

## Evaluation of the Nutritional and Storage Quality of Meatballs Formulated with Bee Pollen

Sadettin Turhan\*, Fehmi Yazici, Furkan Turker Saricaoglu, Mustafa Mortas, and Huseyin Genccelep  
*Department of Food Engineering, Ondokuz Mayıs University, 55139 Samsun, Turkey*

### Abstract

In this study, the nutritional and storage quality of meatballs formulated with different levels (0, 1.5, 3.0, 4.5 and 6.0%) of bee pollen were investigated during storage at 41°C for 9 d. Protein content of meatballs increased, while moisture content decreased with increased pollen. The addition of pollen improved cooking loss but decreased the redness (Hunter *a* value) and sensory scores. Textural parameters (hardness, springiness, gumminess, and chewiness) were affected by pollen addition and the hardness and gumminess values of meatballs decreased as the pollen content increased. While C18:0 content of meatballs slightly decreased with pollen addition, C18:2n-6c, C18:3n-3, C20:5n-3, and PUFA contents increased. The PUFA/saturated fatty acids (P/S) ratio increased from 0.05 in the control to 0.09 in meatballs with 6.0% pollen. The n-6/n-3 ratio decreased from 11.84 in the control to 3.65 in the meatballs with 6.0% pollen. The addition of pollen retarded the lipid oxidation and inhibited the bacterial growth in meatballs. The pH, redness, TBA value and total aerobic mesophilic bacteria, coliform bacteria and *S. aureus* counts values changed significantly during storage. The results suggest that bee pollen could be added to enhance the nutritional and storage quality of meatballs with minimal changes in composition and/or sensory properties.

**Keywords:** meatball, bee pollen, nutritional quality, storage quality

### Introduction

Meatballs are common meat products processed from lower-value trimmed red meat to produce a high-value meat product (Dzudie *et al.*, 2004). Like any other food, these products also contain elements (fat, cholesterol, salt, nitrite, phosphate, residues from environmental pollution or the use of pharmaceuticals, contaminants from disinfectants or detergents, toxic compounds formed during cooking, etc.) which in certain circumstances and in inappropriate proportions have a negative effect on human health (Jimenez-Colmenero *et al.*, 2001). This unfortunate situation derives mainly from the content of fat, saturated fatty acids, and cholesterol and their association with cardiovascular diseases, some types of cancer and obesity (Bilek and Turhan, 2009; Fernandez-Gines *et al.*, 2005). There are diverse possible strategies for developing healthier meat products. Reformulation of meat products is one of the strategies (Arihara, 2006; Fernandez-

Gines *et al.*, 2005; Jimenez-Colmenero *et al.*, 2001). Much attention in recent years has been focused on this application to improve nutritional and storage quality of meatballs (Aleson-Carbonell *et al.*, 2005; Bilek and Turhan, 2009; Choe *et al.*, 2011; Huang *et al.*, 2005; Johnston *et al.*, 2005; Sanchez-Escalante *et al.*, 2003; Turhan *et al.*, 2007, 2009).

Bee pollen is a fine powder-like material produced by flowering plants pollen, mixed with nectar and bee secretions and gathered by honey-bees (Graikou *et al.*, 2011). This apicultural product has been used in the human diet for many centuries, due to its nutritional properties and health benefits (Freire *et al.*, 2012; Negri *et al.*, 2011; Rzepecka-Stojko *et al.*, 2012). It is the source of numerous biologically active substances. The most important groups of chemical compounds in bee pollen are the following: proteins and amino acids, carbohydrates, lipids and fatty acids, vitamins, minerals, phenolic compounds, flavonoids, phytosterols and phytochemicals (Morais *et al.*, 2011; Rzepecka-Stojko *et al.*, 2012). Phytochemicals, such as phenolic compounds are considered beneficial for human health since they decrease the risk of degenerative diseases by reducing oxidative stress and inhibiting macromolecular oxidation. They have been shown to possess

\*Corresponding author: Sadettin Turhan, Department of Food Engineering, Engineering Faculty, Ondokuz Mayıs University, 55139 Samsun, Turkey. Tel: +90-362-312-1919/1506; Fax: +90-362-457-6034, E-mail: [sturhan@omu.edu.tr](mailto:sturhan@omu.edu.tr)

free-scavenging and metal chelating activity in addition to their reported anticarcinogenic properties. Bee pollen has also been successfully used for the treatment of some cases of benign prostatitis and for oral desensitization of children who have allergies (Morais *et al.*, 2011). The biological activity of bee pollen is related to its high antioxidative potential due to the presence of polyphenols, including flavonoids (Rzepecka-Stojko *et al.*, 2012).

There is little information on the use of bee products in meat products. Johnston *et al.* (2005) studied the antioxidant capabilities of clover and wildflower honeys in delaying lipid oxidation in cooked and reheated ground beef patties stored in refrigerated and frozen states, and suggested honey may be a natural alternative to phosphates to delay lipid oxidation. Nagai *et al.* (2006) reported the effect of honey species (honey, propolis, royal jelly) on storage of meat, and they showed that honey species exhibited the inhibition of bacterial growth. To the best of our knowledge, there are no reports on the usage of bee pollen in meatballs to improve nutritional and storage quality of product. Therefore, the object of the present study was to evaluate the nutritional (proximate composition, cooking loss, textural and sensorial properties, fatty acid composition) and storage quality (pH, color, TBA value, microbiological properties) of meatballs formulated with different levels (0, 1.5, 3.0, 4.5 and 6.0%) of bee pollen.

## Materials and Methods

### Materials

Ground beef (moisture 63.45%, protein 15.20%, fat 18.93% and ash 0.92%), beef fat (moisture 12.97% and fat 86.32%), bee pollen (moisture 21.86%, protein 21.90%, fat 5.33%, ash 2.45%, carbohydrate 48.46%, Hunter *L* 50.58, *a* 8.22 and *b* 26.98), salt and garlic powder were used as raw materials. Ground beef and beef fat were purchased from a butcher shop. Bee pollen was obtained from Fanus Gıda ve Organik Ürünler San. Tic. Ltd. Sti.,

Turkey and used in the form of flour after being ground in a mortar. Salt and garlic powder were purchased from a local market. The fatty acid composition (g/100 g fatty acids) of beef fat and bee pollen was determined by gas chromatography. Beef fat contained C10:0 (0.05), C12:0 (0.05), C13:0 (0.02), C14:0 (2.99), C14:1 (0.45), C15:0 (0.44), C16:0 (28.04), C16:1 (2.04), C17:0 (1.61), C17:1 (0.67), C18:0 (31.17), C18:1n-9c,t (29.07), C18:2n-6t (0.14), C18:2n-6c (2.67), C18:3n-3 (0.23), C20:0 (0.20), C20:1n-9 (0.08), C20:2 (0.02), C20:3n-6 (0.02), C20:4n-6 (0.04) and C22:0 (0.03). Bee pollen contained C6:0 (0.18), C8:0 (0.16), C10:0 (0.40), C12:0 (0.41), C14:0 (0.73), C14:1 (0.12), C15:0 (0.15), C15:1 (1.52), C16:0 (25.63), C16:1 (0.25), C17:0 (0.28), C18:0 (3.73), C18:1n-9c,t (12.92), C18:2n-6c (22.11), C18:3n-3 (25.87), C20:0 (0.98), C20:1n-9 (0.28), C20:2 (0.21), C20:3n-3 (0.97), C22:0 (2.26), C23:0 (0.56) and C24:0 (0.28). All chemicals were used the analytical grade.

### Preparation of meatballs

Ground beef was divided into five batches. The first batch was used as control, and the other batches were supplemented with different levels of bee pollen (1.5% bee pollen for the second batch, 3.0% bee pollen for the third batch, 4.5% bee pollen for the fourth batch and 6.0% bee pollen for the fifth batch) and adjusted to a fat content of 20% by the addition of beef fat. In addition, 15% stale bread, 1.5% salt and 1% garlic powder were added to each batch (Table 1). Batches of 1 kg of each formulation were mixed by hand with gloves for 5 min, weighed into 30-35 g portions, and shaped by hand with gloves. The meatballs were then packaged in polyamide bags (Aksedef Plastic, Turkey) and stored at 4±1°C a home-type refrigerator for 9 d. Samples were randomly drawn for analysis at the evaluation periods. The pH, color, TBA value and microbial growth were analysed on 1, 3, 6 and 9 d of storage. Proximate composition, cooking loss, texture profile analysis, sensory properties and fatty acid composition of samples were evaluated on 1 d of

**Table 1. Formulation of meatballs formulated with different levels of bee pollen**

Ingredients (% w/w)	Bee pollen level (%)				
	Control	1.5	3.0	4.5	6.0
Ground beef	76.0	74.2	72.4	70.6	68.8
Beef fat	6.5	6.8	7.1	7.4	7.7
Bee pollen	-	1.5	3.0	4.5	6.0
Salt	1.5	1.5	1.5	1.5	1.5
Garlic powder	1.0	1.0	1.0	1.0	1.0
Stale bread	15	15	15	15	15
Total	100	100	100	100	100

storage. All analyses were performed in triplicate.

#### **Total phenolic content and antioxidant activity**

The concentration of phenolic compounds in bee pollen was determined by the Folin-Ciocalteu method as described by Singleton and Rossi (1965). Briefly, 100 L of Folin-Ciocalteu reagent was added to 20 L of ethanol extract. After 5 min, 300 L of 7.5 g/100 mL sodium carbonate was added to extract. The extract were incubated for 2 h at room temperature and the absorbance was measured with a UV-VIS spectrophotometer (Helios gamma, Thermo Spectronic, USA) at 760 nm against a blank reagent. The quantity of total phenolic content in the sample was calculated as gallic acid equivalent.

For ferric reducing antioxidant power (FRAP) determination, extract was mixed with 0.95 mL ferric-TPTZ reagent (prepared by mixing 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl<sub>3</sub> in the ratio 10:1:1, respectively) and measured at 593 nm (Helios gamma, Thermo Spectronic, USA). FeSO<sub>4</sub> was used as a standard and antioxidant power was expressed as mmol/g (Gao *et al.*, 2000).

Radical scavenging activity (DPPH) of pollen extract was determined according to Morais *et al.* (2011) with a slight modification. Briefly, 50 µL of the extract were added to 1 mL of DPPH methanol solution (100 µM) and left to stand for 30 min at room temperature followed by absorbance measurement of the resulting solutions at 515 nm using a spectrophotometer (Helios gamma). This activity is given as percent DPPH scavenging activity that is calculated as DPPH scavenging activity (%) = [(control absorbance – extract absorbance) / (control absorbance)] × 100. The extract concentration providing 50% of DPPH (EC<sub>50</sub>) was calculated by interpolation from the graph of DPPH percentage against extract concentration.

#### **Proximate composition and cooking loss**

Moisture, protein (N×6.25), fat and ash contents of meatballs were determined according to AOAC (2000) procedure. Carbohydrate contents were calculated by difference. For cooking loss, meatballs were cooked in a preheated electrical grill (Arcelik Midi Firin, Turkey) for a total of 10 min, 6 min one side and 4 min the other side. The weight of three meatballs per batch was measured at room temperature, before and after cooking to calculate cooking loss.

#### **Texture profile analysis**

Texture profile analysis (TPA) was performed accord-

ing to the procedure of Saricaoglu and Turhan (2013) using a Texture Analyzer (TA-XT Plus, Stable Micro Systems, UK) with a maximum force of 2 kg. An adaptor, a cylinder with a diameter of 36 mm, was used. The meatballs were put in a cylindrical container with a high of 15 mm and a diameter of 30 mm. The adaptor was 20 mm above the sample, and then declining at rate of 30 mm/min. The adaptor was backed up to the original position after penetrating 10 mm in depth. The movement of the adaptor was repeated once for completing the measurements. Parameters of TPA, namely hardness, springiness, cohesiveness, gumminess and chewiness were computed. Four different meatballs from each batch were analyzed.

#### **Sensory evaluation**

Meatballs were cooked as previously described and served warm to a trained consumer panel of 8-10 volunteers from the Department of Food Engineering at Ondokuz Mayıs University in Turkey. Each sample was coded with randomly selected 3-digit numbers. The sensory evaluations were performed by the panelists under fluorescence lighting. Panelists were instructed to cleanse their palates between samples using water. Each treatment from each replication was evaluated in one separate session given a total of three sessions. Appearance evaluation was performed only on raw samples, while flavor, hardness and juiciness were done on cooked samples. The appearance, flavor, hardness and juiciness of the meatballs were scored using a 9 point Hedonic scale, where 1 represented dislike extremely and 9 represented like extremely. Each attribute was discussed and tests were initiated after the panelists were familiarized with the scales. The overall acceptability was calculated taking into account appearance, flavor, hardness, and juiciness (each with 25%).

#### **Fatty acid composition**

Total lipids were extracted by the method of Bligh and Dyer (1959). Fatty acid composition was determined after methylation (ISO, 1978) by GC-MS (Shimadzu model of QP2010 Plus, Shimadzu Corporation, Japan) using a Teknokroma TR-CN 100 column (60 m × 0.25 mm I.D., 0.20 µm) (Teknokroma, Spain). The temperature of the injector port and detector was held at 250°C. The injected volume was 1.0 µl. The carrier gas was helium at a pressure of 200 kPa. The split used was 1:100. The temperature of the column was held at 90°C for 7 min, raised to 240°C at 5°C/min and finally held at 240°C for 10 min. Fatty acids were identified by comparison of their retention times

with those of authentic standards (Supelco 37 Components FAME Mixture, Cat. No. 18919-1AMP, USA) and reported as the percentage of total fatty acids determined.

#### **pH, color and TBA value**

pH values of meatballs were measured using a digital pH-meter (Cyberscan PC 510, Singapore) equipped with a combination pH electrode (Sensorex, S175CD Spear Tip, USA) calibrated in buffers at pH 4.01 and 7.00 (Mettler Toledo, USA) at 25°C. The average of three measurements was used.

The surface color of meatballs was measured using the Hunter Lab system with a colorimeter (Minolta CR 300), calibrated with a white tile (Minolta calibration plate, No. 21733001,  $Y=92.6$ ,  $x=0.3136$ ,  $y=0.3196$ ) at 2 observation angle with a C illuminant source. Three raw meatballs per batch were randomly selected and three readings were taken from each meatball surface. Hunter *L* (lightness; 100=white, 0=black), *a* (redness; +, red; -, green), *b* (yellowness; +, yellow; -, blue) values were recorded.

TBA value was determined according to the water vapour distillation method of Tarladgis *et al.* (1960), and expressed as milligram of malondialdehyde (MDA) per kilogram of sample.

#### **Microbiological analysis**

For microbiological analysis, meatball samples (10 g) were transferred aseptically into individual stomacher bags, containing 90 mL of sterile saline solution (NaCl 0.85%) and homogenized in a stomacher for 1 min. For each sample, appropriate serial decimal dilutions were prepared in saline solution (NaCl 0.85%) and the surface plate method was used for enumeration.

The microbiological analyses made on the samples were: total aerobic mesophilic bacteria (TAMB) determined on Plate Count Agar (PCA, Merck) incubated at 28°C for 3 d, coliform bacteria on 3M Petrifilm Coliform Count Plate (Bioser, Barcelona, Spain) incubated at 37°C for 24 h and *S. aureus* on 3M Petrifilm *S. aureus* Count Plate (Scharlau, Spain) incubated at 37°C for 24 h. Results were expressed as  $\text{Log}_{10}\text{CFU/g}$ .

#### **Statistical analysis**

The experiment was replicated three times. The data obtained from the nutritional (proximate composition, cooking loss, textural properties, sensory scores and fatty acid composition) and storage parameters (pH, color, TBA value and microbiological properties) were analyzed by one-way and two-way ANOVA, respectively, using

the SPSS statistical package program (SPSS Inc., USA). Significant differences among the means were compared using the Duncan's Multiple Range test. A significance level of 0.05 was chosen.

## **Results and Discussion**

#### **Total phenolic content and antioxidant activity of bee pollen**

Phenolic compounds are abundantly found in bee pollen and they may contribute directly to antioxidant activity, because of their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Negri *et al.*, 2011). The results showed that the phenolic content of pollen was found at the value of  $10.32\pm 1.01$  mg of gallic acid equivalents per gram of pollen. Even though the values for total phenolic content were low compared with other reports on pollen samples from around the world, for example, Poland (Leja *et al.*, 2007) and Portugal pollens (Morais *et al.*, 2011), but there were samples with similar total phenolic compound content (Graikou *et al.*, 2011). This result could be attributable to botanical and geographical origin, season, and extraction procedures of pollen samples.

Antioxidant activity of pollen is generally related to its high phenolic content (Leja *et al.*, 2007), however, according to report of Negri *et al.* (2011) no correlation between the amount of total phenols and antioxidant activity was found. Thus, qualitative traits may be more important to determine the observed antioxidant effect than the total content of phenols (Negri *et al.*, 2011). The results showed that FRAP and the DPPH  $\text{EC}_{50}$  values of pollen were  $26.59\pm 2.40$  mmol/g and  $0.48\pm 0.04$  mg/mL, respectively. The FRAP values reported by Graikou *et al.* (2011) in Greek pollen and the DPPH  $\text{EC}_{50}$  values reported by Morais *et al.* (2011) in Portugal pollen were different than our results which could be attributable the above mentioned factors.

#### **Proximate composition and cooking loss**

The proximate composition and cooking loss values of meatballs formulated with different levels of bee pollen are given in Table 2. Data indicated that the highest moisture content was determined for control meatballs ( $p < 0.05$ ). The moisture contents of samples decreased as the pollen content increased ( $p < 0.05$ ). The lowest protein content was determined for control meatballs as 15.58% and protein contents of meatballs formulated with pollen ranged from 16.46 to 18.94%. The protein contents of

**Table 2. Proximate composition, cooking loss, texture profile analysis and sensory scores of meatballs formulated with different levels of bee pollen**

	Bee pollen level (%)				
	Control	1.5	3.0	4.5	6.0
<i>Proximate composition (%)</i>					
Moisture	58.03±0.43 <sup>a</sup>	57.12±0.24 <sup>b</sup>	56.33±0.38 <sup>c</sup>	56.05±0.50 <sup>c</sup>	55.00±0.46 <sup>d</sup>
Protein	15.58±0.21 <sup>d</sup>	16.46±0.08 <sup>c</sup>	16.96±0.10 <sup>c</sup>	17.87±0.66 <sup>b</sup>	18.94±0.73 <sup>a</sup>
Fat	20.00±0.01	20.65±0.29	20.78±0.41	20.43±0.08	19.94±0.48
Ash	2.35±0.03	2.19±0.06	2.29±0.09	2.23±0.08	2.35±0.01
Carbohydrate	4.03±0.61	3.58±0.32	3.64±0.55	3.42±0.92	3.77±1.16
Cooking loss (%)	39.94±0.31 <sup>a</sup>	36.49±0.30 <sup>b</sup>	33.05±0.20 <sup>c</sup>	32.29±0.13 <sup>c</sup>	27.04±0.92 <sup>d</sup>
<i>Texture profile analysis</i>					
Hardness (N)	42.52±2.53 <sup>a</sup>	39.18±1.23 <sup>b</sup>	38.85±1.91 <sup>b</sup>	37.39±1.39 <sup>b</sup>	33.61±0.45 <sup>c</sup>
Springiness (cm)	0.58±0.03 <sup>d</sup>	0.78±0.01 <sup>b</sup>	0.93±0.01 <sup>a</sup>	0.66±0.03 <sup>c</sup>	0.58±0.03 <sup>d</sup>
Cohesiveness	0.17±0.01	0.17±0.01	0.16±0.01	0.17±0.01	0.16±0.01
Gumminess (N)	7.50±0.54 <sup>a</sup>	6.79±0.42 <sup>ab</sup>	6.33±0.57 <sup>b</sup>	6.35±0.57 <sup>b</sup>	4.97±0.36 <sup>c</sup>
Chewiness (N.cm)	4.34±0.28 <sup>b</sup>	5.31±0.36 <sup>a</sup>	5.93±0.55 <sup>a</sup>	4.20±0.28 <sup>b</sup>	2.88±0.24 <sup>c</sup>
<i>Sensory scores</i>					
Appearance	7.83±0.37 <sup>a</sup>	6.45±0.12 <sup>b</sup>	5.66±0.34 <sup>c</sup>	4.92±0.16 <sup>d</sup>	4.16±0.15 <sup>e</sup>
Flavor	7.55±0.34 <sup>a</sup>	6.10±0.45 <sup>b</sup>	5.56±0.11 <sup>c</sup>	4.44±0.16 <sup>d</sup>	3.04±0.04 <sup>e</sup>
Hardness	6.16±0.15 <sup>a</sup>	6.00±0.08 <sup>a</sup>	5.91±0.26 <sup>a</sup>	5.54±0.03 <sup>b</sup>	4.68±0.18 <sup>c</sup>
Juiciness	6.74±0.14 <sup>a</sup>	6.02±0.15 <sup>b</sup>	5.93±0.03 <sup>b</sup>	5.35±0.50 <sup>c</sup>	4.43±0.14 <sup>d</sup>
Overall acceptability	7.07±0.25 <sup>a</sup>	6.14±0.16 <sup>b</sup>	5.77±0.13 <sup>c</sup>	5.06±0.12 <sup>d</sup>	4.08±0.02 <sup>e</sup>

Values are means standard deviation of three replicates.

<sup>a-e</sup>Means in the same row with different superscript letters are different ( $p<0.05$ ).

Appearance, flavor, hardness and juiciness were scored using a 9 point scale, where 1: dislike extremely and 9: like extremely.

The overall acceptability was calculated from these four sensory properties (each with 25%).

meatballs increased as the pollen content increased ( $p<0.05$ ) and this could be attributable to the protein content of pollen, 21.90%. The fat contents of meatballs were close to adjusted level ( $p>0.05$ ). The moisture, protein and fat contents of all meatballs were within the limits of the Turkish Uncooked Meatball Standard (TSE, 1992). The ash and carbohydrate contents of meatballs were not significantly changed ( $p>0.05$ ) by the addition of pollen.

Cooking loss occurs by loss of fat and evaporation of moisture. The highest cooking loss was from the control sample because of the high loss of fat and moisture during cooking ( $p<0.05$ ). The cooking loss of the samples decreased with more pollen addition ( $p<0.05$ ) because of its high ability to keep moisture and fat in matrix. Similar to our findings, various researchers also reported that non-meat ingredients such as lemon albedo (Aleson-Carbonell *et al.*, 2005), flaxseed flour (Bilek and Turhan, 2009), rice bran (Huang *et al.*, 2005), okara (Turhan *et al.*, 2007, 2009) and hazelnut pellicle (Turhan *et al.*, 2005) reduced the cooking loss of meatballs.

### Texture profile analysis

Texture profile analysis of meatballs formulated with different levels of bee pollen is given in Table 2. Data

indicated that all textural parameters studied were affected by pollen addition ( $p<0.05$ ), except cohesiveness. Meatballs formulated with pollen were less hard and less gummy ( $p<0.05$ ) than the control. The hardness and gumminess values of meatballs decreased as the pollen content increased ( $p<0.05$ ). Addition of pollen apparently modifies structure of meatballs resulting in decreased hardness and gumminess. In addition, hardness and gumminess reduction could be related to water holding ability of pollen. Also, cooking loss values of meatballs supported this conclusion. Similar to our findings, several researchers reported that the addition of non-meat ingredients to meat batter decreased the hardness and/or gumminess of these meat products (Aleson-Carbonell *et al.*, 2005; Kassama *et al.*, 2003). Springiness and chewiness values of meatballs containing 3.0% pollen were the highest and increasing the level of pollen decreased the springiness and chewiness values of meatballs ( $p<0.05$ ). This may be partially attributed to physical and chemical characteristics of pollen. However, an exact explanation of the findings is not clear.

### Sensory evaluation

Sensory scores for parameters such as appearance, fla-

avour, hardness, juiciness and overall acceptability were significantly affected ( $p < 0.05$ ) by the addition of pollen (Table 2). The highest appearance, flavour, juiciness and overall acceptability scores were recorded for the control meatballs. Generally, the sensory scores of meatballs decreased as the pollen content increased ( $p < 0.05$ ) and this decrease was higher with more than 4.5% pollen addition. Therefore, 4.5% of pollen is considered optimum for use as an enhancer to the nutritive value in meatballs. With increasing of non-meat additives in meat products contents, decreasing of sensory scores were also reported some researchers (Bilek and Turhan, 2009; Huang *et al.*, 2005; Turhan *et al.*, 2005).

### Fatty acid composition

The fatty acid composition of meatballs formulated with different levels of bee pollen is given in Table 3. The SFAs with the highest concentration in these meatballs were palmitic and stearic acid, while the most abundant was oleic acid, with a concentration of 31.97-33.27 g/100 g. MUFAs and PUFAs together accounted for 39.71-41.10% of the total fatty acids. Of PUFAs, C18:2n-6c was the most abundant (2.52-4.07 g/100 g). These results are consistent with the fatty acid composition reported elsewhere in ground meat products (Bilek and Turhan, 2009; Yilmaz and Daglioglu, 2003).

Data in Table 3 indicated that C18:0, C18:2n-6c, C18:

**Table 3. Fatty acid composition of meatballs formulated with different levels of bee pollen (g/100 g fatty acids)**

	Bee pollen level (%)				
	Control	1.5	3.0	4.5	6.0
C10:0	0.05±0.02	0.05±0.02	0.05±0.01	0.05±0.02	0.06±0.01
C12:0	0.05±0.02	0.06±0.01	0.07±0.02	0.06±0.01	0.07±0.02
C13:0	0.01±0.00	0.01±0.00	0.01±0.01	0.01±0.00	0.01±0.00
C14:0	2.91±0.11	2.90±0.10	2.99±0.10	3.23±0.49	3.23±0.38
C15:0	0.46±0.05	0.49±0.05	0.49±0.05	0.47±0.04	0.46±0.05
C16:0	27.74±1.56	27.40±1.35	28.12±1.53	28.17±1.04	28.38±0.85
C17:0	1.32±0.10	1.23±0.09	1.16±0.11	1.17±0.11	1.14±0.09
C18:0	27.49±0.92 <sup>a</sup>	27.51±0.95 <sup>a</sup>	26.22±0.55 <sup>ab</sup>	25.47±1.16 <sup>b</sup>	25.27±1.20 <sup>b</sup>
C20:0	0.20±0.05	0.22±0.05	0.21±0.06	0.20±0.06	0.20±0.06
C22:0	0.03±0.01	0.04±0.01	0.03±0.01	0.03±0.01	0.04±0.02
C23:0	0.01±0.00	0.03±0.01	0.03±0.01	0.02±0.01	0.02±0.01
ΣSFA <sup>1)</sup>	60.27±0.48	59.94±0.70	59.39±1.80	58.89±1.25	58.88±2.27
C14:1	0.39±0.12	0.43±0.10	0.45±0.09	0.43±0.13	0.45±0.13
C15:1	ND <sup>5)</sup>	0.01±0.00	0.02±0.01	0.01±0.01	0.02±0.01
C16:1	2.20±0.30	2.08±0.19	2.15±0.14	2.19±0.19	2.02±0.16
C17:1	0.74±0.20	0.75±0.10	0.63±0.11	0.77±0.19	0.81±0.11
C18:1n-9c,t	33.27±1.01	33.13±1.01	32.95±1.02	32.94±1.00	31.97±1.42
C20:1n-9	0.17±0.05	0.24±0.06	0.24±0.05	0.24±0.05	0.27±0.10
C22:1n-9	ND	0.01±0.01	0.01±0.00	0.01±0.00	0.02±0.01
C24:1	0.02±0.01	0.02±0.01	0.02±0.01	0.03±0.01	0.03±0.01
ΣMUFA <sup>2)</sup>	36.80±1.21	36.69±1.14	36.48±1.27	36.65±1.20	35.59±1.84
C18:2n-6t	0.10±0.01	0.12±0.01	0.12±0.01	0.12±0.01	0.10±0.01
C18:2n-6c	2.52±0.13 <sup>c</sup>	2.78±0.17 <sup>c</sup>	3.24±0.23 <sup>b</sup>	3.43±0.14 <sup>b</sup>	4.07±0.14 <sup>a</sup>
C18:3n-3	0.23±0.05 <sup>c</sup>	0.28±0.06 <sup>c</sup>	0.58±0.05 <sup>b</sup>	0.66±0.09 <sup>b</sup>	1.02±0.28 <sup>a</sup>
C20:2n-3	0.02±0.01	0.03±0.01	0.02±0.01	0.02±0.01	0.02±0.01
C20:3n-6	0.03±0.01	0.05±0.01	0.07±0.01	0.08±0.01	0.10±0.06
C20:5n-3	ND	0.07±0.01 <sup>c</sup>	0.08±0.02 <sup>c</sup>	0.13±0.01 <sup>b</sup>	0.18±0.04 <sup>a</sup>
ΣPUFA <sup>3)</sup>	2.91±0.18 <sup>c</sup>	3.35±0.22 <sup>c</sup>	4.12±0.29 <sup>b</sup>	4.46±0.14 <sup>b</sup>	5.51±0.43 <sup>a</sup>
P/S <sup>4)</sup>	0.05±0.01 <sup>c</sup>	0.05±0.01 <sup>c</sup>	0.07±0.01 <sup>b</sup>	0.08±0.01 <sup>b</sup>	0.09±0.01 <sup>a</sup>
n-6/n-3	11.84±2.05 <sup>a</sup>	8.54±1.85 <sup>b</sup>	5.16±0.56 <sup>c</sup>	4.58±0.58 <sup>c</sup>	3.65±0.57 <sup>d</sup>

Values are means standard deviation of three replicates.

<sup>a-d</sup>Means in the same row with different superscript letters are different ( $p < 0.05$ ).

<sup>1)</sup>SFA=Saturated fatty acids.

<sup>2)</sup>MUFA=Monounsaturated fatty acids.

<sup>3)</sup>PUFA=Polyunsaturated fatty acids.

<sup>4)</sup>P/S=Polyunsaturated fatty acids/saturated fatty acids.

<sup>5)</sup>ND=No detected.

3n-3, C20:5n-3 and PUFA contents of meatballs were affected by pollen addition ( $p<0.05$ ). While C18:0 content of meatballs slightly decreased with pollen addition, C18:2n-6c, C18:3n-3, C20:5n-3 and PUFA contents increased ( $p<0.05$ ). The low amount of C18:0 and the high amount of C18:2n-6c, C18:3n-3, C20:5n-3 in bee pollen is responsible for these effects. From a nutritional perspective,  $\alpha$ -linolenic acid (C18:3n-3) content of control meatballs was 0.23 g/100 g, and ranged between 0.28 and 1.02 g/100 g for meatballs with added pollen. In the human diet, the recommended adequate intake for  $\alpha$ -linolenic acid is 2.22 g/day based on a 2000 kcal diet (Simopoulos *et al.*, 1999). Therefore, 100 g portion of meatballs with added pollen would provide between 13 and 46% of this recommended intake.

P/S and n-6/n-3 ratios are thought to be important in relation to the nutritional value of foods for human health

(Bilek and Turhan, 2009; Lopez-Lopez *et al.*, 2011). The recommended healthy P/S ratio is above 0.4 (Lopez-Lopez *et al.*, 2011). In the present study, the addition of pollen to meatballs slightly increased the P/S ratio ( $p<0.05$ ), but this increase was lower than recommended value. Higher P/S ratios were reported by Ansorena and Astiasaran (2004) in dry-fermented sausages by adding linseed oil and by Bilek and Turhan (2009) in beef patties by adding flaxseed flour. The effect of the n-6/n-3 ratio is probably of more significance than the P/S ratio in term of human nutrition (Bilek and Turhan, 2009). There is abundant evidence associating a higher n-6/n-3 ratio with the promotion of pathogenesis of many diseases, including CVD and cancer, and lower ratios with a suppressive effect. Therefore, the dietary recommendation is to reduce this value to less than 4 for prevention of CVD (Lopez-Lopez *et al.*, 2011). In the present study, n-6/n-3 ratio decreased

**Table 4. pH, the Hunter Lab attributes and TBA value of meatballs formulated with different levels of bee pollen**

	Bee pollen level (%)	Storage time (d)			
		1	3	6	9
pH	Control	6.58±0.02 <sup>aA</sup>	6.57±0.03 <sup>BA</sup>	5.98±0.03 <sup>AB</sup>	5.56±0.04 <sup>BC</sup>
	1.5	6.51±0.02 <sup>BB</sup>	6.61±0.01 <sup>AA</sup>	5.70±0.05 <sup>DC</sup>	5.76±0.07 <sup>AC</sup>
	3.0	6.48±0.02 <sup>BB</sup>	6.59±0.01 <sup>abA</sup>	5.79±0.03 <sup>CC</sup>	5.47±0.02 <sup>CD</sup>
	4.5	6.34±0.03 <sup>CB</sup>	6.49±0.03 <sup>CA</sup>	5.96±0.05 <sup>abC</sup>	5.71±0.02 <sup>AD</sup>
	6.0	6.37±0.01 <sup>CA</sup>	6.40±0.02 <sup>DA</sup>	5.91±0.04 <sup>BB</sup>	5.59±0.02 <sup>BC</sup>
Hunter <i>L</i> <sup>1)</sup>	Control	53.47±3.01 <sup>ab</sup>	55.29±1.03 <sup>a</sup>	52.48±1.05 <sup>b</sup>	55.77±0.42 <sup>a</sup>
	1.5	55.59±2.62 <sup>a</sup>	56.07±1.15 <sup>a</sup>	56.60±0.45 <sup>a</sup>	54.71±0.42 <sup>a</sup>
	3.0	54.47±1.32 <sup>a</sup>	53.19±0.55 <sup>b</sup>	55.45±0.34 <sup>a</sup>	55.47±2.14 <sup>a</sup>
	4.5	52.00±1.06 <sup>ab</sup>	52.43±0.98 <sup>b</sup>	50.66±0.69 <sup>c</sup>	51.71±0.13 <sup>b</sup>
	6.0	49.81±0.86 <sup>BA</sup>	51.47±1.35 <sup>BA</sup>	47.53±1.51 <sup>DB</sup>	49.49±0.35 <sup>CA</sup>
Hunter <i>a</i> <sup>2)</sup>	Control	9.94±1.49 <sup>AB</sup>	8.82±0.99 <sup>AB</sup>	11.74±0.33 <sup>AA</sup>	9.58±0.03 <sup>AB</sup>
	1.5	6.79±0.64 <sup>BD</sup>	8.69±0.61 <sup>AC</sup>	10.66±0.25 <sup>AA</sup>	9.70±0.24 <sup>AB</sup>
	3.0	7.15±0.78 <sup>BB</sup>	6.02±0.62 <sup>BC</sup>	9.23±1.18 <sup>BA</sup>	7.53±0.28 <sup>BB</sup>
	4.5	6.59±0.21 <sup>BB</sup>	4.95±0.32 <sup>bcC</sup>	7.38±0.39 <sup>CA</sup>	7.47±0.37 <sup>BA</sup>
	6.0	6.39±0.63 <sup>BB</sup>	4.34±0.48 <sup>CC</sup>	7.68±0.19 <sup>CA</sup>	7.41±0.33 <sup>BA</sup>
Hunter <i>b</i> <sup>3)</sup>	Control	11.74±0.72 <sup>CA</sup>	11.56±0.48 <sup>CA</sup>	10.54±0.62 <sup>CB</sup>	10.19±0.12 <sup>DB</sup>
	1.5	15.05±0.50 <sup>b</sup>	15.57±0.37 <sup>b</sup>	15.61±0.62 <sup>b</sup>	14.74±0.51 <sup>c</sup>
	3.0	18.10±1.09 <sup>a</sup>	16.67±0.92 <sup>b</sup>	17.26±0.76 <sup>a</sup>	17.21±0.93 <sup>b</sup>
	4.5	18.64±1.43 <sup>a</sup>	18.28±0.50 <sup>a</sup>	17.89±0.46 <sup>a</sup>	18.08±0.74 <sup>ab</sup>
	6.0	18.92±0.53 <sup>AA</sup>	19.27±0.87 <sup>AA</sup>	17.39±0.15 <sup>AB</sup>	18.71±0.23 <sup>AA</sup>
TBA <sup>4)</sup>	Control	0.44±0.04 <sup>D</sup>	0.58±0.05 <sup>AC</sup>	0.66±0.03 <sup>AB</sup>	0.79±0.01 <sup>AA</sup>
	1.5	0.41±0.08 <sup>B</sup>	0.48±0.03 <sup>BB</sup>	0.56±0.03 <sup>BA</sup>	0.60±0.01 <sup>BA</sup>
	3.0	0.38±0.06 <sup>C</sup>	0.44±0.02 <sup>CB</sup>	0.54±0.03 <sup>BA</sup>	0.55±0.04 <sup>CA</sup>
	4.5	0.38±0.03 <sup>C</sup>	0.40±0.04 <sup>cdC</sup>	0.45±0.01 <sup>CB</sup>	0.53±0.01 <sup>CA</sup>
	6.0	0.34±0.01	0.36±0.03 <sup>d</sup>	0.36±0.06 <sup>d</sup>	0.38±0.02 <sup>d</sup>

Values are means standard deviation of three replicates.

<sup>a-d</sup>Means in the same column with different superscript letters are different ( $p<0.05$ ).

<sup>A-D</sup>Means in the same row with different superscript letters are different ( $p<0.05$ ).

<sup>1)</sup>Hunter *L*=Lightness (100=white, 0=black).

<sup>2)</sup>Hunter *a*=Redness (+, red; -, green).

<sup>3)</sup>Hunter *b*=Yellowness (+, yellow; -, blue).

<sup>4)</sup>TBA=Thiobarbituric acid (mg MDA/kg sample).

from 11.84 in control meatball to 3.65-8.54 in meatballs with added pollen as a consequence of the C18:3n-3 and C20:5n-3 increment. The addition of pollen to meatballs obviously improved their nutritive value. Lower n-6/n-3 ratios were reported by Ansorena and Astiasaran (2004) in dry-fermented sausages by adding linseed oil, by Bilek and Turhan (2009) in beef patties by adding flaxseed flour and by Valencia *et al.* (2008) in fresh pork sausages by adding linseed oil, fish oil and natural antioxidants.

### pH, color and TBA value

pH, the Hunter Lab attributes and TBA value of meatballs formulated with different levels of bee pollen are given in Table 4. pH values of meatball samples were affected by both pollen addition and storage time ( $p < 0.05$ ). While on 1 and 3 d of storage, in general, pH values of samples decreased as the pollen content increased, on the other days of storage, different effects were observed. Various researchers also reported that non-meat ingredients such as oat bran (Yilmaz and Daglioglu, 2003), honey (Johnston *et al.*, 2005), okara (Turhan *et al.*, 2007, 2009) and pumpkin (*Cucurbitamoschata* Duch.) leaf extract (Choe *et al.*, 2011) changed pH value of meat products. In general, pH value of control sample decreased during storage, while pH values of meatballs formulated with bee pollen slightly increased until the 3 d of storage and then, began to decrease ( $p < 0.05$ ). The decrease in pH of meatballs during storage might be caused by the action of bacteria, which brings about a breakdown of the carbohydrate into organic acids, mainly lactic acid causing a decrease in pH (Kumar *et al.*, 2007).

In all storage times, in general, Hunter *L* values (lightness) of meatballs decreased ( $p < 0.05$ ) as the pollen content increased however, Hunter *L* values of samples did not change ( $p > 0.05$ ) during storage except meatballs added with 6% pollen. Hunter *a* (redness) values of meatball samples were affected by both pollen addition and storage time ( $p < 0.05$ ). In general, Hunter *a* values of samples decreased as the pollen content increased. This might be a result of a decrease in red meat or an increase in pollen in the formulation. Turhan *et al.* (2005) and Yilmaz and Daglioglu (2003) obtained similar results in beef burgers with hazelnut pellicle and in meatball with oat bran added, respectively. The changes in Hunter *a* value of meatballs during storage are not clear. Hunter *b* values (yellowness) also showed a contrary trend to Hunter *a* values. The control meatballs had the lowest Hunter *b* values and Hunter *b* values of meatballs increased ( $p < 0.05$ ) as the pollen content increased. These results are in agreement with

those of the previous studies (Jimenez-Colmenero *et al.*, 2003; Turhan *et al.*, 2007, 2009). Only, Hunter *b* values of control samples and meatballs with added 6% pollen changed during storage ( $p < 0.05$ ).

TBA value is one of the most widely used tests for evaluating the lipid oxidation of meat and meat products. TBA values of meatball samples were affected by both pollen addition except 1 d of storage and storage time except meatballs added with 6% pollen ( $p < 0.05$ ). On 3, 6 and 9 d of storage, the highest TBA value was determined in control samples ( $p < 0.05$ ) and in general, TBA value of samples decreased as the pollen content increased ( $p < 0.05$ ). TBA values of meatballs increased during storage ( $p < 0.05$ ) and the increase in control sample was faster and higher level than in the meatballs formulated with pollen. On 9 d of storage, the increase in TBA value of control and meatballs formulated with 1.5, 3.0 and 4.5% was 80, 46, 45 and 40%, respectively. TBA values of the meatballs formulated with 6% remained relatively constant during storage ( $p > 0.05$ ). These results indicate that bee pollen is effective in retarding the lipid oxidation of cold stored meatballs. These results are also supported by phenolic content and antioxidant activity of bee pollen. Antioxidant capacity of bee pollen is related to its phenolic compounds. The high ability of phenolic constituents to neutralize the active oxygen species is strongly associated with their structure, such as the conjugated double bonds and the number of hydroxyl groups in the aromatic ring, mostly attributed to flavonoids and cinnamic acid derivatives (Leja *et al.*, 2007). Similar to our findings, Freire *et al.* (2012), Graikou *et al.* (2011), Morais *et al.* (2011), Negri *et al.* (2011) and Rzepecka-Stojko *et al.* (2012) reported bee pollens have strong antioxidant capacity. Johnston *et al.* (2005) also reported that clover and wildflower honeys delayed lipid oxidation in ready-to-eat ground beef patties.

### Microbiological analysis

Results of the microbiological analyses of the meatballs formulated with different levels of bee pollen during the 9 d storage time are presented in Table 5. The counts of all determined microbiological indicators were affected by both pollen addition, except 1 d of storage for *S. aureus* and storage time ( $p < 0.05$ ). On 1 and 3 d of storage, the highest total aerobic mesophilic bacteria (TAMB) count was observed in control samples, while on 6 and 9 d of storage, the highest TAMB count was observed control and meatballs added with 1.5% pollen. In general, TAMB counts of samples decreased as the pollen content increa-



**Table 5. The microbiological properties of meatballs formulated with different levels of bee pollen (Log CFU/g)**

	Bee pollen level (%)	Storage time (d)			
		1	3	6	9
TAMB <sup>1)</sup>	Control	6.45±0.11 <sup>aC</sup>	6.80±0.04 <sup>aB</sup>	8.05±0.08 <sup>aA</sup>	8.19±0.07 <sup>aA</sup>
	1.5	6.36±0.02 <sup>bD</sup>	6.71±0.05 <sup>bC</sup>	8.05±0.04 <sup>aB</sup>	8.12±0.02 <sup>aA</sup>
	3.0	6.29±0.03 <sup>bD</sup>	6.47±0.03 <sup>cC</sup>	7.87±0.03 <sup>bB</sup>	7.96±0.04 <sup>bA</sup>
	4.5	6.26±0.04 <sup>bD</sup>	6.43±0.03 <sup>cC</sup>	7.70±0.10 <sup>cB</sup>	7.95±0.02 <sup>bA</sup>
	6.0	6.26±0.03 <sup>bD</sup>	6.40±0.03 <sup>cC</sup>	7.68±0.03 <sup>cB</sup>	7.94±0.08 <sup>bA</sup>
Coliform bacteria	Control	5.98±0.04 <sup>aD</sup>	6.39±0.06 <sup>aC</sup>	6.58±0.03 <sup>aB</sup>	6.71±0.04 <sup>aA</sup>
	1.5	5.87±0.02 <sup>bC</sup>	6.28±0.05 <sup>bA</sup>	6.12±0.09 <sup>bB</sup>	5.86±0.08 <sup>bC</sup>
	3.0	5.75±0.04 <sup>cBC</sup>	5.69±0.06 <sup>cC</sup>	6.07±0.06 <sup>bCA</sup>	5.82±0.03 <sup>bCB</sup>
	4.5	5.75±0.06 <sup>cB</sup>	5.73±0.06 <sup>cB</sup>	5.98±0.04 <sup>cdA</sup>	5.76±0.04 <sup>cB</sup>
	6.0	5.73±0.05 <sup>cB</sup>	5.73±0.03 <sup>cB</sup>	5.96±0.03 <sup>dA</sup>	5.66±0.04 <sup>dC</sup>
<i>S. aureus</i>	Control	4.58±0.09 <sup>C</sup>	5.38±0.02 <sup>aB</sup>	5.77±0.06 <sup>aA</sup>	5.83±0.03 <sup>aA</sup>
	1.5	4.49±0.05 <sup>C</sup>	5.13±0.03 <sup>bB</sup>	5.27±0.02 <sup>bA</sup>	5.26±0.04 <sup>bA</sup>
	3.0	4.48±0.05 <sup>D</sup>	5.02±0.04 <sup>cB</sup>	5.10±0.01 <sup>cA</sup>	4.63±0.03 <sup>cC</sup>
	4.5	4.46±0.04 <sup>B</sup>	5.00±0.03 <sup>cA</sup>	5.02±0.01 <sup>dA</sup>	4.25±0.05 <sup>dC</sup>
	6.0	4.47±0.03 <sup>B</sup>	5.00±0.04 <sup>cA</sup>	5.01±0.02 <sup>dA</sup>	4.26±0.03 <sup>dC</sup>

Values are means standard deviation of three replicates.

<sup>a-d</sup>Means in the same column with different superscript letters are different ( $p < 0.05$ ).

<sup>A-D</sup>Means in the same row with different superscript letters are different ( $p < 0.05$ ).

<sup>1)</sup>TAMB=Total aerobic mesophilic bacteria.

sed and on 9 d of storage, the lowest count was observed in meatballs added with 3.0, 4.5 and 6% pollen. In general, TAMB counts of meatballs increased during storage. Microbial spoilage of meat usually occurs when TAMB count reaches  $10^8$  CFU/g (Ozturk *et al.*, 2010). TAMB counts of control and meatballs added with 1.5% pollen exceeded 8 Log CFU/g on 6 d of storage, while TAMB counts of meatballs added with 3.0, 4.5 and 6.0% approached this value on 9 d of storage.

In all storage times, the highest coliform bacteria and *S. aureus* counts were in the control meatballs and the lowest in the samples added with pollen. In general, these bacteria counts decreased as the pollen content increased and the decrease was higher than TAMB counts. Coliform bacteria and *S. aureus* counts of meatballs fluctuated during storage. It is obvious from the present studies that bee pollen inhibited the bacterial growth. The antibacterial effect of pollen could be attributed mainly to the high content of flavonoids such as quercetin and kaempferolglucosides which are known to possess antibacterial activity (Graikou *et al.*, 2011). The antimicrobial activity of pollen was also confirmed by several researchers (Basim *et al.*, 2006; Graikou *et al.*, 2011; Morais *et al.*, 2011).

## Conclusions

The results of this study indicate that bee pollen addition had a significant effect on the nutritional and storage

quality of meatballs. The addition of pollen improved cooking loss, PUFA content, and P/S and n-6/n-3 ratios. Moreover, the addition of pollen minimized lipid oxidation and inhibited microbial growth during storage time. However, addition of pollen decreased sensory scores and Hunter *a* values. Generally, the sensory scores of meatballs decreased with more than 4.5% pollen addition. Thus, bee pollen could be added to enhance the nutritional and storage quality of meatballs with minimal changes in composition and/or sensory properties.

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