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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 (Sarbazi *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; Majzoobi *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

Titratable 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

Titratable Acidity

Using the Iranian oak extract caused a significant ($P< 0.05$) decrease in titratable acidity (Table 2). The cheese samples containing the oak extract (T1) exhibited a lower titratable acidity (0.27±0.01%) than the control (T0) samples (0.35±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±0.01%) after 60 days of storage at 4°C in comparison with T1 (0.4±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}

Characteristics	Day treatment	0	15	30	45	60
Salmonella Listeria coagulase-positive staphylococci *Staphylococcus aureus*	T0	Negative	Negative	Negative	Negative	Negative
	T1	Negative	Negative	Negative	Negative	Negative
Escherichia coli	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}	3.2×102±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}

Characteristics	Day treatment	0	15	30	45	60
Titratable 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; Razaei *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; Mallatov *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 ± Standard deviation).[a]

Characteristics treatment	Flavor	Aroma	Color	Texture	Overall acceptability
T0	2.8±0.224^{b}	3±0.189^{b}	5±0.173^{a}	4±0.574^{a}	3±0.265^{b}
T1	4.8±0.224^{a}	4±0.447^{a}	2.4±0.173^{b}	4.4±0.574^{a}	4.8±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.

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عصاره الکلی (*Quercus persica* Jaub. & Spach) به عنوان یک نگهدارنده طبیعی فراسودمند 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 نمونه های پنیر تولیدی طی نگهداری 60 روزه در 4 درجه سانتی گراد مورد ارزیابی قرار گرفت. نتایج نشان داد که نمونه ی 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±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 اسید های چرب زنجیر کوتاه در پنیر طی زمان نگهداری متغیر بود ولی تغییر معناداری در محتوای اسید های چرب زنجیر بلند مشاهده نگردید. میزان اسید های 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 اسید های چرب طی زمان نگهداری مشاهده نگردید. نتایج ارزیابی میکروبی حاکی از پایین تر بودن معنادار شمارش کلی¬میکروبی، کلی¬فرم¬ها 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 فیزیکو شیمیایی پنیر کوزه تولیدی، می تواند زمان ماندگاری آن را افزایش دهد.