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Application of microencapsulated propolis extract in yoghurt production

Yasemin Taşdemir[1], Evren Gölge[2]*

[1]Department of Food Engineering, İstanbul Technical University, İstanbul, Turkey; [2]Department of Nanotechnology Engineering, Sivas Cumhuriyet University, Sivas, Turkey

***Corresponding Author:** Evren Gölge, Department Of Nanotechnology Engineering, Sivas Cumhuriyet University, 58010 Sivas, Turkey. Email: egolge@cumhuriyet.edu.tr

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ORIGINAL ARTICLE

Abstract

Extract of ethanolic propolis was microencapsulated in spray dryer before being used in stirred-style yoghurt manufacturing at quantities ranging from 0.5% to 2%. Samples were stored for 21 days for analysis. A statistically significant drop in pH, total phenolic content, and antioxidant activity was identified during 21 days of storage. Increase in the concentration of microencapsulated propolis extract (MPE) demonstrated increasing effects of syneresis, titration acidity, total phenolic content, and antioxidant activity. Contrarily, L* and a* values decreased significantly with increase in b* value. Generally, 2% MPE samples were less preferred in terms of flavor, smell, consistency, and the overall impression.

Keywords: antimicrobial; encapsulation; propolis; yoghurt

Introduction

Propolis is a particular resinous combination with antiviral, antibacterial, antifungal, antioxidant, anti-cancer, and anti-inflammatory properties. Bees collect it from tree cones, leaves, young shoots, shells, and buds as well as other plant oils, pollen, resin, and waxy components. These substances are harmonized through metabolic secretions of honeybees (*Apis mellifera L.*). It is a unique source of a wide variety of bioactive natural products, such as polyphenols, flavonoids, caffeic acid, and esters (Laskar *et al.*, 2010). The potential applications of propolis as a natural antioxidant and antimicrobial in the pharmaceutical and food industries appear to be promising. However, because of its strong flavor and aroma as well as its limited solubility in water, the use of propolis in foods is limited. Propolis involves valuable chemical substances, such as flavonoids, quinine, apigenin, acacetin, quercetin, kaempferide, kaempferol-7,4-dimethyl ether, ermanin, galangin, pinocembrin, pinobanksin, pinobanksin-3-acetate, pinostrobin, pecto-linaringenin, luteolin, isopentyl ferulate, p-coumaric acid benzyl ester, caffeic acid, prenyl kaffeate, 3-methylbut-2-enyl caffeate, caffeic acid phenyl ester, methyl kaffeate, diterpenoid clerodan, and volatile compounds. Propolis is not only bacteriostatic but also bactericidal; therefore, both growth inhibition and bacterial degradation are achieved. It prevents the spread of pathogens and helps the host's immune system to eradicate bacteria. This condition is critical for an effective therapeutic approach to combat infectious diseases (Koç *et al.*, 2007; Mirzoeva *et al.*, 1997; Santos *et al.*, 2020).

In literature, some attempts are discovered of using propolis in yoghurt as a functional ingredient or replacement to an antimicrobial additive (potassium sorbate). Elkassas *et al.* (2023) used varying amounts of water extracts of crude propolis in milk and yoghurt samples. Santos *et al.* (2019) used the 0.05% (w/v) ethanolic extract of Brazilian red propolis to replace potassium sorbate in conventional probiotic yoghurt samples involving 10% strawberry pulp.

Encapsulation is defined as the coating of microorganisms, cells, enzymes, and other food components with a wall material based on carbohydrates or proteins in all

three physical states (Desai and Park, 2005; Jafari *et al.*, 2022; Tavares *et al.*, 2022). This technology allows the coated material to be retained in the microcapsules and to be released in a controlled manner with a controlled speed (Wang *et al.*, 2011). Although most of microencapsulates have a round shape. It is known that microencapsulation affects the composition of wall material as well as the physicochemical properties of the coated material and the shape of microencapsulates. The carrier materials to be used in the encapsulation of food products should be suitable for food production and should form a barrier around the active substance. Thanks to this technology, it is possible to provide aroma and flavor differentiation to mask ingredients with bad odor and taste and to increase stability and bioavailability. The encapsulation process can also be used to immobilize the cells or enzymes in food production processes, such as fermentation and metabolite production (Baysan *et al.*, 2019; Tavares *et al.*, 2022). Enrichment of yoghurt with various functional compounds is presented in many studies (Chaikham 2015; Francisco *et al.*, 2018; Ghorbanzade *et al.*, 2017; Kailasapathy 2006; Özcan and Altun 2013; Sultana *et al.*, 2000).

The purpose of this research was to study the functional benefits of encapsulated propolis extract in daily food intake. It also revealed the possibility of using propolis as an antibacterial agent in manufacturing of yoghurt and to examine the effect of propolis on the bioactive properties of produced yoghurt samples.

Materials and methods

Chemicals and consumables

Propolis was obtained from a local farm facilitating in Sivas province. Ethanol (80% v/v; Sigma-Aldrich, Germany), NaOH (Merck, Germany), phenolphthalein (Merck), Folin-Colin reagent (Sigma-Aldrich, China), Na_2CO_3 solution (Sigma-Aldrich, China), 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (Merck), thermophilic starter culture, freeze-dried lactic culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) YoFlex YC-X16 (Christie Hansen, Denmark), and commercial brand pasteurized milk (Sütaş, 3% protein and 3% fat) were obtained from the local market.

Preparation of propolis extract and microencapsulation

Approximately 14 g of propolis was dissolved in 100 mL of ethanol (Sigma-Aldrich, Germany) for 24 h and filtered under vacuum using Büchner funnel. To remove remaining wax, the ethanolic extract was kept in a freezer (-20°C) for 10 h and centrifuged twice (Sigma, 2-16PK, Germany) at 5°C, at 4500 rpm for 10 min. The supernatant was removed and stored at 4°C. For microencapsulation, the laboratory-scale Buchi B-290 (Flawil, Switzerland) mini spray drier in TUBITAK Marmara Araştırma Merkezi (İstanbul, Turkey) was used. Maltodextrin (DE 14-17) was used as a coating agent. Emulsion ratio was adjusted as 1:3 (on dry basis). The feed flow rate was 4 mL/min, the air inlet temperature was 120°C, and the outlet temperature varied between 65°C and 70°C. Encapsulation was performed for 8 h and the resultant microencapsulated propolis extract (MPE) were stored at 4°C in a refrigerator (Bostancı *et al.*, 2023; Busch *et al.*, 2017).

Preparation of MPE-added yoghurt

The pasteurized milk was heated to 42°C and YC-X16 yoghurt culture (3 g/100 mL) was added to produce stirred-type yoghurt. Consequently, varying amounts of MPE were added (0%, 0.5%, 1%, and 2%) as shown in Table 1. The samples were allowed to incubate at 42°C for 8 h until pH 4.6 was reached. The samples were stored in 250-mL plastic closed-lid containers at 4°C and analyzed on day 0, 7, 14, and 21.

Physical and chemical analyses

The dry matter content of the samples was determined by the oven method at 105°C (930.15; Association of Official Analytical Chemists [AOAC], 1990). The pH values were measured by Sentix 41 electrode by hand-type WTW 315i set (Weilheim, Germany) pH meter. Titratable acidity was determined by titration with the adjusted 0.1-N NaOH solution with phenolphthalein. The results were measured in terms of gram lactic acid in 100-g sample (942.15; AOAC, 1990). In syneresis analysis, 25 g of yoghurt samples were taken and filtered for 4 h through filter paper. The liquid collected was measured

Table 1. Sample formulations.

Sample tag	Pasteurized milk	YC-X16 culture	Microencapsulated propolis extract (MPE)
Control	1 L	30 g	None
0.5% MPE	1 L	30 g	5 g
1% MPE	1 L	30 g	10 g
2% MPE	1 L	30 g	20 g

in volumetric cylinder. Antioxidant activity was determined using to the DPPH method. Results were obtained in terms of IC_{50} (Aqil *et al.*, 2006). Total phenolic compounds in yoghurt samples were determined according to the Folin–Ciocalteau method. The results were measured according to the standard curve of gallic acid (mg GAE/100 g; Woisky and Salatino, 1998).

Color analysis

Minolta–Hunter colorimeter model CM-3500d Minolta spectrophotometer (Minolta, Japan) was used for color analysis of yoghurt samples in terms of L*, a*, and b* values.

Microbiological analysis

Yeast and mold counts were determined by pour plate technique using potato dextrose agar (incubation at 22°C for 5 days; Marshall, 1992).

Sensory analysis

The randomly coded yoghurt samples were placed on neutral white background and presented to panelists in daylight conditions. Eight panelists familiar with yogurt (five females and three males, aged 20–55 years) evaluated yogurt samples on day 1, 7, 14, and 21 of storage. Fresh water was used for mouth neutralization. Each sample was evaluated by panelists in terms of flavor, consistency, smell, and overall impression. A 10-point hedonic scale was used for evaluation, with "1" being the worst and "10" denoting the best sample. The scores given by panelists to each sample were noted separately. The consistency of samples was judged by gentle mixing of yogurt with a spoon and by tasting. A spoonful of yogurt was taken and spread on tongue to evaluate consistency (Kramer and Twigg, 1970).

Statistical analysis

All yoghurt samples were analyzed in two replicates. Analysis of variance (ANOVA) was performed using SPSS 22.0 and the significance of changes during storage was calculated by Tukey's range test ($p < 0.05$).

Results and discussion

Physical and chemical analyses

No significant difference was observed in the total dry matter content of the samples during storage period as shown in Table 2 ($p < 0.05$). Because the samples were kept in closed containers, it was expected that there would be no significant change in the total dry matter content. The increasing amount of MPE resulted in an increase in the total dry matter content ($p < 0.05$). Yadav *et al.* (2018) and Paseephol *et al.* (2008) showed that there was no significant difference in the total dry matter content of yoghurts during storage.

pH values of the samples decreased with storage and this change was found to be statistically significant ($p < 0.05$; Table 2). The highest pH values were observed on day 1 of storage; however, pH decreased on subsequent days, resulting in increased acidity. On the other hand, the increasing MPE ratios caused no significant differences in pH values (Table 2). This decrease in pH values helped in the inhibition of pathogenic bacteria. The regulation of pH in yoghurt is very important as it affects syneresis with excessive thrust effect. Decrease in pH also influences the decomposition of casein micelle and the reconstruction of a three-dimensional (3D) protein network (Santos *et al.*, 2019).

The effect of MPE ratios on titratable acidity was statistically significant (p <0.05; Table 2). Acidity increased with increase in storage time. These values were consistent with the expected results. During storage, acidity is a natural result of bacterial process in yoghurt. Since lactose breaks down into lactic acid and the formation of fatty acids results in the hydrolyzation of fats present in yoghurt. Similar results were also reported in literature (Aportela- Palacios *et al.*, 2005; Güven *et al.*, 2005; Santos *et al.*, 2019).

Syneresis is one of the essential factors while assessing the consistency of yoghurt. Different MPE amounts caused significant variations in the syneresis amount of samples as shown in Table 2. A statistically significant decrease was observed in the syneresis levels during storage ($p < 0.05$). In literature, this occurrence is explained by drop in pH during storage, which may have a contracting effect on casein micelle matrix by release of more serum (Estrada *et al.*, 2011; Ghorbanzade *et al.*, 2017; Salvador and Fiszman, 2004; Staffolo *et al.*, 2004). Similar changes in pH values and amount of syneresis during storage are observed in several studies (Aportela-Palacios *et al.*, 2005; Ghorbanzade *et al.*, 2017; Tosun *et al.*, 2011).

A significant increase in total phenolic content was observed with increase in the concentration of MPE ($p < 0.05$; Table 3). The first day of storage had the highest phenolic content, while the last day had the lowest value. Many studies discovered that propolis was high in phenolic compounds (Baysan *et al.*, 2021, Elkassas *et al.*, 2023; Jansen-Alves *et al.*, 2019). Therefore, increase in the content of phenolic compounds was an expected

Table 2. Physicochemical properties of MPE-added yogurt (n = 4).

	Day			
Sample	**0**	**7**	**14**	**21**
DMC (g/100 g)				
Control	10.76 ± 0.40^{a,A}	10.55 ± 0.44^{a,A}	10.80 ± 0.54^{a,A}	10.60 ± 0.40^{a,A}
0.5% MPE	11.20 ± 0.18^{a,B}	10.79 ± 0.68^{a,B}	10.98 ± 0.25^{a,B}	11.06 ± 0.03^{a,B}
1% MPE	11.61 ± 0.17^{a,C}	11.23 ± 0.64^{a,C}	11.22 ± 0.27^{a,C}	11.08 ± 0.22^{a,C}
2% MPE	12.45 ± 0.46^{a,D}	11.51 ± 0.64^{a,D}	11.75 ± 0.04^{a,D}	11.61 ± 0.39^{a,D}
SA (mL/100 g)				
Control	49.60 ± 0.00^{a,A}	43.43 ± 0.00^{b,A}	29.78 ± 0.02^{c,A}	28.04 ± 0.06^{d,A}
0.5% MPE	49.93 ± 0.06^{a,B}	33.88 ± 0.01^{b,B}	30.28 ± 0.00^{c,B}	24.97 ± 0.00^{d,B}
1% MPE	50.00 ± 0.00^{a,C}	49.08 ± 0.74^{b,C}	30.29 ± 0.00^{c,B}	29.26 ± 0.00^{d,C}
2% MPE	49.75 ± 0.00^{a,D}	39.65 ± 0.01^{b,D}	40.05 ± 0.00^{c,C}	26.30 ± 0.00^{d,D}
pH				
Control	4.31 ± 0.00^{a,A}	4.16 ± 0.00^{b,A}	4.10 ± 0.00^{c,A}	4.09 ± 0.00^{d,A}
0.5% MPE	4.31 ± 0.00^{a,A}	4.25 ± 0.00^{b,A}	4.21 ± 0.00^{c,A}	4.06 ± 0.05^{d,A}
1% MPE	4.32 ± 0.00^{a,A}	4.25 ± 0.00^{b,A}	4.22 ± 0.00^{c,A}	4.18 ± 0.00^{d,A}
2% MPE	4.39 ± 0.00^{a,A}	4.24 ± 0.00^{b,A}	4.17 ± 0.00^{c,A}	4.14 ± 0.00^{d,A}
TA (g/100 g)				
Control	0.74 ± 0.00^{a,A}	0.80 ± 0.00^{b,A}	0.82 ± 0.01^{c,A}	0.85 ± 0.00^{d,A}
0.5% MPE	0.75 ± 0.01^{a,B}	0.82 ± 0.01^{b,B}	0.85 ± 0.00^{c,B}	0.83 ± 0.00^{d,B}
1% MPE	0.74 ± 0.00^{a,AB}	0.78 ± 0.01^{b,C}	0.81 ± 0.00^{c,C}	0.82 ± 0.00^{d,C}
2% MPE	0.73 ± 0.02^{a,AB}	0.73 ± 0.00^{b,D}	0.84 ± 0.00^{c,D}	0.90 ± 0.02^{d,D}

^{a,b,c,d}Significant differences between rows at $p < 0.05$. ^{A,B,C,D}Significant differences between columns at $p < 0.05$.
DMC: dry matter content; SA: syneresis analysis; TA: titratable acidity.

Table 3. Total phenolic content and antioxidant activity of MPE-added yogurt.

	Day			
Sample	**0**	**7**	**14**	**21**
TPC (mg GAE/100 g) (n = 4)				
Control	33.81 ± 0.15^{a,A}	26.08 ± 0.44^{b,A}	24.65 ± 0.53^{c,A}	11.57 ± 0.05^{d,A}
0.5% MPE	57.73 ± 0.06^{a,B}	28.58 ± 0.24^{b,B}	25.05 ± 0.49^{c,AB}	14.47 ± 0.24^{d,B}
1% MPE	67.01 ± 0.33^{a,C}	29.12 ± 0.12^{b,C}	25.45 ± 0.86^{c,B}	15.99 ± 0.24^{d,C}
2% MPE	76.78 ± 0.13^{a,D}	30.07 ± 0.00^{b,D}	26.13 ± 0.82^{c,C}	23.38 ± 0.02^{d,D}
AA (IC_{50} = mg/mL) (n = 2)				
Control	200.02 ± 0.35^{a,A}	347.51 ± 0.61^{b,A}	489.69 ± 0.07^{c,A}	549.98 ± 0.03^{d,A}
0.5% MPE	184.75 ± 0.21^{a,B}	295.77 ± 0.56^{b,B}	435.75 ± 0.09^{b,B}	534.88 ± 0.34^{c,B}
1% MPE	120.68 ± 0.02^{a,C}	243.69 ± 0.11^{b,C}	244.43 ± 0.06^{c,C}	474.13 ± 0.69^{d,C}
2% MPE	112.15 ± 0.08^{a,D}	198.41 ± 0.13^{b,D}	204.34 ± 0.06^{c,D}	312.97 ± 0.07^{d,D}

^{a,b,c,d}Significant differences were observed between rows at $p < 0.05$. ^{A,B,C,D}Significant differences were observed between columns at $p < 0.05$.
TPC: total phenolic content; AA: antioxidant activity; GAE: Gallic acid equivalent.

outcome with addition of propolis. Total phenolic content was expected to decrease with storage because of increasing chemical or enzymatic oxidation of phenolic compounds. Santos *et al.* (2019) reported decrease in phenolic compounds in yoghurt containing Red Brazilian propolis and 10% strawberry pulp during a storage period of 28 days. The quantity of total antioxidants and phenolic compounds found in propolis depended on collection time, area, and vegetation of the region where it was collected (Ahn *et al.*, 2007).

Scibisz *et al.* (2012) investigated the effect of prebiotic culture on the stability of anthocyanins in yoghurt. The authors reported that anthocyanins were more stable

in the samples containing YC-X16 culture, rather than the samples containing *Bif. Animalis*, subsp. *Lactis, Lactobacillus acidophilus* and *Lactobacillus Paracasei*, subsp. *Paracasei.*

At the beginning of storage, the 2% MPE sample had the highest antiradical activity, while the control sample had the lowest value (Table 3). A lower IC_{50} value indicates higher antiradical activity. As the used MPE ratio increased, the antioxidant activity of the samples increased due to significant decrease in IC_{50} values. In other words, a significant reduction in antioxidant activity of the samples was observed during storage period ($p < 0.05$). This result was parallel to the total phenolic substance analysis of the samples and they supported each other. The increased antioxidant activity of propolis-added yoghurt is a positive function that can improve yoghurt's nutritional value. Reduced total phenolic content and antioxidant activity levels after storage were consistent with the results of prior research (Santos *et al.*, 2019).

Color analysis

In Table 4, as the MPE content increased, L* value decreased ($p < 0.05$). Some darkening of yoghurt was observed with increased amount of MPE content. However, a* value increased with increase in MPE content but decreased during storage ($p < 0.05$). The effect of storage period on b* value was found to be significant in all samples ($p < 0.05$). Value of b* increased with increase in MPE content. Since the MPE extracts were yellow, an increase in +b* value was expected. Comunian *et al.* (2017) obtained a* values from 8.36 to 8.94 and b* values from –0.62 to –0.47 over a 21-day storage period for yoghurt enriched with microcapsules of echium oil. However, these values were substantially different from those obtained for yoghurt with propolis microencapsulates in the current study.

Microbiological analysis

In the microbiological analysis, the control and samples containing MPE showed yeast and mold counts of <10 cfu/mL during a 21-day storage period. Yoghurt is susceptible to microbial spoilage because of its high water activity. In MPE-containing yoghurt samples, the growth of mold and yeast was greatly hindered. Santos *et al.* (2019) observed that yeast and mold counts in Red Brazilian propolis-containing yoghurt and potassium sorbate-containing control group were <10 cfu/mL for a 28-day storage period. Similarly, in an *in vitro* study investigating the antibacterial activity of propolis and *Arnica montana,* different microorganisms were used, and the ethanolic extract of propolis showed an inhibitory effect against all microorganisms tested (Koo *et al.*, 2000). Moreno *et al.* (1999) investigated the antibacterial effects of Argentinepropolis, in which the alcoholic extracts of propolis collected from different regions were used and no growth was observed in half of the microorganism species used.

Table 4. Changes in L*, a*, and b* values of MPE-added yogurt (n = 4).

	Days			
Sample	**0**	**7**	**14**	**21**
L* value				
Control	$74.54 \pm 0.42^{a,A}$	$60.84 \pm 0.09^{b,A}$	$63.25 \pm 0.27^{c,A}$	$68.00 \pm 0.54^{d,A}$
0.5% MPE	$72.77 \pm 0.11^{a,A}$	$59.85 \pm 0.26^{b,B}$	$62.67 \pm 0.05^{c,B}$	$67.60 \pm 0.07^{d,A}$
1% MPE	$68.48 \pm 0.36^{a,B}$	$59.85 \pm 0.26^{b,B}$	$59.02 \pm 0.16^{c,C}$	$62.95 \pm 0.16^{d,B}$
2% MPE	$65.53 \pm 2.37^{a,C}$	$57.19 \pm 0.02^{b,C}$	$57.20 \pm 0.18^{b,D}$	$57.27 \pm 0.16^{b,C}$
a* value				
Control	$-0.80 \pm 0.04^{a,A}$	$-1.67 \pm 0.01^{b,A}$	$-1.52 \pm 0.01^{c,A}$	$-1.62 \pm 0.02^{d,A}$
0.5% MPE	$-0.51 \pm 0.02^{a,B}$	$-1.59 \pm 0.00^{b,B}$	$-1.52 \pm 0.01^{c,A}$	$-1.47 \pm 0.01^{d,B}$
1% MPE	$-0.45 \pm 0.02^{a,C}$	$-1.40 \pm 0.01^{b,C}$	$-1.51 \pm 0.02^{c,A}$	$-1.45 \pm 0.00^{d,B}$
2% MPE	$-0.19 \pm 0.02^{a,D}$	$-1.31 \pm 0.02^{b,D}$	$-1.43 \pm 0.01^{c,B}$	$-1.42 \pm 0.05^{c,B}$
b* value				
Control	$0.33 \pm 0.01^{a,A}$	$4.86 \pm 0.02^{b,A}$	$5.38 \pm 0.01^{c,A}$	$5.60 \pm 0.00^{d,A}$
0.5% MPE	$1.24 \pm 0.17^{a,B}$	$5.85 \pm 0.00^{b,B}$	$6.07 \pm 0.00^{b,B}$	$7.19 \pm 0.03^{c,B}$
1% MPE	$1.86 \pm 0.05^{a,C}$	$5.90 \pm 0.01^{b,C}$	$7.38 \pm 0.01^{c,C}$	$7.59 \pm 0.03^{d,C}$
2% MPE	$3.26 \pm 0.24^{a,D}$	$7.18 \pm 0.03^{b,D}$	$7.26 \pm 0.00^{b,D}$	$8.39 \pm 0.11^{c,D}$

^{a,b,c,d}Significant differences were observed between rows at $p < 0.05$.
^{A,B,C,D}Significant differences were observed between columns at $p < 0.05$.

Shori *et al.* (2012) reported an increase in the bacterial count of *Lactobacillus* spp from 1.4×10^6 log cfu/mL to 2.3×10^6 log cfu/mL on day 7 of storage; however, it gradually decreased to 1.4×10^6 log cfu/mL on day 21 of storage for plain cow milk yoghurt.

Scibisz *et al.* (2012) prepared blueberry yoghurt with YC-X16 culture. The authors observed that 8.5 log cfu/g cell counts for *Lactobacillus sp.* did not differ significantly after 8 weeks of storage at 4°C. Hoxha *et al.* (2023) reported an increase from 8.55 log cfu/g to 8.75 log cfu/g in 7 days, but the count decreased to 8.47 log cfu/g at the end of a storage period of 28 days for plain cow milk yoghurt samples.

Sensory analysis

The sensory properties of the samples were evaluated according to hedonic test mean values. As shown in Table 5, no significant difference occurred in terms of flavor during storage period ($p < 0.05$). Results were statistically different for control and 0.5%, 1%, and 2% MPE-containing samples. In addition, 2% MPE-containing samples were less preferred. In terms of smell, the samples on day 1 and 21 were statistically different from the samples on day 7 ($p < 0.05$). Smell in the case of control group was more preferred, compared to 0.5% and 1% MPE-containing samples, and Smell of 2% MPE-containing samples was least preferred.

Consistency of the samples on day 7 was statistically different. Addition of MPE had no statistically significant effect on the consistency of yoghurt samples. In terms of the overall impression, no statistically significant effect was observed in different groups during storage ($p < 0.05$). The control and 0.5% MPE-containing samples were the most preferred ones, compared to other groups ($p < 0.05$).

Elkassas *et al.* (2023) reported that yoghurt samples containing 2% water-extracted propolis (WEP) had the highest color and appearance sensory scores for 15-day storage. Moreover, 1% WEP-containing samples had better scores for body and texture, taste, and the overall acceptability. Finally, no significant differences were observed between control and WEP-containing groups. In many studies conducted with enriched yoghurt with various functional ingredients, control groups were more preferred (Baba *et al.*, 2018; Ghorbanzade *et al.*, 2017; Tan and Korel, 2007).

Conclusions

For decades, yogurt has been recognized as an excellent nutritional food. Conventional yogurt has fewer biological effects, such as anti-obesity, anticancer, and antidiabetic properties, because it lacks several nutrients, such as flavonoids, anthocyanins, iron, and phenolics. Consequently, addition of natural functional ingredients

Table 5. Results of hedonic test.

		Days			
Sensory parameter	**Sample**	**1**	**7**	**14**	**21**
Flavor	Control	$7.38 \pm 0.52^{a,A}$	$7.38 \pm 1.19^{a,A}$	$7.63 \pm 0.92^{a,A}$	$7.50 \pm 0.93^{a,A}$
	0.5% MPE	$6.63 \pm 0.92^{b,A}$	$7.25 \pm 0.46^{b,A}$	$7.38 \pm 1.41^{b,A}$	$5.50 \pm 1.93^{b,A}$
	1% MPE	$7.50 \pm 1.60^{c,A}$	$4.88 \pm 1.36^{c,A}$	$4.75 \pm 1.67^{c,A}$	$4.88 \pm 0.64^{c,A}$
	2% MPE	$4.50 \pm 0.98^{d,A}$	$4.63 \pm 1.06^{d,A}$	$4.50 \pm 0.53^{d,A}$	$4.75 \pm 1.49^{d,A}$
Smell	Control	$6.63 \pm 0.92^{a,A}$	$7.38 \pm 1.41^{a,B}$	$7.50 \pm 1.60^{a,A,B}$	$7.50 \pm 0.93^{a,A}$
	0.5% MPE	$4.88 \pm 1.13^{b,A}$	$7.38 \pm 1.41^{b,B}$	$7.25 \pm 0.89^{b,A,B}$	$4.88 \pm 1.25^{b,A}$
	1% MPE	$5.50 \pm 1.20^{b,A}$	$7.50 \pm 1.31^{b,B}$	$4.63 \pm 0.92^{b,A,B}$	$5.50 \pm 1.51^{b,A}$
	2% MPE	$4.88 \pm 0.64^{c,A}$	$4.38 \pm 1.19^{c,B}$	$4.50 \pm 1.60^{c,A,B}$	$4.63 \pm 1.41^{c,A}$
Consistency	Control	$7.50 \pm 2.05^{a,A}$	$8.00 \pm 1.41^{a,B}$	$7.63 \pm 2.92^{a,A}$	$7.63 \pm 1.40^{a,A}$
	0.5% MPE	$5.63 \pm 1.77^{a,A}$	$6.75 \pm 2.12^{a,B}$	$4.75 \pm 2.25^{a,A}$	$6.75 \pm 2.05^{a,A}$
	1% MPE	$6.13 \pm 1.95^{a,A}$	$5.50 \pm 1.92^{a,B}$	$4.75 \pm 2.25^{a,A}$	$5.88 \pm 1.64^{a,A}$
	2% MPE	$5.25 \pm 1.83^{a,A}$	$6.50 \pm 1.51^{a,B}$	$4.50 \pm 1.77^{a,A}$	$5.50 \pm 1.19^{a,A}$
Overal ımpression	Control	$7.50 \pm 1.60^{a,A}$	$7.50 \pm 0.93^{a,A}$	$7.63 \pm 1.60^{a,A}$	$7.38 \pm 1.06^{a,A}$
	0.5% MPE	$6.50 \pm 1.41^{a,A}$	$7.13 \pm 1.64^{a,A}$	$7.50 \pm 1.41^{a,A}$	$7.13 \pm 1.36^{a,A}$
	1% MPE	$7.38 \pm 1.51^{b,A}$	$6.63 \pm 1.77^{b,A}$	$4.75 \pm 0.71^{b,A}$	$4.63 \pm 0.92^{b,A}$
	2% MPE	$4.50 \pm 1.60^{c,A}$	$4.25 \pm 1.39^{c,A}$	$4.50 \pm 1.41^{c,A}$	$4.63 \pm 1.69^{c,A}$

^{a,b,c,d}Significant differences between rows at $p < 0.05$.
^{A,B,C,D}Significant differences between columns at $p < 0.05$.

to yogurt improves its physicochemical properties and biological activity. Sensitivity of nutraceuticals is strongly influenced by a number of internal and external factors, such as food composition, structure, and physicochemical characteristics, as well as environmental factors, such processing technique, storage conditions, and processing environment. Propolis is a natural bee product whose functional properties and beneficial effects on human health are demonstrated in experimental studies and must be encapsulated to increase technological utilization possibilities and stability. The MPE used in yoghurt has shown a strong property to suppress microbial development. Furthermore, it enhances the probiotic capacity of yoghurt, as it does not associate adversely with lactic acid bacteria. Increase in MPE ratio caused no significant effect on pH of the samples. A significant ascent in total phenolic content was observed with increase in MPE concentration. The antioxidant activity of samples increased with increase in the MPE ratio employed, because of significant decrease in IC_{50} values. Some darkening of yoghurt color was observed with increased amount of MPE content. Increased values of a* and b* were observed with increase in MPE content. The control and MPE-containing samples showed yeast and mold counts of <10 log cfu/mL during a 21-day storage period. Generally, 2% MPE-containing samples were less preferred in sensorial analysis.

The findings of this study indicate that encapsulated propolis could be an alternative for use in various food products. Alternative microencapsulation methods or utilization of various flavoring and/or coloring agents could help to mask the unwanted sensory properties of propolis extract depending on the type of food product.

Conflict of interest

Authors declared no conflict of interests.

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