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Research Article

Yogurt supplemented with bee pollen: Physicochemical and sensory properties, and in vitro pollen digestibility

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Abstract

This work aimed to formulate yogurt with different levels of bee pollen; determine the in vitro bee pollen (BP) digestibility; and evaluate the yogurt's physicochemical properties, sensory acceptance, and microbial counts during 33 days of refrigerated storage. The multifloral BP composition was also analyzed. The composition of the BP was 21.3% protein, 2.2% fat, 65.1 carbohydrates, 3.1% ash, and 7.8% moisture. The yogurts were supplemented with 1% to 7.5% BP and 4% of bee honey. Yogurt added with 5% of BP was selected for tests during storage and in vitro digestibility analysis. As expected, BP enhanced the protein content of yogurt up to 4.5%, provided yellow color (reducing luminosity (L*) and increasing yellowness (b*)), and increased the titratable acidity by 0.18%. In vitro BP digestibility improved up to 99% after grinding and fermentation, whereas ground BP suspended in milk reached a value of 88%. At the end of the storage, yogurt supplemented with BP had a luminosity of 61.6 and a microbial count of ~8.3 $\log_{10}$ CFU/g. After 33 days of storage, the pH dropped by ~0.3 units and sensory acceptability decreased by one unit. The incorporation of BP in yogurt formulation improves its nutritional quality.

1. Introduction

Bee pollen (BP) is recognized as nutritionally rich in protein (7-40%), lipids (1-18%), carbohydrates (24-60%), and bioactive compounds (phenolic compounds, flavonoids, and anthocyanins) [1]. The composition of BP primarily depends on flower type and region of production. Bee pollen provides health benefits such as decrease lipids and cholesterol levels in blood serum, hypoglycemic and anti-atherosclerotic effects, reduce symptoms of allergic diseases (rhinitis and asthma), improves male reproductive functions, relieves constipation, and release oxidative stress and inflammation of nerves [1, 2]. However, BP direct ingestion does not ensure the availability of nutrients and bioactive compounds due to its outer wall known as exine, which provides chemical resistance [3]. In fact, if raw BP is chewed and swallowed by humans, only 10-15% of the nutrients are utilized whereas grinding process increases its bioavailability up to 60-80% [1]. Fermentation (commercial yogurt starters) and

enzymatic hydrolysis (serine peptidase) improve digestibility of proteins up to 84.8% and 89.7%, respectively [3]. The daily intake of BP for an adult can range from 20 to 40 g [1].

Yogurt is a fermented milk produce by the symbiotic cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* [4]. Lactic acid bacteria are capable to enhance the digestibility of protein in food and, in consequence, improve the nutritional value [5]. Previous studies have demonstrated that lactic acid fermentation improves digestibility of proteins from plant-based such as maize-based porridge [6], quinoa flour [7], soybean flour [8], and sorghum flours [9]. In the last decade, various studies have been conducted to assess the incorporation of bee pollen into yogurt formulation and evaluate the composition; physicochemical, sensory, microbial, and rheological properties; antioxidant capacity, microstructure, amino acids, and probiotic growth and viability [10-12]. As mentioned above, BP provides important nutrients and bioactive compounds with positive impact on human health; moreover, often it is recognized as complete food [13]. Thus, the study of BP incorporating into yogurt is valuable for industrial applications. Scarce information about BP supplementation >1%, color and in vitro digestibility of BP into yogurt fermentation has been reported. Therefore, the aim of this work was to formulate a yogurt with BP and honey and assess its physicochemical properties, microbial counts, sensory acceptance, and BP in vitro digestibility in yogurt. In addition, BP composition was evaluated.

2. Materials and methods

2.1 Materials

Commercial bee pollen collected in Puebla, Mexico, was acquired from local producers. Multifloral bee honey (produced from the nectar of more than one type of flower) was also purchased from local producers. The starter culture yogurt was a lyophilized commercial mixture (YO-MIX, Danisco, Madison, WI) donated by Alcatraz distributor (Puebla, Puebla, Mexico). The mixture contains *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Whole milk was acquired at a local supermarket in Puebla, Puebla, Mexico.

2.2 Proximate composition analysis of bee pollen

Bee pollen grains were ground in a mortar before the proximate analysis. Moisture content was determined using 5 g of BP by weighing the difference in an oven at 105±2 °C. Proteins were quantified following the Micro-Kjeldahl method using 0.2 g of BP and a factor conversion of 6.25. Fat was determined in 5 g of BP by Soxhlet extraction with petroleum ether for 6 h. For ashes, method 942.05 AOAC [14] were followed. Total carbohydrate was calculated by difference with the previous macro-components.

2.3 Preparation of yogurt with bee pollen

Each liter of whole milk (33 g/L of fat and 31 g/L of protein) was added with 10, 25, 50, or 75 g ground BP, 40 g bee honey, and 0.2 g of starter culture. The level of bee honey was chosen based on the previous report by Metry and Owayss [10] which improved the culture viability and sensory acceptability of a yogurt with bee honey. The inoculated milk was distributed in portions of 100 mL in polypropylene cups and incubated at 42 °C until pH of 4.6 was reached. Then, cups were cooled to 5 °C for 24 h. After the cooling time, the storage time was considered equal zero day. Yogurts were ranked with preliminary sensory evaluation by 15 semi-trained panelists from university staff and postgraduate students. Yogurt formulated with 5% of BP was selected for further tests and stored for analysis. Cups containing yogurts were stored at 5 °C, and at 7, 11, 18, 25, and 33 days, three cups were taken to determine the viability of starter culture, color, pH, and titratable acidity. Meanwhile, the level of acceptability was determined by sensory evaluation at 0, 11, and 33 days of storage. In addition, yogurt without BP and bee honey (control) was also prepared and stored under the same conditions for physicochemical and microbiological analysis.

2.4 Physicochemical analysis of yogurt

Yogurt with BP (5%) and control were analyzed to determine the protein content using the Kjeldahl method and fat following the method 989.05 AOAC [14]. Total reducing sugars were analyzed in yogurt with BP using the Miller method utilizing 3,5-dinitrosalycilic acid reading the absorbance at 570 nm in a spectrophotometer (Genesys 20, Thermo Fisher Scientific). These tests were determined only at the beginning of the storage. Two commercial brands of yogurts (one sweet plain yogurt (CY) and one with bee honey (CYH)) were also analyzed for total reducing sugars.

The pH, titratable acidity, and color were measured during the refrigerated storage of both yogurts. Yogurt's pH was measured by electrode immersion with a pH meter (Orion Star A211, Thermo Scientific Inc., Singapore). 10 g of yogurt was used to determine the percentage titratable acidity by titration with 0.1 N NaOH using phenolphthalein as indicator. Results were expressed as lactic acid (g/100 g of yogurt). The color of yogurt was measured in 20-mL samples in triplicate using a colorimeter (CR 400, Konica Minolta Inc., Japan) calibrated to measure reflectance. The parameters were measured using the CIELAB scale (lightness (L*), red-green color (a*), and yellow-blue color (b*)). The Euclidean distance between the yogurt during the storage and yogurt at zero days (ΔE) was calculated using the following equation.

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

2.5 In vitro digestibility of pollen in yogurt

In vitro digestibility of yogurt with BP was determined using the method previously reported by Zuluaga et al. [3] with some modifications. Briefly, 30 g of dry yogurt was mixed with 150 mL of 0.002% pepsin in HCl 0.075 N solution and kept in agitation for 16 h at 45 °C. Following, the mixture was filtered, and the protein content of the digestible portion was determined by Kjehldahl method. To assess the effect of fermentation on BP, whole milk mixed with 5% of ground BP was analyzed as previously described. In vitro digestibility is the ratio between digested protein per 100 g of the total protein content of the yogurt.

2.6 Determination of microbial viability during storage

Microbial counts were determined in yogurt with BP and control. One gram of yogurt was diluted in 9 mL of sterile peptone water (0.1%) and appropriate ten-fold dilutions were plated on MRS agar for *S. thermophilus* and *L. bulgaricus* counts. Inoculated plates were incubated anaerobically for 72 h at 37 °C.

2.7 Sensory evaluation during storage

Sensory tests were carried out with yogurt with BP (5%) at 0, 11, and 33 days of storage. Thirty untrained panelists evaluated the overall acceptability. Panelists were selected from university staff and students, who regularly consumed yogurt. A 1-9 hedonic scale was used to qualify the sample, in which a score of 9 represents the attribute most liked and a score of 1 represents the attribute most disliked. Scores around 6 were considered acceptable.

2.8 Statistical analysis

Statistical analysis of the data was performed by ANOVA and Tukey's mean comparison tests ($p<0.05$) using MINITAB statistical package (ver. 17), to identify differences in starters viability, pH, color, and sensory evaluation parameters during the storage.

3. Results and discussion

3.1 Bee pollen composition

Table 1 presents the proximal composition of BP used in this study. Values of moisture and ash were in the range expected for this product 2% to 9% and 1.5%-3.2%, respectively [1, 15, 16]. Bee pollen commonly contains1-13% fat [17] hence values obtained in this study are in the previously reported range. Regarding proteins and carbohydrate content, the levels were similar to those reported by Feás et al. [18] for 22 samples of organic BP (proteins ranged from 19.1 to 27.1% and carbohydrates values were 61.2-70.6%). Darwish et al. [15] recorded a very similar protein content (21.09%) of BP from Alexandria, Egypt. Therefore, the BP used in this study fulfills the typical composition of this food.

Table 1. Bee pollen composition was used in this study.

Component	Value (%)
Moisture	7.8±0.29
Ash	3.1±0.03
Fat	2.2±0.08
Proteins	21.3±0.63
Carbohydrates	65.1±0.66

3.2 Physicochemical analysis of yogurt with bee pollen

Table 2 displays the physicochemical properties of yogurt with BP and control. The addition of BP (5%) increased the proteins by 45% in this study. This improvement was higher than those reported by Chinelate et al. [19] for yogurt with 5% of BP (~18%) when they compared it with control yogurt. In contrast, fat in yogurt was reduced 17% when BP was incorporated, whereas yogurt formulated with 5 % of BP by Chinelate et al. [19] increased fat by 3.6%. Rosero and Herrera [20] reported a similar value of fat (2.76%) for yogurt added with 1% of BP and 3.75% of bee honey. Differences could be attributed to BP and milk composition. Despite the increases being dependent on raw material composition, the protein level was increased significantly ($p<0.05$) in this study. Titratable acidity (TA) was higher in yogurt with BP (+0.18%) despite the pH were lower than the control

Table 2. Physicochemical properties of yogurt with and without bee pollen.

Parameters	Yogurt with bee pollen (5%)	Yogurt control
pH	4.63±0.01	4.38±0.01
Titratable acidity (%)	0.96±0.04	0.78±0.06
Color		
L*	55.85±0.36	74.25±0.69
a*	-1.35±0.10	-2.19±0.03
b*	28.07±1.12	6.92±0.05
Proteins (%)	4.5±0.26	3.1±0.10
Fat (%)	2.6±0.08	3.13±0.12
Total reducing sugars (g/L)	19.6±0.14	ND

yogurt (0.25 units). The addition of bee products (BP, bee bread, or/and royal jelly) at 1% (individual or mixed) increased TA and reduced the pH of fermented milk [15]. In contrast, the supplementation of low amounts of BP (≤0.8%) did not modify the yogurt's pH [21]. Bee pollen influenced the TA of yogurt, since could be provided levels of titratable acidity ranged from 189 to 323 meq/kg [22]. In addition, BP and bee honey provided more sugars to LAB during fermentation, resulting probably in more lactic acid production. L* parameter was lower than those reported for yogurt with 1% of BP and 5% of bee honey (L*= 85.70; [20]) or 3% of BP (L*= 79.98; [22]), the difference is attributed to the amount of BP incorporated into yogurt. The a* (red-green color) parameter decreased and b* (yellow-blue color) boosted due to BP contributed to green and yellow color of yogurt. Similar values of a* and b* were reported for yogurt with 3% of BP [22]. The amount of BP added to yogurt is probably the main reason for the differences. The color parameters a* and b* of the control yogurt were similar to previously reported for natural yogurts, which ranged from 6.87 to 8.04 for b* value and -2.19 to -2.73 for a* value [23]. The L* value was lower than luminosity of probiotic yogurts (L*= 83.12) [23]. The total reducing sugars in commercial yogurts were 14.7 and 16.2 g/L for commercial sweet plain yogurt and commercial yogurt with bee honey, respectively. Hartati et al. [24] reported a high value (29.9 g/L) of total reducing sugars for a yogurt added with 6% of date (*Phoenix dactylifera*). Yogurt supplemented with BP (5%) had a value of total reducing sugars similar to commercial yogurts analyzed in this study.

Figure 1 presents the pH and titratable acidity (TA) during the storage of yogurts. The average value of TA for control yogurt was 0.87% whereas for yogurt with BP was 0.97%. Bee pollen added sugars to yogurt, and consequently more lactic acid was produced during fermentation. Fuenmayor et al. [22] reported that BP contained 19.5% of fructose, 13.6% of glucose and 6.7% of sucrose. Post-acidification during the storage was evident in control yogurt since increments of titratable acidity were ~0.15% after 33 days of storage, whereas yogurt added with BP remained constant values of TA and pH. Atallah [17] reported higher increments of TA for control yogurt (without BP) than for yogurt added with 0.8% of BP. In contrast, Rosero and Herrera [20] observed an increase of 0.35% of titratable acidity after 30 days of storage for yogurt contained 1% of BP and 3.75% of bee honey. Camacho-Bernal et al. [25] mentioned that bee honey (2-7%) or BP (0.8%) addition reduced the acidity.

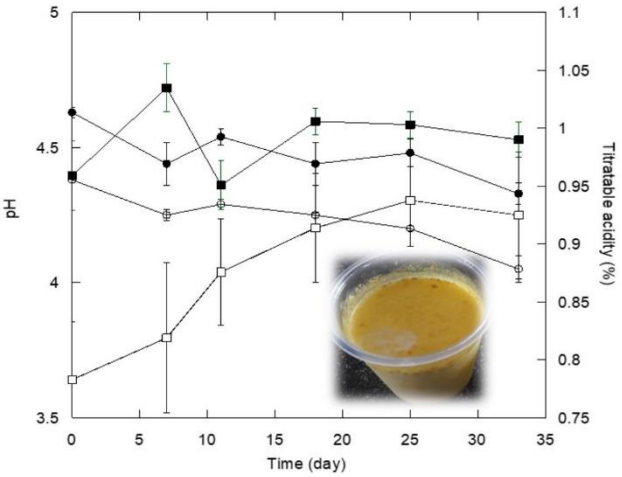

Figure 1. pH and titratable acidity (%) in control yogurt (○ pH, □ titratable acidity) and yogurt with 5% bee pollen (● pH, ■ titratable acidity).

Table 3 exhibits the color parameters of yogurts formulated in this study. Control yogurt remained steady color during storage. In yogurt supplemented with BP, the L* and a* parameters increased slightly for 11 days, then values dropped. Luminosity increases may be due to discoloration or changes in pollen pigments. For instance, b* values displayed increases during storage trended to yellow tones whereas a* to green tones. L* and a* changes during storage influenced the ΔE behavior, thus it increased at day 11 and slowly reduced till the end of storage. Rosero and Herrera [20] did not observe color changes in yogurt with BP (1%) stored by 30 days.

Table 3. Color parameters of yogurts during refrigeration

Parameters	Yogurt with 5% of bee pollen					
	0 d	7 d	11 d	18 d	25 d	33 d
L*	55.85±0.36e	58.23±0.80d	64.89±0.60a	62.21±0.62b	60.70±0.20c	61.66±0.61bc
a*	-1.35±0.10a	-1.46±0.13ab	-1.62±0.07b	-1.52±0.15ab	-1.48±0.03ab	-1.46±0.14ab
b*	28.07±1.12c	30.92±0.80b	31.18±0.50b	31.10±1.35b	31.69±0.57b	34.61±0.89a
ΔE	----	3.89±1.01d	9.65±0.77a	7.29±1.13bc	6.17±0.96c	8.85±1.12ab
	Control yogurt					
L*	74.25±0.69c	77.65±0.54a	73.82±0.53c	76.06±1.11b	72.12±0.24d	75.03±0.31bc
a*	-2.19±0.03ab	-2.42±0.02e	-2.34±0.02d	-2.13±0.04a	-2.23±0.03bc	-2.27±0.06c
b*	6.92±0.05b	7.18±0.05a	7.15±0.06a	6.79±0.14b	6.83±0.09b	7.24±0.17a
ΔE	----	3.42±0.50a	0.55±0.27c	1.83±1.10b	2.13±0.56b	0.92±0.49bc

3.3 In vitro digestibility of yogurt with bee pollen

The in vitro digestibility of BP incorporated in yogurt achieved 99.01±1.0% whereas BP mixed with milk reached 88.3±1.1%. In vitro digestibility was favored by mechanical grinding since exine was fractured in the BP granule. Fermentation improved the in vitro digestibility since BP achieved ~100% of protein digestibility. This is not surprising because protein in fermented milk is reported as totally digestible [26], and in this case, BP protein also is transformed into ~100% in vitro digestible. It attributes to proteolytic activity of starter bacteria (lactic acid bacteria) which results in high levels of peptides and amino acids [26, 27]. More details about lactic acid bacteria metabolism and protein degradation can be revised in Wang et al. [27]. Zuluaga et al. [3] did not improve the in vitro digestibility of BP fermented using a starter culture (*S. thermophilus*, *Lactobacillus lactis*, and *L. bulgaricus*) after thermal treatment (10 min at 121 °C). They fermented the BP in water at a ratio of 1:1 for 72 h at 37 °C; the inoculum was previously prepared using 1 g of BP in 9 mL of MRS broth, and 1 mL from activated culture (10^8 CFU/mL) in saline solution incubated by 48 h at 37 °C. They reported ~83% of digestibility of BP after thermal treatment. In other study, enzymatic treatment of BP using an endo-protease (Protamex™) under constant shaking (200 rpm) for 4 h at 37 °C and sterilized at 121 °C for 15 min achieved in vitro digestibility up to 93% [28]. High in vitro protein digestibility of BP in yogurt was obtained due to excellent hydrolysis of proteins by the starter culture. Previous studies have shown lactic acid fermentation increased the in vitro protein digestibility in soybean flours from 85.5% to 93.7% and sorghum flours from 64% to 85% after 48 h fermentation with LAB-consortium from maize or sorghum fermented [8, 9]. The partial degradation of complex storage proteins into more simple and soluble compounds allows protein digestibility improving [29]. The enzymatic treatment of the isolated pea protein and fermented with LAB (*Lactobacillus plantarum* CKDHC 0801 and *Lactobacillus brevis* KCCM 11509) showed higher in vitro digestibility (57.4%) than isolated pea protein (35%) [30].

3.4 Microbial viability during storage

Figure 2 displays the counts of lactic acid bacteria during the refrigerated storage (5 °C) of yogurts. Microbial counts reached levels ~7 $\log_{10}$ UFC/g at the end of fermentation and were increased gradually up to ~9 $\log_{10}$ UFC/g. Bacteria growth could be attributed to their residual activity and to bee honey oligosaccharides which exhibit prebiotic effects stimulating the lactobacilli counts [25, 31, 32]. Yerlikaya [11] reported higher microbial counts for *Lactobacillus acidophilus* and *S. thermophilus* after fermentation of yogurts (with 2% of BP or without BP, which ranged from 8.0 to 10 $\log_{10}$ CFU/mL). After 21 days, in control yogurt counts of *L. acidophilus* remained constant at 9.3 $\log_{10}$ CFU/mL and *S. thermophilus* increased slightly up to 10.14 $\log_{10}$ CFU/mL. In yogurt with BP (2%) slight decreases (~0.7 $\log_{10}$ CFU/mL) were observed for both bacteria at the end of 21 days [11].

3.5 Sensory analysis during storage

Average scores of overall acceptability were 6.6±1.0, 6.3±1.3, and 5.5±1.8 for 0, 11 or 33 days of refrigerated storage. The acceptability level of yogurt corresponded to "like little". Darwish et al. [15] obtained similar scores in taste and texture (6.9-7.0) for fresh fermented milk added with 1% BP. After 11

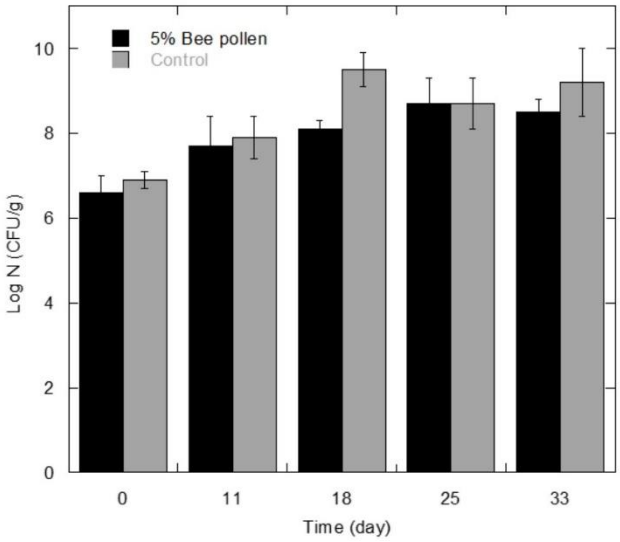

Figure 2. Lactic acid bacteria count during refrigerated storage (5 °C) of control yogurt and yogurt with 5% of bee pollen.

days, the level of acceptability did not change ($p>0.05$) whereas at 33 days of storage the level of acceptability was declined ($p<0.05$). Panelists commented at 33 days that yogurt had bitter taste, it is probably attributed to free amino acids increased during storage. It is well known that fermentation process of BP elevated free amino acids levels as a result of the activity of proteolytic enzymes [1]. Yerlikaya [11] exhibited the release of amino acids in yogurt supplemented with 2% of BP. El-Kholy et al. [33] assessed the sensory attributes of yogurt enriched with date palm pollen grains (0.75%) during the storage. The overall acceptability of yogurt remained unchanged after 15 days, which is similar to this work. In other study, a yogurt supplemented with 2% of BP obtained similar scores in general acceptability attribute (~6.5) from the beginning to after 21 days of storage [11]. According to previous reports [11, 33] and the data obtained in this study, the suggested shelf life for yogurt with BP considering the sensory acceptability is 15-20 days.

4. Conclusions

The yogurt supplemented with 5% bee pollen was the best accepted by panelists. The grinding and fermentation processes improved the in vitro bee pollen digestibility up to 99% which was higher than ground bee pollen suspended in milk (88%). Bee pollen enhanced the protein content of yogurt. The incorporation of bee pollen into yogurt improves their nutritional quality. To expand the knowledge of yogurt supplemented with BP, future research directions could include amino acids composition during the storage, instrumental texture analysis, sensory analysis every week or 5 days during the storage and confirm the health benefits through in vivo studies.

Authors' contributions

Conceptualization, V.C.A. and E.M.L.; Methodology, V.C.A. and E.M.L.; Formal Analysis, V.C.A.; M.E.M.L and E.M.L.; Writing – Original Draft Preparation, E.M.L.; Writing – Review & Editing, E.M.L.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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