: %2 bee bread.

Table 4. The effect on syneresis values during storage in yoghurt experimental groups.

	Days				
Group	**1st day**	**7th day**	**14th day**	**21st day**	**28th day**
A	14.47 ± 0.31^{ya}	$13.44\pm0,15^{yb}$	11.35 ± 0.50^{yd}	12.31 ± 0.02^{zc}	12.33 ± 0.31^{xc}
B	17.66 ± 0.07^{xa}	13.73 ± 0.19^{yb}	12.82 ± 0.56^{xc}	12.52 ± 0.38^{zc}	12.55 ± 0.60^{xc}
C	17.86 ± 0.01^{xa}	15.62 ± 0.32^{xb}	9.77 ± 0.35^{zkc}	9.97 ± 0.10^{yc}	8.79 ± 0.48^{yd}
D	17.62 ± 0.11^{xa}	15.60 ± 0.06^{xb}	8.80 ± 0.59^{kc}	9.26 ± 0.82^{yc}	8.87 ± 0.52^{yc}
E	17.76 ± 0.15^{xa}	15.19 ± 0.14^{xb}	10.38 ± 0.56^{yzc}	9.12 ± 0.01^{yd}	9.50 ± 0.41^{ycd}

a, b, c, d (→) Significant differences exist between the days indicated by different letters on the same line (P<0.05). x, y, z, k (↓) Significant differences exist between groups that are indicated by different letters in the same column (P<0.05). A: Control *L. acidophilus* LA5 (-), B: Control *L. acidophilus* LA5 (+), C: %0.5 bee bread, D: %1 bee bread, E: %2 bee bread.

Physicochemical Parameters: The physicochemical parameters, including pH, acidity, dry matter, ash, milk fat, and water holding capacity, were not significantly affected by adding bee bread (P>0.05). After analyzing the protein ratios of the yoghurt experiment groups, no significant difference was observed between the groups on the 21st and 28th days of the storage period (P>0.05). The group with the greatest protein ratio among all the groups was identified as E, with a statistical significance of (P<0.05). Protein levels in all groups increased over the last days of storage. (Table 3). As a result of examining the syneresis values of the various yoghurt experiment groups, it was found that the maximum syneresis occurred on the first day of storage (P<0.05). The evaluation indicated that there was a decrease in syneresis as the storage period increased, with a statistical significance of (P<0.05). The maximum syneresis value was observed on the initial day of study and in group C. The groups A and B demonstrated the highest syneresis value on the 21st and 28th days, while the groups containing bee bread showed a comparatively lower syneresis value (Table 4).

Microbiological Analysis: The addition of bee bread positively affected the growth of *S. thermophilus* and *L. bulgaricus*. Additionally, the activity of *L. acidophilus* LA-5 remained stable at approximately 10^7 kob/g throughout the storage period, with statistical significance (P<0.05). The results of the microbiological analysis are presented in Table 5.

Color Analysis: The L* values of the yoghurt experiment groups are presented in Table 6. During the initial analysis, it was observed that the L* value in group A was significantly greater than that in group E (P<0.05). There is an increase in the storage period towards the end compared to the initial days (P>0.05). The a* values of the yoghurt experimental groups are shown in Table 6. Group E had the greatest values up until the 14th day of storage, as shown by statistical analysis (P<0.05). During other analysis days, despite the apparently increased numbers, the statistical difference is not significant (P>0.05). Control groups A and B exhibited the lowest values on the 1th, 7th, and 14th days of storage, as assessed with statistical significance (P<0.05). While several groups exhibited statistical differences over different days, these variations could not be explained by the duration of storage. Upon analyzing the b* values of the different yoghurt experiment groups, it was noted that group E had the highest values (P<0.05). During the past two days of analysis, there was a significant difference in concentration between groups C and D (P<0.05). The control groups had significantly lower values compared to the other groups (P<0.05) (Table 6).

Table 5. *S. thermophilus, L. bularicus*, and *L. acidophilus* LA-5 bacteria growth values (log_{10} cfu/g) during storage in yoghurt experimental groups.

***S. thermophilus* (log cfu/g)**					
Group	**1st day**	**7th day**	**14th day**	**21st day**	**28th day**
A	8.24 ± 0.24^{ya}	7.90 ± 0.09^{ya}	6.84 ± 0.25^{yb}	7.01 ± 0.07^{yb}	7.04 ± 0.02^{zb}
B	7.84 ± 0.19^{za}	8.03 ± 0.19^{ya}	7.99 ± 0.42^{xa}	7.48 ± 0.12^{xya}	7.77 ± 0.26^{ya}
C	8.87 ± 0.12^{xa}	8.15 ± 0.24^{xybc}	7.81 ± 0.42^{xbc}	7.74 ± 0.31^{xyc}	8.38 ± 0.06^{xab}
D	8.65 ± 0.17^{xa}	8.32 ± 0.40^{xya}	8.03 ± 0.16^{xab}	7.19 ± 0.99^{yb}	8.09 ± 0.05^{xyab}
E	8.82 ± 0.07^{xa}	8.64 ± 0.23^{xab}	8.39 ± 0.13^{xb}	8.41 ± 0.12^{xab}	8.45 ± 0.29^{xab}
***L. bulgaricus* (log cfu/g)**					
Group	**1st day**	**7th day**	**14th day**	**21st day**	**28th day**
A	6.28 ± 0.05^{zc}	6.56 ± 0.09^{za}	6.94 ± 0.12^{zb}	6.39 ± 0.32^{zc}	5.58 ± 0.02^{kbc}
B	7.75 ± 0.14^{ya}	7.58 ± 0.15^{ya}	7.07 ± 0.21^{yb}	6.63 ± 0.33^{yb}	6.05 ± 0.03^{zc}
C	8.05 ± 0.22^{xya}	7.69 ± 0.31^{ya}	7.06 ± 0.06^{yb}	6.84 ± 0.33^{yb}	6.78 ± 0.19^{yb}
D	8.14 ± 0.19^{xa}	7.89 ± 0.14^{ya}	6.99 ± 0.16^{yb}	7.13 ± 0.04^{yb}	7.23 ± 0.23^{yb}
E	8.18 ± 0.10^{xab}	8.42 ± 0.13^{xa}	8.08 ± 0.09^{xab}	8.09 ± 0.04^{xab}	7.76 ± 0.40^{xb}
***L. acidophilus* LA-5 (log cfu/g)**					
Group	**1st day**	**7th day**	**14th day**	**21st day**	**28th day**
A	-	-	-	-	-
B	7.88 ± 0.11^{za}	7.87 ± 0.10^{ya}	7.28 ± 0.04^{yb}	7.37 ± 0.08^{yb}	6.47 ± 0.08^{zc}
C	7.98 ± 0.05^{za}	7.74 ± 0.24^{ya}	7.24 ± 0.16^{yzb}	6.79 ± 0.02^{kc}	6.91 ± 0.02^{yc}
D	8.33 ± 0.15^{ya}	7.77 ± 0.18^{yb}	7.05 ± 0.08^{zc}	7.18 ± 0.08^{zc}	7.01 ± 0.07^{yc}
E	8.73 ± 0.15^{xa}	8.25 ± 0.13^{xb}	$7.75\pm0.11x^{c}$	7.67 ± 0.07^{xc}	7.70 ± 0.10^{xc}

a, b, c (→) Significant differences exist between the days indicated by different letters on the same line ($P<0.05$). x, y, z (↓) Significant differences exist between groups that are indicated by different letters in the same column ($P<0.05$). A: Control *L. acidophilus* LA5 (-), B: Control *L. acidophilus* LA5 (+), C: %0.5 bee bread, D: %1 bee bread, E: %2 bee bread.

Table 6. The effect of storage on L*, a*, b* color levels in different groups of yoghurt experiments.

L* value					
Group	**1st day**	**7th day**	**14th day**	**21st day**	**28th day**
A	92.66 ± 2.39^{xa}	91.81 ± 3.08^{xa}	95.50 ± 0.37^{xa}	94.57 ± 0.53^{xya}	93.57 ± 0.51^{xa}
B	88.44 ± 1.95^{xyab}	87.33 ± 3.97^{xb}	87.28 ± 4.02^{yb}	95.51 ± 0.79^{xa}	94.09 ± 1.22^{xab}
C	86.45 ± 4.30^{xya}	84.85 ± 4.74^{xa}	86.64 ± 4.21^{ya}	91.24 ± 0.53^{xya}	89.10 ± 1.71^{ya}
D	88.44 ± 0.29^{xya}	86.92 ± 1.09^{xa}	89.99 ± 1.44^{xya}	87.12 ± 6.30^{ya}	91.03 ± 1.13^{xya}
E	84.49 ± 1.60^{ya}	83.69 ± 1.76^{xa}	86.51 ± 1.19^{ya}	$87.42\pm2,77^{xya}$	88.59 ± 1.59^{ya}
a* value					
Group	**1st day**	**7th day**	**14th day**	**21st day**	**28th day**
A	1.60 ± 0.18^{za}	1.54 ± 0.45^{za}	1.60 ± 0.07^{za}	1.34 ± 0.11^{ya}	1.21 ± 0.17^{za}
B	0.93 ± 0.02^{zb}	1.35 ± 0.28^{zab}	1.24 ± 0.07^{zb}	1.37 ± 0.06^{yab}	1.77 ± 0.28^{zka}
C	2.45 ± 0.07^{ya}	2.97 ± 0.54^{ya}	3.16 ± 0.71^{ya}	2.45 ± 0.14^{ya}	2.67 ± 0.42^{yza}
D	3.09 ± 0.25^{ya}	2.97 ± 0.28^{ya}	3.45 ± 0.05^{ya}	3.58 ± 0.89^{xa}	3.80 ± 0.96^{xya}
E	4.98 ± 0.53^{xab}	4.49 ± 0.19^{xabc}	5.06 ± 0.10^{xa}	4.20 ± 0.15^{xc}	4.28 ± 0.23^{xbc}
b* value					
Group	**1st day**	**7th day**	**14th day**	**21st day**	**28th day**
A	4.23 ± 0.51^{za}	3.53 ± 0.09^{ka}	4.37 ± 1.46^{za}	3.30 ± 0.38^{ka}	3.04 ± 0.13^{ka}
B	4.73 ± 0.59^{zb}	5.89 ± 0.11^{za}	3.89 ± 0.12^{zc}	2.24 ± 0.09^{ld}	2.88 ± 0.14^{kd}
C	9.61 ± 0.29^{yb}	13.40 ± 0.59^{ya}	9.76 ± 0.03^{yb}	8.01 ± 0.10^{zc}	9.42 ± 0.05^{zb}
D	11.50 ± 1.38^{yb}	13.42 ± 0.49^{ya}	9.96 ± 0.16^{ybc}	9.27 ± 0.01^{yc}	9.79 ± 0.14^{ybc}
E	18.58 ± 0.01^{xa}	18.27 ± 0.15^{xa}	16.51 ± 0.30^{xb}	13.86 ± 0.03^{xd}	15.76 ± 0.04^{xc}

a, b, c, d (→) Significant differences exist between the days indicated by different letters on the same line ($P<0.05$). x, y, z, k, l (↓) Significant differences exist between groups that are indicated by different letters in the same column ($P<0.05$). A: Control *L. acidophilus* LA5 (-), B: Control *L. acidophilus* LA5 (+), C: %0.5 bee bread, D: %1 bee bread, E: %2 bee bread.

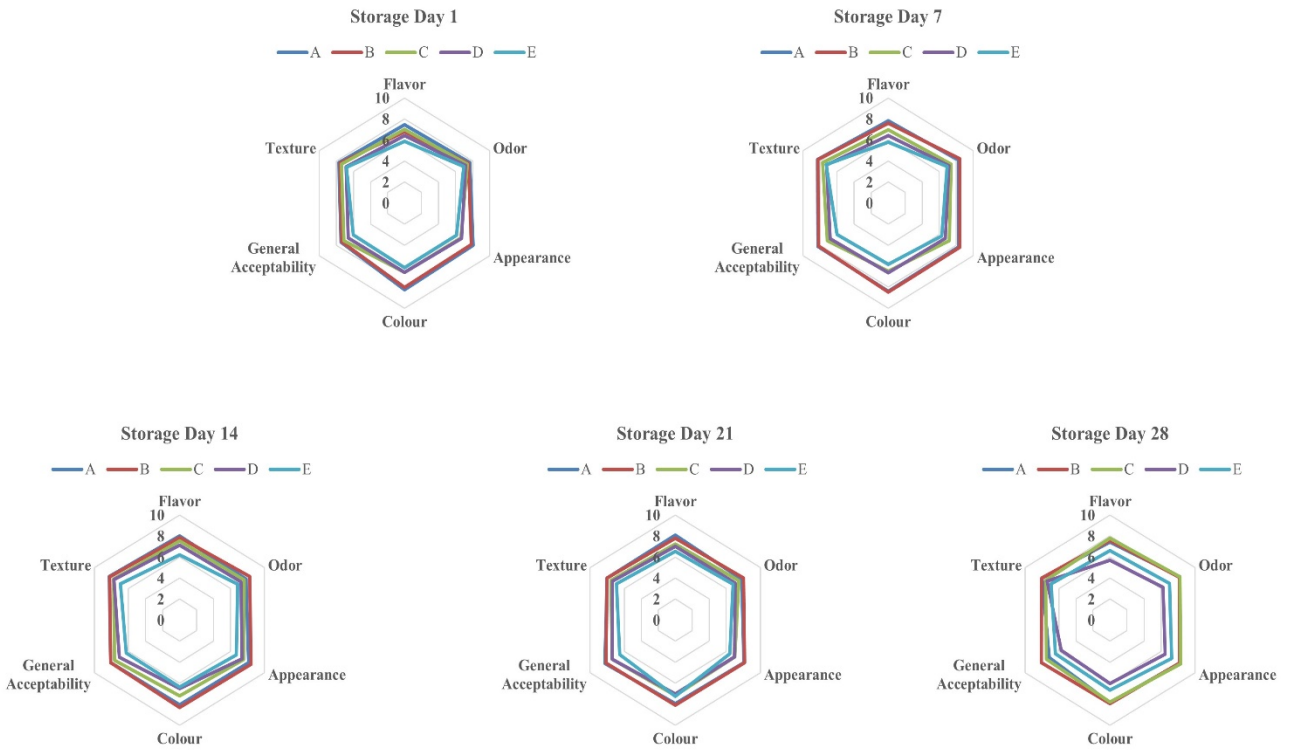

Figure 2. A graphical representation showing the sensory analysis values observed on the 1st, 7th, 14th, 21st, and 28th days of storage.

Sensory Analysis: Following the conclusion of a sensory analysis, consumers indicated that they favored the group that was provided with 0.5% bee bread. The results of the sensory evaluation are shown in Figure 2.

Discussion and Conclusion

Bee bread has been identified as a significant protein source (19.96/100g). Additionally, it is rich in total free sugar (18 grams per 100 grams), macroelements, microelements, polyunsaturated fatty acids, tocopherol, and natural antioxidants. Furthermore, it was ascertained that bee bread exhibited antioxidant properties and demonstrated efficacy against all examined bacteria and fungi (5). Similar to this study, Bakour et al. (4) identified fructose (118 mg/g) and glucose (57 mg/g) as primary components. In a study conducted in Romania, Dranca et al. (14) identified the presence of gluconic acid, formic acid, acetic acid, propionic acid, and butyric acid. Bakour et al. (4) identified the presence of oxalic acid in bee bread in another study. According to a recent investigation conducted by Bayram et al. (7), an analysis of phenolic components in pollen and bee bread demonstrated elevated levels of protocatechuic acid, 2,5-dihydroxybenzoic acid, and kaempferol in bee bread. The kaempferol content of the data obtained from this study shows similarities. In a survey conducted by Bakour et al. (4), it was observed that the α-tocopherol content measured 10.5 µg/g, while the δ-tocopherol content measured 0.40 µg/g, exceeding the levels observed in the present study. In a study conducted by Hryniewicka et al. (19), the researchers determined that the α-tocopherol content of bee bread was measured to be 80±30 µg/g. Differences in values could potentially be attributed to variations in botanical provenance. In the context of this research, it is important to determine the existence of tocopherol in bee bread. GC/MS SPME aroma profile study by Kaškonienė et al. (25) found 32 components in bee bread and honey. Dimethylsulfide, acetic acid, furfural, nonan, and 1-heptadekene are 20.0%, 13.4%, 9.8%, 10.4%, and 13.9%, respectively. According to the findings of Bakour et al. (4), the mineral content of bee bread in this study exhibited comparable values. Specifically, the mineral content of bee bread was found to be as follows: calcium (Ca) at 1.98 mg/g, iron (Fe) at 0.273 mg/g, potassium (K) at 3.38 mg/g, magnesium (Mg) at 0.61 mg/g, sodium (Na) at 0.142 mg/g, zinc (Zn) at 0.0331 mg/g, phosphorus (P) at 2.51 mg/g, and manganese (Mn) at 0.026 mg/g. In a previous study conducted by Andjelkovic et al. (1), the primary mineral identified as potassium (K), with phosphorus (P), calcium (Ca), and magnesium (Mg) following as secondary minerals. The primary origin of mineral substances within bee bread is from flower pollen, which is a significant mineral reservoir in both nectar and water. According to Andjelkovic et al. (1), geographical conditions can influence the mineral substance content. Ivanišová et al. (21) found that 15.78 mg TEAC/g was the highest antioxidant activity in bee bread samples collected from

five distinct localities within Ukraine. The IC_{50} value of bee bread was calculated to be 1.414 mg/mL in this study.

According to the findings of Khider et al. (26), adding 1% pollen to yoghurts resulted in a decrease in syneresis, an improvement in texture, and a pleasant aroma. The individual stated that the rheological characteristics and the presence of advantageous bacteria in fermented beverages were altered upon the addition of bee pollen. The study conducted by Yerlikaya (47) found no discernible adverse consequences associated with the incremental addition of pollen. Another study investigated the impact of different pollen rates (0%, 5%, 1%, 2.5%, and 3%) on the bio-functional properties of yoghurt produced from cow, sheep, and goat milk. Research findings have indicated that the inclusion of pollen in yoghurts increases their antioxidant capacity and total phenolic content. Furthermore, enhancements were observed in the sensory attributes of yoghurt, including taste, aroma, visual appeal, and texture. According to a study conducted by Karabagias et al. (24), it has been suggested that incorporating bee pollen into yoghurts could potentially serve as a cost-effective means of producing functional food products, thereby holding significant promise for future applications. A research study using bee bread as an additive determined that the pH level exhibited greater intensity than the control group.

The color attribute of foods is regarded as a significant factor in determining consumer acceptance (29). The observed disparity in L* values between groups A and E in this study may be attributed to the absence of bee bread in the first group. According to Ozcan et al. (35), there is a negative correlation between adding pollen to yoghurt and the L* value, indicating a decrease in the L* value as the amount of pollen increases. The higher b* and a* values observed in group E could potentially be attributed to the excess concentration of bee bread. The control groups in both color groups were found to have lower values compared to the other groups. The potential explanation for this could be the lack of bee bread within these groups. In their study, Ozcan et al. (35) conducted an evaluation that revealed that the inclusion of pollen resulted in a reduction of b* and a* values. The observed differences are believed to have originated from the related structural differences between bee bread and pollen. Insufficient research has been conducted on using bee bread to produce yoghurt.

This study targeted to investigate the potential impact of bee bread on probiotic bacteria during the storage period of yoghurt. Upon analysis of the acquired data, it was discovered that the experimental groups, which were supplemented with bee bread, exhibited a notable enhancement in the population of *L. acidophilus* LA-5. In an additional study, the impact of integrating pine honey into yoghurt at varying concentrations (2%, 4%, 6%) on the activity of *L. acidophilus* was assessed. The study findings revealed a notable reduction in the population of microorganisms during the preservation procedure, specifically a lower count of *L. acidophilus* compared to the mentioned study. The highest recorded count was determined to be 7.70 log cfu/g. It can be said that bee bread demonstrates a greater impact on probiotic activity compared to pine honey (12). This study suggests that the inclusion of bee bread generally resulted in higher probiotic activity in the respective groups. The maintenance of probiotic activity is estimated to be sustained at approximately 10^7 colony-forming units per gram (cfu/g). According to the findings of Demirci et al. (13), it is recommended that the concentration of probiotic bacteria should be no less than 10^7 colony-forming units per gram (cfu/g) in order to produce beneficial health effects. In their study, Panesar et al. (37) observed that the probiotic microorganisms *L. acidophilus* and *B. bifidum* maintained their continuity in probiotic yoghurt containing *Aloe vera* even after storage.

The data collected from the panelists during the sensory analysis conducted in this study generally exhibited values of 5 or higher. The panelists generally rated Group C (0.5%), one of the experimental groups that received bee bread supplementation, as more acceptable. Certain storage durations and sensory characteristics have been observed to produce a higher preference for groups containing added probiotic bacteria than those without. The observed improvements in sensory parameters can be attributed to the introduction of probiotic bacteria, as suggested by Atallah (3). According to a study conducted by El-Kholy et al. (15), yoghurts that incorporated nano-encapsulated pollen were found to have satisfactory sensory attributes. Insufficient sensory analysis data is available for producing yoghurt using bee bread. Hence, the significance of this study cannot be overstated.

In conclusion, the study findings indicate that bee bread possesses significant importance as a bee product due to its composition, which includes high levels of sugar content, organic acids, tocopherols, mineral substances, phenolic components, volatile components, and antioxidant substances. The presence of natural components in the composition of bee bread has been found to have advantageous impacts on human health. Bee bread, because of its high content of mineral and phenolic components, maintains the potential for the development of food products or food additives. Studies can be conducted to explore the innovative aspects of the antioxidant activity observed in bee bread. The study has demonstrated that adding bee bread during yoghurt production can effectively maintain probiotic activity. The inclusion of bee bread in yoghurts has been found to be positively perceived by consumers as a health-promoting

product. This study presented significant data regarding the utilization of bee bread within the food industry.

Acknowledgements

This study was derived from the Ph.D. thesis of the first author.

Financial Support

This research has been supported within the context of project no: 0770-DR-21/2017K12-41003 by The Scientific Research Projects Committee of Burdur Mehmet Akif Ersoy University.

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

The experiments were conceived and planned by NK and OY. NK and OY conducted the experiments. NK and OY conceived and executed the simulations. NK and OY contributed to the interpretation of the results. NK managed the composition of the manuscript. All authors provided constructive feedback and contributed to developing the research, analysis, and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study does not present any ethical concerns.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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DOI: 10.33988/auvfd.1326701