Original article

Effect of Encapsulated Propolis on Microbial Quality and Antioxidant Activity of Yoghurt

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Abstract. Yoghurt, a functional food, has been gained much attention on its production and consumption in the world. It is a fermented dairy product that contains lots of nutritional components. Some manufacturers have used some additives to extend the shelf life of yoghurt. Propolis could be alternative food additive instead of synthetic ones. Propolis is a resinous mixture collected by honeybees for hive protection. In this study functional yoghurt fortified with microencapsulated propolis was prepared. Ethanol extract of propolis was encapsulated by using sodium alginate, pectin and gelatin separately. The effect of microencapsulated propolis on physicochemical characteristics and microbial quality of yoghurts was tested. Especially the effect of propolis on unwanted microorganisms during storage was studied. Our results clearly showed that fortified yoghurt samples were better in terms of microbial quality during storage. It could be concluded that propolis could be a natural preservative for extending the shelf life of yoghurt.

Key words: Encapsulation, Yoghurt, Propolis, Functional Food

1. Introduction

Yoghurt, obtained by the lactic acid fermentation process, is a fermented milk product with highly valuable nutritional value. Yoghurt contains the essential nutritional components required for human nutrition. Chemical composition of yoghurt strictly depends on the properties of milk and the process applied (1; 2; 3). Chemically yoghurt is similar with milk but in terms of nutritional value and protein content yoghurt is better. Yoghurt attracts a great deal of attention of nutrition specialist because of its high content of calcium, phosphorus, riboflavin, thiamine, cobalamin, folate, niacin, magnesium and zinc (4). It is stated that a food should be advantageous in terms of health (curative or preventive for a certain disease) besides it's nutritionally richness in order to be considered as a functional food. Yoghurt can be classified as a functional food when these characteristics are taken in the consideration (5).

Propolis is a resinous substance collected from different parts of the plants by honey bees. It is a natural mixture with its specific strong odor, not readily soluble in water, viscous and sticky. Propolis possesses antioxidant, antimicrobial, anti-inflammatory and antitumor activity because it contains different class of compounds like volatiles and phenolic compounds (6; 7; 8). Although propolis is a natural preservative, the usage of propolis in food industry is quite limited. This is due to the resinous nature (not readily soluble in water), its ethanol solubility, its specific strong smell and taste. It is suggested that encapsulation of propolis active compounds may overcome whole or some of the mentioned problems and enhance the availability of propolis for food industry (9). It is well stated in literature that some unfavorable effects developing during production or storage such as decrease in functionality in a product, development of bad smell, deterioration of structure and decrease in activity could be reduced or prevented by encapsulation. Moreover,

encapsulation may also contribute the moisture content control, improved antioxidant activity, preservation and bioavailability of active ingredients (10; 11). It is obvious that obtained powdered forms of propolis hold different physical and chemical characteristic after encapsulation with different capsulating material.

There are many studies on propolis usage in foods as preservative but there are certain limitations for this purpose. The limitations arise from the properties of propolis such as its ethanol solubility, strong taste and aroma. In experimental scale it is possible to add ethanol extract of propolis in foods during production process but it is not possible to add it in industrial scale. The strong smell and taste of propolis also limits its usage in foods as preservative. Encapsulation of propolis ethanol extract could overcome these limitations mentioned above. Main aim of this study was the production of yoghurt by using powdered form of propolis with distinct physical and pharmacologic future obtained after encapsulation with different encapsulating material. Especially, the effect of antioxidant and antimicrobial feature of propolis on the characteristic of yoghurt was tested.

2. Material and Methods

2.1. Preparation of Propolis Extract and Microcapsules

Raw propolis sample was collected from Koyunköy district Bilecik city, Turkey during harvest season of 2018 by propolis traps. Frozen propolis sample was grounded and 25 g of powdered propolis sample was mixed with 250 ml 70% of ethanol and shaken for 24 h under controlled speed. Then the mixture was filtered with Buchner doing vacuum with a Whatman no 1 filter paper. Obtained clear filtrate was used as propolis extract. Chemical composition of propolis extract was determined by using GC-MS technique with the method described in earlier published paper (12).

Preparation of microcapsules was carried out by using ionic gelation and solvent evaporation techniques separately. Sodium alginate, pectin and gelatin were used as encapsulating agent separately. Alginate-propolis micro beads were prepared according to earlier reported method (13). Gelatin microbeads were prepared according to previous study (14). Preparation of pectin-propolis microbeads was carried out by using ionic gelation and solvent evaporation method. 2% of pectin solution (50 mL) was prepared in a beaker. 25 ml of propolis extract was diluted to 50 mL with ethanol and $CaCl_2$ (0.05 M) was dissolved into this mixture. Pectin solution was dropped through a syringe into this solution for obtaining pectin-propolis microbeads. Obtained beads were separated by filtering and dried at 60 °C under vacuum. Filtrate was examined in terms of total phenolics in order to determine encapsulation efficiency. Encapsulation efficiency was calculated by using the equation given below

EE% = (A-B)/A*100 where;

A: Total phenolic content of propolis extract, mg GAE

B: Total phenolic content of filtrate, mg GAE

2.2. Preparation of Yoghurt with Encapsulated Propolis

Functional yoghurts were produced by the addition of different concentration (0.05 and 0.1% (w/v) of encapsulated propolis samples and propolis alcohol extract (0.05; 0.1% (v/v) into Pasteurized milk. A control sample of yoghurt was also produced. Dry matter of raw milk was standardized by evaporation and pasteurized at 90 °C for 5 min in dairy plants. Pasteurized milk was cooled down at 45°C, taken into sterile packages and mixed with propolis and then inoculated with 3% (v/v) yoghurt starter culture (YO-MIX 572 and YO-MIX 601, DANISCO) containing *Streptococcus salivarius subsp. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*. Inoculated milk was incubated at 45 °C until reaching pH 4.7 and then stored in a refrigerator at 4 °C.

2.3. Determination of Physicochemical Properties

Yoghurt samples were stored for 30 days at 4 °C and sampled out at 1st, 7th, 15th and 30th days' separately. Viscosity, water activity, pH and color of the samples were determined during storage. Fat, protein, total solids, and ash contents of yoghurt samples were measured by AOAC Official Method 2000.18; ISO-8968-1; ISO 6731; AOAC Official Method 945.46

respectively at first day (15; 16; 17; 18). All tests were performed in triplicate. Viscosity value of yoghurt samples was determined as centipoise (cP) unit in a viscosity measuring device (AND, SV-10 Sine-Wave Vibro) operating with a tuning-fork vibration method at a frequency of 30 Hz, at 25 ± 0.5 °C (A&D Company Limited, 2005) . The water activity was determined by water activity device (Novasina LabSwift-aw). The pH value of each yoghurt sample was measured with a pre-calibrated pH meter (InoLab pH 720 model, WTW, GmbH, Germany). L (lightness-whiteness), a (redness-greenness or redness) and b (yellownessyellowness or blueness) values for color analysis of samples were measured using color determination device (CR- 400, Minolta Company, Osaka, Japan) standardized with white ceramic calibration plate (Konica Minolta 13433102; L = 97.65, a = -10, b = -0.14) for calibration. Measurements were carried out at 20 ± 2 °C.

2.4 Determination of Microbiological Analysis during Storage

2.4.1. Determination of Yoghurt Culture

The viable cell counts of *Lactobacillus* species and *Streptococcus* and *Lactococcus* members of each yoghurt sample at 1st, 7th, 15th and 30th days' were measured separately. Samples were analyzed within 2 hours after being brought to the laboratory under cold chain under aseptic conditions. DeMan Rogosa Sharp Agar (MRS Agar) and MRS Broth (Merck, Germany) was used for counting *Lactobacillus* species. M17 Agar and M17 Broth (Merck, Germany) media were used for counting of *Streptococcus* and *Lactococcus* members. Cultivation of bacteria was carried out by using the spreading plate method. MRS agars were incubated in anaerobic conditions (19) using Anaerocult C (Merck, 116275) at 37 °C for 48 hours. M17 agars were incubated at 30 °C for 48 hours in aerobic conditions [20].

2.4.2. Determination of Unwanted Microorganisms of Yoghurt

In order to determine unwanted microorganisms of yoghurt samples on the 1st, 15th and 30th days, *Coliform*, *Escherichia coli*, molds and yeasts, *Bacillus cereus*, coagulase positive *Staphylococcus aureus* and *Salmonella* entities were investigated following the ISO 4832:2006; ISO 6611:2004; ISO 21871:2006; ISO 6888:1999; ISO 6579: 2003 methods instructions, respectively (21; 22; 23; 24; 25). The results were calculated directly as colony forming unit per g sample (log CFU/g).

2.5. Statistical Analysis

The yoghurt samples were all normally distributed and data's were expressed as means and standard errors of the mean. The difference between yoghurt sample groups on chemical composition of yoghurts at first day was tested using One Way ANOVA with 0.05 level of significance. The difference between the chemical compositions of yoghurt samples at first day was tested using one-way ANOVA with 0.05 level of significance. The effects of propolis groups and time effects on viscosity, Water activity, pH and The L, a and b color indexes were investigated using GLM analysis. Moreover, the number of colonies obtained at the end of the incubation procedures performed on the 1st, 7th, 15th and 30th days of storage for MRS Agar and M17 Agar were examined using GLM analysis. Duncan multiple comparison tests were applied in order to compare differences between group means for ANOVA and GLM analysis but Dunn's test for Kruskal-Wallis test. All statistical tests were performed at 5% level of statistical significance by IBM SPSS statistics 20.0

3. Results and Discussion

3.1. Physicochemical Properties of Yoghurt Samples during Storage

At first day the chemical composition of yoghurt samples (fat, protein, total solids, and ash) was analyzed. The results were summarized in Table 1. The addition of encapsulated propolis with different encapsulant affected significantly ($p<0.05$) dry matter, protein and ash of yoghurt samples. Differences are due to propolis and encapsulant used for propolis encapsulation. The presence of propolis had insignificant influence on fat

Table 1. Mean and standard errors of the chemical composition of yogurts at First day

Group	Dry matter	Fat	Protein	Ash
1	16.80±0.006[d]	3.50±0.058	4.86 ±0.026 [d]	1.21±0.006 [a]
2	16.72±0.003 [e]	3.47±0.033	5.17±0.010 [b]	1.12±0.006 [bc]
3	16.67±0.006 [f]	3.47±0.033	4.85±0.006 [d]	1.15±0.028 [b]
4	16.85±0.006 [c]	3.47±0.033	4.82±0.021 [d]	1.21±0.010 [a]
5	16.86±0.006 [bc]	3.47±0.033	4.50±0.025 [e]	1.20±0.006 [a]
6	16.87±0.006 [ab]	3.50±0.057	5.26±0.010 [a]	1.15±0.031 [a]
7	16.88±0.003[a]	3.47±0.033	4.95±0.010 [c]	1.14±0.006 [b]
8	16.85±0.003 [c]	3.53±0.033	5.18±0.015 [b]	1.09±0.006 [c]
9	16.86±0.003 [bc]	3.50±0.057	4.96±0.015 [c]	1.08±0.006 [c]

1: Control group, 2: 0.05% EEP, 3: 0.10% EEP, 4: 0.05% AMP, 5: 0.10% AMP, 6: 0.05% GMP, 7: 0.10% GMP,
8: 0.05% PMP, 9: 0.10% PMP (EEP: Ethanolic propolis extract, AMP: Alginate microencapsulated propolis, GMP: Gelatin microencapsulated propolis, PMP: Pectin microencapsulated propolis)
Different letters in the same column show significant difference between groups (p<0.05)
*: Values in a column which do not share a common superscript are statistically different. P<0.05

content in the yoghurt. The highest protein content was found in yoghurt fortified with gelatin encapsulated propolis.

Viscosity analysis, as an important parameter in terms of quality criteria of yoghurt samples (26) was carried out in all samples during the storage period. The viscosity changes of the yoghurt samples over 30 days of storage were reported in Table 2. The mean viscosity value of the control group yoghurt sample was determined as 2981,083 cP however, the viscosity values vary between 2793 and 3227 cP during the storage period. When the mean of viscosity values were considered during the storage period, it was determined that the viscosity values increased in direct proportion to the amounts added in the yoghurt samples produced by adding alginate, gelatin and pectin. As a matter of fact, it is known that stabilizers such as pectin, gelatin and alginate are added to milk in the production process in order to improve the properties including mouthfeel, viscosity/consistency, appearance, texture in yoghurts and to prevent whey separation (26).

Bchir et. al., (2019) and Hanou et. al., (2016) reported that the increase in viscosity values in Spirulina which is a cyanobacterium species and ginger powder added yoghurt was due to the interactions with protein molecules and an increase in the amount of dry matter, respectively (27; 28). It is well known fact that viscosity values increase in proportion with the increasing amount of dry matter in yoghurt (26). Similarly, it is suggested that the viscosity of yoghurt or perceived thickness increases with an increase proportional to the total solids content (29; 30). From this point of view, it is thought that the increase in viscosity values observed in proportion with the contribution percentages of the alginate, gelatin and pectin additive groups may be associated with the increase of protein interactions and/or dry matter contents of these substances. However, there was a decrease in viscosity values of group 2 and group 3 samples produced with the addition of alcohol. In all sample groups, it is observed that viscosity values tend to increase until the 15th day of storage period, but there is a decrease in viscosity values between the 15th and 30th days. It is known that the viscosity and stability increase due to the increasing acidity and decreasing pH values during the storage period and it is directly affected in the resistance to serum separation.

The water activity changes of the yoghurt samples belonging to the sample groups during 30 days of storage were reported in Table 3. The mean water activity value of the control group yoghurt sample was determined as 0.968 during the shelf life. Except for alcohol extract supplemented groups, water activity values of all fortified yoghurt sample groups were higher than

Table 2. Viscosity values changes in yoghurt sample groups during 30 days of storage

Sample Group	Day of storage				Mean	Std. Error
	1	7	15	30		
1	2793.333±4.148^{q*}	3056.000±4.148^{v*}	3227.000±4.148^{u*}	2848.000±4.148^{w*}	2981.083	2.074^{g*}
2	2565.000±4.148^{qq}	2762.000±4.148^{x}	3028.000±4.148^{y}	2624.000±4.148^{zz}	2744.750	2.074^{h}
3	2491.000±4.148^{rr}	2694.000±4.148^{yy}	2905.000±4.148^{z}	2540.000±4.148^{xx}	2657.500	2.074^{i}
4	3420.000±4.148^{l}	3390.000±4.148^{m}	3528.000±4.148^{h}	3320.000±4.148^{o}	3375.500	2.074^{e}
5	3264.000±4.148^{s}	3558.000±4.148^{e}	3774.000±4.148^{a}	3542.000±4.148^{fg}	3573.500	2.074^{b}
6	3245.000±4.148^{t}	3355.000±4.148^{n}	3514.000±4.148^{i}	3295.000±4.148^{p}	3352.250	2.074^{f}
7	3394.000±4.148^{m}	3551.000±4.148^{ef}	3680.000±4.148^{b}	3466.000±4.148^{j}	3522.750	2.074^{c}
8	3280.000±4.148^{r}	3414.000±4.148^{l}	3536.000±4.148^{gh}	3349.000±4.148^{n}	3394.750	2.074^{d}
9	3444.000±4.148^{k}	3572.000±4.148^{d}	3780.000±4.148^{a}	3605.000±4.148^{c}	3600.250	2.074^{a}

1: Control group, 2: 0.05% EEP, 3: 0.10% EEP, 4: 0.05% AMP, 5: 0.10% AMP, 6: 0.05% GMP, 7: 0.10% GMP,
8: 0.05% PMP, 9: 0.10% PMP (EEP: Ethanolic propolis extract, AMP: Alginate microencapsulated propolis, GMP: Gelatin microencapsulated propolis, PMP: Pectin microencapsulated propolis)
*: Values in a column which do not share a common superscript are statistically different. P<0.05

Table 3. Water activity values changes in yoghurt sample groups during 30 days of storage

Sample group	Days of analysis				Mean	Std. Error
	1	7	15	30		
1	0.977± 0.00^{1i*}	0.968±0.001^{kl*}	0.965±0.001^{lm*}	0.962±0.001^{mn*}	0.968	0.001^{e*}
2	0.979±0.001^{hi}	0.964±0.001^{lm}	0.962±0.001^{mn}	0.958±0.001^{n}	0.966	0.001^{f}
3	0.976±0.001^{ij}	0.964±0.001^{lm}	0.959±0.001^{n}	0.959±0.001^{n}	0.965	0.001^{f}
4	0.990±0.001^{ab}	0.987±0.001^{bcde}	0.986±0.001^{bcdef}	0.980±0.001^{ghi}	0.986	0.001^{b}
5	0.986±0.001^{bcdef}	0.983±0.001^{efgh}	0.980±0.001^{ghi}	0.972±0.001^{jk}	0.980	0.001^{d}
6	0.988±0.001^{bcd}	0.985±0.001^{cdef}	0.980±0.001^{ghi}	0.980±0.001^{ghi}	0.983	0.001^{c}
7	0.988±0.001^{bcd}	0.986±0.001^{bcdef}	0.982±0.001^{fgh}	0.976±0.001^{ij}	0.983	0.001^{c}
8	0.994±0.001^{a}	0.990±0.001^{ab}	0.984±0.001^{defg}	0.984±0.001^{defg}	0.988	0.001^{a}
9	0.989±0.001^{bc}	0.988±0.001^{bcd}	0.984±0.001^{defg}	0.983±0.001^{efgh}	0.986	0.001^{ab}

1: Control group, 2: 0.05 % EEP, 3: 0.10 % EEP, 4: 0.05 % AMP, 5: 0.10 % AMP, 6: 0.05 % GMP, 7: 0.10 % GMP, 8: 0.05 % PMP, 9: 0.10 % PMP (EEP: Ethanolic propolis extract, AMP: Alginate microencapsulated propolis, GMP: Gelatin microencapsulated propolis, PMP: Pectin microencapsulated propolis)
*: Values in a column which do not share a common superscript are statistically different. P<0.05

the control group. In alcohol extract added yoghurt samples, mean water activity values were determined as 0,966 and 0,965 for 0.05% and 0.10% addition groups, respectively. It was found that water activity values showed a decreasing tendency in all sample groups during storage. Tayar et. al., (1995) examined the general properties of agar, gelatin and sodium caseinate, as stabilizers, added yoghurts (31). Similar to the present findings, the researchers found that in control and all experimental groups, water activity values decreased during the storage of fourteen days.

pH value of yoghurt samples on the 1st, 7th, 15th and 30 days of storage was given in Table 4. Average pH values of the control group yoghurt samples were determined as 4.203 during the shelf life. Except for gelatin supplemented groups, pH values of all fortified yoghurt sample groups were higher than the control group at first day and at the end of storage.

Table 4. pH value changes in yoghurt sample groups during 30 days of storage

Sample group	Days of analysis				Mean	Std. Error
	1	7	15	30		
1	$4.470\pm0.022^{ef*}$	$4.210\pm0.022^{no*}$	$4.150\pm0.022^{opr*}$	$3.980\pm0.022^{tu*}$	4.203	0.011^{e*}
2	4.640 ± 0.022^{a}	4.450 ± 0.022^{fg}	4.320 ± 0.022^{ijkl}	4.240 ± 0.022^{mn}	4.300	0.011^{c}
3	4.620 ± 0.022^{ab}	4.460 ± 0.022^{fg}	4.340 ± 0.022^{hijk}	4.210 ± 0.022^{no}	4.335	0.011^{b}
4	4.540 ± 0.022^{cd}	4.400 ± 0.022^{ghi}	4.260 ± 0.022^{lmn}	4.000 ± 0.022^{tu}	4.413	0.011^{a}
5	4.560 ± 0.022^{bc}	4.420 ± 0.022^{fgh}	4.260 ± 0.022^{lmn}	4.100 ± 0.022^{rs}	4.408	0.011^{a}
6	4.380 ± 0.022^{ghi}	4.250 ± 0.022^{mn}	4.140 ± 0.022^{pr}	3.960 ± 0.022^{v}	4.183	0.011^{e}
7	4.360 ± 0.022^{ghij}	4.280 ± 0.022^{klmn}	4.170 ± 0.022^{op}	3.950 ± 0.022^{v}	4.190	0.011^{e}
8	4.480 ± 0.022^{def}	4.310 ± 0.022^{jklm}	4.210 ± 0.022^{no}	3.980 ± 0.022^{tu}	4.245	0.011^{d}
9	4.530 ± 0.022^{cde}	4.340 ± 0.022^{hijk}	4.240 ± 0.022^{mn}	4.040 ± 0.022^{st}	4.288	0.011^{c}

1: Control group, 2: 0.05 % EEP, 3: 0.10 % EEP, 4: 0.05 % AMP, 5: 0.10 % AMP, 6: 0.05 % GMP, 7: 0.10 % GMP, 8: 0.05 % PMP, 9: 0.10 % PMP (EEP: Ethanolic propolis extract, AMP: Alginate microencapsulated propolis, GMP: Gelatin microencapsulated propolis, PMP: Pectin microencapsulated propolis)

*: Values in a column which do not share a common superscript are statistically different. $P<0.05$

In gelatin added yoghurt samples, the average pH value was determined as 4.183 and 4.190 for the 0.05% and 0.10% addition group, respectively. Similar to the water activity values, pH value tends to decrease during storage during all sample groups. It is thought that lactic acid bacteria played a major role in the decrease of pH values, which were high at the beginning of storage, especially until the 7th day of storage, and this was due to the significant increase of lactic acid bacteria in yoghurt in this period. Lee and Lucey (2010) have suggested that yoghurts, especially produced with the addition of protein-containing additives, increase the dry matter content in the raw material, as well as an increase in the buffering capacity that requires additional acid development by starter cultures, resulting in a significant pH decrease at the beginning of storage (26). This decrease may be most likely due to the higher level of production of lactic acid during storage (32). Similarly, De Brabandere & De Baerdemaeker (1999) stated that a sigmoidal pH decrease has occurred in the set and stirred type yoghurts since the beginning of fermentation (33). Seo et. al., (2009) investigated the physicochemical, microbiological, rheological and sensory properties of chitosan-added yoghurt. The researchers emphasized that the pH of the samples decreased proportionally during the storage (34). Tarakçı (2010) reported that yoghurts produced by adding different amounts of kiwi marmalade increased the titratable acidity of the control and all experimental groups during the 21-day storage period. However, similar to the present study findings, it was stated that pH values tend to decrease continuously throughout the storage. This decrease might be due to the acid production in the experimental yoghurts during storage as a result of the fermentation of lactose by the action of the starter cultures (35).

The changes in the L, a and b indexes of yoghurt color observed during the 30 days of storage were reported in Table 5.

The L rating refers to a range from black (0) to white (100) and indicates the lightness characteristics of the samples. a value is used to express the redness and greenness properties of the samples. The L and a indexes were not statistically different according to storage day ($p>0.05$). The a value of -80 to 0 indicates green, and 0 to 100 indicates red. The value of b is also a value that is used in determining the degree of yellowness and blueness, and the range of -100 and 0 indicates the degree of blue and the range of 0 to 70 indicate the degree of yellowness (36).

In the control group samples, the L values were observed to be 86.85, 91.17, 93.19 and 89.95 on the 1st, 7th, 15th and 30th days respectively. When obtained data's for all sample groups were evaluated, It

Table 5. L, a and b color properties changes in yoghurt sample groups during 30 days of storage

		Days of analysis					
	Sample group	1	7	15	30	Mean	Std. Error
L Values	1	86.85	91.17	93.19	89.95	90.293	0.246^{c*}
	2	87.80	92.14	94.53	91.48	91.490	0.246^{ab}
	3	88.62	92.85	94.6	92.44	92.133	0.246^{a}
	4	85.14	89.47	90.68	86.48	87.943	0.246^{e}
	5	84.38	88.52	90.31	86.29	87.379	0.246^{e}
	6	88.02	92.49	94.04	90.77	91.333	0.246^{b}
	7	87.22	91.91	93.32	90.05	90.627	0.246^{c}
	8	85.52	90.16	91.89	87.74	88.830	0.246^{d}
	9	84.82	88.78	90.34	87.24	87.798	0.246^{e}
a Values	1	-2.26	-2.82	-2.65	-2.8	-2.637	0.074^{a*}
	2	-3.53	-3.87	-3.57	-3.6	-3.645	0.074^{de}
	3	-3.59	-3.97	-3.79	-3.63	-3.749	0.074^{e}
	4	-3.08	-3.57	-3.4	-3.5	-3.389	0.074^{c}
	5	-3.13	-3.77	-3.39	-3.59	-3.473	0.074^{cd}
	6	-2.72	-2.94	-2.83	-2.9	-2.851	0.074^{a}
	7	-2.44	-2.82	-2.71	-2.84	-2.703	0.074^{a}
	8	-2.91	-3.34	-3.11	-3.28	-3.163	0.074^{b}
	9	-3.02	-3.61	-3.38	-3.52	-3.385	0.074^{c}
b Values	1	11.26	11.68	10.94	13.1	11.746	0.105^{c*}
	2	13.09	13.77	12.94	14.15	13.490	0.105^{a}
	3	13.39	13.78	13.1	14.46	13.683	0.105^{a}
	4	10.08	11.12	9.76	12.62	10.898	0.105^{d}
	5	9.79	10.44	9.94	12.03	10.554	0.105^{e}
	6	11.61	12.24	11.31	13.52	12.172	0.105^{b}
	7	11.46	11.74	11.02	13.18	11.853	0.105^{c}
	8	10.26	11.24	10.04	12.88	11.107	0.105^{d}
	9	10.04	10.94	9.71	12.38	10.768	0.105^{de}

1: Control group, 2: 0.05 % EEP, 3: 0.10 % EEP, 4: 0.05 % AMP, 5: 0.10 % AMP, 6: 0.05 % GMP, 7: 0.10 % GMP, 8: 0.05 % PMP, 9: 0.10 % PMP (EEP: Ethanolic propolis extract, AMP: Alginate microencapsulated propolis, GMP: Gelatin microencapsulated propolis, PMP: Pectin microencapsulated propolis)

*: Values in a column which do not share a common superscript are statistically different. P<0.05

was determined that L values increased as whiteness until the 15th day of storage, but tend to decrease between the 15th days and 30th days. When both the group averages of the analyzed days and the 1st, 7th, 15th and 30th days were considered separately, L values of the 2nd and 3rd groups (ethanol propolis extract added) and the 6th and 7th group (gelatin microencapsulated propolis added) were observed to increase in comparison with the control group. However, it was determined that L values decreased in the 4th and 5th groups (alginate microencapsulated propolis added) and in the 8th and 9th (pectin microencapsulated propolis added) groups. Kumar and Mishra (2004) likewise stated that gelatin addition caused an increase whereas pectin addition caused a decrease in L parameters of yoghurts in the research findings, where they

detected the physicochemical effects of the stabilizer addition in mango soy-added yoghurts (37). In the control group samples, the a values were determined as -2,26, -2,82, -2,65 and -2,8 on the 1st, 7th, 15th and 30th days respectively. When the a values of the sample groups for storage period were examined, it was determined that there was an increase in greenness in all groups between the 1st and 7th days, a decrease between the 7th and 15th days, and an increase in the 15th and 30th days excluding the 3rd group samples. In the control group samples, the b values were determined as 11.26, 11.68, 10.94 and 13.10 on the 1st, 7th, 15th and 30th days respectively. The average b values of all samples for the first day were determined as 11.26; 13.09; 13.39; 10.08; 9.79; 11.61; 11.46; 10.26 and 10.04 respectively. In terms of b values, the highest averages were found in groups 2 and 3 with alcohol extract addition, the lowest averages in groups 4 and 5 with alginate addition. Oroian et. al., (2011) determined the average values of L, a and b of yoghurt samples sold in Spain as 89.9, -2.14 and 8.12, respectively. The researchers determined these values in fruit added yoghurts as 78.64, 9.29 and 4.55 respectively (38). Tarakçı (2010) examined the effects of the addition of kiwi marmalade on the color parameters on the 1st, 7th, 14th and 21st days of storage in yoghurts. Similar to the present study findings, the averages of the control group L, a and b values were reported as 89.87, -2.69 and 10.81 respectively. However, it was suggested that the increase in the rate of marmalade in yoghurt decreased the L and b values while causing an increase in the a value (35). Çayır (2007) stated that the L, a and b values of apricot-added probiotic yoghurts were determined as 85.31±1.32, 2.73±0.22 and 11.65±0.62 on the first day of storage. The researcher also stated that the increase in the amount of apricot puree caused a decrease in the L value of yoghurts and an increase in a and b values [39]. Damian (2013) examined the effect of addition of different dietary fibers on the rheological properties on yoghurts and the L, a and b values of control group were reported as 97.02±0.45, -2.24±0.98 and 12.61±0.67 respectively. The researcher also reported an increase in a and b values and a decrease in L value for 1% apple fiber added yoghurt where as a decrease in L and a values and an increase in b value for 1% inulin added yoghurt (40).

3.2. Determination of Microbiological Analysis during Storage

3.2.1. The changes of yoghurt starter culture during storage

Dominant organisms in starter cultures, such as *Lactococcus lactis*, Lactobacillus sp., *Streptococcus thermophilus*, Bifidobacterium sp. and Leuconostoc sp., perform 3 basic biochemical transformations (glycolysis, proteolysis and lipolysis) during fermentation in terms of their effects on milk components (1). The optimum growth temperature of Streptococcus subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus, the thermophilic lactic acid bacteria in yoghurt fermentation, is between 40-45 °C. During the incubation between these temperatures, lactose is transformed into lactic acid, which is the primary factor in decreasing the pH with the effect of bacterial fermentation, and the pH decreases from 6.7 to ≤ 4.6.

Acidification around the isoelectric point of the casein at pH 4.6 levels with a decrease in electrostatic repulsion between casein molecules results in the formation of a three-dimensional network of casein clusters and chains. This means that the number and activities of yoghurt bacteria have very important functions in yoghurt formation, especially casein interactions [26]. DeMan Rogosa Sharp Agar (MRS Agar) was used for counting the viable *Lactobacillus* species, one of the lactic acid bacteria. However, M17 Agar was planted in the counting of viable *Streptococcus* and *Lactococcus* members. The findings of the classical cultural cultivation methods and the number of colonies obtained at the end of the incubation procedures performed on the 1st, 7th, 15th and 30th days of storage on both media were given in Table 6 and 7.

The number of viable colonies on MRS Agar were found statistically different on the 1st, 7th, 15th and 30th days of storage (p<0.05). When the number of colony forming units on the 1st, 7th, 15th and 30th days of storage in DeMan Rogosa Sharp Agar was evaluated, the number of *Lactobacillus* species was determined as 7.61; 8.35; 7.47 and 6.14 log CFU/g for the control group respectively. *Lactobacillus* numbers were found to be higher in the 8th and 9th group samples produced by adding pectin on all days of storage

Table 6. Classical cultural analysis results of the samples by MRS Agar by days (log CFU/g)

Sample group	Days of analysis			
	1	7	15	30
1	7.610±0.051^{ijk}	8.353±0.051^{d}	7.470±0.051^{kl}	6.143±0.051^{p}
2	7.570±0.051^{jk}	8.233±0.051^{de}	7.350±0.051^{l}	6.050±0.051^{p}
3	7.573±0.051^{jk}	7.907±0.051^{fg}	7.333±0.051^{l}	5.900±0.051^{r}
4	7.707±0.051^{hij}	8.533±0.051^{c}	7.573±0.051^{jk}	6.427±0.051^{o}
5	7.710±0.051^{hij}	8.627±0.051^{bc}	7.603±0.051^{ijk}	6.400±0.051^{o}
6	7.743±0.051^{ghi}	8.713±0.051^{b}	7.907±0.051^{fg}	6.800±0.051^{n}
7	7.763±0.051^{ghi}	8.870±0.051^{b}	8.020±0.051^{f}	6.727±0.051^{n}
8	7.843±0.051^{gh}	8.970±0.051^{b}	8.183±0.051^{e}	7.123±0.051^{m}
9	7.883±0.051^{fg}	9.127±0.051^{a}	8.240±0.051^{de}	7.127±0.051^{m}

1: Control group, 2: 0.05 % EEP, 3: 0.10 % EEP, 4: 0.05 % AMP, 5: 0.10 % AMP, 6: 0.05 % GMP, 7: 0.10 % GMP, 8: 0.05 % PMP, 9: 0.10 % PMP (EEP: Ethanolic propolis extract, AMP: Alginate microencapsulated propolis, GMP: Gelatin microencapsulated propolis, PMP: Pectin microencapsulated propolis)
*: Values in a column which do not share a common superscript are statistically different. P<0.05

Table 7. Classical cultural analysis results of the samples by M17 Agar by days (log CFU/g)

Sample group	Days of analysis			
	1	7	15	30
1	8.300±0.085	8.423±0.085	8.123±0.085	7.743±0.085
2	8.240±0.085	8.277±0.085	8.017±0.085	7.460±0.085
3	8.220±0.085	8.173±0.085	7.940±0.085	7.430±0.085
4	8.343±0.085	8.640±0.085	8.400±0.085	8.027±0.085
5	8.360±0.085	8.720±0.085	8.467±0.085	8.043±0.085
6	8.400±0.085	8.740±0.085	8.513±0.085	8.190±0.085
7	8.433±0.085	8.800±0.085	8.560±0.085	8.240±0.085
8	8.393±0.085	8.683±0.085	8.420±0.085	7.943±0.085
9	8.413±0.085	8.713±0.085	8.430±0.085	8.023±0.085

1: Control group, 2: 0.05 % EEP, 3: 0.10 % EEP, 4: 0.05 % AMP, 5: 0.10 % AMP, 6: 0.05 % GMP, 7: 0.10 % GMP, 8: 0.05 % PMP, 9: 0.10 % PMP (EEP: Ethanolic propolis extract, AMP: Alginate microencapsulated propolis, GMP: Gelatin microencapsulated propolis, PMP: Pectin microencapsulated propolis)
*: Values in a column which do not share a common superscript are statistically different. P<0.05

compared to the other groups. When the increasing and decreasing tendency of *Lactobacillus* numbers by days was evaluated, it was observed that bacterial growth was rapid and numbers increase between 1 and 7 days of storage in all sample groups. However, it was determined that bacterial growth was restricted and a decrease in number occurred in the process until the 30th day after the storage. When the number of colony forming units on M17 Agar for the 1st, 7th, 15th and 30th days was evaluated the number of *Streptococcus* and *Lactococcus* members were determined as 8.30; 8.42; 8,12 and 7,74 log CFU/g for the control group respectively. When the 1st and 30th days of storage was evaluated in terms of *Streptococcus* and *Lactococcus* numbers; sample groups produced with the addition of gelatin (groups 6 and 7) were observed as the highest, while alcohol extract supplemented yoghurt groups (groups 2 and 3) were observed as the lowest bacterial

groups. An increase in *Streptococcus* and *Lactococcus* numbers up to 7 days of storage was observed in all groups except for the 3rd sample group with 0.10% alcohol extract. However, between 7th day and 30th day of storage, it was determined that bacterial growth was limited and decreased in number.

The positive effect of regular yoghurt consumption on health is associated with the presence of high concentrations of live lactic acid bacteria in yoghurt. This has led to the approach of determining the minimum levels of lactic acid bacteria in yoghurts throughout the shelf life in some countries. The viability of lactic acid bacteria in yoghurt is directly affected by the chemical composition and especially acidity of yoghurt (41). It is known that Streptococcus thermophilus initiate lactic acid fermentation in yoghurt formation and their growth in yoghurt is stimulated by the milk casein-derived proteolytic activities of Lactobacillus bulgaricus (42). This situation could be the main reason for the detection of higher number of bacteria in M17 Agar plates in the first stage of fermentation as observed in the present study findings. However, the initial increase observed in the changes of the number of bacteria obtained for all groups and the decrease for the storage period from the results of classical cultural analysis performed on both groups of media could be explained with the development of acidity and the decrease in a_w values.

3.2.2. Determination of Unwanted Microorganisms during Storage

The presence of unwanted microorganism shows the hygienic quality of yoghurt. The criteria specified in the Turkish Food Codex Fermented Dairy Communiqué have been taken into consideration. In this study, the presence and number of *Coliform* bacteria, *Escherichia coli*, mold and yeast, *Bacillus cereus*, Coagulase positive *Staphylococcus aureus* and *Salmonella* microorganisms were determined. Results presented in Table 8 indicated that addition of encapsulated propolis into yoghurt affected *Coliform* bacteria, *Escherichia*

Table 8. Counts of other microorganisms of the analyzed during storage (log CFU/g)

Samples Group	Storage time (days)	*Coliform*	Mold and yeast	*B. cereus*	Coagulase + *S. aureus*
1	1	ND	1.30	1.77	1.30
2		1.32	1.84	ND	1.60
3		1.07	2.00	1.30	1.60
4		1.14	2.14	1.30	ND
5		1.23	2.20	1.77	1.30
6		1.64	2.04	1.77	1.60
7		2.11	2.07	1.30	1.60
8		2.22	2.04	2.34	1.30
9		2.25	2.04	2.44	ND
1	15	ND	2.04	ND	ND
2		ND	2.07	ND	ND
3		ND	2.32	ND	ND
4		ND	1.95	1.30	ND
5		ND	1.77	ND	ND
6		ND	2.53	ND	1.00
7		ND	2.41	1.30	ND
8		ND	2.51	ND	ND
9		ND	1.95	ND	ND

Table 8 (*Continued*)

Samples Group	Storage time (days)	*Coliform*	Mold and yeast	*B. cereus*	Coagulase + *S. aureus*
1	30	1	2.44	1.30	ND
2		1	3.69	ND	ND
3		ND	3.20	ND	ND
4		ND	3.15	ND	ND
5		ND	2.47	ND	ND
6		ND	3.69	ND	ND
7		ND	2.80	ND	ND
8		ND	3.69	ND	ND
9		ND	3.59	ND	ND

1: Control group, 2: 0.05 % EEP, 3: 0.10 % EEP, 4: 0.05 % AMP, 5: 0.10 % AMP, 6: 0.05 % GMP, 7: 0.10 % GMP, 8: 0.05 % PMP, 9: 0.10 % PMP (EEP: Ethanolic propolis extract, AMP: Alginate microencapsulated propolis, GMP: Gelatin microencapsulated propolis, PMP: Pectin microencapsulated propolis

coli, *Bacillus cereus*, Coagulase positive *Staphylococcus aureus* and *Salmonella* but there was no effect on yeasts and molds during storage.

There are lots of studies about antimicrobial activity of propolis [43; 44; 45; 46]. Propolis has been reported to exhibit high bacteriostatic effect and stability compared with sodium benzoate, sorbic acid and potassium sorbate. The effect of propolis concentration as 7.8; 31.25 and 31.25 mg/mL were reported on the inhibition of *Staphylococcus aureus, Escherichia coli* and *Aspergillus niger* respectively (47). Yang et. al., (2009) stated that the concentration of propolis was effective on the shelf life of yoghurts, adding high concentration of propolis had better antibacterial effect. Propolis solution added at the rate of 0.05% was reported to preserve the quality of the yoghurt with increased shelf life (48). The possibility of *Coliform* group bacteria to be present in yoghurt is lower than other dairy products due to its heat treatment and high acidity. The presence of *coliform* microorganisms in yoghurt samples indicates that yoghurt production was carried out without taking necessary hygiene measures (49; 50). No *coliform* bacteria were found in all samples in analysis on day 15 and the *coliform* bacteria were detected (log cfu/mL) only in control group and group 2 at the end of storage. *Escherichia coli* were not detected in the yoghurt samples examined in our study. In our study, *Staphylococcus aureus* was detected in most of the yoghurt samples examined on the first day. However, on the 15th day, *Staphylococcus aureus* was only detected 75% reduced rate in gelatin 0.05% encapsulated yoghurt. *Staphylococcus aureus* was not detected in any samples on the 30th day. These results are in agreement with those of Santos et. al., (2019) who observed coagulase-positive *Staphylococcus aureus* was less than 1.0 log CFU/ mL and Gao et. al., (2011) who found the inhibitory effect of propolis added to yoghurt for *Staphylococcus aureus* and *Escherichia coli* bacteria (43; 51). The presence of *Staphylococcus aureus* in yoghurt samples indicates that adequate hygienic precautions were not taken during production and there may be a personnel-related contamination (52). *Bacillus cereus* was detected in all our yoghurt groups at first day and it was detected only in alginate encapsulated (0.05%) propolis and gelatin encapsulated (0.1%) propolis yoghurt on 15 d and only in the control group on 30 d. *Salmonella* spp. was not found in any of the yoghurt samples examined in this study. Similar results were obtained by Santos et. al., (2019) who did not find *Salmonella, Coliform* and *Escherichia coli* bacteria in all samples during the 28 days storage period in the red propolis added yoghurt study (43). Our findings could be explained by the antimicrobial activity of propolis. Not detecting the *Coliform*, *Bacillus cereus* and coagulase-positive *Staphylococcus aureus* on 30th day could be related to more released phenolic and flavonoid compounds from the microbeads during the storage period.

Molds and yeasts create a breeding ground for bacteria that cause spoilage by using some of the lactic acid that provides the formation of acidity in

yoghurt (53). On the 1st day, the number of yeasts and molds in the samples of yoghurt was determined as the least number in the control group and yoghurt fortified with alcohol extract. The number of molds and yeasts in yoghurts fortified with encapsulated propolis was initially high according to Turkish Food Codex Fermented Dairy Communiqué (10^1-10^2 cfu/g) (54). On day 15th, yoghurt with pectin encapsulated propolis (group 9) and yoghurt with alginate encapsulated propolis (group 4 and 5) were showed decrease in number of yeasts and molds, while an increase was observed in other samples. However, when we evaluated the results of day 30, the excess number of molds and yeasts in all yoghurts was remarkable. These results are nearest similar to Güney (2016) who remarked the importance the dose of added propolis into fruit yoghurt for molds and yeasts and found increase in number of molds and yeasts in all groups, especially highest in control group on the 7th day (55). These results are not in agreement with those of Yiyang (2005) who observed that adding 0.1% of propolis into milk before sterilization increased the shelf life of milk and prevented significantly mold growth in yoghurt during its shelf life (56). According to the findings of our study, antifungal effect of propolis was not seen sufficiently in yoghurt samples. In addition, there are quite a lot of molds and yeasts in other yoghurt samples compared to the control group on the 30th day. According to previous studies propolis has an antifungal feature (8; 57; 46). *Absidia, Alternaria, Aspergillus, Micelia, Monilia, Mucor, Penicillium, Pullaria* and *Rhizopus* are among the most common molds in yoghurts that are not produced and stored under suitable conditions. Geotrichum candidum (Oidium lactis) is known as yoghurt mold or milk mold (58). According to Gajger et. al., (2017), the effect of propolis on yeast is related to the dose and antimicrobial effect depends on the phenolic and flavonoid content of propolis (59). Of course, it is very important that the additives used should be hygienic. Ertem and Çakmakçı (2018) produced a new functional yoghurt using L. acidophilus with 5% Gobdin and evaluated properties of them. They did not detect *Coliform* bacteria, yeast and mould in all yoghurts during the storage periods because of using the pasteurized gobdin (60).

4. Conclusion

In this study, physicochemical, microbiological, of functional yoghurts prepared by adding different microencapsulated propolis and propolis alcohol extract were determined during storage. The evaluation of propolis-added yoghurt prepared with different encapsulants is the first study to our knowledge. In our study, propolis active ingredients were encapsulated for preventing the pungent odor of propolis. Microencapsulation of propolis active compounds had one more advantage as increasing antioxidant activity during storage, since the antioxidant compounds could be released slowly into yoghurt. Our results also showed it noteworthy that addition of propolis into yoghurt did not affect the growth of yoghurt culture negatively and an increase was noticed in some groups compared to the control group. In addition, it was observed that addition of propolis was highly effective during the shelf life of yoghurt in terms of unwanted microorganisms. However, the antifungal effect of propolis was not enough. This situation demonstrated the necessity to reduce the microbial load of the encapsulated propolis by applying UV or heat treatments before adding it to the yoghurt milk. Further studies are required to obtain optimum encapsulated propolis forms applicable to milk industry. It can be concluded that addition of propolis in microencapsulated forms as nutritional and functional additive to yoghurt will bring an innovative approach to the functional food market.

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