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Effects of Mozafati, Piaroum, Zahedi date extracts and their combination on the chemical, microbial and sensory properties of farmed rainbow trout (*Oncorhynchus mykiss*) fillets during refrigeration (4˚C)

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Abstract

Rainbow trout fillet is susceptible to microbial and oxidative spoilage. Therefore, it is essential to use preservatives to extend its shelf life. Date extract has significant antibacterial and antioxidant properties. This research was conducted to study the effect of aqueous date extracts on chemical, microbial and sensory properties of farmed rainbow trout during refrigeration. Total phenolic and flavonoid content were determined through Folin-Ciocalteu and colorimetric method. The first step of study was performed for determination the antimicrobial activity of date extracts against the inherent flora of fish fillet, lactic acid bacteria, *Pseudomonas* and *Enterobacteriaceae* by disc diffusion method. In parallel with antimicrobial tests, sensory evaluation was also performed for choosing the best concentration of extracts in order to applying on fish fillets. In the second step of study, fish fillet samples were immersed in date extracts (3% w/v for 5 minute), packaged in zip–bags and stored at 4 ^{o}C over a period of 17 days. The extract-free fillet was used as control. The samples were analyzed for microbiological (mesophilic, lactic acid bacteria, *Pseudomonas* and *Enterobacteriaceae* counts) and chemical (PV, TBARS and TVB-N) parameters. The 5-point hedonic method was carried out for sensory evaluation by 30 trained panelists. Analyses were conducted at 2 h after preparation and 1, 3, 5, 7, 9, 11, 13, 15 and 17 days of storage. Phenolic and flavonoids contents showed no significant differences between date extracts (p>0.05). Total bacterial counts, *Enterobacteriaceae*, *Pseudomonas*, TBARS, PV and TVB-N showed no significant difference in test samples (p>0.05). These parameters were within an acceptable range up to 15 days for test samples while the control samples had a shelf life of 5 days. Samples preserved by Piaroum extract had the longest shelf life while samples preserved by the combination of extracts had the shortest. According to the results, the Piaroum, Zahedi, Mozafati date extracts and their combination could be used as natural preservatives for trout fillet shelf-life extension.

Keywords: Chemical composition, Date, Extract, Farmed rainbow trout, Microbial quality, Sensory evaluation.

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Introduction

Spoilage of food products can be occurred due to chemical, enzymatic or microbial activities. One-fourth of the world's food supply and 30% of landed fish are lost through microbial activity alone. Chemical deterioration and microbial spoilage are responsible for loss of 25% of fishery products every year (Baird-Parker, 2000). Around 4-5 million tons of trawled fish are lost every year due to enzymatic and microbial spoilage because of improper onsite storage (Unklesbay, 1992). With world population growing and the need to store and transport of food, it is a great attention to increase the shelf life and maintains nutritional value of food products. Preservation techniques can improve the quality of fish and fish products and increase their shelf life (Ghaly *et al.*, 2010). These techniques include low temperature storage (Ashie *et al.*, 1996), controlling water activity (Abbas *et al.*, 2009), phenolic antioxidants (Davidson, 1993), using the preservatives and lactic acid bacteria (Doores, 2005). Some scientific reports suggested that excessive consumption of synthetic preservatives might have negative effects on human health (Jay, 2013). Reports on health risks linked to chemical preservatives in foods have made consumers return to fresh organic products.

Rainbow trout (*Oncorhynchus mykiss*) is a native species of North America and Russia which has been widely farmed as a recreational and food fish around the world (Rahimzade *et al*., 2019). Trout is regarded as marketable and premium fish in the fishing industry. The rainbow trout is a valuable commercial species. The good nutritional value and distinctive taste of its flesh, caused that it has attracted by many consumers. It makes a major contribution to the food supply not merely because of its high nutritional value but also because of being rich in unsaturated fats, which are necessary for a healthy diet. Fresh fish are highly perishable compared with other food products. Their spoilage is associated with decreased marketability and consumer concerns.

Cultivation of palm trees has gone back to 4000 BC. Date constitutes one of the most significant species within palm family. Date embrace around 200 genera along with 2500 species. There are about 400 species of dates growing in Iran (Ashraf and Hamidi Esfahani, 2011). Iran is the second major producer of dates with 14% of total world date production. Nevertheless, there has, so far, been no extracts made from dates (El Hadrami and Al-Khayri, 2012). Millions of people throughout centuries have consumed dates as staple food. Various kinds of dates such as Mozafati (*Phoenix dactylifera*), Piaroum (*P. dactylifera*) and Zahedi (*P. dactylifera*) have also certain biogenic characteristics that distinguish them from one another. Since dates have dietary fiber and phenolic compounds, they can be consumed as functional food (Hadrami and Al-Khayri, 2012). Beside high consumption of fresh date, up to now there is a few studies about using the date extract as natural product in food products.

Date extract is a natural substance that possesses antioxidant and antimicrobial properties (Ashraf and Hamidi Esfahani, 2011). Present research was conducted to study the effect of aqueous date extracts of Mozafati, Piaroum and Zahedi and their

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combination on farmed rainbow trout fish quality, chemical, microbial and sensory properties during refrigeration.

Materials and methods

Extraction of date aqueous extract

For preparation of date extracts, the dates (100 g) were first immersed in distilled water (200 ml) for 72 h in the dark at the refrigeration temperature. Then, the solution was mixed with a mixer and filtered using filter paper No. 1. The suspension was centrifuged using a refrigerated centrifuge at 3000 rpm for 15 min. The supernatant was pasteurized at 65 °C for 30 min and finally dried by rotary evaporator at 60 ^{o}C. The extracts were stored at refrigeration until use (Mehdipour *et al.*, 2017).

Biochemical analysis of date extracts

The chemical composition of the date extracts was evaluated by determining the total flavonoid and total polyphenolic contents. The total flavonoid content was measured using the colorimetric method and the quercetin standard linear equation (y=0.27x-0.22) by Nano drop (Thermo science) at a wave length of 410 nm. The total polyphenolic content was determined using the colorimetric Folin-Ciocalteu method and the gallic acid standard linear equation (y=0.2x-0.1) by Nano drop at a wave length of 760 nm (Salmanian *et al.*, 2013). The standard reference method was applied to determine the moisture content (Iranian National Standardization No 672, 2015). The tests were repeated three times.

The potential usefulness of date extract as antimicrobials for fish preservation

This step was conducted at two stages including microbial and sensory tests. To detect the sensitivity of the natural flora of trout to date extracts, a homogenate from chilled trout stored for 10 days at 5 ^{o}C, was obtained. The homogenate was prepared by mixing 25 g of fish flesh with 225 ml of buffered 0.1% peptone water and homogenized for 5 min. Then, 10 µl of this homogenate was inoculated on Muller Hinton agar and the Plates were refrigerated for 2 h. Then, 10 µl of Mozafati, Piaroum and Zahedi extracts were transferred on each disc and the discs were placed on Muller Hinton agar. Plates were incubated at 37 ^{o}C for 72 h. This experiment was repeated three times (Gómez-Estaca *et al.*, 2010).

The antibacterial activity of date extracts also studied against *Pseudomonas*, *Enterobacteriaceae* and lactic acid bacteria. The mentioned bacteria were cultured in nutrient broth and incubated at 37 ^{o}C for 24 h. After this time, their turbidity was compared with MacFarland 0.5. Then, 10 µl of bacteria was spread on the Muller Hinton Agar and the Plates were refrigerated for 2 h. Then, 10 µl of 1–5% concentrations of Mozafati (MDE), Piaroum (PDE) and Zahedi (ZDE) extract was transferred on each disc and the discs were placed on Muller Hinton agar. Plates were incubated in 37 ^{o}C for 72 h. This step was repeated three times.

Sampling

70 kg of farmed trout caught in spring were used for this study. The fish were chilled down to zero ^{o}C under an icy cover (the ratio of ice to fish was 2:1). Before

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being processed, fish were washed with chlorinated water. Then, the heads were cut; the fins and viscera were taken out. The cleaned fish were washed again.

Fish storage trial

The current study was included one control group and four treatment groups. The treatment groups were trout fillets immersed in date aqueous extracts namely Mozafati date extract (MDE), Piaroum date extract (PDE), Zahedi date extract (ZDE) and their combination (MPZE). They were kept immersed for 5 minutes. The extracts were provided at a concentration of 3% (3 g powder in 100 ml water). The fillets (with skin, deboned and headless) were packaged in zipper bags in 200 g pieces. The packages were refrigerated at a temperature of 4 ^{o}C for seventeen days. These samples were processed three times. The extract-free fillet was used as control sample. Its packaging and storage processes were similar to those of the experimental samples.

Bacterial analysis

The microbial quality of the experimental and control samples was evaluated by determining the total bacterial counts, *Enterobacteriaceae*, lactic acid bacteria and *Pseudomonas* counts. *Pseudomonas* bacteria (Institute of Standards and Industrial Research of Iran No.4791, 1998) and lactic acid bacteria (Institute of Standards and Industrial Research of Iran No 17164, 2014) were cultured using surface method on Cetrimide agar and MRS agar, respectively. The total bacterial counts (Andrews and Hammak, 2003; Maturin, 2001) and *Enterobacteriaceae* counts (Center Food Safety, 2014) were determined using pour-plate and double-layer-plate on the Plate count agar and VRBG agar, respectively. Sampling was carried out 2 h after, 1, 3, 5, 7, 9, 11, 13, 15 and 17 days after the beginning of the process of refrigeration. Each step of the tests was repeated three times.

Chemical analysis

The chemical parameters including peroxide value (Iranian National Standard No 493, 2003), TVB-N (Iranian National Standard No 5625, 2002) and TBARS (Iranian National Standard No 10494, 2006) were measured for the test and control samples. Sampling for these tests was the same as in the previous step.

Sensory analysis

Sensory scores of tissue, odor, color, taste and overall acceptance were determined for the test sample and control samples. The 5-point hedonic method was used for sensory evaluation (Gilbert, 2013). Numbers 1, 2, 3, 4 and 5 show poor, average, good, very good and excellent quality, respectively. Sensory tests were performed at two stages. The first stage was conducted to choose the most effective concentration of date extracts from a sensory point of view. The next one was performed during refrigeration. This step was performed by 30 evaluators (15 men and 15 women aged 25 – 30 years). Sensory tests were carried out on the test and control samples three times at each sampling time.

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Statistical analysis

The results of microbial, chemical, sensory tests, Total flavonoid and total polyphenolic contents were analyzed by SPSS 17 Software. One-way, two-way, Tukey and T-tests were used in our study.

Results

Results of total flavonoid and total polyphenolic contents of the date extracts were shown in Table 1. As can be seen, the total flavonoid and polyphenolic contents of the Piaroum extract were higher than those of the Zahedi and Mozafati extracts (p>0.05). Nevertheless, there was no significant difference between the date extracts with respect to the total flavonoid and polyphenolic contents (p>0.05).

Table 1: Total flavonoid (Querstin 100ml^{-1}) and total polyphenolic contents (Gallic acid 100ml^{-1}) of the date extracts (Values are mean+standard deviation).

Index \ Date extract	Mozafati	Piaroum	Zahedi
Total flavonoids (Querstin 100 ml^{-1})	2.15±0.02^{a}	3.22± 0.03 ^{a}	2.26±0.05^{a}
Total polyphenols (Gallic cid 100 ml^{-1})	1.87±0.03^{a}	2.49±0.01^{a}	1.88±0.02^{a}

Different letters in the same columns indicate significant differences (p<0.05). Same letters in the same columns indicate no significant differences (p>0.05).

Table 2 shows the effects of different concentrations (1 – 5%) of date extracts on lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonas* bacteria, and fish flesh flora by disc diffusion method. Significant differences were observed between different concentrations (p<0.05). 5% and 1% concentrations of Mozafati, Piaroum and Zahedi extracts showed most and least antibacterial effects on lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonas* bacteria and fish flesh flora.

Table 2: Effects of different concentrations (1–5%) of date extracts on lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonas* bacteria, and fish flesh flora by disc diffusion method (mm) (Values are mean + standard deviation).

Date extract	Mozafati extract (%)					Piaroum extract (%)					Zahedi extract (%)				
Concentration	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Lactic acid bacteria	18±1.94e	22±1.19d	27±2.25c	30±2.19b	32±1.99a	17±1.35E	20±2.29d	24±1.97c	28±2.23b	31±2.34a	18±2.31d	21±1.76c	25±1.25b	27±1.15a	28±2.12a
Enterobacteriaceae	17±2.13d	21±1.67c	25±2.27b	26±2.16b	29±1.78a	19±1.26d	22±2.32c	26±1.92b	27±1.94b	29±2.64a	20±2.54d	23±1.61c	27±1.19b	29±1.61a	30±2.91a
Pseudomonas bacteria	19±2.41d	23±2.31d	26±1.91c	28±2.13b	30±1.67a	18±1.47d	22±1.98c	27±1.83b	29±1.38a	30±2.53a	19±2.28d	22±1.43c	26±2.21b	27±1.73b	29±2.43a
fish flesh flora	16±1.89e	19±1.65d	20±1.56c	22±1.34b	24±2.36a	15±1.94d	17±2.34c	20±2.15b	22±1.77a	23±1.99a	14±2.11d	16±1.57c	19±2.27b	20±1.30b	22±2.35a

Different letters in the same columns indicate significant differences (p<0.05). Same letters in the same columns indicate no significant differences (p>0.05).

Sensory evaluation of trout treated with different concentrations (1–5%) of date extracts were showed in Table 3. Different concentrations showed significant differences on the sensory evaluation of trout fillet (p<0.05). 3% concentration showed the best sensory evaluation (color, odor, taste, texture and overall acceptance) compared with the other samples. Therefore, 3% concentration was considered for fish fillet preservation.

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Table 3: Sensory evaluation of trout treated with different concentrations (1–5%) of date extracts (Values are mean+standard deviation).

Date extract	Mozafati extract (%)					Piaroum extract (%)					Zahedi extract (%)				
Concentration	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Taste	3.11±1.24^{b}	3.15±1.71^{b}	4.96±1.18^{a}	2.64±1.32^{c}	2.43±1.21^{c}	3.26±2.12^{b}	3.54±1.99^{b}	4.98±1.34^{a}	2.55±1.41^{c}	2.49±1.76^{c}	3.36±1.89^{b}	3.41±1.81^{b}	4.91±1.32^{a}	2.25±1.17^{c}	2.19±1.13^{c}
Odor	5±1.87^{a}	5±1.56^{a}	5±1.27^{a}	5±1.38^{a}	5±1.12^{a}	5±2.14^{a}	5±1.92^{a}	5±1.46^{a}	5±1.42^{a}	5±1.56^{a}	5±1.41^{a}	5±193^{a}	5±1.47^{a}	5±1.21^{a}	5±1.43^{a}
Color	5±1.67^{a}	5±1.34^{a}	5±1.94^{a}	5±1.92^{a}	5±1.15^{a}	5±1.16^{a}	5±1.07^{a}	5±1.24^{a}	5±1.39^{a}	5±1.74^{a}	5±1.30^{a}	5±1.48^{a}	5±1.72^{a}	5±1.36^{a}	5±1.78^{a}
Texture	5±1.59^{a}	5±1.93^{a}	5±1.89^{a}	5±1.79^{a}	5±1.34^{a}	5±1.37^{a}	5±1.76^{a}	5±1.57^{a}	5±1.14^{a}	5±1.38^{a}	5±1.13^{a}	5±1.42^{a}	5±1.63^{a}	5±1.42^{a}	5±1.91^{a}
Overall acceptance	3.16^{b}	3.34^{b}	4.95^{a}	2.72^{c}	2.62^{c}	3.12^{b}	3.17^{b}	4.99^{a}	2.59^{c}	2.14^{c}	3.19^{b}	3.32^{b}	4.89^{a}	2.17^{c}	2.15^{c}

Different letters in the same columns indicate significant differences (p<0.05). Same letters in the same columns indicate no significant differences (p>0.05).

Acceptable limit of *Enterobacteriaceae*, total bacterial counts and *Pseudomonas* counts are 4, 7 and 6 log CFU.g^{-1}, respectively (Erkan, 2007; Center for Food Safety, 2014). The samples were ranked as follows in descending order, from the highest to the lowest bacterial count: Control, Mozafati, Zahedi, Piaroum and their combination. As the refrigeration time passed, from the initial to the last sampling stage, bacterial population counts increased in all the samples significantly (p<0.05). *Pseudomonas*, *Enterobacteriaceae* and the total bacterial counts were within an acceptable range for 15 days of the 17-days refrigeration. Lactic acid bacteria were within an acceptable range in control samples for 9 days, samples preserved by combination of extracts for 11 days, samples preserved by Mozafati and Zahedi extracts for 13 days and samples preserved by Piaroum extract for 15 days. Lactic acid bacteria was higher in samples treated by combination extracts, Mozafati, Zahedi and Piaroum extracts, respectively. Bacterial population counts were lower in the groups of Mozafati, Piaroum, Zahedi, and their combination, compared with the control group (p<0.05). Total bacterial counts, *Enterobacteriaceae* and *Pseudomonas* counts showed no significant difference in samples preserved by Mozafati, Piaroum, Zahadi extracts and their combination (Tables 4 and 5).

Table 4: Total bacterial counts and *Enterobacteriaceae* counts of date extract treated trout and control samples during refrigeration (log CFU g^{-1}) (Values are mean + standard deviation).

Index	Total bacterial counts					Enterobacteriaceae				
Sampling time										
Treatment	MDE	PDE	ZDE	MPZDE	Control	MDE	PDE	ZDE	MPZDE	Control
2 h	3.19±1.17gA	3.16±1.95fA	3.12±1.45iA	3.11±1.36hA	3.95± 1.12eA	2.15±1.21fA	2.17±1.32fA	2.23±1.14fA	2.11±1.18fA	2.46±1.27fA
1 day	3.23±0.96g	3.21±1.12f	3.18±1.13hi	3.18±1.67gh	4.12±1.19e	2.36±1.23f	2.43±1.58ef	2.55±1.88ef	2.39±1.57ef	2.85±1.15f
3 days	3.56±1.23fg	3.55±1.16ef	3.51±1.26gh	3.52±1.54fg	5.16±1.53d	2.54±1.89ef	2.68±0.93e	2.64±1.19e	2.47±0.91e	3.47±1.13e
5days	3.89±1.12f	3.90±0.96e	3.93±.93fg	3.87±1.84f	6.87±1.97c	2.86±1.78de	2.95±0.99de	2.89±1.7de	2.69±0.97de	3.89±1.34e
7 days	4.17±1.45ef	4.13±0.85de	4.15±1.29ef	4.10±1.43e	8.93±2.45b	3.12±1.73cd	3.18±1.13cd	3.18±1.5cd	2.96±1.42cd	4.86±1.56d
9 days	4.56±1.36de	4.54±0.79cd	4.59±1.27de	4.62±1.87d	9.14±2.26b	3.23±1.38c	3.49±1.46bc	3.34±1.24c	3.21±1.31c	5.16±1.69d
11 days	4.98±1.14d	4.99±1.39c	4.91±1.57d	4.89±1.59d	9.24±1.24b	3.47±1.48bc	3.56±1.98b	3.45±1.43c	1111	6.32±1.87c
13 days	5.74±1.89c	5.78±1.54b	5.83±1.37c	5.74±1.29c	9.35±1.35ab	3.78±1.51ab	3.74±1.41ab	3.73±1.92bc	3.45±1.99b	7.56±1.59b
15 days	6.99±1.54b	6.95±1.46b	6.94±1.46b	6.97±1.47b	9.65±1.16a	3.93±1.39a	3.83±1.76a	3.95±1.29b	3.69±1.77ab	7.93±1.31ab
17 days	7.78±1.78a	7.53±1.67a	7.61±1.97a	7.58±1.89a	9.76±2.15a	4.18±1.17a	4.16±1.72a	4.43±1.16a	4.10±2.25a	8.12±2.15a

Different letters in the same columns indicate significant differences (p<0.05). Same letters in the same columns indicate no significant differences (p>0.05).

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Table 5: *Pseudomonas bacteria* and Acid lactic bacteria counts of control and test samples during refrigeration (log CFU g^{-1}) (Values are mean + standard deviation).

Index / Sampling time	*Pseudomonas bacteria*					Acid lactic bacteria				
Treatment	**MDE**	**PDE**	**ZDE**	**MPZDE**	**Control**	**MDE**	**PDE**	**ZDE**	**MPZDE**	**Control**
2 h	2.87±0.96gA	2.93±1.80fA	2.96±1.71eA	2.85±1.72fA	3.34±1.67gA	3.14±1.24fA	2.17±1.33gA	3.18±0.94gA	3.36±0.56gA	2.16±0.83gA
1 day	2.91±0.93g	2.95±1.81f	3.11±1.63e	2.96±1.84f	3.73±1.56fg	3.19±1.47f	2.29±1.34g	3.42±0.99g	3.52±0.81g	2.23±0.97g
3 days	3.87±0.97f	3.97±1.93e	3.91±1.74d	3.71±1.33f	4.12±1.46f	4.21±1.78e	2.75±1.18f	4.53±0.96f	5.21±0.92f	2.78±0.93f
5days	4.35±0.91e	4.29±1.19e	4.37±173cd	4.25±1.32e	5.95±1.45e	4.87±1.29d	3.84±1.88e	5.16±1.36e	5.91±1.18e	3.90±2.12e
7 days	5.16±0.92d	5.11±1.31d	4.85±1.21c	4.91±1.87d	7.45±1.81d	5.36±1.35c	5.14±1.91d	5.81±1.82d	6.32±1.51de	5.12±1.97d
9 days	5.48±1.12cd	5.35±1.23cd	5.24±1.91c	5..27±1.96cd	8.12±1.48c	5.89±1.11b	5.76±1.32c	6.13±1.52d	6.57±1.63cd	6.78±1.44c
11 days	5.67±1.42bc	5.69±1.87bc	5.49±1.82bc	5.46±1.98bc	8.97±2.68b	6.17±2.27b	6.14±2.34c	6.43±2.56cd	6.98±1.17bc	7.15±1.63c
13 days	5.86±1.76b	5. 76±1.54b	5.73±1.90b	5.68±2.89b	9.13±2.73b	6.75±1.99a	6.53±2.87b	6.87±2.27bc	7.14±1.56ab	7.73±1.84b
15 days	5.97±1.89b	5.95±1.86b	5.96±1.89b	5.89±1.51b	9.45±2.71ab	7.14±2.14a	6.94±2.83ab	7.12±2.13b	7.28±1.45a	7.95±1.72ab
17 days	7.10±1.10a	7.13±1.24a	7.19±1.28a	7.17±1.15a	9.83±1.12a	7.21±1.13a	7.13±1.27a	7.84±1.23a	7.48±1.34a	8.32±1.38a

Different letters in the same columns indicate significant differences (p<0.05). Same letters in the same columns indicate no significant differences (p>0.05).

Acceptable range of TBARS, TVB-N, peroxide are 1 mg.kg^{-1}, 25 mg.100 g^{-1}, 5 meq.kgoil^{-1}, respectively (Gill, 1990; Kilincceker *et al.*, 2009; Seifzadeh, 2014). As the refrigeration time passed, from the initial to the last sampling stage, PV value, TBARS value and range for 5 days. PV and TBARS showed no significant difference in the test samples during the first 3 days. The amount of chemical parameters was lower in the groups treated by date extracts compared with the control group (p<0.05). However, these parameters showed no significant difference in samples treated with Mozafati, Piaroum, Zahedi extracts and their combination (p>0.05).

TVB-N increased in all the samples significantly (p<0.05). The chemical parameters were within an acceptable range for 15 days. In control samples, PV and TBARS values were within an acceptable (Tables 6 and 7).

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Table 6: PV and TBARS of control and date extract treated trout during refrigeration (Values are mean + standard deviation).

Index	PV value (meq kgoil $^{-1}$)					TBARS (mg kg^{-1})				
Sampling time / Treatment	MDE	PDE	ZDE	MPZDE	Control	MDE	PDE	ZDE	MPZDE	Control
2 h	0.21±0.14aA	0.24±0.17aA	0.26±0.18aA	0.11±0.14aA	0.43±0.22aA	0.008±0.01aA	0.007±0.03aA	0.009±0.04aA	0.006±0.05aA	0.009±0.14aA
1 day	0.24±0.12a	0.29±0.13a	0.34±0.23a	0.14±0.11a	0.88±1.39b	0.013±0.12a	0.011±0.04a	0.015±0.08a	0.009±0.02a	0.058±0.15b
3 days	0.64±0.35ab	0.67±0.37ab	0.71±0.25ab	0.42±0.14ab	2.96±1.61c	0.056±0.04ab	0.045±0.02ab	0.041±0.05a	0.039±0.09ab	0.17±0.18c
5days	0.95±0.46b	1.15±0.16bc	0.99±0.46bc	0.69±0.17b	4.85±1.73d	0.093±0.08b	0.084±0.06b	0.091±0.09b	0.073±0.13b	0.86±0.73d
7 days	1.39±0.24cd	1.48±0.29cd	1.46±0.21c	0.95±0.19b	6.57±1.49e	0.11±0.02b	0.10±0.01b	0. 14±0.11b	0.099±0.32b	1.12±0.21de
9 days	1.84±0.42d	1.89±0.38d	1.96±0.12d	1.24±0.51b	6.85±1.64d	0.39±0.13b	0.36±0.11b	0.48±0.37b	0.34±0.28bc	1.34±0.49e
11 days	1.63±0.19d	1.65±0.27d	1.61±0.31de	1.17±0.43b	6.53±1.12de	0.53±0.11b	0.87±0.14d	0.55±0.38b	0.73±0.32c	1.56±0.57ef
13 days	1.41±0.28d	1.55±0.22d	1.45±0.39e	1.09±0.71b	6.31±1.39e	0.67±0.35bc	0.89±0.19d	0.64±0.76b	0.78±0.29c	1.98±0.86fg
15 days	1.84±0.41d	1.87±0.31d	1.93±0.52f	0.96±0.58b	6.14±1.53e	0.91±0.23cd	0.98±0.28d	0.87±0.53bc	0.86±0.57c	2.34±0.96g
17 days	1.57±0.49d	1.51±0.89d	1.74±0.79f	0.73±0.29b	5.89±1.53e	1.25±0.23d	1.21±0.54d	1.18±0.41c	1.11±0.62c	2.88±0.41h

Different letters in the same columns indicate significant differences (p<0.05). Same letters in the same columns indicate no significant differences (p>0.05).

Table 7: TVB- N of control and date extract treated trout during refrigeration (Values are mean + standard deviation)

Sampling time	Treatment				
	MDE	PDE	ZDE	MPZDE	Control
2 h	11.28±1.67aA	11.31±1.27aA	11.39±2.34aA	11.45±1.96aA	11.51±1.72aA
1 day	11.93±1.12b	12.37±1.33b	12.16±1.88b	12.31±1.56b	12.63±1.97b
3 days	13.14±1.56c	13.98±1.69c	13.94±1.94c	13.75±1.97c	16.89±1.12c
5days	15.95±1.68d	15.38±1.94d	15.84±1.67d	15.72±1.62d	21.91±1.53d
7 days	17.39±1.39e	17.83±1.76e	17.18±1.96e	17.41±1.53e	25.57±1.73e
9 days	18.84±1.78f	19.98±1.89f	19.79±1.53f	19.84±1.92f	27.72±1.99f
11 days	20.34±1.99g	21.73±1.47g	21.49±1.32g	21.59±1.24g	28.89±2.34g
13 days	22.11±2.38h	22.61±1.53h	23.15±1.79h	22.95±2.43h	30.87±2.36h
15 days	24.84±2.21i	24.72±1.68i	24.94±1.93i	23.91±2.35i	31.89±2.31i
17 days	27.84±1.35j	26.94±1.97j	27.35±1.38j	26.18±1.31j	33.16±1.14j

Different letters in the same columns indicate significant differences (p<0.05). Same letters in the same columns indicate no significant differences (p>0.05).

Sensory evaluation of control and date extract preserved trout during refrigeration was shown in Tables 8 and 9. The samples were ranked as follows in descending order, from having the best to the worst sensory traits: Piaroum, Zahedi, Mozafati, combination of extracts and control. Color showed no significant differences in samples treated by Mozafati and Piaroum for 9 days, but in samples in preserved by Zahedi and their combination extracts for 7 days. During samples refrigeration, color maintained a good quality for 15 days. Odor and taste had no significant difference in Mozafati, Piaroum, Zahedi and their combination for 9, 11, 7 and 7 days, respectively. Texture and taste had no significant difference samples treated by Mozafati, Piarpum and Zahedi extracts for 7 days, but in samples treated by their combination was 5 days. Taste was weaker in samples treated by the combination of date extracts compared to the other samples (p>0.05). Color, odor, texture and taste had no significant differences in control samples for 4-5 days p>0.05). Overall acceptance, texture, taste and odor were within an acceptable range for control samples 5 days, samples treated by combination of extracts 11 days, (Mozafati and Zahedi extracts 13 days and Piaroum extract 15 days. Surface slime layer was formed on the fish fillets treated by date extracts after 15 days.

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Table 8: Sensory evaluation (Color, odor and texture) of date extract preserved trout and control during refrigeration.

Index	Color					Odor					Texture				
Treatment	MDE	PDE	ZDE	MPZDE	Control	MDE	PDE	ZDE	MPZDE	Control	MDE	PDE	ZDE	MPZDE	Control
Sampling time															
2 h	5±1.93a	5±1.19a	5±1.98a	5±1.23a	5±1.67a	5±1.87a	5±1.59a	5±2.23a	5±2.56a	5±1.73a	5±1.16a	5±1.51a	5±2.19a	5±1.79a	5±2.16a
1 day	5±1.57a	5±1.36a	5±1.78a	5±.213a	5±1.36a	5±1.66a	5±1.47a	5±1.78a	5±2.43a	5±1.49a	5±1.12a	5±2.45a	5±2.21a	5±2.45a	5±1.19a
3 days	5±1.52a	5±1.87a	5±1.56a	5±1.96a	4.68±1.49ab	5±1.56a	5±1.17a	5±2.16a	5±1.95a	5±1.28a	5±1.52a	5±2.17a	5±1.38a	5±2.93a	5±1.67a
5days	5±1.73a	5±2.24a	5±1.46a	5±1.94a	4.32±2.12b	5±1.78a	5±1.58a	5±1.79a	5±2.18a	4.59±1.99b	5±2.18a	5±1.75a	5±2.58a	5±2.34a	4.81±2.36a
7 days	4.87±1.82a	4.89±2.16a	4.67±2.28ab	4.83±2.25a	2.89±1.16c	4.95±2.17a	4.92±2.78a	4.74±2.87ab	4.78±2.69a	2.94±1.42c	4.91±1.98a	4.74±1.56ab	4.83±1.84a	4.37±2.28b	2.69±1.49b
9 days	4.52±1.91ab	4.63±1.56a	4.35±2.26b	4.52±2.16b	2.53±1.13cd	4.73±2.18ab	4.87±1.87a	4.31±1.68bc	4.23±1.79b	1.98±1.14d	4.34±2.24b	4.23±2.37bc	4.51±2.52a	3.89±1.59c	1.94±1.34c
11 days	4.36±1.92b	4.39±2.26b	4.23±2.25bc	4.12±2.30c	2.11±1.56de	4.12±1.89bc	4.56±2.34a	4.12±2.67c	3.61±1.78c	1.68±1.36d	3.95±1.67bc	3.86±1.70cd	3.71±1.89b	3.53±1.63c	1.86±1.25c
13 days	3.85±1.86c	3.80±1.45c	3.76±1.98c	3.75±1.99cd	1.95±1.56e	3.68±1.67c	3.42±1.89b	3.45±1.79d	1.76±1.23d	1.57±1.12de	3.79±2.15c	3.52±2.35d	3.63±2.47c	1.98±1.11d	1.57±1.24cd
15 days	3.12±1.39d	3.45±2.12c	3.15±1.18d	3.42±1.24d	1.84±1.33e	2.56±1.65d	3.26±1.10b	2.37±1.19e	1.54±1.13d	1.49±1.15e	1.56±1.21d	3.19±1.41d	1.78±1.56d	1.62±1.34d	1.49±1.22d
17 days	1.67±1.17e	1.87±1.12d	1.84±1.37e	1.82±1.45e	1.56±1.12e	1.67±1.14e	1.57±1.34c	1.63±1.24f	1.62±1.15d	1.32±1.13e	1.48±1.12d	1.78±1.19e	1.63±1.27d	1.58±1.13d	1.32±1.21d

Different letters in the same columns indicate significant differences (p<0.05). Same letters in the same columns indicate no significant differences (p>0.05).

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Table 9: Sensory evaluation (Taste and overall acceptance) of control and test samples during refrigeration.

Index		Taste					Overall acceptance				
Treatment / Sampling time		MDE	PDE	ZDE	MPZDE	Control	MDE	PDE	ZDE	MPZDE	Control
2 h		5±1.12a	5±1.43a	5±1.97a	4.85±1.23a	5±1.78a	5±1.38a	5±1.31a	5±1.71a	5±1.14a	5±1.41a
1 day		5±1.19a	5±1.24a	5±1.48a	4.82±1.46a	5±1.59a	5±1.49a	5±1.42a	5±1.92a	5±1.78a	5±1.67a
3 days		5±1.36a	5±1.76a	5±1.45a	4.59±1.78a	5±1.65a	5±1.15a	5±1.62a	5±1.93a	5±1.54a	5±1.19a
5days		5±1.14a	5±1.35a	5±1.18a	4.36±1.81ab	4.95±1.64a	5±1.15a	5±1.67a	5±1.54a	5±1.87a	4.90±1.48a
7 days		4.69±1.17ab	5±1.13a	4.78±1.54a	4.17±1.93bc	2.73±1.83b	4.78±2.13ab	4.69±2.45a	4.72±1.87ab	4.55±1.49a	1.78±1.34b
9 days		4.39±1.32bc	4.86±1.25ab	4.39±1.19b	3.89±1.81cd	1.78±1.26c	4.51±1.24b	4.19±2.14b	4.34±2.14bc	3.98±1.68bc	1.56±1.35b
11 days		4.11±1.29cd	4.47±1.16bc	3.94±1.32bc	3.67±1.92d	1.58±1.39c	4.13±2.17c	3.89±2.13bc	4.12±2.28c	3.45±1.89c	1.45±0.98b
13 days		3.78±1.28d	4.16±1.12c	3.59±1.26c	1.78±1.32e	1.54±1.24c	3.483±2.78c	3.55±1.99c	3.59±1.49d	1.78±1.18d	1.34±1.21bc
15 days		1.68±1.31e	3.92±1.17c	1.73±1.16d	1.46±1.47ef	1.34±1.13cd	1.52±1.45d	3.45±1.97c	1.47±1.37e	1.59±1.29d	1.12±1.10c
17 days		1.35±1.39e	1.47±1.21d	1.23±1.3e	1.12±1.24f	1.21±1.12d	1.48±1.23d	1.64±1.17d	1.28±1.11e	1.23±1.34d	1.11±1.13c

Different letters in the same columns indicate significant differences (p<0.05). Same letters in the same columns indicate no significant differences (p>0.05).

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Discussion

Total flavonoid and polyphenolic content of date extracts were 2.15 – 3.22 Q.100 ml^{-1} and 1.87 – 2.49 GA.100 ml^{-1}, respectively (Table 1). Chaira *et al.* (2009) found that the polyphenolic content in aqueous and ethanol extracts of 10 Tunisian date varieties did not exceed 9.70 mg GAE.100 g^{-1}. Rastgar *et al.* (2016) reported that the flavonoid content in Piaroum date was 25 mg Q $100g^{-1}$ at the Tamar stage. Mansouri *et al.* (2005) estimated polyphenolic contents of seven different varieties of ripe dates (Akerbouche, Deglet-Nour, Ougherouss, Tafiziouine, Tantbouchte, Tazerzait and Tazizaout) using the Folin-Ciocalteu method. They reported that the total phenolic content was in the range of 2.49 to 8.36 mg GAE $100g^{-1}$. The difference in the polyphenolic and flavonoid contents of different date extracts could be originated from differences of date varieties, moisture content, harvest season, experimental method, extraction method and palm farm (Al-Farsi *et al.*, 2005; Salmanian *et al.*, 2014; Odeh *et al.*, 2014).

The antibacterial activity of date extracts increased with increasing the concentration from 1 to 5% (Table 2). However, according to table 3 the concentration of 3% showed the best sensory scores. Therefore, the concentration of 3% selected step 2 of study.

The total bacterial counts as well as the counts for *Pseodomonas*, *Enterobacteriaceae* and lactic acid bacteria increased significantly in all the samples during storage period (Table 4). The total bacterial counts as well as the counts for *Pseudomonas* bacteria and *Enterobacteriaceae* showed a decrease in the treated samples compared to the control samples. Date extracts has different antibacterial compounds such as poly phenols, flavonoids and the antimicrobial property of date extracts previously was reported by Rauha *et al*. (2000) and Baliga *et al.* (2011). Phenolic compounds are not the only antibacterial agent in date extracts. Gaballa and Helmann (2007) reported that the antibacterial property of Piaroum extract is attributed to phenolic compounds and heat-sensitive siderophore with a molecular weight of less than 5 kDa. Another factor that can be effected the antimicrobial activity of date extracts is presences of probiotic bacteria. Seifzadeh *et al*. (2019) reported that the bacterial flora in Piaroum extract belonged to *Bacillus subtilis*, in Mozafati to *Lecunostoc mesenteroeides* and in Zahedi extract to *Pediococcus parvalus.* All these bacteria were probiotic. The higher decrease in the bacterial population of the samples treated with the combination of extracts compared with the other treated samples could be originated from the synergetic effect of all antibacterial agents such as flavonoids, pseudo-surfactants, polyphenols and probiotic bacteria of date extracts. Bacterial counts showed no significant difference in the test samples. Since, these components showed no significant differences in studied date extracts. Sani *et al*. (2017) detected antimicrobial activity of date palm on some members of *Enterobacteriacea.* found Nasiri *et al*. (2016) showed dipping the fish in the aqueous extract of myrtle reduced the psychrophilic bacterial count immediately after the treatment and

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significantly retarded the microbial deterioration of treated fishes during chilled storage. These results are similar to the results of the current study. Saleh and Otaibi (2013) evaluated effects of aqueous, ethanol, and ether extracts of three date varieties (Sheshi, Khulase and Rezaz) in three maturation stages (Biser, Rutab and Tamer) on bacterial population in minced camel meat. Their study revealed that the ethanol and aqueous extracts of Rezaz dates at the Biser stage had the strongest antimicrobial effect on the bacterial population. The effectiveness of the date extracts in decreasing the bacterial population in the present study is consistent with similar research on minced camel meat (Biglari, 2009).

TVB-N is widely used as fish spoilage index. Based on table 5, the amount of TVB-N was lower in treated samples compared with the control samples during the storage period. This can be attributed to lower microbial counts in treated samples that explained in the last section (Ashraf and Hamidi Esfahani, 2011).

A suitable index for determination of progress in lipid/fat oxidation and production of carbonyl compounds is the measurement of TBARS. As shown in table 5, peroxide and TBARS decreased in the samples treated by date extracts compared with control samples. It can be due to the antioxidant activity of date extracts. TBARS value showed significant difference in test and control samples during storage period. The primary product of lipid oxidation, hydroperoxide, may break into secondary products such as aldehydes which can increase TBARS value (Seifzadeh, 2014). Biglari (2009) indicated that Bam Mozafati (*Phoenix dactylifera*) and Kharak (*Phoenix dactylifera*) date extracts inhibited lipid oxidation in minced chicken meat and concentration of 4% was the best. The effectiveness of the date extracts in decreasing the PV and TBARS in the present study is consistent with similar research on minced chicken meat.

As shown in Table 6, sensory evaluation including taste, odor, texture, color and overall acceptance had better quality in samples treated by date extracts compared with control samples. This could be due to the effects of the date extracts. A decrease in sensory factors was observed in test and control samples during storage period. Production of carbonyl compounds from oxidation process in fish meat causes some changes in its sensory properties such as taste, color and smell. Aldehydes produced from oxidation process can react with proteins. This compounds react with pigments and other molecules of fish fillets. These compounds finally could lead to color loss and bad smell of product. Also, *Pseudomonas* bacteria and some species of *Enterobacteriaceae* have lipase enzymes which can intensify the process of color change. Besides, these species can produce surface slime layer which leads to a decrease in sensory factors.

The results of the present study revealed that date extracts showed appropriate antibacterial activity and up to concentration of 3% had good effect on sensory scores of trout fillets. During refrigeration conditions the treatment groups, including trout fillets immersed in Mozafati, Piaroum, Zahedi extracts and their combination, had a good microbial quality under. The best microbial quality and longest shelf life of the test samples

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were observed in samples preserved by Piaroum, Zahedi, Mozafati and their combination extracts, respectively. According to the results, the treatment preserved by Piaroum extract is recommended for the preservation of trout fillet in food industry.

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