



## Determination of the effects of propolis ethanolic extract on some properties of fruit yoghurt during storage

Propolis etanol ekstraktının depolama süresince meyveli yoğurtların bazı özellikleri üzerine etkilerinin belirlenmesi

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### Ö Z E T / A B S T R A C T

**Aims:** The aim of this study was to determine the chemical and microbiological properties of propolis ethanolic (PEE) extract added fruit yoghurt during a storage period.

**Methods and Results:** PEE (in different ratios: 0.01%, 0.03%, 0.10%, 0.20% and control=0.00%) added fruit yoghurt was stored at +4 °C for 28 days. Dry matter, protein content, pH, titratable acidity, DPPH inhibition and total phenols were analysed on the first and 28th days of storage. Microbiological analyses of yoghurts were also carried in first and seventh days. Titratable acidity values were increased while pH values decreased at the end of the storage period in all samples. DPPH inhibition and total phenols amounts were increased in line with the amount of added PEE. It was observed that added propolis amount did not affect total aerobic mesophilic flora ( $p>0.05$ ). During the storage period, lactic acid bacteria (LAB) increased in all the groups and the control group had the highest bacteria count. The number of yeast and mould increased in all the groups.

**Conclusions:** Our results indicated that PEE does not adversely influence the mechanism of yoghurt formation. We also found that propolis increased the nutritional benefits by increasing the antioxidant capacity of yoghurt.

**Significance and Impact of the Study:** In this study PEE has been added to fruit yoghurts in different proportions. It has been observed that the nutritional properties and antioxidant content of yoghurts have increased. It was considered that propolis can be used as a natural food additive.

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## INTRODUCTION

Propolis is produced by bees from buds and the exudates of various trees and plants such as birch, poplars, oaks, willows, conifers and many others (Bankova, et al., 2000; Freires et al., 2016). It is a natural remedy that has been in use for centuries (Castaldo and Capasso, 2002) and is widely applied in traditional medicine thanks to its pharmacological benefits of anticancer (Mouse et al.,

2012), antioxidant (Kumazawa et al., 2004), antiviral (Almutairi et al., 2014), anti-inflammatory and antimicrobial properties (Banskota et al., 2002; Bittencourt et al., 2015; Popova et al., 2005). Propolis is recently used in confectionery, biopharmaceuticals, cosmetics and is available as capsule, extract, cream, and powder (Castaldo and Capasso, 2002; Oses et al., 2016). Due to high antioxidative activity and biological properties, propolis

is useful in foods. Propolis is as a natural preservative and a source of bioactive compounds for foods and drinks that help improve shelf-life and consumer health (Duman and Ozpolat, 2015; Gutiérrez-Cortés and Suarez Mahecha, 2014).

Yoghurt is the most common dairy product (Gyawali and Ibrahim, 2016), consumed for excellent sensory properties, high nutritive, and therapeutic values (Najgebauer-Lejko et al., 2015). It is made by fermenting fresh or reconstituted milk with lactic acid bacteria (Ye et al., 2013) and is considered to be healthy due to high digestibility and bioavailability of protein, energy and calcium (Shori and Baba, 2013). Presence of spoilage bacteria and fungi (especially yeast) makes yoghurt vulnerable unless some precautions are taken. Preservatives would be useful, but most countries do not allow the use of preservatives in yoghurt. There has been increasing interest in the use of natural food additives and the incorporation of health-promoting substances in the diet (Shori and Baba, 2013). Many studies have shown that excessive consumption of synthetic food additives causes adverse effects (Caleja et al., 2016). As alternatives to synthetic preservatives, natural preservatives have the potential to reduce microbial growth or numbers in yoghurt (Penney et al., 2004). Propolis is a good natural preservative against yeast and spoilage microorganisms because low concentrations of propolis solution have an inhibitory effect on the multiplication of normal bacteria while having almost no influence on *Bifidobacterium* and *Lactobacillus* (Gao et al., 2011).

The present study aims to determine the properties of fruit yoghurt to which dry apricot pulp and different proportions of PEE (P1=0.01%, P2=0.03%, P3=0.10%, P4=0.20% and control=0.00% propolis) were added during a storage period. Propolis has a bitter and

undesirable taste by most people. In the preliminary trials, we conducted these ratios. Because when the ratios exceeded 0.20%, it caused some problems in colour, taste and acceptability. We also added dried apricot pulp both to improve the taste and to enhance the colour.

## MATERIALS and METHODS

### *Propolis extract*

The raw propolis was collected from local beekeepers and stored in the dark. 30 g of propolis was extracted for a week with 100 mL of 70% ethanol at room temperature and then filtered to obtain the extract (Silici and Kutluca, 2005).

### *Production of yoghurt*

Raw cow's milk (about 15L) was obtained from the local farm in the city of Ordu, Turkey. Yoghurt samples were made from a mixture of cow's milk, sucrose, skimmed milk powder, starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus*, YoFlex Advance 2.0 DVS, Chr. Hansen, Denmark) and propolis solution in the percentages shown in Table 1. The mixture was heated up to 90 °C, kept for five minutes (Miocinovic et al., 2016), then cooled to 45 °C and inoculated with 1% (w/w) starter culture. Fermentation was set at 44±1 °C until pH was reached to 4.7 (about 3 hours). The pH values were measured with a pH-meter (Thermo Scientific, Orion 3 Star, USA). Yoghurt samples were cooled at the room temperature (in 30 minutes) and poured into plastic cups (100 g), with added dry apricot pulp (10%), stirred and then stored at 4 °C ±1. Three yoghurt samples in 5 groups were analysed, for each day.

Table 1. Percentages of the materials used for yoghurt preparation

Yoghurt Groups	Sucrose (%)	Skimmed milk powder (%)	Starter culture (%)	Dry apricot pulp (%)	PEE (%)
Control	4	2	1	10	0
P1	4	2	1	10	0.01
P2	4	2	1	10	0.03
P3	4	2	1	10	0.10
P4	4	2	1	10	0.20

### *Chemical analysis*

Analyses were carried out at the Apiculture Research Institute Directorate (Ordu, Turkey). The dry matter content of yoghurt was determined by drying samples at 105±1° C overnight to constant weight (Helrich, 1990).

The protein analyses were performed with a protein-nitrogen analyzer (LECO FP-528, USA). Samples were heated to destruction in a combustion tube at high temperatures (900-1200° C) in an oxygen atmosphere according to Dumas principle (Anonymous, 2002). The

pH was determined with a glass electrode attached to the pH-meter (Thermo Scientific, Orion 3-Star, USA). The titratable acidity was measured by titrating 5 g of yoghurt sample and 5 mL distilled water mixture with 0.1 N NaOH solution using phenolphthalein as the indicator (Bradley et al., 1992).

DPPH assay was performed according to Shori and Baba (2013). Briefly, an aliquot of the yoghurt samples was mixed with DPPH solution (Sigma-Aldrich, Germany). The mixture was shaken thoroughly and allowed to stand at room temperature. The constant absorbance readings at 517 nm were recorded and the inhibition of DPPH oxidation (%) was calculated as follows (Shori and Baba, 2013):

$\% \text{ Inhibition} = \frac{(\text{AbsControl} - \text{AbsExtract})}{\text{AbsControl}} \times 100$

The total phenolic contents of the extracts were determined to employ the methods involving Folin-Ciocalteu Reagent. A portion of 300  $\mu\text{L}$  from each sample was diluted into 4.3 mL distilled water and 100  $\mu\text{L}$  Folin-Ciocalteu reagents were added. After 3 min, 20%  $\text{Na}_2\text{CO}_3$  has added to 300  $\mu\text{L}$  portions and the mixture was vortexed and incubated for 30 min. Absorbance was then read on a UV-vis spectrophotometer (Lambda-25, PerkinElmer, USA) at 760 nm. Gallic acid was used as the standard. The results were expressed as mg gallic acid (GAE)/g sample material (Kucuker et al., 2014).

### **Microbiological analysis**

The microbiological analyses were carried out with automated TEMPO® system (bioMerieux, France) for determining the total aerobic mesophilic bacteria, lactic acid bacteria and yeast-mould. 10 g yoghurt sample was placed in 90 mL of buffered peptone water and then homogenized in a stomacher bag with a lateral filter. The obtained filtrate was taken to perform further dilutions in buffered peptone water (Kunicka, 2007). 1 mL properly diluted filtrate was transferred to TEMPO® culture media (AC: Aerobic mesophilic total flora, LAB: Lactic acid bacteria, YM: Yeasts and moulds). At the end of incubation time and temperature program, the system gave the number of microorganisms by reading positive wells and performed statistical analysis with the use of the Most Probable Number (MPN) method. Results were expressed as log colony-forming unit (log cfu).

### **Statistical Analysis**

All the results were analyzed with SPSS Statistics V20. First, analysis of variance (two-way ANOVA) was performed, next Duncan's multiple range test was used to differentiate treatment means at 5% level of significance.

## **RESULTS and DISCUSSION**

### **Determination of changes in yoghurt**

Dry matter content, protein amount, pH and titratable acidity of yoghurt were analyzed on the first and 28th days of storage. The results are presented in Table 2. Only the pH value decreased while other values had increased by the end of the storage period.

### **Dry matter content**

The initial dry matter content of yoghurt samples ranged from 16.63 to 17.66% with no statistically significant differences between the treatments (Table 2). While the dry matter content of the control group was highest, P2 group was the lowest on 28th day. Overall, dry matter contents of all the yoghurt samples increased in small amounts at the end of the storage period. Researchers reported different results about dry matter content of yoghurt. While Biberoglu and Ceylan (2013) reported that dry matter contents ranged from 9.98 to 18.46%, Karahan (2016) found 10.22 to 19.13%. Factors such as milk type, added ingredients, production methods and storage conditions can be the reasons for these differences in dry matter.

### **Protein content**

The protein content of the yoghurt samples ranged from 3.09 to 3.22%, showing no statistically significant changes ( $p > 0.05$ ). According to the Turkish Food Codex Fermented Dairy Products Communique, protein content must be above 3% (Anonymous, 2009). All the results found above this limit. Our results agreed with those of Biberoglu and Ceylan (2013) and Tonguc et al. (2013) who reported protein content between 2.91-6.22% and 2.34-2.98%, respectively. In our study, added PEE had no adverse effect on protein content and yoghurt texture.

Table 2. Physicochemical properties of yoghurt

Physicochemical Properties	Storage Time (day)	Yoghurt Samples				
		P1	P2	P3	P4	Control
Dry Matter (%)	1	16.63 ±0.29 <sup>A,a</sup>	16.90 ±0.09 <sup>A,b</sup>	17.45 ±0.24 <sup>A,a</sup>	16.72 ±0.35 <sup>A,a</sup>	17.66 ±0.09 <sup>A,b</sup>
	28	17.35 ±0.14 <sup>B,a</sup>	17.19 ±0.03 <sup>B,a</sup>	18.19 ±0.11 <sup>A,a</sup>	17.28 ±0.08 <sup>B,a</sup>	18.36 ±0.06 <sup>A,a</sup>
Protein Content (%)	1	3.09 ±0.02 <sup>B,a</sup>	3.14 ±0.02 <sup>AB,a</sup>	3.19 ±0.00 <sup>A,a</sup>	3.21 ±0.02 <sup>A,a</sup>	3.16 ±0.0 <sup>AB,b</sup>
	28	3.17 ±0.03 <sup>A,a</sup>	3.16 ±0.00 <sup>Aa</sup>	3.20 ±0.01 <sup>A,a</sup>	3.21 ±0.01 <sup>A,a</sup>	3.22 ±0.01 <sup>A,a</sup>
pH	1	4.36 ±0.01 <sup>B,a</sup>	4.37 ±0.03 <sup>AB,a</sup>	4.44 ±0.01 <sup>A,a</sup>	4.39 ±0.01 <sup>AB,a</sup>	4.38 ±0.00 <sup>AB,a</sup>
	28	4.20 ±0.01 <sup>BC,b</sup>	4.22 ±0.01 <sup>B,b</sup>	4.32 ±0.01 <sup>A,b</sup>	4.25 ±0.01 <sup>B,b</sup>	4.16 ±0.00 <sup>C,b</sup>
Titratable Acidity (% lactic acid)	1	0.86 ±0.01 <sup>A,b</sup>	0.83 ±0.01 <sup>A,b</sup>	0.81 ±0.01 <sup>A,b</sup>	0.87 ±0.02 <sup>A,b</sup>	0.83 ±0.01 <sup>A,b</sup>
	28	0.98 ±0.00 <sup>B,a</sup>	0.95 ±0.01 <sup>B,a</sup>	0.93 ±0.01 <sup>B,a</sup>	0.96 ±0.00 <sup>B,a</sup>	1.06 ±0.00 <sup>A,a</sup>

Control=0%, P1=0.01%, P2=0.03%, P3=0.10% and P4=0.20% PEE added fruit yoghurt. A-C Means with the same letters in a row within the category data are not significant at  $P > 0.05$ . a-b Means with the same letters in a column within the category data are not significant at  $P > 0.05$ .

#### **pH and titratable acidity**

The pH values were found to be in the range of the optimum values (pH 4.0-4.6) recommended by Özdemir and Bodur (1994). During the study, the pH value of the samples did not exceed this optimal value range. The highest pH value was determined on the first day and then decreased by the end of the storage period. The pH of yoghurts decreased to lower pH values possibly as a result of the accumulation of acetic acid, acetaldehyde, formic acid and lactic acid (Amirdivani and Baba, 2011). Decreasing pH values of yoghurt have also been reported by other researchers (Misirlilar et al., 2012; Şenel et al., 2009; Tseng and Zhao, 2013). Titratable acidity is an important quality parameter in the flavour and shelf life of yoghurt which measures the equivalent percentage (%) of lactic acid. Titratable acidity increased in all the groups at the end of storage. Higher titratable acidity may indicate differential microbial population during fermentation and possibly storage (Shori and Baba, 2013). Titratable acidity is limited to min 0.6%, max 1.5% in Turkish Food Codex. Our results met with the Turkish Food Codex and none of the yoghurt samples exceeded the permitted limits, supporting previous research (Atasever, 2004; Şenel et al., 2009).

#### **DPPH inhibition and total phenolics**

The antioxidant properties of yoghurt on the first and 28th days are shown in Table 3. As shown in the table,

the total phenolic content and DPPH inhibition of yoghurt samples were in the range from 2.10 to 4.63 mg GAE/g and 16.52 to 49.70%, respectively. The highest value at the beginning of storage 3.98 mg GAE/g was determined in P4 sample while the lowest value 2.10 mg GAE/g was determined in the control yoghurt.

As expected, total phenolic content and DPPH inhibition of yoghurt samples increased significantly ( $p < 0.05$ ) in accordance with the increased propolis amount. The minimum total phenolic content and DPPH inhibition values were observed in yoghurt without propolis (control group), while maximum values were observed in yoghurt with 0.20% propolis (P4 group). The higher antioxidant activity of propolis-added yoghurt is a desirable characteristic that may enhance the therapeutic values of yoghurt. We observed that the DPPH inhibition and total phenolic values increased at the end of the storage period. This finding was similar to previous studies (Shori and Baba, 2011; Perna et al., 2013; Amirdivani and Baba, 2011). Increasing antioxidant activity may be attributed to the metabolically active yoghurt bacteria even at low temperature (Papadimitriou et al., 2007). Other possible sources of that increase may be proteolysis of milk protein and organic acids as a result of fermentation and post-acidification during storage (Shori and Baba, 2013).

Table 3. Antioxidant properties of yoghurt

Antioxidant Properties	Storage Time (day)	Yoghurt Samples				
		P1	P2	P3	P4	Control
DPPH Inhibition (%)	1	17.13 ±0.47 <sup>C,b</sup>	21.37 ±0.94 <sup>B,b</sup>	39.26 ±0.81 <sup>A,a</sup>	40.72 ±0.69 <sup>A,b</sup>	16.52 ±1.35 <sup>C,a</sup>
	28	20.82 ±0.55 <sup>B,C,a</sup>	27.12 ±0.72 <sup>B,a</sup>	44.64 ±3.28 <sup>A,a</sup>	49.70 ±0.81 <sup>A,a</sup>	19.58 ±0,42 <sup>C,a</sup>
Total Phenolic (mg GAE/g)	1	2.99 ±0,19 <sup>AB,a</sup>	3.13 ±0.16 <sup>AB,b</sup>	3.26 ± 0.22 <sup>AB,b</sup>	3.98 ±0.42 <sup>A,a</sup>	2.10 ±0.46 <sup>B,a</sup>
	28	3.49 ±0.28 <sup>BC,a</sup>	3.91 ±0.04 <sup>AB,a</sup>	4.53 ±0.09 <sup>A,a</sup>	4.63 ±0.23 <sup>A,a</sup>	2.58 ±0.28 <sup>C,a</sup>

Control=0%, P=0.01%, P2=0.03%, P3=0.10% and P4=0.20% PEE added fruit yoghurt. A-C Means with the same letters in a row within the category data are not significant at P > 0.05. a-b Means with the same letters in a column within the category data are not significant at P > 0.05.

### Microbiological properties

The results obtained from the microbiological analysis of yoghurts in 1st and 7th days are shown in Table 4.

Microbial growth continues during storage and the number of viable microorganisms is a critical factor in the final product and nutritional health benefits of yoghurt (Zare et al., 2011). Beginning of spoilage and shelf life can be determined by counting total mesophilic aerobic microorganism (AC). We found that propolis amount did not affect aerobic mesophilic total flora (p>0.05) while storage time was found statistically significant (p<0.05). AC count increased in all the groups and the control group had the highest number with 7.38

log cfu/g in 7th day of storage. The AC number of yoghurt samples ranged from 5.70 log cfu/g to 7.38 log cfu/g. These results are consistent with other studies (Atasoy et al., 2003; Demirkaya and Ceylan, 2013).

Lactic acid bacteria (LAB) play an essential role in fermentation, extending shelf life, imparting beneficial influence on food's nutritional value, and on its healthy (Marhamatizadeh and Sayyadi, 2019). During the storage period LAB number increased in all the groups and control group had the highest bacteria number with 6.28 log cfu/g. Mataragas et al. (2011) reported that lactic acid bacteria number was constant or a little decreased.

Table 4: Microbiological enumeration of yoghurt (log cfu/g)

	Storage Time (day)	Yoghurt Samples				
		P1	P2	P3	P4	Control
AC	1	5.74 ±0.00 <sup>A,b</sup>	5.74 ±0.04 <sup>A,b</sup>	5.84 ±0.03 <sup>A,b</sup>	5.73 ±0.03 <sup>A,b</sup>	5.70 ±0.01 <sup>A,b</sup>
	7	6.79 ±0.02 <sup>A,a</sup>	6.77 ±0.18 <sup>A,a</sup>	6.60 ±0.08 <sup>A,a</sup>	6.96 ±0.07 <sup>A,a</sup>	7.38 ±0.31 <sup>A,a</sup>
LAB	1	5.53 ±0.02 <sup>A,b</sup>	5.37 ±0.02 <sup>A,b</sup>	5.44 ±0.12 <sup>A,a</sup>	5.28 ±0.01 <sup>A,b</sup>	5.45 ±0.15 <sup>A,b</sup>
	7	6.14 ±0.05 <sup>A,a</sup>	5.84 ±0.03 <sup>B,a</sup>	5.67 ±0.06 <sup>B,a</sup>	5.66 ±0.06 <sup>B,a</sup>	6.28 ±0.03 <sup>A,a</sup>
YM	1	1.15 ±0.15 <sup>A,a</sup>	1.30 ±0.10 <sup>A,a</sup>	1.0 ±0.0 <sup>A,b</sup>	1.15 ±0.05 <sup>A,b</sup>	1.35 ±0.15 <sup>A,a</sup>
	7	1.65 ±0.05 <sup>AB,a</sup>	1.55 ±0.05 <sup>B,a</sup>	1.35 ±0.05 <sup>B,a</sup>	1.30 ±0.10 <sup>B,a</sup>	2.0 ±0.10 <sup>A,a</sup>

Control=0%, P=0.01%, P2=0.03%, P3=0.10% and P4=0.20% PEE added fruit yoghurt. A-C Means with the same letters in a row within the category data are not significant at P > 0.05. a-b Means with the same letters in a column within the category data are not significant at P > 0.05. AC: Aerobic mesophilic total flora, LAB: Lactic acid bacteria, YM: Yeasts and moulds.

Alirezalu et al. (2019) reported that post contamination microorganisms such as yeasts and moulds (YM) coupled with undesirable conditions results in the development of off-flavours and other unacceptable changes that eventually yoghurt becomes unconsumable. The number of yeast and mould increased in all the groups. The YM was varied from 1.0 to 2.0 log cfu/g in 7 days. Results in

our study did not exceed the yeast number reported by Dublin-Green and Ibe (2005) as the presence of spoilage in yoghurt.

This study aimed to determine the effect of the PEE on the physicochemical and microbiological features of fruit yoghurt. The results of the present study show that propolis extract does not adversely influence the

mechanism of yoghurt formation. It was also observed, propolis increased nutritional content of yoghurt. Taken together, these findings support the suggestion that in appropriate proportions propolis extract can be used for increasing bioactive properties in fruit yoghurts, without any adverse effects.

## ÖZET

**Amaç:** Bu çalışmanın amacı farklı oranlarda propolis etanol ekstraktı (PEE) katılan meyveli yoğurtların depolama boyunca kimyasal ve mikrobiyolojik özelliklerini belirlemektir.

**Yöntem ve Bulgular:** Farklı oranlarda PEE katılan meyveli yoğurtlar +4 °C'de 28 gün depolanmıştır. Depolamanın birinci ve 28. günlerinde kuru madde, protein, pH, titrasyon asitliği, DPPH inhibisyonu ve toplam fenol içeriği analiz edilmiştir. Ayrıca depolamanın birinci ve 7. günlerinde mikrobiyolojik analizler de gerçekleştirilmiştir. Depolama sonunda tüm yoğurtlarda titrasyon asitliği artarken, pH değeri düşmüştür. DPPH inhibisyonu ve toplam fenol miktarı yoğurtlara katılan propolis miktarı ile bağlı olarak artmıştır. Propolis miktarının aerobik mezofilik toplam flora üzerinde etkisi olmadığı görülmüştür. Depolama süresince laktik asit bakterileri tüm gruplarda artmış ve en yüksek bakteri sayısı kontrol grubunda görülmüştür. Maya ve küf sayısı ise tüm gruplarda artmıştır.

**Genel Yorum:** Yaptığımız çalışmada katılan propolisin yogurt oluşum mekanizmasını olumsuz etkilemediği görülmüştür. Ayrıca propolis yoğurtların besleyici özelliklerini ve antioksidan etkisini arttırmıştır.

**Çalışmanın Önemi ve Etkisi:** Bu çalışmada çok değerli bir arı ürünü olan propolisin farklı oranlardaki etanol çözültüsü meyveli yoğurtlara katılmış ve yoğurtların besleyici özelliklerinin ve antioksidan içeriğinin arttığı görülmüştür. Doğal ürünlere ilginin arttığı günümüzde propolisin doğal bir gıda katkı maddesi olarak kullanılabileceği düşünülmektedir.

**Anahtar Kelimeler:** Propolis, yoğurt, antioksidan etki, mikrobiyolojik özellikler.

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## CONFLICT OF INTEREST

This study is derived from a part of Fazıl GÜNEY's master science thesis. The authors declare that there is no conflict of interest.

## AUTHOR'S CONTRIBUTIONS

The contribution of the authors is equal.

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