

Alcoholic Extract of (*Quercus persica* Jaub. & Spach) as a Functional Natural Preservative to Improve Hygienic Quality of Jug Cheese

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ABSTRACT

The alcoholic extract of Iranian oak (*Quercus persica* Jaub. & Spach) fruit at a final concentration of 0.25% (w/v) was added to cheese-making milk, and some physicochemical, sensory, and microbial properties of Jug cheese were evaluated during 60 days of storage at 4°C. The results showed significantly higher Total Polyphenol Content (TPC), antioxidant activity, and lower acidity for the samples containing the oak extract (T1) compared to the control (T0). At the beginning of the storage time, T1 showed the highest values of TPC (128.83 ± 0.467 mg GAE g⁻¹) and antioxidant activity (97.12 ± 0.095) for DPPH (2,2-DiPhenyl-1-PicrylHydrazyl) radical scavenging activity. The antioxidant activity decreased significantly during the storage period. The flavor, aroma, and overall acceptability scores were higher for T1 than for T0. Short-chain fatty acids content of the cheese varied during the storage period, but no significant change was observed in the content of long chain fatty acids. The amount of butyric, caproic, caprylic, capric, lauric, myristic, palmitic, oleic (trans) and linoleic (cis) fatty acids in T1 were significantly higher than in T0. No significant changes in these fatty acids concentrations were observed during storage period. Total microbial count, Coliforms, mold and yeast were significantly lower in the T1 than in T0. Therefore, the Iranian oak extract as a source of antioxidant and antimicrobial polyphenolic compounds could potentially improve the quality and shelf life of Jug cheese without adversely affecting its sensory and physicochemical properties.

Keywords: Antimicrobial activity, Antioxidant activity, Oak extract, Total polyphenol content.

INTRODUCTION

Traditional Iranian cheeses are among the most commonly used fermented milk products in Iran. They are produced by various processing methods in different regions; therefore, they have different sensory, physicochemical and microbial characteristics based on the processing method used (Mirzaei *et al.*, 2008). Due to climatic diversity and prevalence of traditional farming in Iran, different types of traditional cheeses, such as Shabestar, Jug, Taleshi, Hamedan, Zanjan, Lighvan,

Golpayegan and Kurdistan cheeses are produced in different regions.

Jug is a hard, slightly acidic, and salty cheese with a granular and dry appearance that is produced and consumed in the west parts of Iran. It contains 22.4-22.6% protein, 24-26% fat, 53-55% dry matter, 44-49% moisture, 28-31% Solids-Non-Fat (SNF) and 48-46% fat in dry matter (Hesaami-Rad *et al.*, 2006). Ripening of this cheese occurs under anaerobic conditions while it is kept in warehouses or under soil in special storage conditions for about 3 to 4 months, which results in its physical, chemical and

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microbial changes (Hesaami-Rad *et al.*, 2006). This product is often produced from raw milk of sheep, goat or cow, without using a starter culture. Nowadays, the Jug cheese makers are persuaded to use pasteurized milk, but most of them believe that the raw milk creates a pleasant flavor in the cheese, which is actually due to the activity of proteolytic and lipolytic enzymes that are present inherently in the raw milk or are produced by its microbial flora (Dolci *et al.*, 2008).

The dominant species of lactic microbial flora contributing in the ripening process of Jug cheese are *Lactobacillus* (37.3%) and *Enterococcus* (25.5%) (Ghaderi *et al.*, 2013).

The number of naturally occurring bacteria in raw milk is about 10^3 to 10^7 CFU mL⁻¹ and includes Psychrotrophic bacteria such as *Pseudomonas aeruginosa*, *Alcaligenes* species, *Aeromonas*, Lactic acid bacteria, Gram-positive spore-forming bacilli, *Corynebacterium*, *Micrococci*, and Coliforms. Milk pasteurization reduces the total microbial count of cheese; however, some microorganisms are resistant to these conditions or may be present in the cheese due to post-contamination (Sarbaz *et al.*, 2014). The presence of these microorganisms has been demonstrated in different types of milk products, including cheese during the production, processing and storage stages. Their consumption may also cause poisoning (Hayaloglu and Kirbag, 2007).

One of the methods for increasing the shelf life of cheese is to use preservatives with natural origin without side effects. The *Quercus persica* extract contains some flavonoids, tannins, procyanidins, and significant amounts of bioactive compounds including gallic acid, ellagic acid and hexahydroxyphenyl derivatives (Khosravi and Behzadi, 2006; Kaur *et al.*, 2004; Sakar *et al.*, 2005; Ebrahimi *et al.*, 2010; Ebadi Fathabada *et al.*, 2015), which are assumed to reduce the total microbial count, improve antioxidant activity and ensure the hygienic quality of traditionally produced Jug cheese.

The inhibitory effect of the oak extract on different bacteria has been previously investigated (Ebrahimi *et al.*, 2005; Ebadi Fathabada *et al.*, 2015; Taran *et al.*, 2010; Sadeghian *et al.*, 2012; Bajalan *et al.*, 2014; Sefidgar *et al.*, 2015; Nourafcan *et al.*, 2013). The antimicrobial and antioxidant effects of oak extract and flour on some foods have also been reported (Rabiei *et al.*, 2018; Nedamani *et al.*, 2014; Ghaderi *et al.*, 2013; Majzobi *et al.*, 2013; Hojjati and Jooyandeh, 2017; Mahrous *et al.*, 2014). However, to our knowledge, the potential use of the oak extract on Jug cheese has not been particularly addressed in terms of its effect on the microbial, sensory and physicochemical properties of the cheese. Therefore, this study aimed to examine the potential use of the alcoholic extract of *Quercus persica* in Jug cheese during storage at 4°C.

MATERIALS AND METHODS

The oak fruit was purchased from the local market in Tehran (Iran), and its variety was identified by the Herbarium Department of the Faculty of Agriculture of Shiraz University (Shiraz, Iran). Its scientific name was confirmed as *Quercus persica*. Raw milk, cheese starter culture (Mito), salt and fungal rennet were obtained respectively from Pak (Iran), Mito (Japan), Payandeh (Iran) and Mito (Japan) Companies. All chemicals and microbial cultures needed for the tests were purchased from Merck Company (Germany).

Preparation of the Alcoholic Extract of the Iranian Oak Fruit

First, the outer shell of the oak fruits was separated by an electric mill and sieve. The fruit powder was prepared and kept in a glass container at 4°C until use. The fat and other oleoresins were removed by the Soxhlet method. For this purpose, 200 mL of N-hexane solvent was added to 10 g of the

powdered sample. After 5 h, the solvent was removed and the powder was dried by oven at 100°C. Then, the alcoholic extract was prepared through immersing 20 g of the dried powder in 40 mL of 96% ethanol for 20 hours. Finally, the extract was separated using rotary evaporator (Ika, Germany) at 80°C under vacuum condition (Ghaderi *et al.*, 2013).

Producing Industrial Jug Cheese

Jug cheese was produced in Pak Co. (Tehran, Iran). Cow milk (8.32% SNF and 3.25% fat) was pasteurized (65°C for 30 minutes) and cooled to 32°C and then cheese starter culture and the oak extract with a final concentration of 0.25% (w/v) were added. Then, 0.01 g (w/w) the fungal rennet was added to the milk and stirred well for 5 minutes. After the milk was coagulated (about 50 min), the coagulum was cut at about 1×1×1 cm with a sharp knife to remove the whey. After pressing the coagulum for about 1 h, the molded clots were transferred to 20% pasteurized (80°C for 10 minutes) saturated brine and kept at 20°C for 1 day. After 24 hours, they were taken out of the brine, and coarse-grained dry salt (1% w/w) was sprayed on them twice daily for 3 days. After 3 days, the clots were immersed in 12% brine; then packed in 500 g plastic containers and kept at 4°C for 2 months.

It should be noted that the MIC and MBC concentrations of the oak extract against *Salmonella typhimurium* (PTCC 1709), *Escherichia coli* (ATCC 19118), *Listeria monocytogenes* (PTC), yeast (*Saccharomyces cerevisiae* ATCC 9763), *Staphylococcus aureus* coagulase positive (ATCC 6538), and *Klebsiella pneumonia* (ATCC 700603) were estimated using microdilution method (Sefidgar *et al.*, 2015). Based on the results (data are not shown), the Iranian oak extract at a final concentration of 0.25% (w/v) was used in producing Jug cheese (T1), and its

characteristics were compared with the Jug cheese without Iranian oak extract (T0).

The cheese samples analyses were carried out on 0, 15th, 30th, 45th and 60th days of storage at 4°C. It should be noted that due to the detection of *Escherichia coli* in the control sample on 45th day, the sensory evaluation of the Jug cheese sample containing the Iranian oak extract was performed only on 60th day of storage.

Microbial Analyses

Salmonella typhimurium, coagulase-positive *Staphylococcus aureus*, total microbial population, *Escherichia coli*, Coliform, *Listeria monocytogenes*, mold and yeast were counted according to the Iranian national standards (ISIRI, 2009; ISIRI, 2006; ISIRI, 2014; SIRI, 2005; ISIRI, 2006; ISIRI, 1998; ISIRI, 2006).

Physicochemical Analyses

Titrateable acidity was measured on the basis of standard provided by the Iranian national standard (ISIRI, 2006). Total Polyphenol Content (TPC) was measured by the phenol folin ciocalteu method (McCuea and Shetty, 2005). The antioxidant activity was analyzed based on the scavenging capacity of free radical 2, 2-DiPhenyl-1-PicrylHydrazyl (DPPH) that was according to the method of McCuea and Shetty (2005). The identification of fatty acids was performed according to the standard provided by the Iranian national standard (ISIRI, 1992).

Sensory Analyses

The texture, color, flavor, odor and overall acceptability characteristics of the cheese samples were evaluated by 5 trained panelists using a five-point hedonic method. Number 1 represents the lowest score, and



number 5 represents the highest score (ISIRI, 1999).

Statistical Analysis

The results were analyzed in a completely randomized factorial design. Duncan test at a 95% level was used to compare mean values. Data were analyzed in 3 replications using SAS software version 9.2.

RESULTS AND DISCUSSION

Microbial Analysis Results

Changes in the total microbial count, *Salmonella*, coagulase-positive *Staphylococcus*, *Listeria monocytogenes*, *Escherichia coli*, Coliform, mold, and yeast were influenced by the presence of the oak extract and the time of storage (Table 1). Addition of the oak extract had a significant negative effect ($P < 0.05$) on the total microbial count and the growth of *Escherichia coli*, Coliform, yeast, and mold.

The total microbial number of the control sample (T0) was very high and uncountable, it was higher than the sample containing the oak extract (T1) during 60 days of storage at 4°C. T1 sample exhibited a significant reduction in the total microbial count during storage, and it decreased the count from 3.6×10^2 to 2.6×10^2 CFU g⁻¹.

The results showed that *Salmonella*, coagulase-positive *Staphylococci* and *Listeria monocytogenes* did not grow after 60 days of storage in both T0 and T1 samples, which was in the range announced by the Iranian national standard (ISIRI, 2016). Despite the positive results of *E.coli* count in the T0 sample, after 45 days of storage at the refrigerator, it remained negative for the T1 sample during 60 days of storage. The count of Coliform decreased for both T0 and T1 samples from 46.66 to 8 CFU g⁻¹ during the storage time. Despite no significant changes in total counts of yeast and mold in T0 during 60 days of

storage, they declined from 4.8×10^2 to 10 CFU g⁻¹ in T1.

The observed decrease in the number of microorganisms in the Jug cheese containing the oak extract could be due to the antibacterial compounds, such as tannins and gallic acid, in the oak extract (Sefidgar *et al.*, 2015). Numerous researchers have reported the antimicrobial effect of the oak extract (Khosravi and Behzadi, 2006; Ebrahimi *et al.*, 2005; Ebadi Fathabada *et al.*, 2015; Taran *et al.*, 2010; Sadeghian *et al.*, 2012; Bajalan *et al.*, 2014; Sefidgar *et al.*, 2015; Nourafcan *et al.*, 2013). In addition, the lactic acid produced by the lactic acid bacteria during cheese ripening could exhibit antimicrobial effects (Boddy and Wimpenny, 1992; Sarbazi *et al.*, 2014). Therefore, the reason that the number of *Escherichia coli* was positive for 45 days of storage and turned negative later could be an increase in the acidity during ripening. In accordance with the present results, Aghazadeh Meshgi (2007) confirmed the presence of *Escherichia coli* in west Azerbaijan Jug fresh cheese, whereas, in the ripened cheeses, no pathogenic bacteria were found. Also, the total microbial count decreased during the ripening period.

Physicochemical Analysis Results

Titrateable Acidity

Using the Iranian oak extract caused a significant ($P < 0.05$) decrease in titrateable acidity (Table 2). The cheese samples containing the oak extract (T1) exhibited a lower titrateable acidity ($0.27 \pm 0.01\%$) than the control (T0) samples ($0.35 \pm 0.006\%$) in zero time. During storage time, a significant ($P < 0.05$) change in acidity was observed for both samples. Finally, T0 exhibited higher acidity ($0.67 \pm 0.01\%$) after 60 days of storage at 4°C in comparison with T1 ($0.4 \pm 0.006\%$). It can be attributed to the antibacterial effects of the oak extract that prevents the activity of starter culture bacteria. The results are similar to the results reported by other researchers (Hojjati and Jooyandeh, 2017; Shahrabi *et al.*, 2017;

Table 1. Microbial counts (CFU g⁻¹) changes of Jug cheese containing Iranian oak extract during cold storage (Mean ±Standard deviation).^a

	Day treatment	0	15	30	45	60
Characteristics						
Salmonella	T0	Negative	Negative	Negative	Negative	Negative
Listeria coagulase-positive staphylococci	T1	Negative	Negative	Negative	Negative	Negative
<i>Staphylococcus aureus</i>						
<i>Escherichia coli</i>	T0	Positive	Positive	Positive	Positive	Negative
	T1	Negative	Negative	Negative	Negative	Negative
Coliform	T0	133.33±1.54 ^a	103.33±5.744 ^b	76.66±5.74 ^c	40.0±1.115 ^d	23.33±5.774 ^e
	T1	46.66±5.744 ^d	40.0±1.115 ^d	23.33±5.774 ^e	13.33±5.774 ^{ef}	8±1.023 ^f
Yeast and mold	T0	4.8×10 ² ±3.12 ^a	4.3×10 ² ±2.14 ^a	4.5×10 ² ±3.58 ^a	4.6×10 ² ±1.15 ^a	4.6×10 ² ±1.15 ^a
	T1	Less than 10	Less than 10	Less than 10	Less than 10	Less than 10
Total microbial count	T0	Uncountable	Uncountable	Uncountable	Uncountable	Uncountable
	T1	3.6×10 ² ±5.825 ^{bc}	3.5×10 ² ±5.166 ^{bc}	3.1×10 ² ±5.166 ^b	3.2×10 ² ±5.166 ^b	2.6×10 ² ±5.275 ^a

^a Means with different subscripts differ significantly (P< 0.05). O: Iranian Oak extract (mg mL⁻¹); T0 (Control): O= 0, T1: O= 0.25%.

Table 2. Physicochemical properties changes of Jug cheese containing Iranian oak extract during cold storage (Mean±Standard deviation).^a

	Day treatment	0	15	30	45	60
Characteristics						
Titrateable acidity (Lactic acid %)	T0	0.35±0.006 ^f	0.41±0.01 ^d	0.51±0.015 ^c	0.62±0.025 ^b	0.67±0.01 ^a
	T1	0.27±0.01 ^h	0.31±0.015 ^g	0.35±0.01 ^f	0.37±0.006 ^e	0.4±0.006 ^d
Total phenolic compounds (mg GAE g ⁻¹)	T0	96.33±0.577 ^f	83.6±0.375 ^g	61.93±0.723 ^h	51.26±0.058 ⁱ	45.46±0.02 ^j
	T1	128.83±0.467 ^a	125.8±0.458 ^b	123.27±0.375 ^c	119.73±0.058 ^d	117.26±0.153 ^e
Antioxidant activity (%DPPH radical scavenging activity)	T0	51.66±0.577 ^f	39.66±0.528 ^g	33.26±0.153 ^h	22.13±0.666 ⁱ	18.86±0.586 ^j
	T1	97.12±0.095 ^a	96.0±0.265 ^b	94.8±0.1 ^c	90.76±0.551 ^d	89.36±0.153 ^e

^a Means with different subscripts differ significantly (P< 0.05). O, T0, and T1 as under Table 1.

Roshani *et al.*, 2016) who showed decreasing acidity in cheeses by adding antibacterial ingredients to cheese-making milk. Over the storage time, the increase of acidity could be attributed to proteolysis and lipolysis by cheese starters (Osman *et al.*, 2008; Rotaro and. Clementi, 2008; Sarbazi *et al.*, 2014; Shahrabi *et al.*, 2017).

Total Polyphenol Content and Antioxidant Activity

T0 and T1 samples were also evaluated for Total Polyphenol Content (TPC) and antioxidant activity on the basis of DPPH radical scavenging activity. The cheese



sample enriched with the oak extract (T1) had a higher TPC and DPPH radical scavenging activity than the control ($P < 0.05$). This result could be related to the presence of significant amounts of bioactive compounds in oak such as gallic acid, tannin, ellagic acid, flavonoids, procyanidins and hexahydroxyphenyl derivatives, which exhibit antioxidant activity (Kaur et al., 2004; Sakar et al., 2005; Khosravi and Behzadi, 2006; Nedamani et al., 2014; Ghaderi et al., 2013; Bahrami et al., 2017). This property also led to higher oxidative stability of the product during storage time. The same results were also reported previously for other dairy products supplemented with phenolic compounds (Cho et al., 2020; Kim et al., 2019; Bchir et al., 2019, Trigueros et al., 2014; Sánchez-Bravo et al., 2018; Muniandy et al., 2016; Senadeera et al., 2018; Jeong et al., 2018). The components such as free amino acids, proteins, and low-molecular-weight antioxidants, as well as polyphenols (Helal and Tagliazucchi, 2018; Kim et al., 2019; Senadeera et al., 2018) were responsible for antioxidant activity of control sample. The antioxidant activity of cheese was likely due to the antioxidant properties of metabolites or bioactive peptides produced by lactic acid bacteria during fermentation and cold storage (Cho et al., 2020; Jeong et al., 2018).

Also, a higher stability of total phenolic compounds and a higher antioxidant activity were observed in T1 compared to T0. Therefore, the TPC and DPPH radical scavenging activity of T0 declined by 52 and 63%, respectively, during 60 days of storage. However, these values declined to 8.98 and 7.99%, respectively, for T1. This observation could be attributed to the degradation of polymeric phenolic compounds in the presence of lactic acid bacteria (Kim et al., 2019; Cho et al., 2020; Muniandy et al., 2016) and increased interactions between milk proteins and polyphenols, that is, a part of the total antioxidant capacity may be masked by the interaction (Kim et al., 2019; Bchir et al.,

2019, Arts et al., 2002; Trigueros et al., 2014; Ozdal et al. 2013; Oksuz et al., 2019; Sánchez-Bravo et al., 2018; Helal and Tagliazucchi, 2018). The decreasing trend of antioxidant activity during the storage of the Jug cheese samples in the present study was in agreement with the results of other researchers (Bahrami et al., 2017; Razaeei et al., 2013; Masoodi Tonkaboni et al., 2013).

Identification of Fatty Acids

Both samples were also evaluated for the kind of fatty acids during the storage time, and results are presented in Table 3.

The amount of butyric, caproic, caprylic, capric, lauric, myristic, palmitic, oleic (trans) and linoleic (cis) fatty acids were significantly higher in treated than in the control samples ($P < 0.05$). No significant changes in these fatty acids concentrations were observed during the storage time ($P > 0.05$). No significant difference was found in the amounts of stearic, linoleic (trans), arachidic and linolenic (cis and trans) fatty acids between two samples ($P > 0.05$). Overall, the amounts of oleic (cis) and behenic fatty acids in T1 were lower than in the control ($P < 0.05$).

The results presented in Table 3 show higher levels of short and medium-chain fatty acids with even carbon numbers (C4: 0-C12: 0) in the sample containing the oat extract compared to the control. Short and medium-chain fatty acids with even carbon numbers have significantly low sensory thresholds, and each of them has a distinctive flavor. Free fatty acids can have a positive or negative effect on cheese flavor depending on the concentration and sensory threshold (Collins et al., 2003). The levels of short fatty acids in samples could be an indicator of the microbial lipolytic activity. Many volatile compounds in cheese can be derived from catabolism of fatty acids by cheese microbiota. Lipolysis and catabolism of fatty acids are key ripening processes. The main lipolytic agents in cheese include lipoprotein lipase from raw milk, pregastric

Table 3. Fatty acids (%) changes of Jug cheese containing Iranian oak extract during cold storage (Mean±Standard deviation). ^{az}

Characteristics	Day treatment	0	15	30	45	60
Butyric acid	T0	^b 516.1	^b 523.1	^b 523.1	^b 546.1	^b 553.1
	T1	^a 673.1	^a 666.1	^a 650.1	^a 667.1	^a 660.1
Caproic acid	T0	^b 526.1	^b 533.1	^b 536.1	^b 546.1	^b 553.1
	T1	^a 740.1	^a 733.1	^a 756.1	^a 750.1	^a 760.1
Caprylic acid	T0	^c 050.1	^c 033.1	^c 036.1	^c 043.1	^c 053.1
	T1	^b 220.1	^{ab} 340.1	^{ab} 240.1	^a 260.1	^a 266.1
Capric acid	T0	^c 510.2	^{bc} 520.2	^{bc} 536.2	^{bc} 550.2	^c 560.2
	T1	^a 840.2	^a 833.2	^a 856.2	^a 873.2	^a 873.2
Lauric acid	T0	^c 130.3	^{bc} 146.3	^{bc} 150.3	^{bc} 160.3	^b 166.3
	T1	^a 353.3	^a 350.3	^a 356.3	^a 366.3	^a 376.3
Myristic acid	T0	^b 026.11	^b 02.11	^b 033.11	^b 033.11	^b 043.11
	T1	^a 370.11	^a 3806.11	^a 353.11	^a 376.11	^a 380.11
Palmitic acid	T0	^b 563.29	^b 543.29	^b 570.29	^b 556.29	^b 560.29
	T1	^a 016.30	^a 040.30	^a 043.30	^a 050.30	^a 043.30
Stearic acid	T0	^c 023.10	^{abc} 033.10	^{abc} 033.10	^{ab} 053.10	^a 056.10
	T1	^c 020.10	^{abc} 030.10	^{bc} 026.10	^{abc} 036.10	^{abc} 040.10
Oleic acid trans	T0	^b 133.1	^b 140.1	^b 126.1	^b 140.1	^b 140.1
	T1	^a 636.1	^a 630.1	^a 636.1	^a 653.1	^a 663.1
Oleic acid Cis	T0	^b 660.22	^b 676.22	^b 676.22	^b 670.22	^b 670.22
	T1	^a 153.21	^a 160.21	^a 133.21	^a 150.21	^a 156.21
Linoleic acid Trans	T0	^a 866.0	^a 856.0	^a 860.0	^a 860.0	^a 833.0
	T1	^a 833.0	^a 873.0	^a 863.0	^a 876.0	^a 833.0
Linoleic acid Cis	T0	^{bc} 646.2	^c 636.2	^{bc} 656.2	^{bc} 676.2	^b 683.2
	T1	^a 130.3	^a 130.3	^a 130.3	^a 130.3	^a 130.3
Linolenic acid Trans	T0	^c 043.0	^{bc} 046.0	^{abc} 050.0	^{abc} 050.0	^{abc} 053.0
	T1	^{abc} 060.0	^{abc} 053.0	^{ab} 066.0	^a 070.0	^{ab} 066.0
Linolenic acid Cis	T0	^a 300.0	^a 300.0	^a 266.0	^a 300.0	^a 333.0
	T1	^a 343.0	^a 336.0	^a 326.0	^a 340.0	^a 350.0
Arachidic acid	T0	^a 116.0	^a 120.0	^a 133.0	^a 0/136	^a 150.0
	T1	^{ab} 133.0	^{ab} 130.0	^{ab} 130.0	^{ab} 143.0	^a 150.0
Behenic acid	T0	^a 183.0	^a 183.0	^{ab} 156.0	^{ab} 160.0	^b 146.0
	T1	^b 133.0	^b 136.0	^{ab} 153.0	^b 146.0	^a 156.0

^a Means with different subscripts differ significantly ($P < 0.05$). O, T0, and T1 as under Table 1.

esterase in cheeses made using rennet paste, and enzymes from the starter and non-starter lactic acid microbiota (Collins *et al.*, 2003). A large amount of the natural milk lipase was destroyed by the pasteurization process, and no other lipase was added during the production process. Therefore, the lipolysis activity could be attributed to the effect of selective lipolysis and catabolism of fatty acids by microorganisms; and the observed differences between T0 and T1 could be explained by different microbial contents of the two samples. Due

to the antibacterial and antioxidant activity of the oak extract in T1, the growth of microorganisms and catabolism of fatty acids were prevented; therefore, the amounts of the mentioned fatty acids compared to the control sample were increased. These results were in accordance with the results of other researchers (Denise Silva Paula *et al.*, 2015; Granato *et al.*, 2018; Sarbazi *et al.*, 2014). In addition, results indicated that the percentage of these fatty acids decreases during the ripening of cheese. This could be attributed to the progress of catabolism of



fatty acids (Mallatou *et al.*, 2003; Collins *et al.*, 2003). Also, the decrease in the percentage of long-chain fatty acids, such as oleic acid, linoleic acid, etc. was parallel to the increase in short-chain fatty acids, which is in accordance with the results of Molimard and Spinnler (1996). Mallatou *et al.* (2003) reported that there was no significant difference in lauric acid content during the 40 days of cheese ripening, but a significant decrease was observed on the 60th day of storage. Palmitic and stearic acids are the most important fatty acids in ripened cheeses (Aminifar and Emam-Djomeh, 2014; Mallatou *et al.*, 2003).

Sensory Analyses

The flavor, aroma and overall acceptability scores for T1 were significantly higher than for T0 ($P < 0.05$). There was no significant difference between the texture of the two samples ($P > 0.05$). T1 had a significantly lower color score than the control ($P < 0.05$).

The effect of the alcoholic oak extract and the storage time on the sensory evaluation of cheese samples are shown in Table 4. The results of the sensorial evaluation indicated higher scores for flavor and aroma of T1 compared to T0 ($P < 0.05$) that could be related to the antioxidant activity of the oak extract, which can prevent oxidation of the sample containing the extract. In general, higher overall acceptability scores of T1 compared to the control sample ($P < 0.05$) indicated that it was more acceptable by the panelists. There was no significant difference between the texture of the two samples ($P > 0.05$), although the color score

of T1 was lower than T0 ($P < 0.05$). The variation of the color was due to the initial color of the oak extract, which had a reddish-brown color. Thus, the color could be considered as a limiting factor for the usage of the oak extract as a preservative ingredient in food products. Other researchers also confirmed the negative effect of oak on the color of some oak-fortified food products (Hojjati and Jooyandeh, 2017; Mahrous *et al.*, 2014). However, Majzoobi *et al.* (2013) reported a higher overall acceptability of bread samples containing oak flour compared to the control sample.

CONCLUSIONS

Results indicated that the usage of the alcoholic extract of Iranian oak (0.25% w/v) has the potential to improve the hygienic quality of Jug cheese due to its antibacterial and antioxidant properties. Because of the undesirable effect of the oak extract on the color of the product, it is probably more suitable for usage in flavored, colored food products. It could also be used in an encapsulated form to prevent color changes in products. In conclusion, the oak extract can be successfully employed to improve hygienic quality and the antioxidant properties of Jug cheese and provide sustained antioxidants during storage.

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Table 4. Sensory properties changes of Jug cheese containing Iranian oak extract on the 60th day of cold storage (Mean $\pm$ Standard deviation).^a

Characteristics treatment	Flavor	Aroma	Color	Texture	Overall acceptability
T0	2.8 $\pm$ 0.224 ^b	3 $\pm$ 0.189 ^b	5 $\pm$ 0.173 ^a	4 $\pm$ 0.574 ^a	3 $\pm$ 0.265 ^b
T1	4.8 $\pm$ 0.224 ^a	4 $\pm$ 0.447 ^a	2.4 $\pm$ 0.173 ^b	4.4 $\pm$ 0.574 ^a	4.8 $\pm$ 0.265 ^a

^a Means with different subscripts differ significantly ($P < 0.05$). O, T0, and T1 as under Table 1.

necessary facilities for conducting this research.

REFERENCES

1. Aghazadeh Meshgi, M. 2007. Evolution of Some Microbial and Chemical Properties of West Azerbaijan's Jug Cheese. *Food Sci. Nutri.*, **3**: 80-87.
2. Aminifar, M. and Emam-Djomeh, Z. 2014. Changes of Texture, Microstructure and Free Fatty Acid Contents of Lighvan Cheese during Accelerated Ripening with Lipase. *J. Agr. Sci. Tech.*, **16**: 113-123.
3. Arts, M., Haenen, G., Wilms, L., Beetstra, S., Heijne, C., Voss, H. and Bast, A. 2002. Interactions between Flavonoids and Proteins: Effect on the Total Antioxidant Capacity. *J. Agr. Food Chem.*, **50**: 1184-1187.
4. Bahrami, B., Alizadeh, M. and Hassanzadeh Ochtapeh, H. 2017. Kinetic Analysis of Antioxidant Changes in Domestic Cheese with Haven Extract Made in Clay Jugs during the Proteolysis Progress. *Iranian J. Nutri. Sci. Food Technol.*, **12(2)**: 87-95.
5. Bajalan, I., Javadian, M., Zarinkoob, S. and Dalvand, H. 2014. Antibacterial Activity of the Extract of Oak (*Quercus persica*) Fruits. *Life Sci.*, **3(5)**: 62-65.
6. Bchir, B., Bouaziz, M.A., Blecker, C. and Attia, H. 2019. Physico-Chemical, Antioxidant Activities, Textural, and Sensory Properties of Yogurt Fortified with Different States and Rates of Pomegranate Seeds (*Punica granatum* L.). *J. Texture Stud.*, **6**: 1-9.
7. Boddy, L. and Wimpenny, J. W. T. 1992. Ecological Concepts in Food Microbiology. *J. Appl. Bacteriol.*, **73**: 23-38.
8. Cho, W. Y., Kim, D. H., Lee, H.J., Yeon, S. J. and Lee, C. H. 2020. Quality Characteristic and Antioxidant Activity of Yogurt Containing Olive Leaf Hot Water Extract. *CYTA-J Food*, **18(1)**: 43-50.
9. Collins, Y. F., McSweeney P. L. and Wilkinson, M. G. 2003. Lipolysis and Free Fatty Acid Catabolism in Cheese: A Review of Current Knowledge. *Int. J. Dairy*, **13(11)**: 841-866.
10. Denise Silva Paula, T., Matumoto, P., Laurent, B., Charles, C. and Michel, B. 2015. Effect of Commercial Grape Extracts on the Cheese-Making Properties of Milk. *Dairy Sci.*, **98**: 1552-1562.
11. Dolci, P., Allesandria, V., Zeppa, G., Rantsiou, K. and Cocolin, L. 2008. Microbiological Characterization of Artisanal Raschera PDO Cheese Analysis of Its Indigenous Lactic Acid Bacteria. *J. Food Microbiol.*, **25(2)**: 392-399.
12. Ebadi Fathabada, A., Nabi Shariatifar, B., Mardania, K. and Mohammadpourfard, I. 2015. Study on Antibacterial and Antioxidant Activity of Oak Gall (*Quercus infectoria*) Extracts from Iran. *Int. J. Cur. Sci.*, **14**: 44-50.
13. Ebrahimi, A., Khayami, M. and Nejati, V. 2010. Evaluation of the Antibacterial Activity of *Quercus persica* Jaub & Spach Fruit's Hydroalcoholic Extract in Disc Diffusion Method. *J. Medicin. Plants*, **9(33)**: 26-34.
14. Ghaderi, M., Sadeghi Mahoonak, A., Alami, M., Khomeiri, M. and Mamashloo, S. 2013. Evaluation of Antimicrobial Activity of the Ethanolic Extracts from *Q. branti* and *Q. castaneifolia* Fruit against Some Foodborne Pathogens by Microdilution Method. *J Agric Eng Res.*, **14 (3)**: 83-96.
15. Granato, P., Masuda, T., Hidaka, K., Shinohara, A., Moekawa, T., Taked, Y. and Yamaguchi, H. 2018. On the Effect of Thyme Extract on Cheese Quality Characteristics. *Food Chem.*, **47(29)**: 71-77.
16. Hayaloglu, A. A. and Kirbag, S. 2007. Microbial Quality and Presence of Molds in Kuflu Cheese. *Int. J. Food Microbiol.*, **115(3)**: 376-380.
17. Helal, A. and Tagliazucchi, D. 2018. Impact of *In-Vitro* Gastro-Pancreatic Digestion on Polyphenols and Cinnamaldehyde Bioaccessibility and Antioxidant Activity in Stirred Cinnamon-Fortified Yogurt. *LWT-Food Sci. Technol.*, **89**: 164-170.
18. Hesaami-Rad, R. and Nezhad Razmjoo Akhgar, R. 2006. Persistence of *Escherichia coli* in West Azerbaijan's Jug Cheese: Security, Waste Reduction, Innovation. *16th National Congress of Iran Food Industry (1st Regional Congress, Gorgan Uni. Agric. Sci. Natural Res.*, PP. 23-24.
19. Hojjati, M. and Jooyandeh, H. 2017. Effect of Addition of Oak (*Quercus persica*) Flour to Iranian White Cheese. *Congress of Organic Food Products*, **2(5)**: 2-8.



20. ISIRI. 1992. Milk Fat: Preparation of Fatty Acid Methyl Esters-Test Method. ISIRI No 8818, Karaj, Iran.
21. ISIRI. 1998. Milk and Milk products: Detection of *Listeria monocytogenes*. ISIRI No 4524, Karaj, Iran.
22. ISIRI. 1999. General Method for Sensory Evaluation of Dairy Product. ISIRI No 4691, Karaj, Iran.
23. ISIRI. 2005. Microbiology of Food and Animal Feeding Stuffs: Detection and Enumeration of Presumptive *Escherichia coli* -Most Probable. ISIRI No 2946, Karaj, Iran.
24. ISIRI. 2006. Microbiology of Food and Animal Feeding Stuffs: Horizontal Method for the Enumeration of Coliforms -Colony-Count Technique. ISIRI No 9263, Karaj, Iran.
25. ISIRI. 2006. Microbiology of Food and Animal Feeding Stuffs: Horizontal Method for the Enumeration of Coagulase-Positive Staphylococci (*Staphylococcus aureus* and Other Species). Part 2. "Technique Using Rabbit Plasma Fibrinogen Agar Medium". ISIRI No 6806-2. Karaj, Iran.
26. ISIRI. 2006. Milk and Milk Products: Enumeration of Colony-Forming Units of Yeasts and/or Molds-Colony-Count Technique at 25°C. ISIRI No 10154, Karaj, Iran.
27. ISIRI. 2006. Milk and Milk Products: Determination of Titrable Acidity and Value pH-Test Method. ISIRI No 2852, Karaj, Iran.
28. ISIRI. 2009. Milk and Milk Products: Detection of Salmonella. ISIRI No 4413, Karaj, Iran.
29. ISIRI. 2014. Microbiology of the Food Chain: Horizontal Method for the Enumeration of Microorganisms. Part 1. "Colony Count at 30°C by the Pour Plate Technique". ISIRI No 5272-1, Karaj, Iran.
30. ISIRI. 2016. Microbiology of Milk and Milk Products Specifications and Test Methods. ISIRI No 2406, Karaj, Iran.
31. Jeong, C. H., Ryu, H., Zhang, T., Lee, C. H., Seo, H. G. and Han, S. G. 2018. Green Tea Powder Supplementation Enhances Fermentation and Antioxidant Activity of Set-Type Yogurt. *Food Sci. Biotechnol.*, **27(5)**: 1419-1427.
32. Kaur, R., Gonzalez, L., Bernardo, A., Fresno, J. M. and Tornadijo, M. E. 2004. Microbiological and Physico-Chemical Changes in Genestoso Cheese, a Spanish Acid Curd Variety, throughout Ripening. *J. Food Control.*, **15(4)**: 271-279.
33. Khosravi, A. and Behzadi A. 2006. Evaluation of the Antibacterial Activity of the Seed Hull of *Quercus brantii* on Some Gram-Negative Bacteria. *Pak. J. Med. Sci.*, **22**: 429-432.
34. Kim, D. H., Cho, W. Y., Yeon, S. J., Choi, S. H. and Lee, C. H. 2019. Effects of Lotus (*Nelumbo nucifera*) Leaf on Quality and Antioxidant Activity of Yogurt during Refrigerated Storage. *Korean J. Food Sci. Anim. Resour.*, **39(5)**: 792-803.
35. Mahrous, H., El-Kholy, V. and Elsanhoty, R. 2014. Production of New Synbiotic Yoghurt with Local Probiotic Isolate and Oat and Study Its Effect on Mice. *J. Adv. Dairy Res.*, **2(2)**: 2-7.
36. Majzoobi, M., Mortazavi, H., Yousef, S. and Farahnaki, E. 2013. Effects of Oak Flour on the Properties of Dough and Barbarian Bread. *J. Food Ind. Res. (University of Tabriz)*, **23(2)**: 275-283.
37. Mallatou, A., Sigolo, S. and Pira, G. A. 2011. Comparative Study of Fatty Acid Composition and CLA Concentration in Commercial Cheese. *J. Food Compos. Anal.*, **24**: 55- 61.
38. Mallatou, H., Pappa, E. and Massouras, T. 2003. Changes in Free Fatty Acids During Ripening of Teleme Cheese Made with Ewes', Goats', Cows' or Mixture of Ewes' and Goats' Milk. *Int. Dairy J.*, **13**: 211-219.
39. Masoodi Tonkaboni, P., Hesari, S., Damiri, P. and Mohamadi, P. 2013. Effect of Adding Green Tea Extract on Dough Properties. *Food Nutri. Sci.*, **85(15)**: 33-45.
40. McCuea, P. P. and Shetty, K. 2005. Phenolic Antioxidant Mobilization during Yogurt Production from Soymilk Using Kefir Cultures. *J. Proc. Biochem.*, **40**: 1791-1797.
41. Mirzaei, H., Ghiasi Khosroshahi, A. and Karim, G. 2008. The Microbiological and Chemical Quality of Lighvan Cheese (White Cheese in Brine) Produced in Tabriz. *Iran. J Anim. Vet. Adv.*, **7(12)**: 1594-1599.
42. Molimard, P. and Spinnler, H. 1996. Review Compounds Involved in the Flavor of Surface Mold-Ripened Cheeses Origins and Properties. *Dairy Sci.*, **79(2)**: 169-184.
43. Muniandy, P., Shori, A. B. and Baba, A. S. 2016. Influence of Green, White and Black Tea Addition on the Antioxidant Activity of

- Probiotic Yogurt during Refrigerated Storage *Food Packag. Shelf Life*, **8**: 1–8.
44. Nedamani, E., Mahoonak, A., Ghorbani, M. and Kashaninejad, M. 2014. Antioxidant Properties of Individual vs. Combined Extracts of Rosemary Leaves and Oak Fruit. *J. Agr. Sci. Tech.*, **16**: 1575-1586.
 45. Nourafcan, H., Nasrollahpourm, M. and Bajalan, I. 2013. Antibacterial Activity of Leaves Extract from Oak (*Quercus persica*) Against Some Positive and Negative Bacteria. *Int. J. Farm. Alli. Sci. (IJFAS)*, **2(24)**: 1153-1155.
 46. Oksuz, T., Tacer-Caba, Z., Nilufer-Erdil, D. and Boyacioglu, D. 2019. Changes in Bioavailability of Sour Cherry (*Prunus cerasus* L.) Phenolics and Anthocyanins When Consumed with Dairy Food Matrices. *J. Food Sci. Technol.*, **56(9)**: 4177-4188.
 47. Osman, N. F., Thiboutot, H. E. and Dako, M. 2008. The Impact of Lactic Acid Bacteria on Cheese Flavor. *J. FEMS Microbiol. Rev.*, **87**: 131-148.
 48. Ozdal, T., Capanoglu, E. and Altay, F. 2013. A Review on Protein–Phenolic Interactions and Associated Changes. *Food Res. Int.*, **51(2)**: 954–970.
 49. Rabiei, M., Zarrini, Gh. and Mahdavi, M. 2018. Cytotoxic Effects of Lactobacilli Isolated from Azerbaijan Traditional Cheeses on Colorectal Tumor Cells HCT 116 and Identification of Paramount Strains. *J. Ardabil Uni. Med. Sci.*, **18(2)**: 191-203.
 50. Rzaei, A., Khosrowshahi, A., Zomorodi, Sh. and Malekinajad, H. 2013. Effect of Addition of Sodium Caseinate and Peppermint Extract on Viability of *Lactobacillus casei* and Physicochemical Properties and Antioxidant Activity of Non-Fat Probiotic Yogurt. *Food Ind. Res.*, **23(3)**: 423-434.
 51. Roshani, A., Bahrami, B., Alizadeh, M. and Hassanzadeh Ochtapeh, H. 2016. Effect of Hun Extract on Mozzarella Cheese. *Iranian J. Nutri. Sci. Food Technol.*, **12(2)**: 87-95.
 52. Rotaro, A. and Clementi, F. 2008. Exopolysaccharides from Lactic Acid Bacteria: Structure, Production and Technological Applications. *Italian J. Food Sci.*, **1**: 23-45.
 53. Sakar, Z., Karahan, G. and Aand Aloglu, H. 2005. Changes in the Microbiological and Chemical Characteristics of an Artisanal Turkish White Cheese during Ripening. *LWT- Food Sci. Technol.*, **39(5)**: 449–454.
 54. Sadeghian, I., Hassanshahian, M., Sadeghian, S. and Jamali, S. H. 2012. Antimicrobial Effects of *Quercus brantii* Fruits on Bacterial Pathogens. *Jundishapur J. Microbiol.*, **5(3)**: 465-469.
 55. Sánchez-Bravo, P., Zapata, P., Martínez-Esplá, A., Carbonell-Barrachina, A. and Sendra, E. 2018. Antioxidant and Anthocyanin Content in Fermented Milks with Sweet Cherry is Affected by the Starter Culture and the Ripening Stage of the Cherry. *BEV*, **4(3)**: 57-69.
 56. Sarbazi, M., Hesari, J., Azadmard-Dmirchi, S. and Rafat, S. A. 2014. Effect of Kind of Packaging Container and Storage Temperature on Some Physicochemical and Sensory Properties of Kope. *Tarbiat Modares Uni. Press Food Sci. Technol.*, **11(43)**: 91-101.
 57. Sefidgar, A. A., Taghizadeh, A., Pournajaf, A., Ardebili, A., Omid, S. and Abdian, A. 2015. Evaluation of Antimicrobial Activity of Alcoholic and Aqueous Extracts from Common Hop (*Humulus lupulus*) and Oak (*Quercus castaneifolia*). *Arak Med. Uni. J. (AMUJ)*, **17(93)**: 39-46.
 58. Senadeera, S. S., Prasanna, P. H. P., Jayawardana, N. W. I. A., Gunasekara, D. C. S., Senadeera, P. and Chandrasekara, A. 2018. Antioxidant, Physicochemical, Microbiological, and Sensory Properties of Probiotic Yoghurt Incorporated with Various *Annona* Species Pulp. *Heliyon.*, **4**: 1-19.
 59. Shahrabi, Sh., Fazlara, A. and Zand moghaddam, A. 2017. The Effect of *Satureja khuzestanica* Essential Oil on *E. coli* in Iranian White Cheese. *Food Microbiol.*, **4(1)**: 61-51.
 60. Taran, M., Azizi, E. and Sharifi, M. 2010. Antibacterial and Antifungal Activity of Hydroalcoholic and Etheric Extracts of *Quercus branti*. *J. Microb. Biotechnol.*, **2(4)**: 7-12.
 61. Trigueros, L., Wojdyło, A. and Sendra, 2014. E. Antioxidant Activity and Protein–Polyphenol Interactions in a Pomegranate (*Punica granatum* L.) Yogurt. *J. Agr. Food Chem.*, **62(27)**: 6417-6425.



عصاره الکلی (*Quercus persica* Jaub. & Spach) به عنوان یک نگهدارنده طبیعی فرا سودمند برای بهبود کیفیت بهداشتی پنیر کوزه

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چکیده

در این پژوهش، عصاره الکلی بلوط ایرانی (*Quercus Persia*) با غلظت 0/25 درصد (وزنی/حجمی) به شیر پنیرسازی افزوده شد و برخی ویژگی‌های فیزیکوشیمیایی، حسی و میکروبی نمونه‌های پنیر تولیدی طی نگهداری 60 روزه در 4 درجه سانتی گراد مورد ارزیابی قرار گرفت. نتایج نشان داد که نمونه‌ی حاوی عصاره بلوط (T1) در قیاس با نمونه شاهد (T0) به طور معناداری دارای محتوای پلی فنل کل و فعالیت ضد اکسیدانی بالاتر و اسیدیته پایین تری بود. در شروع زمان نگهداری، بالاترین عدد محتوای پلی فنل کل (128.83 ± 0.467 mg GAE/g) و فعالیت ضد اکسیدانی ($97.12 \pm 0.095\%$) بر اساس فعالیت جذب رادیکال DPPH (2 و 2، دی فنیل - 1- پیکریل هیدرازیل) به T1 اختصاص داشت؛ فعالیت ضد اکسیدانی طی زمان نگهداری به طور معناداری کاهش یافت؛ امتیازات طعم، بو و پذیرش کلی در T1 بیشتر از T0 بودند. محتوای اسیدهای چرب زنجیر کوتاه در پنیر طی زمان نگهداری متغیر بود ولی تغییر معناداری در محتوای اسیدهای چرب زنجیر بلند مشاهده نگردید. میزان اسیدهای چرب بوتریک، کاپروئیک، کاپریلیک، کاپریک، لوریک، میریستیک، پالمیتیک، اولئیک (ترانس) و لینولئیک (سیس) در T1 به طور معناداری بالاتر از T0 بود. هیچ تغییر معناداری در محتوای این اسیدهای چرب طی زمان نگهداری مشاهده نگردید. نتایج ارزیابی میکروبی حاکی از پایین تر بودن معنادار شمارش کلی-میکروبی، کلی-فرم‌ها و کپک و مخمر در T1 در قیاس با T0 بود. به طور کلی، عصاره بلوط ایرانی به عنوان منبع ارزشمندی از ترکیبات پلی فنلی ضد اکسیدانی و ضد میکروبی بدون تأثیر منفی بر خواص حسی و فیزیکوشیمیایی پنیر کوزه تولیدی، می‌تواند زمان ماندگاری آن را افزایش دهد.