



## THE EFFECT OF PACKAGING METHODS AND STORAGE TEMPERATURES ON THE ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF MALATYA CHEESE

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### ABSTRACT

This study analyzed the total phenolic content (TPC), antioxidant, and antimicrobial activity of traditionally produced Malatya cheeses made from cow's milk. Cheeses were ripened either in brine using plastic drums or in dry salted polyethylene bags at 7 and 20°C for 120 days. Results showed that cheeses ripened in polyethylene had higher TPC and antioxidant activity compared to those in plastic drums. The highest TPC and antioxidant activity were found in water-soluble extracts from the dry salted samples ripened at 20°C for 120 days, while the lowest were in cheeses ripened in brine at 7°C for just 2 days. The antimicrobial activity was tested against two bacteria using an agar well diffusion technique, but no antimicrobial effects were observed. Overall, the findings suggest that salting methods, ripening times, and temperatures significantly influence the TPC and antioxidant activity of Malatya cheeses.

**Key words:** Packaging methods, storage temperatures, antioxidant activity, Malatya cheese

### AMBALAJLAMA YÖNTEMLERİ VE DEPOLAMA SICAKLIKLARININ MALATYA PEYNİRİNİN ANTIOKSİDAN VE ANTIMİKROBİYAL AKTİVİTELERİNE ETKİSİ

#### ÖZ

Bu çalışmada inek sütünden geleneksel yöntemle üretilen Malatya peynirlerinin toplam fenolik madde içeriği (TFM), antioksidan ve antimikrobiyal aktivitesi analiz edilmiştir. Peynir örnekleri plastik bidonlar kullanılarak salamurada ya da kuru tuzlu polietilen poşetlerde 7 ve 20°C'de 120 gün süreyle olgunlaştırılmıştır. Sonuçlar, polietilende olgunlaştırılan peynirlerin plastik bidonlarda olgunlaştırılanlara kıyasla daha yüksek TPC ve antioksidan aktiviteye sahip olduğunu göstermiştir. En yüksek TFM ve antioksidan aktivite 20 °C'de 120 gün olgunlaştırılan kuru tuzlanmış örneklerde bulunurken, en düşük aktivite ise 7 °C'de 2 gün salamurada olgunlaştırılan peynirlerin suda çözünür ekstraktlarında tespit edilmiştir. Antimikrobiyal aktivite iki farklı mikroorganizma kullanılarak kuyu difüzyon tekniği ile test edilmiş, ancak herhangi bir etki görülmemiştir. Bu bulgular, tuzlama yöntemlerinin, olgunlaşma sürelerinin ve sıcaklıklarının Malatya peynirlerinin TFM ve antioksidan aktivitesini önemli ölçüde etkilediğini göstermektedir.

**Anahtar kelimeler:** Ambalajlama yöntemleri, depolama sıcaklığı, antioksidan aktivite, Malatya peyniri

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## INTRODUCTION

Malatya cheese is a flat and disc-shaped cheese with a semi-hard texture and a corrugated mass surface due to the special structures of the printing materials (Cakmakci, 2011). This cheese is traditionally produced from sheep's or cow's milk or a mixture of these (Hayaloglu and Karabulut, 2008). Since pasteurization is not applied in traditional production, warm milk is directly fermented and processed into cheese without adding starter culture to milk. However, in recent years, dairy factories operating in Malatya produce Malatya cheese using pasteurized milk and starter culture. Malatya cheese is scalded in water or in whey at 80-90 °C, regardless of whether the milk used in production is pasteurized milk or raw milk. The manufacturing process of Malatya cheese is very similar to Halloumi cheese and cheeses are evaluated in the same category in terms of sensory. However, it is inevitable that there will be some differences both in production and the characteristics of the product (Cakmakci, 2011).

During scalding, some changes occur in the microbiological, biochemical and physical (texture and microstructure) properties of cheese. It is stated that the scalding temperature affects the cheese microflora and decreases in the total number of microorganisms (Ozer et al., 2004).

There are several studies on Malatya cheese so far, and in these studies, the evaluation of Malatya cheese in terms of microbiological quality, chemical properties, mineral content, antioxidant activity, volatile compounds, ripening properties and food safety has been discussed (Hayaloglu and Brenchany, 2007; Hayaloglu et al., 2008; Hayaloglu et al., 2010; Karatekin, 2014; Hayaloglu et al., 2014; Yasar, 2021; Yasar et al., 2021; Kose et al., 2022; Yasar and Kose, 2022). Although there are several studies on some properties of Malatya cheese, there is a study on antioxidant activity (Kose et al., 2022) and no study on antimicrobial properties of Malatya cheese. In this study, the effect of ripening times (2, 30, 60, 90, and, 120 days), ripening temperatures (7 and 20 °C), and salting methods (brining and dry salting) on the antioxidant and antimicrobial activity of

Malatya cheese produced by traditional method were investigated.

## MATERIALS AND METHODS

### Production of Malatya cheeses

According to the cheese making procedure of Hayaloglu et al. (2008), cheese production was carried out in a cheese factory in Malatya. For the production of traditional Malatya cheese, raw cow milk was heated to approximately 32 °C and commercially sold rennet was added and coagulation was carried out in approximately 45 minutes. After coagulation, the clot was cut into 1-2 cm<sup>3</sup> and the serum was separated. After removing at least 1/3 of the whey, approximately 1 kg of curd was taken into special cloths and filtered on its own. Then, tightly connected straining cloths were printed between special printing plates (made by connecting reed rods) and remained in the press for about 2 hours. After pressing, the cheese masses were scalded in their own whey at 85-90 °C for 3-5 minutes. After being pressed again for a very short time after scalding and providing the desired corrugated structure, the molds were brought to room temperature. Some of the obtained cheeses were dry salted and stored in sterile polyethylene bags, and some were stored in 12% (w/v) brine using plastic containers for 120 days (+7 and +20 °C). For each period, the cheeses were stored with approximately 200g of cheese in each package (Figure 1).

### Preparation of water soluble extracts

The extracts was applied by modifying the method given by Kuchroo and Fox (1982). In order to prepare the extracts, 10 g cheese sample was weighed and 20 ml of water was added and homogenized with Ultra Turrax for 1 minute. After the mixture was kept in a water bath at 40°C for 1 hour, it was centrifuged at 10000 rpm for 20 minutes at 4°C. After centrifugation, the upper oil layer was removed with a spatula and the liquid part was filtered using filter paper (Whatman No:1). TPC, antioxidant and antimicrobial activity analyzes were performed by taking the obtained filtrate.

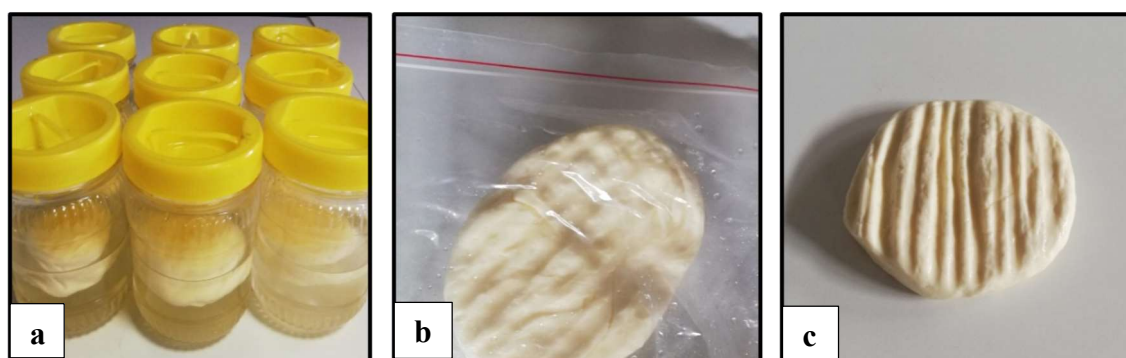


Figure 1. (a) %12 brined Malatya cheeses in plastic containers, (b) Dry salted Malatya cheese in polyethylene bags, (c) Fresh Malatya cheese before packaging

### Total phenolic compounds (TPC)

150  $\mu$ L of sample and 3 mL of sodium carbonate (2%) were placed in the test tubes, and after approximately 2 minutes, 150  $\mu$ L of Folin-Ciocalteu reagent diluted 1:1 with ultrapure water was added to the tubes and mixed. This mixture was then kept at room temperature in a dark place for 45 minutes and readings were taken on a spectrophotometer (UV Mini-1240, Shimadzu, Japan) at 765 nm. Results were given as gallic acid equivalent (GAE) (Yemis et al., 2008).

### Antioxidant activity

#### DPPH test

100  $\mu$ L of water-soluble extracts obtained from Malatya cheeses were placed in tubes, 2.4 mL of the daily prepared DPPH solution was added and mixed thoroughly. Following mixing, the test tubes were incubated for 30 minutes in a dark environment and readings were made on the spectrophotometer at a wavelength of 520 nm. The results were determined from the inhibition of DPPH (%) =  $[1 - (A/A_0)] \times 100$ , where  $A_0$  was the absorbance of the control and  $A$  is the absorbance of the mixture (Brand-Williams et al., 1995).

#### TEAC test

In the study, firstly,  $ABTS^{\bullet+}$  radical was formed by reacting 7 mM  $ABTS^{\bullet+}$  with 2.45 mM potassium persulfate for 12-16 hours in the dark at room temperature. The formed  $ABTS^{\bullet+}$  radical was diluted with ethanol to give an absorbance of  $0.700 \pm 0.05$  at 734 nm, and 2.97 mL of this solution was taken and 30  $\mu$ L of the

extract obtained from the samples was added. After the mixture was kept at room temperature for 6 minutes, the absorbance was measured at 734 nm and the results were expressed as mmol TE/g (Kırca and Ozkan, 2007).

### Antimicrobial activity

Antimicrobial activity of Malatya cheeses were determined using two different microorganisms (*Escherichia coli* ATCC 11303 and *Staphylococcus aureus* ATCC 29213) with an agar well diffusion technique (National Committee for Clinical Laboratory Standard [NCCLS], 1999).

Firstly, a loopful of bacterial colonies from 18-24 hour fresh bacterial cultures were suspended in sterile FTS and the density of the bacterial suspensions was adjusted according to the 0.5 McFarland standard. 100  $\mu$ L of the adjusted bacterial suspensions was taken and spread into Petri dishes containing Müller-Hinton Agar (MHA, Oxoid) using a drigalski loop. 100  $\mu$ L of water-soluble cheese extracts were added to the media with 5 mm diameter wells at certain points. The petri dishes prepared in this way were incubated at 37°C for 24 hours and the inhibition zones formed on the medium were measured with the help of a digital caliper and evaluated in mm. Studies were conducted in 3 parallels and standard antibiotic discs (30  $\mu$ g tetracycline and 10  $\mu$ g ampicillin) were used for comparison as positive controls.

**Statistical analysis**

SAS version 9.4 statistical program was used in the analysis of the data obtained from the study. General linear model (GLM) analysis was performed to define the differences between the means of the groups, and Duncan's multiple comparison test was used to define the differences between the groups. In addition, the t test was used to compare two independent groups.

**RESULTS AND DISCUSSIONS**

While the TPC values of cheese samples stored in plastic packaging at 7°C ranged between 254.88 mg GAE/kg and 646.23 mg GAE/kg, the TPC values of the samples stored at 20°C were found to vary between 265.72 mg GAE/kg and 661.78 mg GAE/kg. The TPC values of cheese samples ripened in polyethylene packaging ranged from 424.05 mg GAE/kg to 695.11 mg GAE/kg at 7°C, and the TPC values of the samples ripened at 20°C ranged between 473.36 mg GAE/kg and 755.11 mg GAE/kg.

As seen in Table 1, it is thought that the compounds formed as a result of glycolysis, lipolysis and proteolysis reactions due to ripening during the preservation of cheeses stored at different temperatures and packages cause an increase in TPC concentration during storage. A similar situation has been reported by Rashidinejad et al. (2015) and Kose and Ocak (2020). Also, the TPC of the cheeses stored in brine in plastic packaging at different temperatures were lower than the TPC of the cheeses stored in dry salted polyethylene packaging. Hala et al. (2010) reported that salting prevents the phenolic compounds from reacting with the Folin-Ciocalteu reagent, making it difficult to determine all of the phenolic compounds and causing a low result. In addition, salting activates the dissolution of Ca<sup>2+</sup>, resulting in an increase in surface charge, volume, and hydration in casein micelles. This situation causes some phenolic compounds to interact with water through hydrogen bonds and causes the values of phenolic compounds to decrease. (Hala et al., 2010).

Table 1. Changes in TPC values during ripening of Malatya cheese samples stored using different packaging materials and different temperatures (mg GAE/kg)

Packaging type	Storage		Storage time (Days)				
	Temperature (°C)		2	30	60	90	120
Plastic	7		254.88±4.68 <sup>D</sup>	442.89±5.56 <sup>C</sup>	466.22±2.22 <sup>CB</sup>	482.99±7.78 <sup>Bz</sup>	646.23±15.5 <sup>A</sup>
	20		265.72±0.50 <sup>E</sup>	457.34±2.23 <sup>D</sup>	472.89±2.22 <sup>C</sup>	578.45±5.56 <sup>Bβ</sup>	661.78±2.22 <sup>A</sup>
Polyethylene	7		424.05±0.05 <sup>Dz</sup>	614±5.56 <sup>Cz</sup>	646.23±4.45 <sup>Bz</sup>	675.11±8.89 <sup>A</sup>	695.11±8.89 <sup>Az</sup>
	20		437.36±2.20 <sup>Dβ</sup>	672.89±4.45 <sup>Cβ</sup>	684±2.22 <sup>Cβ</sup>	712.84±6.72 <sup>B</sup>	755.11±6.67 <sup>Aβ</sup>

<sup>z, β</sup> The difference between the samples of the same package in the same period ( $P < 0.05$ ), <sup>A,B,C,D,E</sup> letters indicate the difference between periods ( $P < 0.05$ ).

Canozer ve Kose (2022) determined that the TPC ratio of traditionally produced Orgu cheese samples ranged from 632.07 mg GAE/kg to 1091.36 mg GAE/kg, and the TPC rate of the industrially produced Orgu cheese samples ranged between 288.86 mg GAE/kg and 930.29 mg GAE/kg. Kara and Kose (2020) determined that the TPC ratio of Herby cheese samples ripened by the brine method ranged from 345.04 mg GAE/kg to 1117.26 mg GAE/kg, and the TPC ratio of Herby cheese samples ripened by the pressing method ranged from 639.11 mg

GAE/kg to 1030.96 mg GAE/kg. When the data obtained in the study were compared with the literature, it was determined that some values were low and some values were similar. It is thought that this difference arises from the storage conditions and maturity level of the cheeses.

The DPPH inhibition rate of the Malatya cheeses stored in plastic packaging at 7°C ranged from 5.44% to 11.01%, and the DPPH inhibition rate of the samples stored at 20°C ranged between

5.71% and 12.19%. Also, the DPPH inhibition rate of the cheese samples stored in polyethylene packaging ranged from 6.95% to 12.61% at 7°C, and the DPPH inhibition rate of the samples stored at 20°C ranged between 8.02% and 13.92%. Although free radical scavenging activity was present in all samples throughout the ripening period, it was relatively low and reached maximum values of up to 13.92%. The weak

radical scavenging capacity may be attributed to the inability of the present peptides to exhibit this activity. In this regard, the donation of a proton or electron is required for the scavenging of free radicals, and if the structure of the amino acids present in the peptide is not adequate, the clearance of radicals will decrease (Ramos et al., 2022).

Table 2. Changes in DPPH inhibition values during maturation of Malatya cheese samples stored using different packaging materials and different temperatures (%)

Packaging type	Storage Temperature (°C)	Storage time (Days)				
		2	30	60	90	120
Plastic	7	5.44±0.00 <sup>Ez</sup>	7.58±0.20 <sup>D</sup>	8.44±0.19 <sup>C</sup>	9.35±0.20 <sup>B</sup>	11.01±0.35 <sup>A</sup>
	20	5.71±0.01 <sup>Dβ</sup>	6.59±0.63 <sup>D</sup>	8.69±0.14 <sup>C</sup>	10.39±0.14 <sup>B</sup>	12.19±0.56 <sup>A</sup>
Polyethylene	7	6.95±0.05 <sup>Ez</sup>	7.45±0.07 <sup>Dz</sup>	10.95±0.13 <sup>C</sup>	11.79±0.19 <sup>B</sup>	12.61±0.14 <sup>A</sup>
	20	8.02±0.02 <sup>Dβ</sup>	8.75±0.20 <sup>Dβ</sup>	11.41±0.07 <sup>C</sup>	12.89±0.42 <sup>B</sup>	13.92±0.35 <sup>A</sup>

<sup>z, β</sup> The difference between the samples of the same package in the same period ( $P<0.05$ ),

<sup>A,B,C,D,E</sup> letters indicate the difference between periods ( $P<0.05$ ).

Canozler (2020) reported that the DPPH inhibition rate of the traditionally produced Orgu cheese samples ranged from 2.19% to 6.90%, and the DPPH inhibition rate of the industrially Orgu cheese samples ranged from 2.65% to 9.56%. Kose and Ocak (2020) reported that the DPPH inhibition rate of Herby cheeses ranged from 2.87% to 9.84%, and the DPPH inhibition rate of Herby cheese samples matured in vacuum packaging ranged from 2.87% to 11.76%. Kara and Kose (2020) reported that the DPPH inhibition rate of Herby cheese samples ripened in brine ranged from 3.60% to 9.59%, and the DPPH inhibition rate of Herby cheese samples

matured by pressing method ranged from 4.31% to 13.05%. Erkaya and Sengül (2015) determined that the DPPH inhibition rate of the water-soluble white cheese extracts varied between 5.10% and 10.38%. When the data obtained in the study was compared with the literature, it was determined that some values were similar, some were low, and some were high. It is thought that this is due to the fact that the antioxidant potential of cheeses varies depending on the type of milk, starter cultures (Stobiecka et al., 2022), storage time, treatment, and packaging type (Kose and Ocak, 2020).

Table 3. Changes in TEAC values (mmol TE/g) during ripening of Malatya cheese samples stored using different packaging materials and different temperatures.

Packaging type	Storage Temperature (°C)	Storage time (Days)				
		2	30	60	90	120
Plastic	7	1.25±0.01 <sup>Ez</sup>	1.72±0.08 <sup>D</sup>	2.46±0.14 <sup>C</sup>	3.11±0.03 <sup>B</sup>	4.09±0.03 <sup>Az</sup>
	20	1.35±0.01 <sup>Eβ</sup>	2.36±0.03 <sup>D</sup>	3.07±0.05 <sup>C</sup>	3.25±0.04 <sup>B</sup>	4.48±0.05 <sup>Aβ</sup>
Polyethylene	7	1.51±0.01 <sup>D</sup>	2.53±0.35 <sup>C</sup>	3.32±0.27 <sup>B</sup>	3.37±0.04 <sup>Bz</sup>	4.62±0.08 <sup>Az</sup>
	20	1.63±0.05 <sup>E</sup>	2.75±0.09 <sup>D</sup>	3.39±0.04 <sup>C</sup>	3.64±0.04 <sup>Bβ</sup>	5.24±0.05 <sup>Aβ</sup>

<sup>z, β</sup> The difference between the samples of the same packaging in the same period ( $P<0.05$ ),

<sup>A,B,C,D,E</sup> letters indicate the difference between periods ( $P<0.05$ ).

The TEAC ratio of Malatya cheeses stored in plastic packaging at 7°C ranged between 1.25 and 4.09 mmol TE/g, and the TEAC ratio of the samples stored at 20°C ranged between 1.35 and 4.48 mmol TE/g. The TEAC ratio of the Malatya cheeses stored in polyethylene packaging at 7°C ranged from 1.51 to 4.62 mmol TE/g, and the TEAC ratio of the samples stored at 20°C ranged between 1.63 and 5.24 mmol TE/g.

According to the obtained data, it was determined that TEAC values increased with time. It is thought that the increase in antioxidant activity in cheese is due to the increase in water-soluble peptide formation due to ripening. Similarly, Gupta et al. (2009) detected that TEAC values increased until the 4th month of storage. In addition, TEAC values of cheese samples ripened in polyethylene packaging were determined to be higher than those ripened in plastic packaging.

The zone diameters of Tetracycline (30 µg) and Ampicillin (10 µg) against *Staphylococcus aureus* ATCC 29213 were found as 38 and 29 mm, respectively. The Ampicillin (10 µg) and Tetracycline (30 µg) produced 21 and 19 mm zone diameters against *Escherichia coli* ATCC 11303, respectively. The water-soluble extracts of traditionally produced Malatya cheeses did not show any antimicrobial activity against *S. aureus* ATCC 29213 and *E. coli* ATCC 11303.

### CONCLUSIONS

The TPC and antioxidant activity of water-soluble extracts of traditionally produced Malatya cheese from raw cow's milk varied depending on the salting methods, ripening temperatures and times. Additionally, it was determined that the extracts did not show any antimicrobial activity against the tested bacteria.

TPC and antioxidant activity of Malatya cheeses stored in plastic packaging in brine were lower than the antioxidant activity of Malatya cheeses stored in dry salted polyethylene packaging. Also, TPC and antioxidant activity of Malatya cheeses ripened at 20°C were higher than those ripened at 7°C. Based on these results, dry salting of cheeses and ripened them in polyethylene packaging at 20

°C for 120 days can be recommended for Malatya cheese with high antioxidant activity.

These findings shed light on the significant impact of processing and storage conditions on the bioactive properties of Malatya cheese. Further research could investigate the underlying mechanisms governing the observed variations and explore additional factors influencing the antioxidant and antimicrobial characteristics of this traditional cheese variety. Such insights can inform strategies to optimize the production and quality of Malatya cheese, improve its nutritional and functional properties, and meet consumer preferences and market demands.

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