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Impact of Bee Pollen Addition on the Quality Characteristics of Probiotic UF-Soft Cheese

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THIS study aimed to evaluate adding bee pollen in different levels (0, 1.0, 2.0 and 3.0%) in the quality characteristics of probiotic UF-soft cheese. Thesamples were analyzed for physicochemical, biochemical, minerals (Iron, Manganese and Zinc), total phenolic, antioxidant activity, free fatty acids, free amino acids, textural,viabilityof ABT probiotic bacteria (*Lactobacillus acidophilus* LA-5, *Streptococcus thermophiles* and *Bifidobacterium* BB-12),and sensory propertiesof UF-soft cheese during the 30 days of storage. Bee pollen addition in UF-soft cheese significantly increased acidity, fat, protein, ash, phenolic compounds (50% more), as well as the antioxidant activity. Moreover, bee pollen-added cheese were characterized by high contentsof Fe, Mn, Zn, free fatty acids, free and total amino acids.Hardness, gumminess, and chewiness of cheese samples with bee pollen were higher than control cheese.The amount of total free amino acids in UF-soft cheese with bee pollen was almost two times higher in comparison with control cheese.Bee pollen addition caused an increase of unsaturated fatty acids, mainly omega-ω_6 and omega-ω_3 in UF-soft cheese.Tyrosine, glutamic,methionine, phenylalanine, isoleucine, and leucine acidswere generally in high proportion in UF-soft cheese with bee pollen.The viability of ABT culture was significantly higher in bee pollen-added cheese than control cheese. Significant improvement was observed in sensory parameters (flavor and texture) for UF-soft cheese with 1.0 or 2.0% bee pollen. It can be concluded that, probiotic UF-soft cheese with 1 or 2% bee pollen maybe proposed as new-functional cheese.

Keywords:UF-soft cheese, Bee pollen, Probiotic cheese, Functionalfood.

Introduction

Natural products are sources of bioactive compounds and they are gaining more and more attention due to their positive effects on human health and possible therapeutic properties that promotes its utilization in the food industry as a functional food ingredient. Beepollenis a natural productknows for its medical properties due to its excellent nutrient profile. Bee pollen is a potential source of vital nutrients such as carbohydrates, proteins, minerals, vitamins, all essential amino acids, ω-з fatty acids (Gercek et al., 2022; Thakur & Nanda, 2018) and phenolic compounds which make it attractive as a functional food ingredient (De-Melo et al., 2016; Thakur & Nanda, 2020). Bee pollen has a great potential in nutritional and biomedicalapplications and it has served to prevent many chronic diseases such as diabetes, obesity, heart muscle diseases and to enhance immunity (Khalifa et al., 2021).So far, a number of pollen-based functional food products have been developed (Denisow & Denisow-Pietrzyk, 2016; Kostić et al., 2020). For instance, adding bee pollen in different levels (0.5, 1.0, 2.5 and 3.0%) to yogurts made from cow, goat, and sheep milk resulted in a food matrix with a higher antioxidant capacity and total phenolic content, in

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addition to improving the taste, smell, appearance, and cohesion of yogurt (Karabagias et al., 2018). Yerlikaya (2014) observed an increase of total solids, proteins, free amino acids groups in the fermented milk beverages supplemented with bee pollen in a concentration range from 2.5 to 20 mg/ml. Several researchers reported the promising results about the incorporation of bee pollen in food products (Khider et al., 2013; Lomova et al., 2014; Zlatev et al., 2018). Zlatev et al. (2018) demonstrated that the addition of bee pollen in yogurt show improved antioxidant capacity, an increase inpolyphenolics content, and significantly improved sensory properties compared to the conventional yogurt.

Considering the positive effects of bee pollen on human health, the aim of this article was to evaluate the effects of bee pollen addition in different levels (0, 1, 2 and 3%) with probiotic culture (ABT) on the physiochemical, textural, free fatty acids (FFAs), free amino acids (FAAs) microbiological and sensory properties of UF-soft cheese during storage for 30 days, in order to provide it with health-promoting substances and phenolic antioxidants.

Materials and Methods

Materials

Four liters of UF buffalo's milk retentate (33.0% total solids, 17.5% fat, 10.35% protein, and 2.20% ash) was obtained from a pilot dairy plant, Faculty of Agriculture, Fayoum University, Egypt. Calcium chloride, potassium sorbate and sodium chloride were used. The ATB- 5- probiotic strains (DVS YC-X11 Yo-Flex) consisted of *Lactobacillus acidophilus* LA-5, *Streptococcus thermophiles* and *Bifidobacterium* BB-12 was obtained from Chr-Hansen's Laboratories, Copenhagen, Denmark. Microbial rennet powder (CHY-MAX, 2280 IMCU/ml) was obtained from Chr. Hansen' Lab., Denmark.Bee pollen was purchased from local market.

Methods

Preliminary study for sensory evaluation

Initial experiments were conducted to determine the acceptable percentages of bee pollen (1-10%). Bee pollen up to 4% was accepted, thus, the 1.0, 2.0 and 3.0% of bee pollen was chosen for UF-soft cheese production.

Bee pollen preparation

Bee pollen was dried at 30°C in electric oven. The dried bee pollen was ground and sieved through a 37 µm mesh, and stored in a freezer at -18°C until used in this study. The chemical composition of Bee pollen is shown in Table 1.

Experimental design and cheese making

By using UF buffalo's milk retentate (4 L, 50°C), it was divided into four equal parts. The first part was left without bee pollen to be served as a control and the last three parts were fortified with bee pollen at the rate of 1.0, 2.0, 3.0% (g/100g) of milk retentate, respectively. All treatments were re-pasteurized at 72°C/15 sec, then cooled to 40°C and inoculated with 2% ABT culture. Then, 0.02% calcium chloride, 0.015% potassium sorbate and 2.0% NaCl were added. The UF-milk retentate batches were immediately renneted (2 g rennet powder/100kg milk retentate). All samples were incubated at 40ºC for complete coagulation and kept under refrigerationat 5±2°C for 30days for further analysis.

Physiochemical analysis

The samples were analyzed for titratable acidity, moisture, fat, protein, water soluble nitrogen,and ashcontents (AOAC, 2019).Total dietary fibre was determined by the enzymaticgravimetric method. Total carbohydrates were calculated by 100 - (moisture + protein + fat + ash + dietary fiber).The pH was measured using a digital pH meter (model Kent EIL 7020, UK). Mineral contents (Iron, manganese and Zinc) were determined by Atomic Absorption Flame Emission Spectrophotometer (Analytik jena ZEEnit 700, Germany).The total volatile fatty acids (TVFA) (0.1N NaOH/100 g cheese) was determined (Kosikowski & Mistry,1977)and the free amino groups (mg leucine/g cheese) were measured by Cd-ninhydrin analysis (Folkertsma & Fox 1992).

Total phenolic and antioxidant activity

By using the Folin-Ciocalteau assay(Ozsoy et al., 2008), the total phenols was measured spectrophotometrically and the results expressed as mg of Gallic acid equivalents (mg GAE/g).

The antioxidant activity was evaluated by DPPH method as described byKaraaslan et al. (2011) and the results were expressed as percentage inhibition of DPPH radical.

Free fatty acids and free amino acids

Free fatty acids and free amino acids were determined by GC-MS according to (Peace & Gilani, 2005).

Textural characteristics

Textural characteristics of cheese samples were evaluated using a Texture Analyzer (Model CT310K Texture Analyzer, USA) according to Gunasekaran & Ak (2002).

Microbiological analysis

The viable count was determined on agar medium 35C/48h, yeasts and molds on PDA medium 25°C/3-5days. *Lactobacilli* groups on MRS agar medium 35°C/48h, *Streptococci* groups on M17 agar 30°C/72h. *Bifidobacteria* on M17 agar 30°C /72h in anaerobic conditions according to(APHA, 2015). Total psychrotrophs on plate count agar medium 5°C/10days. The results expressed as log10 colony forming unit (cfu)/g of cheese.

Sensory evaluation

Cheese samples were evaluated by twenty four panelists using the following scale according to Abdelmontalebet al. (2021): flavor (50 points), body & texture (30 points), color and appearance (20 points).

Statistical analysis

All analyses were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at $P \leq 0.05$ using SPSS software (2007).

Results and Discussion

Quality parameters of Bee pollen

Table 1 presents the chemical composition and bioactive compounds in bee pollen.There were significant quantities of protein (18%), fat (6.7%), carbohydrates (61%), ash (2%) and total dietary fibers (9.6%) contents were recorded in bee pollen used in this study. In addition, it is agood source of phenolic compounds (25.95 mg/100g)), which have the ability to scavenge free radicals. In comparison with the literature, the chemical composition of bee pollen was quite low, indicating that the nutritional and chemical compositionof bee pollen differs depending on the harvested time, geographical origin and plant species (Gercek et al., 2022).

TABLE 1. The average composition of Bee pollen.

Nutrients	Bee pollen
Moisture (%)	4.50
Protein (%)	18.0
Fat (%)	6.70
Carbohydrates (%)	61.0
Ash (%)	2.00
Total dietary fibers (%)	7.60
Iron (ppm)	3.027
Manganese	0.270
Zinc	0.523
Total phenolic content (asmg Gallic acid/100g)	25.95
∑SFA	25.01
∑USFA	40.51
ω_6(C18:2)	14.47
ω_3(C18:3)	18.02
ω_6/ ω_3	0.80
USFA/SFA	1.60
Totalfree amino acids(µg/g)	1207.5

The used bee pollen was found to possess remarkable amounts of beneficial elements such asIron, Manganese and Zinc. Besides, it contained a high concentration of free fatty acids, about 40% of which are unsaturated fatty acids like linolenic (omega-3) and linoleic (omega-6) acids with share of 32.49%. These findings suggest that bee pollen can be potentially used as asource of omega-3. Since a "healthy" product should have a ratioof unsaturated and saturated fatty acids (UFA/SFA) higher than 1.6 (Kostić et al., 2017). The results obtained for the fatty acid profile of the bee pollen used in thisstudy are consistent with those obtained in previous studies in the literature (Gercek et al., 2022).

The major free amino acids in bee pollen was proline (44.8% of the total free amino acid content), followed by tyrosine, glutamic, cysteine and glycine acids at ratios of 542.13, 162.62, 68.05, 54.91, 52.06 µg/g, respectively. Our results for free amino acids agree with(da Silva et al., 2014; Yang et al., 2013). The high content of minerals, phenolic compound, polyunsaturated fatty acid, and free amino acids confirmed the high nutritional quality and biological value of bee pollen and it can be used as a good functional ingredient in UF-soft cheese.

Physicochemicalcharacteristics

Table 2 shows the mean values ofpH, acidity, moisture, fat, protein, ash, carbohydrates and total dietary fiber in control and experimental UF-soft cheese with bee pollen.There were no significant differences ($P\leq0.05$) in pH values between control and experimental UF-soft cheeseat fresh time of storage, while pH significantlydecreased ($P\leq0.05$) in experimental UF-soft cheese when compared to control at the 15th day and till the end of storage time. These results were in line with Özcan et al. (2020) who reported that the addition of bee pollen in dairy products decreased the pH level. On the other hand, the acidity differed significantly ($P\leq0.05$) among all cheese samples during the whole storage period. These differences in acidity and pH values were probably due to the microbial fermentation and the stimulating effect of bee pollen on the microbial growth. These findings were in line with Yerlikaya (2014) and Atallah & Morsy (2017). In addition, the higher amino acids, vitamins and minerals in bee pollen may enhance the growth of lactic acid bacteria and the fermentation of lactose in cheese matrix (Khider et al., 2013; Yang & Li, 2015).

As regards to the higher values of fat, protein, ash, carbohydrates and total dietary fiber obtained in UF-soft cheese with bee pollen were due to the higher total solids occurred upon the addition of bee pollen in the production of UF-soft cheese as Yerlikaya (2014) mentioned the same results. In addition, bee pollen reduced the entrapped water in cheese protein networks due to thehydrophobic interaction between polyphenols and milk proteins during the coagulation of cheese curd as reported byAbd Elhamid & Elbayoumi (2017). The fat, protein total dietary fiber, and ashcontents significantly ($P\leq0.05$) increased during storage in all treatments which might be due to the loss of moisture during storage (Atallah & Morsy, 2017). The moisture contents were significantly higher ($P\leq0.05$) in control cheese when compared to experimental UF-soft cheese, suggesting a possible effect of the bee pollen on the water absorption capacity of the resulting cheese. The moisture content of all cheese samples decreased during the storage periods which in turn affect the dry matter content of such samples. Moisture content decreased in low rate in cheese samples supplemented with bee pollen ($P\leq0.05$) compared to the control cheese, which is evident in cheese samples with higher level of bee pollen (3.0 %). The lower moisture content of cheese samples supplemented with bee pollen is explained by the higher dry matter content of bee pollen incorporated into cheese network due to its high content of fibers (Kostić et al., 2020).

Mineral contents

The mineral contents of bee pollen and different UF-soft cheese samples with bee pollen are presented in Table 3. Bee pollen had a higher content of iron, zinc and manganese which affected such contents in the experimental UF-soft cheese. UF-soft cheese with higher level of bee pollen had the higher values of minerals among all experimental UF-soft cheese.Belina-Aldemita et al., (2019) reported that bee pollen is an excellent source of iron and zinc which support our findings. These results were in line with Atallah (2016) and Atallah & Morsy (2017) who used bee pollen in the production of bio-yoghurt with probiotic bacteria which had higher mineral contents in yoghurt bee pollen samples. Identical results obtained by Özcan et al. (2020).

Biochemical characteristics

Table 4 showed that the addition of bee pollen had a significantlyeffect($P\leq0.05$) on the rate of protein nitrogen breakdown and lipolysis. WSN/TN,TVFAand free amino groups contents were

TABLE 2. Physiochemical characteristics of UF-soft cheese samples containing 0, 1, 2, and 3% bee pollen.

		Treatments			
	Storage period (days)	UF-soft cheese with different levels of bee pollen			
		Control	1.0%	2.0%	3.0%
pH	Fresh	6.17^{a}±0.15	6.23^{a}±0.06	6.23^{a}±0.06	6.24^{a}±0.06
	15	6.07^{a}±0.06	6.00^{a}±0.02	5.87^{b}±0.06	5.67^{c}±0.06
	30	6.03^{a}±0.06	5.77^{b}±0.06	5.70^{b}±0.04	5.53^{c}±0.05
Titratable acidity (%)	Fresh	0.20^{c}±0.01	0.22^{b}±0.01	0.23^{ab}±0.01	0.24^{a}±0.01
	15	0.21^{c}±0.01	0.25^{b}±0.01	0.27^{a}±0.01	0.28^{a}±0.01
	30	0.24^{d}±0.01	0.29^{c}±0.01	0.30^{b}±0.01	0.32^{a}±0.01
Moisture (%)	Fresh	67.21^{a}±0.16	65.55^{b}±0.04	65.16^{c}±0.05	64.47^{d}±0.01
	15	66.72^{a}±0.12	65.07^{b}±0.11	64.99^{b}±0.05	64.26^{c}±0.14
	30	66.11^{a}±0.10	64.87^{b}±0.07	64.73^{b}±0.06	64.13^{c}±0.65
Fat (%)	Fresh	16.92^{c}±0.14	17.33^{b}±0.29	17.50^{b}±0.01	18.00^{a}±0.00
	15	17.33^{b}±0.29	17.87^{a}±0.13	17.92^{a}±0.12	18.25^{a}±0.29
	30	17.67^{c}±0.50	18.00^{c}±0.29	18.67^{b}±0.50	19.33^{a}±0.50
Protein (%)	Fresh	10.29^{c}±0.06	10.59^{b}±0.04	10.46^{b}±0.06	10.72^{a}±0.10
	15	10.40^{d}±0.04	10.78^{a}±0.13	10.65^{b}±0.10	10.93^{a}±0.07
	30	10.50^{c}±0.10	10.85^{ab}±0.06	10.72^{a}±0.04	11.02^{a}±0.06
Ash (%)	Fresh	2.50^{a}±0.10	2.51^{a}±0.01	2.55^{a}±0..05	2.58^{a}±0.01
	15	2.67^{b}±0.03	2.69^{b}±0.03	2.70^{b}±0.03	2.61^{a}±0.01
	30	2.70^{d}±0.05	2.76^{c}±0.04	2.85^{b}±0.01	3.32^{a}±0.04
Carbohydrates (%)	Fresh	2.83^{c}±0.11	3.77^{b}±0.04	3.65^{b}±0.30	4.05^{a}±0.08
	15	2.67^{d}±0.01	3.34^{a}±0.03	3.07^{b}±0.07	2.96^{c}±0.07
	30	2.22^{b}±0.05	2.53^{a}±0.10	2.20^{b}±0.08	2.07^{c}±0.03
Total dietary fibre (%)	Fresh	00	0. 19^{c}±0.30	0.25^{b}±0.17	0. 46^{a}±0.03
	15	00	0.26^{b}±0.37	0.34^{b}±0.28	0.85^{a}±0.28
	30	00	0.43^{b}±0.10	1.14^{a}±0.32	1.19^{a}±0.31

Mean ± SD and different letters in the same row indicate significant differences ($P \leq 0.05$)

TABLE 3. Some mineral contents (ppm) of UF-soft cheeseswith different levels of bee pollen.

	Treatments			
Mineral (ppm)	UF-soft cheese with different levels of Bee pollen			
Control	**1.0%**	**2.0%**	**3.0%**	
Fe	0.352	0.911	0.94	0.989
Mn	0.00	0.02	0.04	0.07
Zn	0.017	0.15	0.212	0.255

significantly higher ($P \leq 0.05$) in experimental UF-soft cheese with bee pollen during storage than control cheese. The greater amounts of smaller peptides and volatile FFAs in UF-soft cheese showed higher hydrolysis of casein molecules and lipolysis in cheese samples because of bee pollen which stimulate the starter culture(Kostić et al., 2020). Moreover, the higher impact of bee pollen on such biochemical characteristics was related to higher protein content in cheese with added bee pollen which might affect the proteolytic activities of soft cheese samples.These results were in agreement with Yerlikaya (2014).

Phenoliccontentand antioxidant activity

The total phenolic content and the antioxidant activity of control and experimental UF-cheese samples are shown in Table 5. All cheese samples with bee pollen possessed high level of total phenolic compound, which significantly increased ($P \leq 0.05$) with increasing the level of bee pollen in UF-soft cheese samples. During storage, the level of total phenols and antioxidant activity increased in all UF-soft cheese samples which could be due to the loss of water from cheese matrix and increase of soluble solids. The higher phenolic content of bee pollen might explain such increase of total phenolic content and the antioxidant activity in UF-soft cheese with bee pollen. It was reported that bee pollen is a perfect food ingredient with relatively higher content of bioactive compounds, total phenols, flavonoids beside the antioxidant activities which make it an attractive for the application in the production of functional food systems (Komosinska-Vassev et al., 2015; Kostić et al., 2020). These results were in line with (Karabagias et al. (2018) and Tatlı Seven et al. (2016)).

Free fatty acids

The fatty acid profile in control and UF-soft cheese with different bee pollen levels at 30 days is shown in Table 6. The most abundant individual FA in UF-soft cheese was palmitic (C16:0; almost 2/3 of total fat), followed to a lesser content of myristic acid (C14:0), and stearic (C18:0), Elaidic acid (C18:1) acids. The most significant change was in Linoleic acid (C18:2) ω_6, and Linolenic acid(C18:2) $\omega_3$contents in UF-soft cheese with bee pollen which increased by 2.40% and 3.6% compared to control cheese, respectively. So, the addition of bee pollen had a major effect on the fatty acid profile. It caused an increase of unsaturated fatty acids, mainly essential fatty acids C18:2 ω_6 and C18:3 ω_3. The higher fat content in the bee pollen sample (Table 1) caused an increase in individual fatty acids. These results are consistent with the lipid profile of the bee pollen (≈40%), which is rich in unsaturated fatty acids (ω_3 ω_6), which account for 32.5% of total fatty acids). Atallah (2018) showed that the fatty acids profile in probiotic yoghurts with royal jelly pollen are higher than the control sample.

TABLE 4. Biochemical characteristics of UF-soft cheese samples containing 0, 1, 2 and 3% of bee pollen.

		Treatments			
	Storage period (days)	UF-soft cheese with different levels of bee pollen			
		Control	1.0%	2.0%	3.0%
WSN/TN (%)	Fresh	17.35±0.57 c	21.69±0.47 b	18.16±0.43 c	23.9±0.52 a
	15	30.04±1.92 b	33.65±0.42 b	27.38±0.47 c	33.93±1.04 a
	30	33.04±0.47 d	35.46±0.52 c	35.86±0.51 b	36.22±0.57 a
Total volatile fatty acids (%)	Fresh	2.13±0.12d	2.53±0.06c	3.73±0.06 b	4.27±0.06 a
	15	2.47±0.24d	2.80±0.10c	4.05±0.13 b	5.88±0.38 a
	30	3.53±0.06d	4.23±0.55c	5.90±1.13 b	8.20±1.56 a
Free amino groups (%) (mg leucine/g)	Fresh	1.99±0.04 d	18.77±0.21 c	24.23±0.59 b	27.28±0.60 a
	15	2.67±0.18 d	19.37±0.12 c	25.60±0.26 b	28.87±0.12 a
	30	3.16±0.04 d	24.29±0.52 c	27.27±0.40 b	30.13±1.15 a

Mean ± SD and different letters in the same row indicate significant differences ($P \leq 0.05$)

TABLE 5. Phenolic content and antioxidant activity of UF-soft cheese samplescontaining 0, 1, 2 and 3% of bee.

Parameters	Storage period (days)	Treatments			
		UF-soft cheese with different levels of bee pollen			
		Control	1.0%	2.0%	3.0%
Total phenolic content (mg of Gallic acid/g)	Fresh	0.12±0.01^{d}	0.37±0.01^{c}	0.53±0.08^{b}	0.68±0.01^{a}
	15	0.15±0.01^{d}	0.43±0.02^{c}	0.58±0.03^{b}	0.74±0.01^{a}
	30	0.20±0.04^{d}	0.59±0.02^{c}	0.73±0.03^{b}	0.93±0.02^{a}
Antioxidant activity (%)	Fresh	15.10±0.01^{d}	45.12±0.03^{c}	47.23±0.06^{b}	50.24±0.02^{a}
	15	18.30±0.05^{d}	52.38±0.07^{c}	58.56±0.06^{b}	62.12±0.03^{a}
	30	25.35±0.04^{d}	55.92±0.05^{c}	59.21±0.7^{b}	63.95±0.01^{a}

Mean ± SD and different letters in the same row indicate significant differences ($P \leq 0.05$)

TABLE 6. Free fatty acids of UF-soft cheesesamplescontaining 0, 1, 2 and 3% of bee pollen at 30days of storage.

Fatty acids	Treatments			
	UF-soft cheese with different levels of bee pollen			
	Control	1.0%	2.0%	3.0%
Saturated fatty acids (SFAs %)				
Butyric acid(C4:0)	3.53	3.44	3.37	3.49
Caproic acid(C6:0)	1.89	2.07	1.82	1.91
Caprylic acid(C8:0)	0.91	1.10	1.16	1.18
Capric acid(C10:0)	1.92	2.22	2.40	2.43
Lauric acid(C12:0)	2.31	2.78	3.02	3.03
Myristic acid(C14:0)	11.51	13.88	14.00	14.67
Palmitic acid(C16:0)	37.00	37.72	38.08	38.29
Stearic acid(C18:0)	8.80	9.08	10.59	10.77
□SFAs	67.87	72.29	76.26	76.77
Unsaturated fatty acids (USFAs %)				
Palmitoleic acid(C16:1)	1.69	1.86	2.21	2.24
Elaidic acid(C18:1)	17.78	17.79	19.84	20.22
Linoleic acid(C18:2) $□_6$	1.44	1.62	1.68	1.75
Linolenic acid(C18:3) $□_3$	0.23	0.63	0.58	0.59
Arachidonic acid (C20:4)	1.85	1.13	0.86	0.89
□USFAs	23.00	23.03	25.17	25.69

Free amino acids (FAAs)

The concentrations of individual and total free amino acids determined in control and UF-soft cheese with different levels of bee pollen are given in Table 7. The major free amino acids, which accounted for about 55% of total FAAs, found in UF-soft cheese with bee pollen were tyrosine followed by glutamic, leucine, methionine, and phenylalanine acids. The content of glutamic, tyrosine, phenylalanine, isoleucine and lysine acids were higher in the UF-soft cheese with bee pollen compared to the control sample. Compared with control, the treatments with bee pollen presented two times a larger quantity of total free amino acids; this could be attributed to the higher content of FAAs in bee pollen samples as previously reported by Abdelmontaleb et al. (2021). The high free amino acids in cheese contribute to improving the flavor of UF-soft cheese and then increasing its marketing place and enhancing the nutritional value of cheese.

Texture characteristics

Data in Table 8 demonstrated textural parameters of control and UF-soft cheese samples with different levels of bee pollen. The results showed that the bee pollen has an obvious effect on the textural characteristics of cheese samples.Hardness, gumminess, and chewiness of treated sampleswere greater than control cheese, but springiness has an opposite trend.This might be related to the internal bonds formed within the cheese matrix due to the increased hardness of such cheese samples. Moreover, the interactions between casein micelles and bee pollen might influence the textural characteristics of cheese.The increase in hardness, gumminess and chewiness in UF-soft cheese could be attributed to the addition of bee pollen which had higher total solids and lower moisture content when compared to control cheese. Low moisture content and high rate of proteolysis in UF-soft cheese resulted in increased textural hardness, gumminess, and cohesiveness. Proteolysis disrupts the structural integrity of the protein matrix resulting in declined cohesiveness. On the other hand, springiness and cohesiveness were lower in experimental UF-soft cheese than in control cheese which might be due to the structural changes occurred in cheese network when bee pollen is added into cheese. These findings were in line with Atallah & Morsy (2017).

TABLE 7. Free amino acidsof UF-soft cheesesamplescontaining 0, 1, 2 and 3% bee pollen at 30 days of storage.

Free amino acid (µg/g)	Bee Pollen	Treatments			
		UF-soft cheese with different levels of bee pollen			
		Control	**1.0%**	**2.0%**	3.0%
Aspartic	34.35	18.54	19.53	21.02	21.05
Glutamic	68.05	34.43	54.83	61.28	65.01
Serine	27.92	15.35	17.96	18.98	19.87
Histidine	32.14	16.53	19.28	21.20	22.73
Arginine	27.94	12.76	14.20	15.38	16.68
Glycine	52.06	17.58	20.28	17.35	19.08
Threonine	37.19	26.20	28.00	27.51	29.92
Alanine	23.30	10.67	11.07	11.97	12.12
Tyrosine	162.62	6.08	128.78	143.45	155.22
Cystine	54.91	0.04	0.07	0.65	0.85
Methionine	19.34	1.35	15.52	16.72	18.48
Phenylalanine	31.18	2.22	25.04	29.02	29.52
IsoLeucine	13.01	3.68	8.06	9.00	12.35
Leucine	38.51	0.68	30.74	33.70	36.07
Lysine	42.85	34.26	36.59	40.71	41.34
Proline	542.13	1.82	2.20	4.53	10.27
Total	1207.5	202.19	432.15	432.15	495.56

Microbiological analysis

Table 9 illustrates the effect of bee pollen (0, 1, 2, and 3%) on the microbiological characteristics of UF-soft cheese during storage period. Generally, the total viable counts (T.C), the number of Lactobacilli, Streptococci and Bifidbacteria groups in UF-soft cheesesamples with bee pollen were significantly higher ($P \leq 0.05$) than control cheese during refrigerated storage.This could be attributed to the stimulation effect of bee pollen on the growth of such bacterial groups. These findings were quite consistent with previously published in the literature (Atallah (2016). Zlatev et al. (2018) reported that bee pollen-supplemented milk had lower fermentation time due to the higher content of polysaccharides in bee pollen which enhance the growth of starter cultures. Moreover, the occurring proteolysis in cheese could provide the essential growth factors such as nitrogenous compounds which enhance the survival of the probiotics in the product (Yerlikaya, 2014).

Yeasts and mold appeared in lower numbers at the end of storage in all experimental UF-soft cheese with bee pollen when compared to control cheese. This might be related to the antimicrobial effect of bee pollen due to its higher content of phenolic compounds and the inhibition is depended on the concentration of bee pollen(Yerlikaya, 2014). Psychrotrophs were not detected in all UF-soft cheese samples at fresh time of storage. While it increased at the end of storage period with higher numbers had been presented in experimental UF-soft cheesesamples.

Sensory characteristics

Table 10 presents the sensory evaluation of control and experimental UF-soft cheese with different levels of bee pollen during refrigerated period. Generally, the panellists gave high scores for all the sensory attributes of UF-soft cheesesamples with 1 or 2% bee pollen. Thus, got high scores for their flavour, texture, and overall acceptability. A value of colour and appearance was significantly lower in UF-soft cheese with bee pollen than control cheese, indicating that bee pollen addition had negative effects on colour and appearance.The improvement in sensory parameters found in experimental UF-soft cheeses was probably due to lower moisture, more acidity, high rate of proteolysis and lipolysis which contribute the product’s odour and flavour. These were in line withKhider et al. (2013)and (Yerlikaya (2014)).

TABLE 8. Textural characteristics of UF-soft cheesesamplescontaining 0, 1, 2 and 3% of bee pollen at the end of storage period.

Parameters	Treatments			
		UF-soft cheese with different levels of bee pollen		
	Control	1.0%	2.0%	3.0%
Hardness (N)	4.89	6.50	11.7	15.11
Cohesiveness	0.59	0.59	0.58	0.53
Gumminess (N)	2.91	3.86	6.24	8.79
Springiness (mm)	0.71	0.70	0.66	0.64
Chewiness (N*mm)	2.03	2.57	4.42	5.59

TABLE 9. Microbiological characteristics of UF-soft cheesesamplescontaining 0, 1, 2 and 3% of bee pollen.

Microbial counts (log cfu /g)	Storage period (days)	Treatments			
			UF-soft cheese with bee pollen		
		Control	1.0%	2.0%	3.0%
Total bacterial counts	Fresh	4.59c±0.13	6.26b±3.54	6.35b±1.62	6.88a±2.98
	15	4.90c±1.11	6.36b±3.01	6.48b±2.03	6.91a±2.79
	30	5.12b±1.34	7.24a±3.38	7.33a±2.34	7.43a±2.97
Streptococci groups	Fresh	5.90d±1.06	6.66c±2.21	6.85b±1.68	8.41a±2.85
	15	6.58c±1.56	7.58b±3.58	7.72b±2.30	8.31a±2.43
	30	6.99b±2.25	7.07ab±2.87	7.20ab±2.48	7.42a±2.84
Lactobacilli groups	Fresh	6.58c±0.0	7.60b±2.38	7.29ab±0.0	7.99a±3.52
	15	7.24b±2.14	7.78a±1.18	7.71a±0.63	8.01a±3.31
	30	7.25b±2.53	7.37ab±2.94	7.42ab±1.74	7.80a±3.08
Bifidobacteria groups	Fresh	5.46d±1.71	6.35c±3.68	6.60b±1.69	7.52a±3.38
	15	6.41d±1.71	7.13c±3.11	7.46b±2.51	7.79a±3.03
	30	5.99d±1.02	6.60c±2.83	6.82b±2.26	7.07a±2.76
Molds and Yeasts	Fresh	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
	15	3.53±2.04	0.00±0.0	0.00±0.0	0.00±0.0
	30	4.82a±1.34	3.71b±0.67	3.69b±0.62	3.65b±0.39
Psychrotrophs	Fresh	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
	15	2.18c±1.27	2.34b±0.61	2.48a±1.06	2.56a±0.91
	30	2.71c±2.03	2.87b±1.04	2.97b±1.10	3.00a±1.09

Mean ± SD and different letters in the same row indicate significant differences ($P≤0.05$)

TABLE 10. Sensorial characteristics of UF-soft cheesesamplescontaining 0, 1, 2 and 3% of bee pollen.

Parameters	Storage (days)	Treatments			
		UF-soft cheese with different levels of bee pollen			
		Control	1.0%	2.0%	3.0%
Flavour(50)	Fresh	48.30a±0.57	49.00a±2.00	48.70a±1.00	45.90b±1.52
	15	47.60a±0.57	47.60a±0.00	48.30a±0.57	45.30b±2.08
	30	45.10b±0.57	47.20a±1.00	47.90a±1.00	41.70c±1.00
Body & Texture(30)	Fresh	28.20b±0.57	28.90a±0.57	29.20a±1.15	28.80a±1.00
	15	28.40a±1.15	29.00a±0.57	29.40a±0.57	29.00a±1.15
	30	28.90b±0.57	29.40a±0.57	29.70a±0.57	29.70a±0.00
Colour& Appearance(20)	Fresh	19.80a±0.57	19.70a±0.57	18.70b±2.08	17.40c±1.52
	15	19.50a±1.15	18.70b±0.57	17.40c±0.57	16.30d±1.52
	30	19.50a±1.15	18.70b±0.57	17.40c±0.57	16.30d±1.52
Total score(100)	Fresh	96.80a±1.15	97.60a±2.00	96.60a±4.00	92.30b±2.89
	15	95.50a±1.73	95.30a±0.57	95.10a±0.57	90.40b±4.51
	30	92.80b±0.57	95.30a±0.00	95.00a±1.52	87.70c±1.15

Mean ± SD and different letters in the same row indicate significant differences ($P≤0.05$)

Conclusion

The results of this study concluded that, the addition of bee pollenhas positive effects on the quality characteristics of UF-soft cheese in terms of its functional properties. A significant increase in protein, minerals, free fatty acids, and free amino acids contents than that in control cheese. In addition, bee pollen increased the antioxidant activity, total free amino acids (two times higher) andunsaturated fatty acids (mainly omega-ω_6 and omega-ω_3) in UF-soft cheese when compared with control cheese. A significant viability of probiotic cultures was observed in the UF-soft cheese supplemented with bee pollen. The most preferred UF-soft cheesewas those supplemented with 1% bee pollen, followed by 2% bee pollen.UF-soft cheese with 3% bee pollen had undesirable effects on flavour score and consumer acceptability.

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تأثير إضافة حبوب لقاح النحل على خصائص الجودة للجبن الأبيض الطرى الحيوى

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الهدف من هذه الدراسة هو تقييم إضافة حبوب لقاح النحل بمستويات مختلفة (٠ ، ١ ، ٢ ، ٣٪) في إنتاج الجبن الطري المصنع بالترشيح الفائق، وتحليل العينات علي فترات ١، ١٥، ٣٠ يوم من حيث الخصائص الفيروكيميائية، والتحلل البيوكيميائية، المعادن ، الفينول الكلي ، النشاط المضاد للأكسدة ، الأحماض الدهنية الحرة ، الأحماض الأمينية الحرة ،القوام ، حيوية بكتيريا ABT ، الخصائص الحسية للجبن الطري. واوضحت النتائج أن إضافة حبوب لقاح النحل إلى الجبن الطريإلى زيادة الحموضة، الدهون، البروتين، الرماد، WSN/TN٪، TVFAs٪ والمركبات الفينولية (زيادة٥٠٪ فأكثر) بالإضافة إلى زيادة نشاط مضادات الأكسدة. وتميزت الجبن المضاف إليها حبوب لقاح النحل بمحتوى أعلى بكثيرمن الحديد، المنجنيز، الزنك من الجبن الكنترول.وكانت كمية الأحماض الأمينية الحرة في الجبنمع حبوب لقاح النحل أعلى مرتين تقريبًا مقارنة بالجبن الكنترول،واحتوت الجبن حبوب لقاح علي أحماض التيروزين، الجلوتاميك، الميثيونين، الفينيل ألانين، الأيزولوسين، والليوسين بنسب أعلى من الجبن الكنترول.وأدت إضافة حبوب لقاح النحل إلى زيادة الأحماض الدهنية غير المشبعة، وخاصة أوميجا ٦وأوميجا ٣، وقد لوحظ تحسن كبير في الصفات الحسية (النكهة والقوام) للجبن الطريالمحتوي علي ١ أو ٢٪ حبوب لقاح النحل. لذا يمكنا الاستنتاج أن الجبن الطري مع ١ أو ٢ ٪ من حبوب لقاح النحل ربما يُقترح كجبن وظيفي جديد.

الكلمات الدالة: الجبن الطري، حبوب لقاح النحل ، الأغذية الوظيفية.