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Antioxidant potential of cinnamon, ajowan and zataria essential oils in grape seed oil

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Abstract

In order to inhibit the oxidation of lipids, improve the oxidative stability of foods and to minimize the hazard risk to human health, antioxidants are added to food materials in industrial processing. In this work, the antioxidant potential of cinnamon (*Cinnamomum zeylanicum), ajowan (Carum copticum)* and zataria (*Zataria multiflora* Boiss.) essential oils (EOs) at different concentrations (0, 1 and 1.5%) on free fatty acid content (FFA), peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) of grape seed oil stored at 60ºC was evaluated. Ajowan treated samples (1.5%) showed the lowest (1.02%) and zataria treated samples (1%) expressed the highest (1.19%) FFA value among EO-treated samples. Samples treated with 1.5% cinnamon showed the lowest PV (69.5 meq O_2/ kg) at the end of the storage period. Following control, the highest PV was seen in samples treated with zataria (1%). Grape seed oil samples treated with 1 and 1.5% cinnamon showed the lowest TBARS values during the whole storage period (one month). TBARS of zataria treated samples increased slightly toward the end of storage and a similar trend in TBARS was observed for samples treated with ajowan. The antioxidant activity of EOs in grape seed oil followed in descending order was cinnamon, ajowan, and zataria.

Keywords*:* Ajowan, Antioxidant activity, Cinnamon, Grape seed oil, Zataria

Introduction

Antioxidants inhibit the initiation or propagation of oxidizing chain reactions, which involve absorption and neutralization of free radicals, quenching singlet and triplet oxygen, and decomposing peroxides (Velioglu *et al.*, 1998). However, the adverse effects of some synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are mostly used in food industry, have already been demonstrated (Reische *et al.*, 1998). Therefore, government agencies authorities and consumers are concerned about the safety of these foodstuffs such as edible oils and fats with synthetic additives.

Essential oils (EOs) are considered as natural antioxidant and antimicrobial substances, obtained from herbs. Main component of herbal EOs and extracts consist of a mixture of terpens, terpenoids, phenolic and other aromatic and aliphatic compounds (Bakkali *et al.*, 2008), however it is clear that their composition can vary extensively depending on the source of origin and growth conditions.

Cinnamon (*Cinnamomum zeylanicum*) is belonging to the family *Lauraceae*. Its EO has shown antioxidant properties and antiradical potential. The main components of cinnamon EO are eugenol and cinnamaldehyde (Ozcan and Arslan, 2011; Perdones *et al.*, 2014).

Carum copticum (ajowan), belonging to the *Apiaceae* family, is an aromatic annual plant. Ajowan grows in Iran, India, Pakistan and Egypt and its fruits have been used extensively in Iranian folk and traditional medicine to treat gastrointestinal, rheumatic and inflammatory disorders. The most important compound of ajowan's EO is thymol (Mahboubi and Kazempour, 2011; Oskuee *et al.*, 2011). *Carum capticum* fruits due to favorite taste, are used by Iranian people from ancient times.

As spice and preservative of foods specially meat

Zataria (*Zataria multiflora* Boiss.) as EO or as an extract is widely used in many food

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products in Iran. The high antioxidant capacity of zataria is due to the phenolic compounds, carvacrol and thymol and also the major non-phenolic compounds, linalool and p-cymene (Akrami e*t al.,* 2015; Basti *et al.*, 2016).

As high levels of unsaturated fatty acids in grape seed oil enhance the formation of oxidized off flavors, food industry has an interest in new approaches that allow edible oil products to be stored with less oxidative deterioration (Jang *et al.*, 2015). The aim of this study was to investigate the antioxidant activity of cinnamon, ajowan and zataria EOs in grape seed oil.

Materials and Methods

Grape seed oil

The red grape seed oil with no antioxidants added was obtained from a local oil extraction shop in Babolsar (Mazandaran, Iran).

Essential oils

The EOs were obtained from Barij Company (Kashan, Iran) and were stored in airtight dark glass vials at 4°C.

Gas Chromatography/Mass Spectroscopy of EOs

The EOs were analyzed by gas chromatography (GC; Thermo Quest 2000, Finnigan, U.K.). The chromatograph was equipped with a DB5 capillary column (30 m× 0.25 mm ID× 0.25 mm film thickness) and the data were acquired under the following conditions: initial temperature 50°C; program rate 2.5°C, final temperature 265°C and injector temperature 250°C. The carrier gas was helium and the split ratio was 120 ml/min. The EOs were also analyzed by GC mass spectroscopy (MS) (Thermo Quest) using the same capillary column and analytical conditions indicated earlier. The MS was run in the electron ionization mode, using ionization energy of 70 eV. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards (Adams, 2001). In order to calculate the relative retention indices, alkanes were used as the reference points.

DPPH radical-scavenging activity

The 2,2-diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging assay was used to evaluate the antioxidant potential of the EOs (Shimada *et al.*, 1992). One milliliter of each EO at known concentration (0, 0.1, 0.3, 0.5 and 1% in dimethyl sulfoxide) was added to 0.25 ml of a DPPH methanolic solution. This mixture was then shaken vigorously and left at room temperature for 30 min, in darkness. The absorbance of the solution was measured at 515 nm and corresponded to the ability of the EO to reduce the stable radical DPPH to the yellow colored diphenyl picryl hydrazine. Absorption of a blank sample containing the same amount of methanol and DPPH solution was considered as negative control.

$$\%\text{DPPH scavenging} = [\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}/\text{Absorbance}_{\text{control}}] \times 100 \quad (1)$$

Sample preparation

Two different concentrations of each EO (1.0 and 1.5 %, v/v) were added to grape seed oil and the samples were stored for 30 days at 60°C, in darkness. Experiments were conducted at 5 day intervals.

Free fatty acid (FFA)

FFA content, expressed as percentage of oleic acid in the sample, was measured via the acidimetric titration of the Bligh and Dyer extracts after adding ethanol and using phenolphthalein as an indicator, following AOCS method (AOCS, 1994).

Peroxide value (PV)

To measure the hydro peroxide content of the samples, 5.0± 0.05 g grape seed oil was taken along with 30 ml mixture (2:3, v/v) of chloroform and acetic acid into a 250 ml flask (Mehenbacher *et al.*, 1997). To the above mixture, 0.5 ml fresh saturated aqueous potassium iodide solution was added. The flask was shaken vigorously for about 1 min. Then, 30 ml distilled water was added and mixed thoroughly with the solution and titrated against 0.1 N and 0.01 N sodium thiosulphate solution (in order to accurate titration), 0.5 ml soluble starch solution was used as an

indicator. Also, a blank was prepared with no oil sample. PV was determined according to the following equation:

PV (milliequivalents of peroxide/ Kg oil sample)= $[(V_s-V_b) \times N \times 10^3] / w$ (2)

Where V_s is the volume (ml) of sodium thiosulphate solution used for the sample, V_b that of the blank, N the normality of sodium thiosulphate solution and W the weight of the oil sample in grams.

Determination of thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances are formed as a byproduct of lipid peroxidation which can be detected by the TBARS assay using thiobarbituric acid as a reagent (Kristensen and Skibsted, 1999). The thiobarbituric acid (TBA) reagent was prepared immediately before use by mixing equal volumes of freshly prepared TBA (0.025 M) (preparation into solution by neutralizing with NaOH) and H_3PO_4 (2M) / citric acid (2M). Measurements were performed at 532 nm (red pigment) and 450 nm (yellow pigment) and the results are expressed as absorbance units in one gram of oil sample.

Statistical analysis

The one-way ANOVA was performed to analyze the chemical parameters and significant differences were determined by using Tukey test. The analyses were performed in SPSS 22 and MS Excel programs. All determinations were performed in triplicate.

Results and Discussion

Spices, aromatic plants and culinary herbs are interesting for their high content of bioactive metabolites and compounds that may exert beneficial effects on human health. According to several studies, these compounds exhibit antiradical and antioxidant potential, mainly through hydrogen donating to reactive radicals (Velioglu *et al.*, 1998; Basti *et al.*, 2016). Twenty six compounds were identified for zataria EO; the main constituents were carvacrol (50.53%) and thymol (14.70%). Also, 15 and 31 compounds were detected for cinnamon and ajowan Eos respectively. The main compounds present in cinnamon EO were cinnamaldehyde (71.5%) and eugenol (3.08%), and those in ajowan EO were thymol (62.45%), (Z)- p-cymene (18.01%) and γ-terpinene (15.89%).

It seems that high degree of antioxidant and antiradical activities of cinnamon EO found in the present work probably derived from the hydrogen donating potential exhibited by a wide range of its constituents, especially cinnamaldehyde and eugenol (Ozcan and Arslan, 2011; Perdones *et al.*, 2014).

The main compounds of ajowan EO are reported to be terpenes such as thymol, (Z)- *p*-cymene and γ-terpinene, as determined in this work and other studies (Mahboubi and Kazempour 2011; Oskuee *et al.*, 2011). The relative antioxidant activity of ajowan suggests that this plant can have the promising potential to be used as a natural antioxidant and flavoring compound in food industry to avoid food spoilage and oxidation while increasing the safety of the food products during the processing and also during the storages at various conditions.

The major components of zataria EO were carvacrol and thymol. In our results, the amount of carvacrol was less than other studies (Akrami *et al.*, 2015; Basti e*t al.*, 2016) that may explain the lower antioxidant activity of this EO in comparison with the other studies. The different amount of chemical compounds in the EOs might be related to harvest season, geographical situation, grinding conditions and genetic parameters (Peter, 2004).

Antioxidant potential of the EOs was evaluated by DPPH assay (Fig. 1). In the present study it has been found that cinnamon EO at concentration of 1% was able to reduce the stable radical DPPH to 1, 1-diphenyl-2-picrylhydrazine up to 76.51%, followed by ajowan and zataria EOs with 54.20 and 26.35% inhibitory activity, respectively.

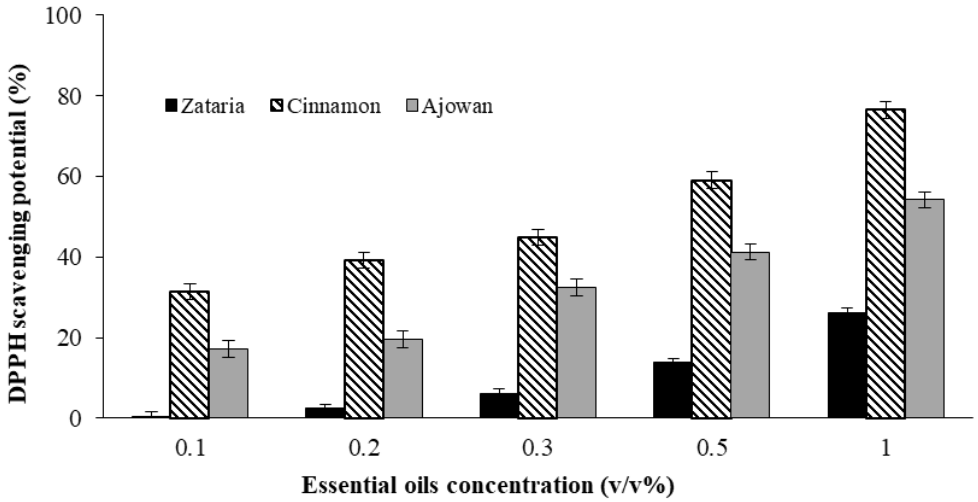

Fig. 1. The effect of different concentrations of EOs (Zataria, cinnamon and ajowan) on free-radical scavenging

The EOs were capable of scavenging DPPH free radicals in a dose-dependent manner through hydrogen-donation converting it to the nonradical hydrazine form. The potential of scavenging DPPH radicals was determined as the cinnamon> ajowan> zataria. The DPPH radical scavenging activities were 76.51%, 54.20 % and 26.35% for cinnamon, ajowan and zataria, respectively, which suggests that the components within cinnamon EO are more efficient radical-scavenging components. The results obtained in this work showed that the antiradical activity may be related to the presence of cinnamaldehyde, carvacrol, thymol and eugenol in essential oils. Cinnamon essential oil had significantly higher persistent antioxidant activity, probably due to considerably high content of cinnamaldehyde (Ozcan and Arslan, 2011).

FFA contents of treated grape seed oil samples are shown in Fig. 2. There was a significant difference between FFA of EO-treated samples with the control ($P<0.05$). Ajowan (1.5%) treated samples showed the lowest (1.02± 0.06 %) and zataria (1%) treated oil samples expressed the highest (1.19± 0.04%) FFA value among EO-treated samples ($P<0.05$).

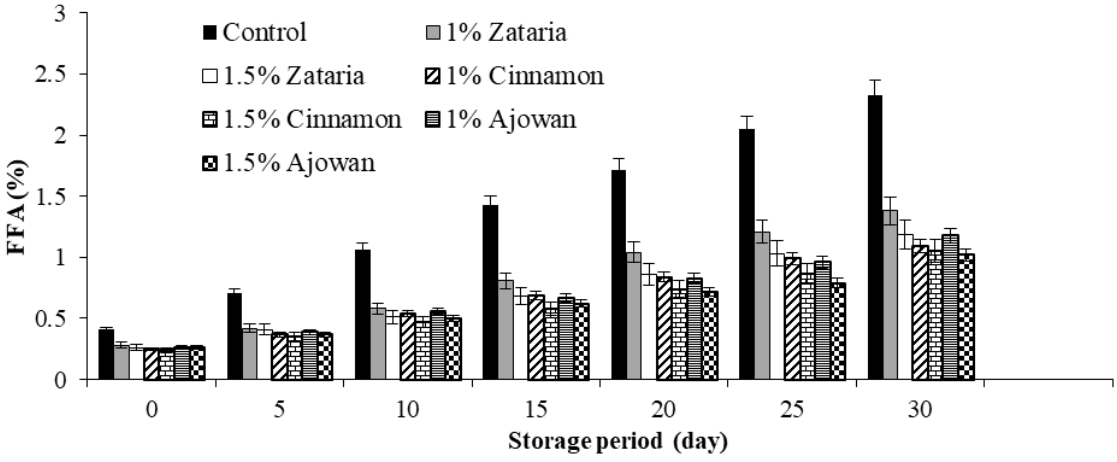

Fig. 2. The effect of type and different concentrations of EOs (Zataria, cinnamon and ajowan) and storage time on the FFA (mg/g oil sample) of grape seed oil

FFAs are produced by the hydrolysis of oils and fats as the result of exposure to various factors such as storage, processing, heating or frying. As FFAs are less stable than triglycerides, they are more prone to start oxidation and to turning rancid. In the present study, the oil samples treated with antioxidants had higher oxidative stability and lower free fatty acid content than the control sample. FFA contents especially in the control and samples treated with 1% zataria EO showed a considerable increase. As it is obvious from the results, cinnamon EO at both concentration of 1 and 1.5% and ajowan EO at 1.5% were more inhibitory against FFA increase, in comparison with other treatments. In a study conducted by Villa-Ruano et al. (2013) anti-lipase effect of 30 medical plants was confirmed.

The effects of type and concentration of EOs and storage time on the PV of grape seed oil at 60°C are illustrated in Fig. 3.

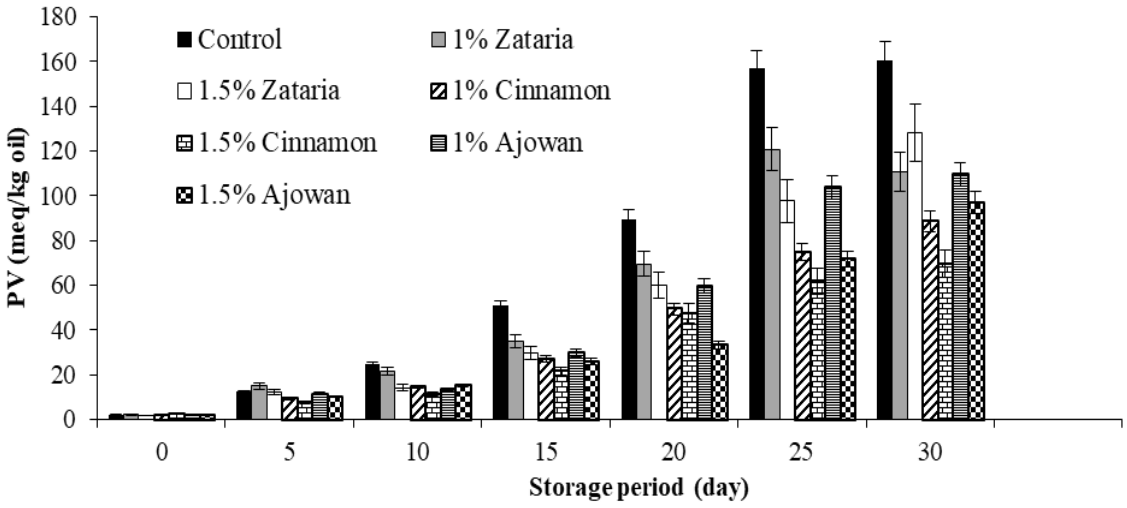

Fig. 3. The effect of type and different concentrations of EOs (Zataria, cinnamon and ajowan) and storage time on the PV (meqO_2/ kg oil) of grape seed oil

After 4 weeks, significant increase in PV was determined in all samples but oil sample treated with 1.5% cinnamon EO showed the lowest PV (69.5 meqO_2/ kg) at the end of the storage period ($p<0.05$). Among EO- treated oil samples, the highest PV was seen in samples treated with 1% of zataria EO ($p<0.05$).The essential oils of cinnamon and ajowan showed more inhibitory effect on PV increase and oxidative deterioration in grape seed oil. Reduction of PV within the last days of storage can be explained by an increase in the rate of hydro peroxide destruction during such period. The same results were obtained by other researchers who observed an increase in PV of soybean oil samples during the first 2 months of storage at 20, 30 and 40ºC and then decrease in PV to the end of the 6-month storage period (Dolati *et al.*, 2016). The PV in control sample was still increasing so the destruction of hydro peroxides didn't result in PV reduction.

Amount of secondary lipid oxidation products were quantified using the TBARS method. The changes of TBARS in the oil samples stored at 60°C are shown in Fig. 4. Grape seed oil samples treated with 1 and 1.5% cinnamon EO, in comparison with other samples, showed the lowest TBARS values during the whole storage period ($p<0.05$). BARS of zataria treated samples increased slightly toward the end of storage and a similar trend in TBARS was observed for samples treated with ajowan EO.

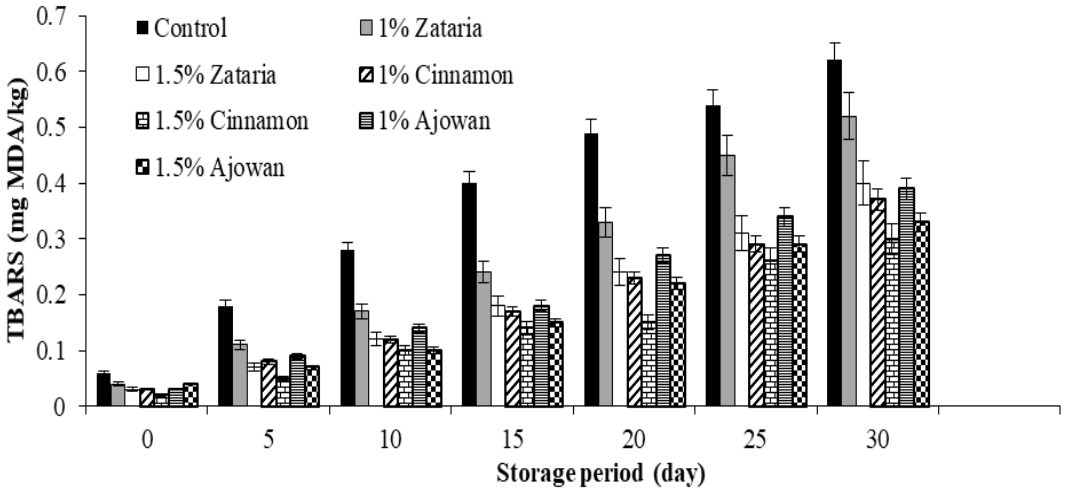

Fig. 4. The effect of type and different concentrations of EOs (Zataria, cinnamon and ajowan) and storage time on the TBARS (mg Malondialdehyde /kg oil) of grape seed oil

Although the primary lipid oxidation products are peroxides but they provide little information about the nature of the oxidative processes during storage of the oils and fats. Lipid peroxides are degraded to products of secondary lipid oxidation. TBARS value of all samples increased significantly with the advancement of storage period. High potential of cinnamon EO in inhibiting TBARS increase in food samples is reported in several studies (Schmidt, 2006; Ozcan and Arsalan, 2011). Shahsavari *et al.* (2008) observed good antioxidant activity from zataria EO at concentration of 0.6% in soybean oil. Also Hashemi *et al.* (2014) reported that the antioxidant effect of 0.75% ajowan EO in sunflower oil considerably restrained TBARS value. In this work, in comparison with other studies, the concentration of 1.5% (v/v) of all three EOs had the highest inhibitory effect on the formation of primary and secondary oxidation products. Zataria and ajowan EOs expressed stronger antioxidant activities in comparison with similar EOs in our work. This may be due to difference in the total phenolic and other antioxidant compounds of EOs with different origins in different studies.

Conclusions

The results of our study revealed that the herbal EOs express considerable antioxidative activity in grape seed oil samples. The main components of EOs in present study are phenolic and terpene compounds which can act as antiradicals through free radical scavenging pathways. In the present study, the antioxidant activity of EOs followed by cinnamon> ajowan> zataria pattern. The concentration of 1.5 % (v/v) of cinnamon EO was the most effective in decreasing peroxide and secondary oxidation products formation rate in grape seed oil samples.

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پتانسیل آنتی‌اکسیدانی اسانس‌های دارچین، زنیان و آویشن شیرازی در روغن هسته انگور

مریم عزیزخانی[1*]- فاطمه آدینه‌پور[2]

تاریخ دریافت: 1397/02/30
تاریخ پذیرش: 1398/05/26

چکیده

در فرآیند صنعتی مواد غذایی، به‌منظور ممانعت از اکسیداسیون چربی‌ها، بهبود پایداری اکسیداتیو مواد غذایی و به حداقل رساندن آسیب به سلامت افراد آنتی‌اکسیدان‌ها به مواد غذایی اضافه می‌شوند. در این پژوهش، توانایی آنتی‌اکسیدانی اسانس‌های دارچین (*Cinnamomum zeylanicum*)، زنیان (*Carum copticum*) و آویشن شیرازی (*Zataria multiflora* Boiss.) در غلظت‌های مختلف (صفر، 1 و 1/5%) بر میزان اسیدهای چرب آزاد، عدد پراکسید و ترکیبات واکنش‌دهنده با تیوباربیتوریک اسید در روغن هسته انگور در 60 درجه سانتی‌گراد ارزیابی گردید. نمونه‌های تیمار شده با اسانس زنیان (1/5%) پایین‌ترین (1/02%) و نمونه‌های تیمار شده با اسانس آویشن شیرازی (1%) بالاترین (1/19%) میزان اسید چرب آزاد را در میان نمونه‌های تیمارشده با اسانس نشان دادند. نمونه‌های تیمارشده با 1/5 اسانس دارچین دارای پایین‌ترین اندیس پراکسید (69/5 $meqO_2/kg$) در انتهای دوره نگهداری بودند. پس از نمونه کنترل، بالاترین اندیس پراکسید در نمونه‌های حاوی اسانس آویشن شیرازی (1%) مشاهده گردید. نمونه‌های روغن هسته انگور تیمارشده با 1 و 1/5% اسانس دارچین واجد کمترین اندیس تیوباربیتوریک اسید در کل دوره نگهداری (یک ماه) بودند. اندیس تیوباربیتوریک اسید نمونه‌های دارای اسانس آویشن شیرازی تا انتهای دوره نگهداری با شیب ملایمی افزایش یافت و تغییرات مشابهی نیز در نمونه‌های تیمارشده با زنیان مشاهده شد. فعالیت آنتی‌اکسیدانی اسانس‌ها در روغن هسته انگور به‌ترتیب نزولی دارچین، زنیان، آویشن شیرازی بود.

واژه‌های کلیدی: آویشن شیرازی، دارچین، روغن هسته انگور، زنیان، فعالیت آنتی‌اکسیدانی

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Effects of the bath and probe ultrasound treatment on the antioxidant activity of phenolic extract from oregano (*Origanumvulgare*L.) leaves

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Abstract

Ultrasound assisted extraction (UAE) has attracted growing interest, due to its great advantages for the extraction of bioactive compounds from plant species. Ultrasound efficiency is affected by various factors such as extraction temperature, extraction time, intensity of ultrasound waves, and type of ultrasound system. Therefore, this study aimed to investigate the process conditions of two ultrasonic systems (bath and probe) to determine the highest extraction efficiency of phenolic and flavonoid compounds in oregano leaves and the best ultrasound extraction conditions. The effects of different combinations of the ultrasonic variables include bath (40-60 °C; 30-60 minutes) and probe (40-60°C; 5-25 minutes and ultrasound amplitude: 20- 40%) were studied using ethanol-water (50:50v/v) solvent. The antioxidant activities of the extracts were then evaluated by the 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and reducing power tests.The best conditions for extraction of phenolic compounds and flavonoids were obtained using ultrasonic probe system at 50 ° C for 15 minutes and amplitude of 40%. The maximum total phenol and flavonoid were 473.22 ± 25.9 µg of gallic acid equivalent and 46± 1.24 µg of quercetin equivalent per mg of dry extract, respectively. The results showed that the UAE method had a considerable effect on the extraction of bioactive compounds from oregano leaves, and also the probe system had a higher efficiency than the bath system.

Keywords: Ultrasonic bath, Ultrasonic probe, Oregano leaf, Phenolic Content, Antioxidant activity.

Introduction

Antioxidants are compounds that inhibit or delay the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidation chain reaction. In recent years, many herbs especially vegetables and medicinal plants, have been identified as important sources of natural antioxidants.The extracts of some of these plants can be used as an alternative to the food preservatives due to their antioxidant and antibacterial activity (Diem Do *et al.*, 2014). Among chemical constituents of these plants, phenolic metabolites are considered a good sources of natural antioxidants in the diet. Phenolic compounds comprise phenolic acids (hydroxybenzoic and hydroxycinnamic acids), polyphenols (hydrolyzable and condensed tannins), flavonoids, and lignin. Antioxidant activity of plant phenolic compounds is mainly due to their reducing properties and chemical structure, which can play an important role in neutralizing the free radicals, chelating transition metals, and quenching the singlet and triplet oxygen through the destruction of peroxides (Chun *et al.*, 2005).

Aromatic plants of *Lamiaceae* family such as oregano, rosemary, thyme and mint have shown strong antioxidant activity due to the high content of phenolic antioxidants. Meanwhile, oregano is known as a native plant of Mediterranean regions, Irano-Siberian and Euro-Siberian. It has long been used in traditional medicine to treat the various diseases such as digestive disorders, menstrual problems, spasms, colds and severe coughs (Zhang *et al.*, 2014; Vazirian *et al.*, 2015). The antioxidant and antibacterial activity of this plant is very strong that can explain its use in traditional medicine (Zhang *et al.*, 2014; Lemhadri *et al.* 2004; Li *et al.* 2016).The antibacterial activity of oregano is due to its high content of essential oil, and soluble phenolic compounds are responsible for its

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antioxidant activity and consequently the positive therapeutic effects (Zhang *et al.*, 2014; Li *et al.* 2016).Oregano extract is a good source of flavonoids, such as naringenin and caffeic acid derivatives that rosmarinic acid is the most abundant among the compounds (Zhang *et al.*, 2014; Vazirian *et al.*, 2015).

Phenolic compounds from the plant sources are traditionally extracted using solvent extraction or steam distillation techniques. Traditional methods have many disadvantages such as high solvent consumption, long extraction time, and lower extraction yield (Dey and Rathod 2013). Therefore, the implementation of novel extraction techniques such as ultrasound assisted extraction, supercritical fluid extraction, subcritical water extraction, and microwave assisted extraction, has been promoted. The ultrasound method has gained higher consideration because of its high efficiency, short time, low solvent consumption, cheap, and easy utilization. The mechanical effects of ultrasound during the cavitation phenomenon lead to more solvent penetration into the cells, and it can improve the mass transfer. Therefore, efficient cellular degradation and effective mass transfer are two major factors that increase ultrasound extraction (Cheok *et al*. 2013; Huang *et al.* 2009). Many factors such as temperature, time, and intensity of waves, type and composition of solvent, the nature of plant material, and the ratio of solvent to sample affect the ultrasound mechanism.

Many studies have been investigated the effects of different conditions to improve the extraction of compounds by using ultrasound, for example, extraction of phenolic compounds from red grape extract (Morelli and Prado, 2012), extraction of melatonin from *Oryza sativa* (Setyaningsih *et al.*, 2015), and extraction of polyphenols from *Zizyphuslotu* (Hammi *et al.*, 2015). Different studies have also been carried out to investigate the phenolic content and antioxidant activity of oregano leaves and plants in which extraction of plant extracts was performed by different solvents in traditional ways (Zhang *et al.*, 2014; Chishti *et al.*, 2014; Chun *et al.*, 2005). So far, the effect of different conditions (temperature, time and the amplitude of ultrasound) on the content of phenolic compounds and antioxidant activity of oregano has not been investigated. The objective of this study was to determine the best conditions for extraction of oregano phenolic compounds and flavonoids by examining the different conditions such as extraction temperature, extraction time and the type of ultrasound system (bath and probe) in comparison with traditional solvent extraction method.

Materials and Methods

Oregano leaves (*Origanum vulgare*) were purchased from a medicinal herb farm (Arak, Iran) in February 2017.The dried samples were sieved through No. 67, and were packed tightly in two-layer polyethylene bags and kept at -18°C in order to avoid moisture absorption (Rezaie *et al.* 2015).

Chemicals and Reagents

All chemicals used in this study were purchased from Merck Chemicals Co. (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA) company. The chemicals were of an analytical grade.

Preparation of Extracts

To extract the antioxidant compounds of Oregano in each treatment, 10 g of sample was placed in 100 ml of ethanol: water (1: 1 v/v) and mixed well. Solvent extraction was done as described by Maghsoudlou *et al.* (2016). In this method, for the best extraction of antioxidant compounds, the mixture was shacked at ambient temperature and kept in dark place for 24 hours. Also, the ultrasound treatment was done as described by Altemimi *et al.* (2015).In the bath method, Elma Ultrasonic (S30H, Germany) was used with a frequency of 37 kHz, a power of 280 W and an internal diameter of 50× 106× 198 cm. The mixture of sample was subjected to ultrasonic bath at 40, 50 and 60°C for 30, 45 and 60 min. In this method, temperature was controlled and maintained through the circulation of water. In the probe method, the ultrasound cell disruptor (KS-250F, China, Ningbo Zhejiang) was used at

frequency 20 kHz (amplitude 20% and 40%) and power 250W. Probe ultrasound treatments were performed at 40, 50, 60°C for 5, 15 and 25 min, the temperature elevation was controlled during sonication by mixing water and ice. The suspension obtained by extraction of the ultrasound and solvent method was filtered with Whatman No. 1 (Whatman International Ltd, Maidstone, United Kingdom) filter paper. Afterwards solvents evaporated by rotary evaporator (BUCHI, Labortechnik AG, Flawil, Switzerland). Finally, the extracts were kept at -18°C till further experiments (Maghsoudlou *et al.*, 2016).

Measurement of Total Phenolic Content

Total phenol was measured as described by Sfahlan *et al.* (2009) with a slight modification by using a spectrophotometer. Briefly, 2.5 ml of Folin-Ciocalteu reagent was diluted ten times with distilled water, 2 ml of sodium carbonate 7.5% and 50 µl of the extract was mixed well. After heating at 45 °C for 15 min, absorption of the mixture was measured using a spectrophotometer (PG Instrument, Ltd.) at 765 nm. The total phenol content of all samples was expressed as gallic acid equivalents per g of extract using the following linear equation based on the calibration curve:

$Y= 0.0083 X +0.018 \quad R^2= 0.094 \quad (1)$

Where Y is absorbance at 765 nm and X is concentrations of gallic acid equivalents (mg/g) (Sfahlan *et al.*, 2009).

Determination of Total Flavonoid Content

Evaluation of total flavonoid content was performed by aluminum chloride method as described by Nabavi *et al.*, (2012). Briefly, 0.5mL of each plant extract in methanol was separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1Mpotassium acetate and 2.8 mL of distilled water, and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/ Visible spectrophotometer (PG Instrument, Ltd.). The calibration curve was plotted by preparing pure quercetin solutions at concentrations 12.5- 100 mg/ml in methanol. Total flavonoid content was calculated as quercetin per g of extract using the following linear equation based on the calibration curve:

$Y= 0.0060X+ 0.0024 \quad R^2= 0.98 \quad (2)$

Where Y is absorbance at 415 nm and X is concentrations of flavonoids compounds (mg quercetin /g extract).

Measurement of the extract antioxidant activity
DPPH radical scavenging activity

The stable 2, 2'-diphenyl-1-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts (Ebrahimzadeh *et al.* 2010). Different concentrations of each extract were added, at an equal volume, to methanolic solution of DPPH (100 µM). The samples were kept at room temperature in the darkness and after 30 min the absorbance of each sample was measured at 517 nm and the percentage of scavenging activity was calculated from equation 3. The experiment was done in triplicate. IC_{50} values denoted the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

$$\%Inh = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \quad (3)$$

Reducing power

The ability of extracts to reduce iron (ш) was evaluated using the method of Altemimi *et al.* (2015). Extracts (2.5 ml) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide ($K_3Fe(CN)_6$; 10 g/L) and incubated for 30 min at 50 °C. Thereafter 2.5 ml of trichloroacetic acid (10% w/v) was added to the solution and the solution was then centrifuged for 10 min. Finally, 2.5 ml of supernatant was combined with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (1 g/L). The absorbance of samples was measured at 700 nm. Higher absorbance values indicated higher reducing power.

Statistical Analysis

Each experiment was carried out at least in Triplicate. Statistical analysis of data was performed using Microsoft Excel. Analysis of

variance was done using the SPSS21 program with a confidence level of a 0.05, to find any significant difference between treatments. Duncan multiple range test was used for mean separation at $P<0.05$ where treatment effect was significant.

Results and Discussion

The effect of ultrasound variables on total phenolic content, flavonoids, and antioxidant activity

The results of comparisons between means of total phenolic and flavonoid compounds (Table 1), DPPH radical scavenging (Table 2) and reducing power (Table 3) tests in different treatments showed that the effect of ultrasound extraction type as well as extraction conditions (temperature, time and amplitude of ultrasound) were significantly different ($P<0.05$).

The highest yield of oregano phenolic compounds was observed 473.32± 25.9 µg GAE/mg of dry extract in probe system at 50°C, for 15 min and amplitude 40%, followed by probe extract (at 50°C for 5 min at amplitude 40%) and bath extract (at 50°C for 45 min) with the 443.24± 5.87 and 430.81± 6.94 µg/ ml of phenolic contents of dry extracts, respectively.

Among the phenolic compounds, flavonoids are considered as stronger antioxidants. These are derived from diphenylpropanes and a 6-membered heterocyclic ring containing oxygen. Increasing the number of hydroxyl groups has a direct relation with an antioxidant strength of flavonoids (Koda *et al.*, 2008). Therefore, measurement of these compounds is very important to determine the antioxidant properties.

Radical scavenging and reducing power tests are widely used to evaluate hydrogen-donor compounds that inhibit free radicals. Results showed that, antioxidant activity was dose-dependentin in all extracts. By increasing the concentration of phenolic compounds and flavonoids, the probability of hydrogen donation to free radicals followed by the inhibitory strength of the extract was increased due to the increase in the number of hydroxyl groups in the reaction medium (Maghsoudlou *et al.* 2016). So that, by increasing concentration to 40 µg/ ml, DPPH scavenging activity was increased and reached its highest level (79%- 86% in different samples). In all treatments, the obtained extracts at 40 µg/ml concentration showed the highest DPPH radical scavenging activity and then by increasing the concentration, radical scavenging activity of the extracts remained stable or decreased slightly (Table 2).

IC_{50} value of the ultrasound in different treatments and control samples is depicted in Table 1.The IC_{50} values are the concentration of an antioxidant extract that can inhibit 50% of free radicals or have absorption of 0.5 at 700nm. Therefore, the lower IC_{50} values in the samples showed higher antioxidant activity.

Probe treatment at 50°C for 15 min and 40% ultrasound amplitude showed the lowest IC_{50} of DPPH radical scavenging and reducing power (14.21± 0.17 and 102.91± 4.19 µg/ml, respectively), so the highest antioxidant activity was obtained in these conditions, followed by probe treatment at 50°C for 5 min, amplitude 40% and bath treatment at 50°C for 45 min respectively.

Effect of temperature

Temperature is one of the most important factors in the extraction of antioxidant compounds by ultrasound. Generally, in both types of ultrasonic bath and probe systems, at each experiment time, by increasing extraction temperature up to 50°C, the phenolic and flavonoid content, and antioxidant activity of the oregano leaf extracts increased and then at higher temperatures (60°C) reduced. Increasing the efficiency of phenolic and flavonoid compounds up to 50°C can be attributed to the positive effect of temperature increase on improving the extraction efficiency due to the rupture of plant tissue links, increasing the solubility of compounds, increasing the velocity of solvent propagation and mass transfer, and reducing the solvent viscosity and surface tension. On the other hand, at higher temperatures of the ultrasound, the solvent vapor pressure rises as a result of lower surface tension and it causes of entering more solvent vapors to a large number of cavitation bubbles, which are less dispersed and reduced the

intensity of cavitation. Therefore, in this study, the sonochemistry effects probably decreased due to the collapse of cavitation bubbles at 60°C (Capelo Martinez *et al.*, 2009; Deyand Rathod, 2013; Medina-Torres *et al.*, 2017).

Table 1. Phenolic and flavonoid compounds and IC_{50} of oregano leaf extracts antioxidant activity test of different treatments

Extraction treatment		Total phenol (µg gallic acid/mg of dry extract)	Flavonoid (µg quercetin/mg of dry extract)	IC_{50} of DPPH (µg /ml)	IC_{50} of Reducing power (µg /ml)
Probe, 40 °C	5 min, 40%*	394.3± 8.71^{d}	28.5± 1.278^{e}	16.41± 0.27^{bcd}	131.8± 2.58^{def}
	5 min, 20%	310.90± 7.28^{h}	23.18± 0.71^{jk}	20.09± 1.09^{h}	151.5±3.73^{k}
	15 min, 40%	409.35± 14.27^{cd}	31.78± 0.82^{d}	15.19± 0.54^{ab}	124.55±1.65^{c}
	15min, 20%	335.80± 16.58^{g}	25.16±1.27^{hi}	19.64± 1.14^{h}	144.09± 1.92^{ij}
	25min, 40%	389.03± 15.27^{de}	27.09± 1.10^{efg}	17.24± 0.40^{cde}	135.79± 1.62^{fg}
	25 min, 20%	309.44± 12.40^{h}	23.5± 0.46^{j}	19.75± 1.36^{h}	151.32± 3.91^{k}
Probe, 50 °C	5 min, 40%	443.24± 5.87^{b}	39.64± 0.86^{b}	15.35± 0.45^{ab}	118.02± 1.68^{b}
	5 min, 20%	352.16± 7.27^{fg}	25.89± 1.15^{fgh}	18.75± 0.79^{efgh}	140.8± 3.28^{ghij}
	15 min, 40%	473.32± 25.90^{a}	46± 1.24^{a}	14.21± 0.17^{a}	102.91± 4.19^{a}
	15min, 20%	369.72± 3.88^{ef}	26.16± 0.47^{fgh}	17.90± 0.81^{def}	137.51± 2.33^{gh}
	25min, 40%	408.10± 21.62^{d}	28.81± 0.46^{e}	16.28± 0.11^{bc}	130.74± 2.16^{def}
	25 min, 20%	334.30± 12.27^{g}	23.86± 0.50^{ij}	19.79± 0.92^{h}	144.36± 2.58^{ij}
Probe, 60 °C	5 min, 40%	405.23± 14.96^{d}	28.5± 1.02^{e}	16.44± 0.15^{bcd}	129.88± 4.7^{d}
	5 min, 20%	386.89± 13.69^{de}	26.62± 1.24^{fgh}	17.29± 1.17^{cdef}	135.34± 1.07^{efg}
	15 min, 40%	368.79± 8.56^{ef}	26.67± 0.63^{fgh}	17.93± 1.29^{defg}	137.43± 1.71^{gh}
	15min, 20%	352.28± 11.34^{fg}	25.21± 1.09^{hi}	17.97± 0.59^{defg}	139.45± 3.69^{ghi}
	25min, 40%	356.61± 7.42^{fg}	25.63± 1.26^{fgh}	17.93± 1.14^{defg}	139.89± 2.17^{ghij}
	25 min, 20%	308.48± 9.85^{h}	21.83± 0.63^{k}	19.87± 0.66^{h}	153.45± 4.81^{k}
Bath, 40 °C	30 min	305.4± 12.44^{h}	21.72± 1.27^{k}	19.56± 0.42^{gh}	150.74± 4.23^{k}
	45 min	387.62± 15.36^{de}	27.35± 1.47^{ef}	17.26± 1.25^{cdef}	135.94± 2.21^{fg}
	60 min	335.76± 15.60^{g}	25.21± 0.95^{hi}	18.78± 1.57^{efgh}	145.21± 1.09^{j}
Bath, 50 °C	30 min	353.53± 8.26^{fg}	25.68± 0.95^{fgh}	18.75± 0.75^{efgh}	141.92± 3.31^{hij}
	45 min	430.81± 6.94^{bc}	35.89± 0.47^{c}	15.39± 0.15^{ab}	116.95± 3.54^{b}
	60 min	408.06± 15.45^{d}	28.76± 0.80^{e}	16.56± 0.88^{bcd}	130.2±2.77^{de}
Bath, 60 °C	30 min	355.07± 7.67fg	25.47± 1.06^{ghi}	18.91± 0.69^{fgh}	139.28± 1.48^{ghi}
	45 min	409.80± 8.32^{cd}	32.61± 0.36^{d}	16.19± 0.81^{bc}	123.95± 1.62^{c}
	60 min	353.08± 5.80^{fg}	25.42± 0.36^{ghi}	18.75± 0.63^{efgh}	139.83± 4.32^{ghi}
Control	(SSE:ambient temp.,24h)	268.03± 8.58^{i}	17.51± 0.65^{i}	22.50± 0.84^{i}	177.81± 3.14^{l}

Data expressed as means ± standard deviations (n=3). Values with different letters within column indicate significance difference at $P < 0.05$. SSE: Shaker Solvent Extraction.

* %: amplitude of ultrasound waves

The best extraction temperature of oregano bioactive compounds is 50°C which is higher than the optimal temperature reported by Altemimi *et al.* (2015). This researchers announced 40°C as an optimal temperature by investigating the different temperature of ultrasound at 30- 50°C on the recovery rate of spinach extract polyphenols. They explained that the higher temperature of ultrasound can degrade some of the phenolic compounds that are dispersed in the extraction environment and had less thermal stability (Altemimi *et al.*, 2015). Most likely, the reason of the difference in the optimum temperature of the present study with the mentioned researchers is the presence of more heat stable phenolic compounds in oregano.

Table 2. DPPH radical scavenging activity (%) of different concentrations of Oregano leaf extract (µg /ml) in various treatments

Extract concentration / treatment		10	20	40	100	500	1000
Probe, 40 °C	5 min, 40%*	33.14 ±1.21 ^{bcd}	58.27± 2.71^{bc}	82.61± 0.28^{ab}	81.28± 1.11^{bc}	77.77± 1.15^{bcd}	75.39± 1.47^{abc}
	5 min, 20%	25.42± 1.88^{i}	49.37± 2.91^{fgh}	79.46± 1.40^{fg}	78.92±2.21^{b}	75.98±1.72^{def}	72.36±0.82^{def}
	15min, 40%	33.55± 0.97^{bc}	65.26±2.58^{a}	82.05± 0.44^{bcd}	80.86±2.37^{bc}	77.786±1.08^{bcd}	74.81±0.92^{bcd}
	15min, 20%	28.24± 2.89^{efgh}	48.27± 2.89^{gh}	82.10± 1.42^{bcd}	80.39± 2.27^{bc}	77.75± 0.54^{bcd}	73.13± 2.23^{cdef}
	25min, 40%	31.98± 3.02^{cdef}	55.03± 2.19^{cdef}	82.55± 1.46^{bcd}	80.36± 2.24^{bc}	76.21± 0.72^{cde}	74.61± 1.08^{bcd}
	25min, 20%	25.72± 2.70^{hi}	50.44±3.30^{efg}	80.20± 1.16^{def}	78.717± 2.10^{b}	75.66± 1.48^{def}	72.00± 1.71^{def}
Probe, 50 °C	5 min, 40%	36.31± 0.98^{ab}	61.40± 2.36^{ab}	82.61± 0.45^{ab}	81.85± 1.41^{bc}	78.99± 0.64^{a}	75.24± 1.88^{bcd}
	5 min, 20%	27.86± 1.41^{ghi}	52.22± 2.51^{def}	82.01± 0.79^{bcd}	81.21± 1.66^{bc}	77.00± 0.87^{bcde}	74.51± 2.53^{bed}
	15min, 40%	40.59±.1.66^{a}	65.51± 1.21^{a}	84.39± 1.80^{a}	82.56± 0.99^{a}	79.02± 0.9^{a}	76.21± 1.22^{ab}
	15min, 20%	28.10± 1.73^{efgh}	55.80±2.01^{bcde}	82.30± 1.31^{bcd}	81.59± 1.92^{bc}	76.86± 1.13^{bcde}	74.22± 1.86^{bcd}
	25min, 40%	33.72± 2.50^{bc}	58.39± 2.09^{bc}	82.03± 0.96^{bcd}	81.77± 1.56^{bc}	78.66± 2.28^{ab}	74.62± 1.20^{bcd}
	25min, 20%	25.86± 3.055^{h}	50.03± 1.50^{efg}	79.80± 1.48^{efg}	78.98± 1.14^{b}	75.51± 1.08^{def}	71.84± 2.08^{ef}
Probe, 60 °C	5 min, 40%	33.84± 3.66^{bc}	57.34± 3.02^{bcd}	81.40± 1.4^{cdrf}	80.83± 1.41^{bc}	77.73± 0.49^{bcd}	76.81± 1.39^{a}
	5 min, 20%	31.92± 3.36^{cdef}	55.06± 4.74^{cdef}	81.72± 1.00^{cde}	80.42± 2.18^{bc}	77.00± 1.02^{bcde}	73.93± 1.42^{bcd}
	15min, 40%	30.17± 1.40^{cdef}	53.74± 6.63^{cdef}	81.52± 1.18^{cdef}	80.70± 1.66^{bc}	1.02± 1.06^{bcde}	73.56± 1.48^{cde}
	15min, 20%	29.51± 0.83^{cdef}	53.98± 3.21^{cdef}	80.99± 1.21^{def}	80.02± 1.59^{bc}	76.87± 0.33^{cde}	73.46± 2.27^{cde}
	25min, 40%	30.44± 5.37^{cdef}	53.41± 0.83^{cdef}	80.71± 1.21^{cdef}	80.71± 1.60^{bc}	76.80± 2.50^{bcde}	73.73± 2.10^{cde}
	25min, 20%	25.45± 1.74^{i}	50.07± 1.34^{efg}	80.31± 1.63^{cdef}	80.31± 1.63^{bc}	75.73± 0.52^{def}	72.47±0.78^{def}
Bath, 40 °C	30 min	26.09± 3.59^{gh}	50.60± 2.11^{efg}	79.87± 0.97^{efg}	79.34± 1.89^{bc}	75.07± 2.21^{ef}	72.28± 1.33^{def}
	45 min	32.07± 4.03^{bcde}	55.12± 3.80^{cdef}	81.51± 0.35^{cdef}	79.81± 1.55^{bc}	76.88± 1.02^{bcde}	75.03± 2.62^{bcd}
	60 min	26.12± 3.45^{hi}	54.13± 4.12^{cdef}	79.18± 0.48^{g}	79.43± 1.43^{bc}	75.83± 1.63^{def}	72.67± 1.02^{def}
Bath, 50 °C	30 min	27.70± 2.46^{ghi}	52.38± 3.72^{def}	80.87± 0.56^{def}	80.37± 1.30^{bc}	74.65± 0.88^{ef}	72.38± 3.04^{def}
	45 min	36.40± 2.19^{ab}	61.07± 1.26^{ab}	83.96± 0.34^{ab}	81.73± 0.14^{bc}	78.53± 1.09^{abc}	75.21± 1.63^{bcd}
	60 min	32.78± 1.07^{bcde}	57.95± 3.82^{bcd}	82.67± 1.14^{ab}	81.46±0.90^{bc}	78.65±0.88^{ab}	75.31±1.02^{bcd}
Bath, 60 °C	30 min	27.99± 0.31^{efgh}	51.40± 3.14^{efg}	81.81± 1.26^{cde}	80.83± 2.08^{bc}	76.11± 0.78^{de}	72.78± 2.13^{cdef}
	45 min	33.50± 1.84^{bc}	59.26± 2.80^{bc}	81.91± 0.73^{cde}	80.87± 1.00^{bc}	78.60± 0.80^{abc}	75.44± 0.84^{abc}
	60 min	28.63± 0.39^{defg}	51.39± 3.03^{efg}	81.49± 0.18^{cdef}	79.85± 1.77^{bc}	75.80± 1.61^{def}	73.58± 2.35^{cde}
Control (SSE:ambient temp.,24 h)		20.93± 2.41^{j}	45.77± 2.44^{h}	76.79± 0.66^{h}	75.38± 0.53^{c}	73.70± 0.46^{f}	70.35± 1.14^{f}

Data expressed as means ± standard deviations (n=3). Values with different letters within column indicate significance difference at $P < 0.05$. SSE: Shaker Solvent Extraction.

* %: amplitude of ultrasound waves

In the other study, Moralli and Prado (2012) studied the extraction optimization for antioxidant phenolic compounds in red grape jam using ultrasound and reported that 50°C was the best extraction temperature of these compounds with the highest antioxidant activity, which shows the same results to the present study.

Effect of time

It was also observed that the phenolic and flavonoid content, and antioxidant activity of the ultrasonic probe was influenced by the exposure time, so that the phenolic content and antioxidant activity of the extracts increased at a constant temperature of 50°C and 40°C with increasing the time from 5 to 15 min. This phenomenon can be explained by the fact that more time is needed to release bioactive compounds from plant tissues and also increasing the variety of extracted compounds. However, the phenolic content of extracts decreased by increasing the extraction time from 15 to 25 min. Also, at the constant temperature of 60°C, with increasing extraction time we observed a decrease in the amount of phenolic compounds. The findings of Veggi *et al.* (2013) and Hammi *et al*. (2015) confirm these results. They investigated the extraction of Jatoba (*Jatoba bark*) polyphenols by using ultrasound at 50°C. They obtained the higher phenolic content during the first 15 minutes of the extraction process, due to the effect of phenolic concentration gradient between the solvent and the plant material.

At the beginning of the extraction, high phenolic content is located in contact with the solvent on the particle surface and accelerated the release of these compounds. However, after this time, the release of these compounds reduced the concentration gradient, as well as residual polyphenols which are located in the inner and deep parts of the particles are less accessible to the solvent. The reduction process of poly phenolic compounds at 60°C can be attributed to the further degradation of the obtained compounds at high temperature during the time (Cheok *et al*. 2013).

In the bath system, the same as the probe system, the phenolic and flavonoid content increased by increasing the extraction time from 30 to 45 min, and then decreased at 60 min. The difference is that in the bath system, the extraction of polyphenols takes a little more time than ultrasonic probes, which is probably due to lower power, lack of uniformity in the distribution of ultrasound energy, and the loss of produced energy in the fluid environment and the glass wall container and therefore leads to the less cavitation in the sample suspension.

Effect of amplitude

Based on Table 1, amplitude played an important role in intensification of extraction during the extraction of phenolic compounds and flavonoids by probe system. So that, in different combinations of temperature and time, the extraction efficiency of phenolic compounds and antioxidant activity in the upper range of the ultrasound (40%) was higher than the lower range (20%). In a similar study, Carrera *et al.* (2012) reported that the efficiency of tannin anthocyanin extraction using the amplitude (20 and 50%), increased the number of cavities thus improved the extraction process. Increasing the amplitude of ultrasound waves will increase the number of contraction and expansion cycles and the pressure range, resulting in more burst of cavitation bubbles. Hence, the higher ranges increase the effects of sonochemistry (Mason *et al.*, 2002; Cheok *et al*. 2013; Mediana-Torres *et al.*, 2017).

In this investigation, the traditional solvent extraction at ambient temperature for 24 hours as a control had the lowest total phenolic compounds (268.03± 8.58 µg GAE/ mg of dry extract) and the highest IC_{50} in DPPH radical scavenging and reducing power tests. Therefore, ultrasound technique was successful to extract the oregano phenolic compounds in all treatments. This can be attributed to the cavitation of the ultrasound technique, which imposes mechanical effects on the plant cell wall.

Table 3. Reducing power of different concentrations of Oregano leaf extract (µg /ml) in various treatments

Extract concentration / treatment		80	100	200	300	500	1000
Probe, 40 °C	5 min, 40%*	0.420± 0.00 ^{b}	0.448± 0.02^{cde}	0.690± 0.01^{def}	1.165± 1.00^{cde}	1.599± 0.01^{de}	2.647± 0.00^{ef}
	5 min, 20%	0.303± 0.012^{e}	0.388± 0.01^{kl}	0.602± 0.01^{j}	1.006±1.01^{i}	1.445±0.03^{h}	2.551±0.1^{efg}
	15min, 40%	0.445± 0.02^{ab}	0.464±0.02^{bc}	0.740± 0.01^{c}	1.225±0.00^{cd}	1.839±0.11^{c}	2.912±0.11^{c}
	15min, 20%	0.355± 0.03^{d}	0.401± 0.01^{ijkl}	0.639± 0.00^{i}	1.112± 0.00^{fgh}	1.498± 0.01^{gh}	2.582± 0.02^{efg}
	25min, 40%	0.392± 0.01^{c}	0.427± 0.00^{efgh}	0.677± 0.01^{efg}	1.131± 0.01^{fgh}	1.568± 0.01^{def}	2.639± 0.01^{efg}
	25min, 20%	0.303± 0.01^{e}	0.384±0.02^{kl}	0.606± 0.01^{j}	1.041± 0.04^{hi}	1.464± 0.00^{gh}	2.562± 0.09^{efg}
Probe, 50 °C	5 min, 40%	0.444± 0.01^{ab}	0.485± 0.00^{b}	0.785± 0.01^{b}	1.439± 0.01^{b}	2.001± 0.01^{b}	3.054± 0.01^{b}
	5 min, 20%	0.371± 0.00^{cd}	0.412± 0.01^{hijk}	0.653± 0.02^{ghi}	1.189± 0.09^{cde}	1.537± 0.01^{efg}	2.615± 0.00^{efg}
	15min, 40%	0.460±.0.01^{a}	0.512± 0.02^{a}	0.852± 0.04^{a}	1.568± 0.00^{a}	2.140± 0.01^{a}	3.222± 0.05^{a}
	15min, 20%	0.395± 0.01^{c}	0.415± 0.00^{ghij}	0.675± 0.01^{efgh}	1.151± 0.02^{def}	1.572± 0.00^{def}	2.639± 0.02^{efg}
	25min, 40%	0.424± 0.00^{b}	0.445± 0.00^{def}	0.702± 0.02^{de}	1.169± 0.01^{cde}	1.594± 0.01^{de}	2.651± 0.02^{ef}
	25min, 20%	0.364± 0.01^{d}	0.396± 0.00^{jkl}	0.642± 0.02^{hi}	1.113± 0.00^{fgh}	1.492± 0.00^{gh}	2.600± 0.01^{efg}
Probe, 60 °C	5 min, 40%	0.420± 0.01^{b}	0.455± 0.02^{cd}	0.700± 0.02^{de}	1.174± 0.10^{cde}	1.613± 0.02^{de}	2.653± 0.02^{ef}
	5 min, 20%	0.395± 0.02^{c}	0.431± 0.00^{defg}	0.677± 0.00^{efg}	1.128± 0.01^{fgh}	1.572± 0.01^{def}	2.641± 0.01^{ef}
	15min, 40%	0.394± 0.01^{c}	0.422± 0.01^{ghij}	0.668± 0.01^{fghi}	1.149± 0.02^{def}	1.575± 0.01^{def}	2.637± 0.01^{efg}
	15min, 20%	0.374± 0.01^{cd}	0.418± 0.01^{ghij}	0.658± 0.01^{fghi}	1.133± 0.00^{fgh}	1.534± 0.01^{fgh}	2.609± 0.01^{efg}
	25min, 40%	0.375± 0.01^{cd}	0.418± 0.01^{ghij}	0.654± 0.02^{ghi}	1.133± 0.01^{fgh}	1.534± 0.02^{fgh}	2.613± 0.02^{efg}
	25min, 20%	0.303± 0.01^{e}	0.375± 0.01^{l}	0.602± 0.02^{j}	1.066± 0.01^{ghi}	1.467± 0.00^{gh}	2.563± 0.02^{efg}
Bath, 40 °C	30 min	0.306± 0.01e	0.387± 0.011^{kl}	0.608± 0.02^{j}	1.048± 0.00^{ghi}	1.463± 0.01^{gh}	2.494± 0.01^{g}
	45 min	0.391± 0.00^{c}	0.424± 0.01^{fghi}	0.679± 0.02^{efg}	1.128± 0.01^{fgh}	1.57± 0.02^{def}	2.639± 0.02^{efg}
	60 min	0.305± 0.01^{e}	0.394± 0.01^{jkl}	0.638± 0.00^{i}	1.106± 0.01^{fgh}	1.465± 0.02^{gh}	2.529± 0.07^{fg}
Bath, 50 °C	30 min	0.374± 0.01^{cd}	0.409± 0.02^{hijk}	0.647± 0.01^{ghi}	1.137± 0.00^{efg}	1.540± 0.03^{efg}	2.629± 0.01^{efg}
	45 min	0.442± 0.01^{ab}	0.489± 0.02^{bc}	0.793± 0.02^{b}	1.426± 0.03^{b}	2.019± 0.04^{b}	3.062± 0.18^{b}
	60 min	0.420± 0.01^{b}	0.446± 0.02^{cde}	0.706± 0.01^{d}	1.174±0.10^{cde}	1.628±0.12^{d}	2.690±0.14^{e}
Bath, 60 °C	30 min	0.377± 0.01^{cd}	0.416± 0.01^{ghij}	0.660± 0.00^{fghi}	1.181± 0.08^{cde}	1.539± 0.01^{efg}	2.608± 0.02^{efg}
	45 min	0.445± 0.01^{ab}	0.464± 0.02^{bc}	0.745± 0.00^{c}	1.254± 0.12^{c}	1.820± 0.17^{c}	2.849± 0.01^{d}
	60 min	0.374± 0.01^{cd}	0.417± 0.01^{ghij}	0.656± 0.02^{ghi}	1.135± 0.00^{fgh}	1.543± 0.02^{efg}	2.614± 0.01^{efg}

Control (SSE:ambient temp.,24 h)	0.202± 0.02^{d}	0.285± 0.01^{m}	0.558± 0.02^{k}	0.782± 0.02^{j}	1.249± 0.13^{i}	1.948± 0.09^{h}

Data expressed as means ± standard deviations (n=3). Values with different letters within column indicate significance difference at $P < 0.05$. SSE: Shaker Solvent Extraction.

* %: amplitude of ultrasound waves

On the other hand, ultrasound accelerates the hydration and swelling process, causing to grow the plant cell wall cavities, increasing the mass transfer of soluble compounds from plant to solvent, and thus improving the efficiency of phenolic compounds extraction. Probably, the degradation of plant cells increases the solvent penetration rate into the plant tissue after the collapse of cavitation bubbles. Altemimi *et al.* (2015) and Han *et al.*, (2011) reported the same results which confirmed that ultrasound power can play an important role during the dissolution of biologically active compounds of plant tissues. Ultrasound treatments also produced higher polyphenol content in shorter time and thus reduced energy consumption (Veggi *et al.*, 2013).

In this study, the obtained content of oregano extracts phenolic compounds was the highest in ultrasonic probe treatment (50°C, 5 min and amplitude 40%) and its lowest content was unsteady from 268.03 to 473.32 µg GAE/mg of dry extract in traditional solvent extraction, which was higher than the total content of phenol (220 µg GAE/ mg of dry extract) reported by Sahin *et al.* (2004). This diversity of the content and even types of the compounds can be attributed to the difference in geographical area of plant growth due to natural cross-pollination. However, many references were reported rosmarinic acid as the most significant phenolic compound of oregano (Chun *et al.*, 2005).

Based on the results of phenolic and flavonoid compounds, it can be expressed that ultrasonic probe was more efficient than ultrasonic bath due to higher power as well as the power to concentrate energy production on the target sample if other conditions, such as temperature and extraction time, were more favorable than ultrasonic bath. However, this system showed a lower efficiency at the lower ultrasound amplitude (20%), as well as unfavorable time and temperature conditions due to degradation of dispersed bioactive compounds. Therefore, efficient extraction of phenolic compounds with an ultrasound system requires the selection of optimal conditions

Correlation between Amounts of Phenolic Compounds and Flavonoids with Antioxidant Activity

There was a significant negative linear correlation between the polyphenol content with IC_{50} of the DPPH radical scavenging and the reducing power, and between the flavonoids with IC_{50} of the DPPH radical scavenging and IC_{50} of the reducing power, meanwhile there was a positive significant correlation between IC_{50} of DPPH radical scavenging and reducing power tests (Table 4).The negativity of this equation means that as the polyphenol content increases, IC_{50} decreases and as a result, antioxidant activity increases (Maghsoudlou *et al.*, 2016).

Conclusions

In all conditions, ultrasound treatments have been able to increase the extraction efficiency of oregano leaves phenolic and flavonoid compounds compared to solvent extraction. The best conditions for extraction of phenolic and flavonoid compounds, and antioxidant activity were obtained by probe system at 50 °C for 15 min and ultrasound amplitude 40%. In addition, oregano leaf extract indicated high antioxidant activity based on DPPH free radical scavenging and reducing power tests. Therefore, oregano leaf extract can be used as good sources of natural antioxidants to improve the oxidative stability of food. Extraction of oregano antioxidants by ultrasound significantly reduced the extraction time from 24 h in solvent extraction to 15 and 45 min in ultrasonic probe and bath, respectively.

.Table 4. Line equations and correlation coefficients between the amount of phenolic and flavonoid compounds with antioxidant activity of extracts

Methods	r (Pearson's correlation coefficients)	R^2	Line equation
(A) Total phenolic compounds (µg gallic acid/mg of dry extract)			
IC_{50} of DPPH (µg/ml)	- 0.973	0.94	y = - 0.0386x + 32.114
IC_{50} of reducing power (µg /ml)	- 0.967	0.93	y = - 0.2862x + 242.96
(B) Total flavonoids (µg quercetin/mg of dry extract)			
IC_{50} of DPPH (µg /ml)	- 0.883	0.78	y = - 0.2906x + 25.875
IC_{50} of reducing power (µg /ml)	- 0.935	0.87	y = - 0.2975x + 200.69
(C) Relationship between antioxidant activity assays			
IC_{50} of DPPH and Reducing power (µg /ml)	0.960	0.92	y = 0.1287x+ 0.188

The results of this study confirm the effectiveness of the ultrasound method for extracting the polyphenols with high antioxidant activity.

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اثر فرآیندهای حمام و پروب فراصوت بر فعالیت آنتی‌اکسیدانی عصاره فنولی برگ‌های پونه کوهی (*Origanum vulgar* L.)

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چکیده

استخراج به کمک فراصوت به دلیل مزایای بسیار آن در استخراج ترکیبات زیست‌فعال از نمونه‌های گیاهی، مورد توجه زیادی قرار گرفته است. کارایی اولتراسوند تحت تاثیر عوامل مختلفی نظیر دمای استخراج، زمان استخراج، شدت امواج و نوع سیستم فراصوت متفاوت است. بنابراین، این مطالعه با هدف بررسی شرایط فرآیند دو نوع سیستم فراصوت(حمام و پروب) برای تعیین بالاترین راندمان استخراج ترکیبات فنولی و فلاونوئیدی برگ پونه کوهی و بهترین شرایط استخراج انجام شد. اثر ترکیب‌های متفاوت متغیرهای اولتراسوند شامل حمام (دما، 60-40 درجه سانتی‌گراد ؛ زمان، 60-30 دقیقه) و پروب (دما، 60-40 درجه سانتی‌گراد ؛ زمان، 25-5 دقیقه؛ دامنه اولتراسوند، 40-20 درصد) با استفاده از حلال اتانول- آب (50:50) موردررسی قرار گرفت. سپس فعالیت آنتی‌اکسیدانی عصاره‌ها توسط مهار رادیکال DPPH و قدرت احیاکنندگی ارزیابی شد. بهترین شرایط برای استخراج ترکیبات فنولی و فلاونوئیدی با استفاده از سیستم پروب فراصوت در دمای50°C به مدت 15 دقیقه و دامنه 40% به‌دست آمد. حداکثر فنول و فلانوئید به‌ترتیب معادل25/9 ±473/32 میکروگرم گالیک اسید در میلی‌گرم عصاره خشک و 1/24 ±46 میکروگرم کوئرستین در میلی‌گرم عصاره خشک بود. نتایج نشان داد روش فراصوت اثر قابل توجهی بر استخراج ترکیبات زیست‌فعال از برگ‌های پونه کوهی دارد، همچنین سیستم پروب کارایی بالاترینسبت به سیستم حمام داشت.

واژه‌های کلیدی: حمام فراصوت، پروب فراصوت، برگ پونه کوهی، ترکیبات فنولیک، فعالیت آنتی‌اکسیدانی

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Study on rheological, physicochemical and sensory properties of synbiotic ice cream using fibers from some fruit peels and *Lactobacillus casei* LC-01

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Abstract

A nutraceutical food may provide expanded utility beyond its nutritional benefit. These benefits are commonly attributed to the active components of the food. Fruit by-products are rich source of dietary fibers that have beneficial effects on human health. Also they can improve the growth and viability of probiotics in food matrix and therefore suitable to produce synbiotic food products. In this study, the effect of adding fiber obtained from apple, banana and mango peels at levels of 0.5, 1 and 1.5% on physicochemical and sensory features and the viability of *Lactobacillus casei* LC-01 in ice cream during 60 days storage at -18°C was investigated. Based on the results, all ice cream mixes were pseudoplastic fluids. The values of flow behavior index decreased and consistency coefficients increased by increasing the level of all mentioned fibers. The pH and specific gravity of samples containing banana and mango fibers were lower than control and sample with apple fiber. Using fibers had no significant effects on overrun values, whereas viscosity and melting resistance of ice cream samples increased with increasing fiber amounts. The most reduction in *Lactobacillus casei* LC-01 count after freezing and during storage period was associated to control sample and adding all types of examined fibers improved probiotic viability. Minimum cell reduction after freezing and during storage period occurred in sample containing 1.5% mango fiber with 0.03 and 0.48 log cycle respectively. Sensory properties of samples containing apple fiber were good and comparable with control sample.

Key Words: Fiber, Ice cream, Probiotic, Synbiotic

Introduction

The benefits promoted by probiotic bacteria are increasingly explored in various types of foods. However, cell viability in these products is often low and the ability to survive and multiply in the digestive tract strongly influences the benefits that probiotics can produce.

Fruit by-products are rich source of dietary fibers that have beneficial effects on human health. It was demonstrated that some fibers of fruit by-products show functional properties such as water-holding, swelling, gel forming, bile acid binding, and cation-exchange capacities (Lamsal and Faubion, 2009). Among the promising fruit by-products are the peels of apple, banana and passion fruit, mainly because of their content of insoluble and soluble dietary fibers (DF), pectin and fructooligosaccharides. These prebiotics are in fact able to selectively stimulate the growth and activity of the gut microbiota, particularly *lactobacilli* and *bifidobacteria* (Davis and Milner, 2009).

Dietary fiber as by-product from apple, banana or passion fruit processing was shown to increase the viability of *Lactobacillus acidophilus* L10 and *Bifidobacterium animalis* subsp. *lactis*B104, HN019 and B94 and short chain and poly unsaturated fatty acid contents of yoghurt (Do Espírito Santo *et al*., 2012). Citrus fiber presence in fermented milk also enhanced bacterial growth and survival of the tested probiotic *Lactobacillus acidophilus* CECT 903, *Lactobacillus casei* CECT 475 and *Bifidobacterium bifidum* CECT 870(Sendra et al., 2008). Also the addition of açai pulp increased *L. acidophilus* L10, *B. animalis* ssp. *lactis* Bl04 and *B. longum* Bl05 counts at the end of 28 days of cold storage in probiotic yoghurt (Do Espírito Santo et al., 2010).

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Vieira et al. (2017) evaluated the impact of fruit (acerola, orange, passion fruit, and mango) and soybean by-products and amaranth flour on the growth of probiotic and starter microorganisms and also the extent of folate production in MRS media. Orange and passion-fruit by-products were the substrates that most promoted the growth of bacterial population. Also the combination of orange by-product and amaranth flour was the best substrate for production of folate by all tested microorganisms (Albuquerque *et al*., 2016).

Pineapple peel powder was reported to be an effective prebiotic in probiotic yoghurt with improved survival of *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus paracasei* spp. *Paracasei* and acceptable physicochemical properties (Sah *et al*., 2016).

The prebiotic potential of soy milk and yacon flour on *Lactobacillus acidophilus* La-5 was shown in apple and strawberry ice cream respectively (Matias *et al.*, 2016;Parussolo *et al*., 2017).

However negative effect of mango and guava pulp on *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* Bb-12Viability in soy yoghurt and simulated gastrointestinal stress was reported by Bedani et al. (2014).

The objective of this study was to investigate the influence of addition of apple, banana and mango peel powder on the viability of *Lactobacillus casei* LC-01 in probiotic ice cream during 60 days storage at -18˚C. The effect of fiber addition on rheological, physicochemical and sensory properties of ice cream was also conducted.

Materials and Methods

Milk containing 2.5% fat and 8% solid nonfat (Pegah Milk Industry, Mashhad, Iran), cream containing 30% fat (Pegah Milk Industry, Mashhad, Iran), low fat milk powder of 1% fat and 96% total milk solids (Golshad Co, Iran), commercial stabilizer emulsifier blend (Panisol ex, Danisco,Denmark), sugar powder and vanilla were obtained from reputable scientific suppliers.

Apple, banana and mango peels were collected and stored at -25˚C until used. The peels were cut, washed with hot water (90˚C for 5min) and dried at 60˚C until constant weight. Dried peels were milled and sieved to less than 40 µm particles (Larrauri *et al*., 1997).

Activation of probiotic bacteria

Lactobacillus casei LC-01 was activated in MRS broth medium at 37˚C for 24 hours under anaerobic condition. The cells were then collected at 4600g for 5min (Homayouni *et al*., 2008).

Ice cream preparation

Product formulation comprised of 12% SNF, 10% fat, 15% sucrose, 0.4% stabilizer emulsifier blend as well as 0.1% vanillin; total solids being 37.5%. The batch size totaled 500 grams. Apple, banana and mango peel powders were used at 0.5, 1 and 1.5% w/w. Ice cream mixes were prepared according to Marshall and Arbuckle (1999) and the samples were aged at 4˚C for 12 hours. Cell suspension prepared previously was added to the mixes after aging then some of the mixes were collected for rheological and physicochemical analysis and also enumeration of *Lactobacillus casei* LC-01before freezing. The remained mixes were frozen in batch type ice cream maker at -18˚C for 20min followed by packing in 50g containers and storing at -18˚C for completing hardening process.

Rheological measurements

Flow behavior of ice cream mixes was evaluated using rotational viscometer (Brookfield model viscometer DV III ULTRA) equipped with a circulator. Product temperature was controlled at 5±0.5°C by a refrigerated/ heating circulator. Range of shear rate was 0.22-55 s^{-1} and apparent viscosity was expressed in Pa.s at a shear rate of 52.1s^{-1} (Morris, 1983).

Physicochemical analysis

pH of ice cream mixes was determined with Metrohm pH meter (Switzerland). The specific gravity of ice cream mix was determined at

25ºC using a pichnometer according to the method of Muhsenin (1978).

The overrun of ice cream samples was estimated using the formula of Marshal and Arbuckle (1996).

Ice cream melting rate was determined according to Sakurai *et al*. (1996). The ice cream (30 g), at -18ºC was placed on a Buchner funnel at ambient temperature (25°C). The weight of the melted material was recorded after 15 minutes and expressed as percentage weight melted.

Enumeration of *Lactobacillus casei* LC-01

Bacterial enumerations were carried out before and after freezing and at days 7, 15, 30 and 60 in triplicate of each batch. MRS-agar medium was used under anaerobic condition at 37˚C for 72 hours. Samples (1 ml) were diluted with 0.1% sterile peptonated water (9 mL). Afterwards, serial dilutions were carried out, and bacteria were counted, applying the pour plate technique. Cell concentration was expressed as CFU g^{-1} of ice cream.

Sensory evaluation

The sensory evaluation of probiotic ice cream was carried out by 18 trained panelists. Ice cream samples were removed from frozen storage (-18ºC) after 24 hours of hardening and immediately offered to panelists. Ice cream samples were coded with three digit random numbers with all orders of serving completely randomized and while served in odorless plastic cups. A 9-point hedonic scale was employed to determine the degree of liking of the products (9= Extreme like, 5= Neither like nor dislike, 1= Extreme dislike). The samples were rated for color and appearance, flavor/ taste, body/texture, as well as overall acceptability as prescribed by Herald *et al.* (2008).

Statistical Analysis

Data coming from three replications were obtained by applying a one factor completely randomized block design with the outcome of the data being analyzed through MSTAT-C software and by use of Analysis of Variance technique. Significant differences ($P \leq 0.05$) were determined through the Duncan's Multiple Range Test. Excel software was employed for plotting the curves.

Results and Discussion

Flow behavior of ice cream Mixes

According to primary tests, the results obtained from all samples were non-newtonian, time independent fluids, as in conformity with some previous reports (Cottrel *et al*., 1980; Goff and Davidson, 1994; Kaya and Tekin, 2001).

Profiles of shear stress and of viscosity versus shear rate revealed shear thinning behavior of all mixes where viscosity values decreased with increasing shear rate. The reason for such behavior is that in low shear rates, molecules are in irregular arrangements that lead to high viscosity values. With increasing shear rate, these molecules get in more similar directions and consequently intermolecular friction increases while viscosity values decreases (Rha, 1975; Glichsman, 1982).

In the current study, shear stress and shear rate values were fitted using power law model:

$$\tau = k\gamma^n \quad (1)$$

Where, τ is shear stress (Pa), γ stands for shear rate (s^{-1}), k is consistency coefficient (Pa s^n) and n denoting flow behavior index.

Flow behavior indices, consistency coefficients and correlation coefficients (r^2) of the model for each sample are presented in Table1. Values of n less than 1 obtained for all samples indicating the shear thinning behavior.

As it can be seen from Table1, using fibers and increasing their amounts decreased flow behavior indices but it is more significant for mango fiber. The minimum n value (0.31) was associated to the sample containing 1.5% of mango fiber.

According to Chinnan *et al.* (1985) psudoplasticity increased by decreasing n. Also Marcotte *et al.* (2001) mentioned that the changes in flow behavior index is related to molecular size. Similarly, Soukoulis *et al.* (2009) showed that the use of dietary fibers in

ice cream mix formulation significantly increased viscosity and shear thinning behavior.

Table 1. Flow behavior indices (n), consistency coefficients (k) and correlation coefficients (r^2) of power law model for ice cream mixes containing different fibers

	control	apple			banana			mango		
		0.5%	1%	1.5%	0.5%	1%	1.5%	0.5%	1%	1.5%
n	0.51±0.02a*	0.52±0.023a	0.45±0.019ab	0.41±0.017b	0.51±0.02a	0.45±0.019ab	0.39±0.016b	0.41±0.017b	0.42±0.018b	0.31±0.013c
k (pa.s^n)	4.96±0.09e	6.4±0.13d	14.39±0.27dc	17.82±0.3c	8.45±0.17de	18.89±0.33c	31.37±1.65ab	21.43±0.95bc	26.9±1.135b	44.12±2.03a
r^2	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99

*Values in a column which do not share a common letter are statistically different (P= 95%).

Also, it was reported that by incorporating sugar beet fiber at levels of 0.7 to 2% , flow behavior index of ice cream and frozen yoghurt decreased compared to control (Mahdian *et al*., 2014). The minimum consistency coefficient was obtained for control sample and k value was higher in samples containing more amounts of all examined fibers. Maximum k value (44.12 pa.s^n) was associated to 1.5% mango fiber ice cream.

Consistency coefficient being an important parameter for estimating viscous nature of food products(Sopade and Kassum, 1992) and was reported to be in the ranges of 0.0733 to 1.260 Pa s^n and 0.145 to 0.0211 Pa s^n by Muse and Hartel (2004) and by Minhas *et al*. (2002) respectively. The higher consistency coefficients obtained for ice cream mixes in this study, in comparison with the previously reported values, may be attributed to the incorporation of fibers and the effect of carbohydrates on water absorption and viscosity of the samples. Similarly, consistency coefficients of ice cream and frozen yoghurt mixes containing sugar beet fiber were reported to be more than the mixes without any fiber added (Mahdian *et al*., 2014).

Physico-chemical Properties

The physico-chemical properties of ice cream mixes and of the finished ice cream are presented in Table 2.

Table 2. Physico-chemical properties of ice cream mixes and of the finished ice cream containing different fibers

	control	apple			banana			mango		
		0.5%	1%	1.5%	0.5%	1%	1.5%	0.5%	1%	1.5%
pH	6.48±0.18bc*	6.54±0.19ab	6.55±0.19ab	6.6±0.2a	6.56±0.18ab	6.55±0.17ab	6.52±0.21b	6.55±0.18ab	6.41±0.15c	6.31±0.15d
Specific gravity	1.11±0.05a	1.11±0.04a	1.11±0.05a	1.11±0.03a	1.10±0.02d	1.10±0.06d	1.11±0.06b	1.10±0.05e	1.10±0.04d	1.10±0.05c
Viscosity (pa.s)	0.66±0.009c	0.93±0.01c	1.58±0.04bc	1.78±0.05b	1.19±0.04c	2.12±0.06b	3.76±0.08a	2.11±0.07b	2.74±0.08b	4.25±0.09a
Overrun (%)	35.33±1.58b	73.85±2.51a	48.95±1.88b	48.45±1.92b	42.99±2.08b	45.83±1.73b	38.42±2.04b	52.63±2.53ab	46.18±1.93b	35.53±1.57b
Melting resistance (%)	87.5±5.54c	89.2±5.21bc	93±6.14a	93.5±6.64a	88±5.91c	92.7±6.24ab	93±6.71a	89±5.54bc	93±7.06a	94.2±6.53a

*Values in a column which do not share a common letter are statistically different (P= 95%).

No significant differences were observed between pH of control and apple and banana fiber samples (p>0.05).Increasing mango fiber from 0.5%, decreased ice cream pH

significantly and the minimum pH was associated to sample containing 1.5% mango fiber. Similarly Mahdian et al reported a decrease in pH of frozen yoghurt containing sugar beet fiber in comparison with control ice cream without fiber (Mahdian *et al*., 2014).

Specific Gravity (SG) of ice cream mixes were not affected by adding apple fiber but using banana and mango fiber decreased SG significantly ($p<0.05$). The specific gravity of ice cream mix as reported by the authors ranged from 1.05 to 1.12 (Marshal and Arbuckle, 1996).

Using fruit fibers and increasing their amounts increased ice cream mix viscosity in comparison with control. In this way, more viscosity values were obtained for mixes containing banana and mango fiber with maximum viscosity (4.25 pa.s) obtained for the mix with 1.5% mango fiber. The increased viscosity of the fiber-enriched ice cream mixes seems to be caused both by the contribution of the soluble matter to the composition of the aqueous phase and by the contribution of insoluble fibers to the increase of total solids, affecting the three dimensional conformation of the hydrated biopolymers (Soukoulis*et al*., 2009). According to Aime *et al*. (2001) consistency coefficients were positively correlated with the apparent viscosity values ($r^2= 0.910$) so the greater viscosity values obtained for samples containing banana and mango fiber may be the result of higher consistency coefficients for these samples.

Overrun values for ice cream samples containing all fibers were a bit more than control but their differences were not significant ($p>0.05$). In this case, maximum overrun value (73.85%) was associated to 0.5% apple fiber ice cream. In general, as the viscosity increases, the resistance to melting and the smoothness of texture increases, however, this may be deterrent to whipping. High acidity contributes to excess mix viscosity, leading to decrease overrun (Arbuckle, 1986).

Adding fibers more than 1% in probiotic ice cream formulation increased melting resistance significantly ($p<0.05$). The minimum (87.5%) and maximum (94.2%) melting resistance were associated to control and ice cream containing 1.5% mango fiber respectively. Fat destabilization is the most important parameter affecting ice cream melting rate (Muse and Hartel, 2004). Herald *et al*. (2008) reported that increasing ice cream mix viscosity resulted in lower melting rate and improved product smoothness. Similar results were reported about using sugar beet fiber in ice cream and frozen yoghurt where increasing mix viscosity and melting resistance took place with increasing fiber amounts(Mahdian *et al.*, 2014).

***Lactobacillus casei* LC-01 viable counts**

The viable counts of *Lactobacillus casei* LC-01 during 60 days storage at -18˚C in ice cream samples containing apple, banana and mango fibers are presented in Tables 3, 4 and 5 respectively.

Table 3. *Lactobacillus casei* LC-01 viable counts before and after freezing and during storage time in ice cream samples containing apple fiber

	Storage time (day)					
sample	**0**	**1**	**7**	**15**	**30**	**60**
Control	6.25±0.14b*	5.48±0.12c	5.25±0.13c	5.18±0.12c	5.15±0.15c	5.11±0.19c
0.5%	6.65±0.12b	6.43±0.12b	6.18±0.21b	5.75±0.11bc	5.56±0.13c	5.25±0.18c
1%	6.04±0.1b	5.65±0.1b	5.61±0.18b	5.45±0.17c	5.39±0.1c	5.57±0.13c
1.5%	7.56±0.16a	7.41±0.15a	7.48±0.15a	6.53±0.14a	6.95±0.18a	6.71±0.15ab

*Values in a column which do not share a common letter are statistically different (P= 95%).

As it can be seen from the data presented in tables, using all examined fibers in probiotic ice cream and increasing their amounts resulted in protection of *Lactobacillus casei* LC-01 against freezing process and also during storage.

Lactobacillus casei LC-01 cell reduction after freezing, was significant just in control sample ($p<0.05$). Considering the minimum viable probiotic counts needed for creating health benefits (10^6 cfu/g), all samples with 1.5% apple and mango fiber had this standard at the end of storage period. Freezing of ice cream mixes containing 0, 0.5, 1 and 1.5% apple fiber has leaded to reduction of 0.78, 0.22, 0.39 and 0.14 log cycle in *Lactobacillus casei* LC-01 counts respectively. Cell reduction after 60 days of storage in those samples was 1.14, 1.4, 0.48 and 0.85 log cycle respectively. Cell reduction in ice cream samples containing 0, 0.5,1 and 1.5% banana fiber was 0.78, 0.21. 0.46 and 0.41 log cycle after freezing and 1.14, 1,16, 0.63, 1.4 log cycle after storage time respectively

Table 4. *Lactobacillus casei* LC-01 viable counts before and after freezing and during storage time in ice cream samples containing banana fiber

	Storage time (day)					
sample	**0**	**1**	**7**	**15**	**30**	**60**
Control	6.25±0.14b*	5.48±0.13c	5.25±0.12c	5.18±0.15c	5.15±0.18c	5.11±0.12c
0.5%	7.46±0.15a	7.25±0.15a	7.28±0.14a	7.2±0.14a	7.36±0.14a	6.3±0.15b
1%	6.95±0.16a	6.49±0.22b	6.51±0.11b	6.33±0.09b	6.21±0.17b	6.33±0.18b
1.5%	6.93±0.11a	6.52±0.23b	6.9±0.21a	6.96±0.12a	6.69±0.11b	5.53±0.13b

*Values in a column which do not share a common letter are statistically different (P= 95%).

In the case of probiotic ice cream samples with mango fiber, cell reduction rate was less than samples with apple and banana fibers and also control sample. 0.78, 0.19, 0.05 and 0.03 log cycle reduction was obtained after freezing and 1.14, 0.65, 0.67 and 0.48 log cycle after storage time for samples containing 0, 0.5,1 and 1.5% mango fiber respectively.

Table 5. *Lactobacillus casei* LC-01 viable counts before and after freezing and during storage time in ice cream samples containing mango fiber

Storage time (day)						
sample	**0**	**1**	**7**	**15**	**30**	**60**
Control	6.25±0.14b*	5.48±0.12c	5.25±0.16c	5.18±0.15c	5.15±0.14c	5.11±0.13c
0.5%	6.62±0.25b	6.43±0.15b	6.28±0.16bc	6.38±0.16b	6.3±0.12b	5.97±0.11bc
1%	6.57±0.18b	6.52±0.11b	6.29±0.13b	6±0.14b	5.95±0.16bc	5.9±0.15b
1.5%	7.59±0.19a	7.56±0.1a	7.34±0.19a	7.31±0.12a	7.24±0.18a	7.11±0.19b

*Values in a column which do not share a common letter are statistically different (P= 95%).

As it can be seen from the data of Tables 3, 4 and 5, *Lactobacillus casei* LC-01cell reduction after freezing and during 60 days storage at -18˚C was more in control probiotic ice cream with no fiber added and apple, banana and mango peel powder could act as prebiotics which can improve *Lactobacillus casei* LC-01viability in ice cream. In this case mango peel powder had more effect on protection of probiotic cells and minimum cell reduction (0.48 log cycle) was associated to the sample containing 1.5% mango fiber.

Dietary fibers obtained from apple, banana and passion fruit byproducts were tested as prebiotic on the viability of *Lactobacillus acidophilus* L10 and three strains of *Bifidobacterium animalis* subsp. *lactis* in probiotic yoghurt. Apple and banana fibers had more effect on protection of all probiotic bacteria than passion fruit fiber. Also *Lactobacillus acidophilus* showed lower survivability than *Bifidobacteria* at the end of storage period (Do Espírito Santo *et al*., 2012). Previously they had been reported that Açai pulp favored an increase in *Lactobacillus acidophilus* L10, *Bifidobacterium animalis* ssp. *lactis* Bl04 and *Bifidobacterium longum* Bl05 counts at the end of 4 weeks of cold storage (Do Espírito Santo *et al*., 2010).

The functionality of Yacon flour as a source of fructooligosaccharide on the viability of *Lactobacillus acidophilus* NCFM in probiotic

ice cream was shown by Parussolo *et al.* (2017). According to Vieira *et al.* (2017), Orange and passion-fruit by-products were the substrates that most promoted the growth of *Lactobacillus* and *Bifidobacteria*. However, negative effect of mango and guava pulp on *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* Bb-12 Viability in soy yoghurt and simulated gastrointestinal stress was reported by Bedani *et al.* (2014). In the previous work, we concluded that sugar beet fiber can improve the growth and viability of *Lactobacillus acidophilus* La-5 and *Bifidobacterium bifidum* Bb-12 in probiotic frozen yoghurt and ice cream during storage period (Mahdian *et al*., 2014).

Sensory properties

Sensory attributes of probiotic ice cream containing varying levels of apple, banana and mango peel powder are presented in Table 6. No significant differences were observed between flavor and texture scores of all samples and all fiber contained ice cream had flavor and texture acceptability comparable with control ($p>0.05$). The color of probiotic ice cream containing1.5% mango fiber was a bit dark and has the minimum score (4.9). All other samples gained color score comparable with control. Overall acceptability of control ice cream was higher than experimental samples but the difference was not significant with 0.5 and 1% apple fiber and 1.5% banana fiber ice cream ($p>0.05$).

Table 6. Sensory attributes of probiotic ice cream containing varying levels of apple, banana and mango peel powder

	control	apple			banana			mango		
		0.5%	1%	1.5%	0.5%	1%	1.5%	0.5%	1%	1.5%
Flavor	6.9±1.15ab*	7.1±1.15a	6.1±0.71ab	4.5±0.88ab	5.1±1.2ab	5.2±0.56ab	5.8±1.23ab	4.4±0.35ab	5.2±1.33ab	4.2±0.55b
Texture	7±1.2a	6.4±1.26a	6.2±1.22a	6±1.19ab	6.4±1.35a	5.4±1.23ab	6±1.15ab	5.3±0.54ab	5.2±1.2ab	3.8±0.42b
Color	8±1.18a	7.2±1.45ab	6.7±1.28abc	6.2±1.51abc	6.1±1.43abc	5.9±1.19abc	5.8±1.19bc	6.2±1.25abc	6±1.43abc	4.9±0.61c
Overall acceptance	7.7±1.31a	7±1.37ab	6.3±1.41ab	5.4±0.79bc	5.3±1.21bc	5.3±0.73bc	6.1±1.23abc	5.1±0.7bc	5.1±0.6bc	4.1±0.72c

*Values in a column which do not share a common letter are statistically different (P= 95%).

It is reported that using 0.7 and 1% sugar beet fiber in probiotic ice cream formulation had no negative effect on flavor acceptability while decreased color score significantly (Mahdian *et al*., 2014). Also probiotic yoghurt containing 1.3% citrus fiber with good acceptability was produced by Dello Staffolo *et al.* (2004). Dervisoglu and Yazici (2006) reported that using 0.8% citrus fiber had no significant effect on flavor, texture and overall acceptance of ice cream but flavor score decreased when more amounts used. Similar results have been reported on fermented milks by Sendra *et al.* (2008). Regarding the consumer intent to purchase, 74% of the panelists would buy the ice cream with the fiber from orange by-products as fat replacer (Crizel *et al*., 2013).

Conclusion

Probiotic ice cream containing apple, banana and mango peel powder was non Newtonian time independent fluid with flow behavior indices less than 1. Flow behavior indices decreased and consistency coefficients increased with increasing fiber amounts from 0 to 1.5%. pH and specific gravity of samples containing banana and mango fibers were lower than control and sample with apple fiber added. Using fibers had no significant effects on overrun values where viscosity and melting resistance of ice cream samples increased with increasing fiber amounts. Based on obtained results, apple, banana and mango peel powder would act as prebiotics which can improve growth and viability of *Lactobacillus casei* LC-01 in ice cream during freezing and freeze storage. In that case the most promising

prebiotics was mango fiber at level of 1.5%. Sensory properties of samples containing apple fiber were good and comparable with control sample.

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بررسی ویژگی‌های رئولوژیکی، فیزیکوشیمیایی و حسی بستنی سین‌بیوتیک با کاربرد فیبر حاصل از پوست میوه‌جات و باکتری *لاکتوباسیلوس کازئی* LC-01

الهام مهدیان[1*]- رضا کاراژیان[2]

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چکیده

یک غذا دارو می‌تواند اثرات سودمند بیشتری علاوه بر مزایای تغذیه‌ای ایجاد نماید. این اثرات عموما به اجزاء فعال موجود در این غذاها مربوط می‌شود. ضایعات میوه‌جات منبع غنی از فیبرهای رژیمی می‌باشند که اثرات مثبتی بر سلامتی انسان دارند. این فیبرها همچنین می‌توانند رشد و فعالیت باکتری‌های پروبیوتیک را در ماتریکس غذایی بهبود داده و بنابراین برای تولید محصولات غذایی سین‌بیوتیک مورد استفاده قرار گیرند.در این تحقیق اثر افزودن فیبر حاصل از پوست موز، سیب و انبه بر خصوصیات فیزیکوشیمیایی و حسی و همچنین قابلیت زنده‌مانی باکتری *لاکتوباسیلوس کازئی* LC-01 در بستنی در مدت 60 روز نگهداری در دمای 18- درجه سانتی‌گراد بررسی و با نمونه شاهد فاقد فیبر مورد مقایسه قرار گرفت. بر اساس نتایج به‌دست آمده مخلوط همه نمونه‌ها رفتار رقیق‌شونده با برش نشان دادند. با افزایش مقدار هر سه فیبر اندیس رفتار جریان کاهش و ضریب قوام افزایش پیدا کرد. مقادیر pH و وزن مخصوص نمونه‌های حاوی فیبر موز و انبه پایین‌تر از نمونه شاهد و نمونه‌های حاوی فیبر سیب بودند. استفاده از فیبرها اثر مشخصی بر ضریب افزایش حجم نمونه‌های بستنی نداشته اما مقادیر ویسکوزیته و مقاومت به ذوب با افزایش مقدار فیبرها افزایش یافت. بیشترین کاهش جمعیت لاکتوباسیلوس کازئی LC-01 بعد از انجماد و طی دوره نگهداری مربوط به نمونه شاهد بوده و افزودن فیبر قابلیت زنده‌مانی این باکتری را بهبود بخشید. کمترین کاهش جمعیت سلولی بعد از انجماد و ذخیره‌سازی مربوط به نمونه حاوی 1/5 درصد فیبر انبه و به‌ترتیب برابر با 0/03 و 0/48 سیکل لگاریتمی بود. خصوصیات حسی نمونه‌های حاوی فیبر سیب خوب و با نمونه شاهد قابل رقابت بود.

واژه‌های کلیدی: فیبر، بستنی، پروبیوتیک، سینبیوتیک.

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Study on Firmness and texture changes of pear fruit when loading different forces and stored at different periods using artificial neural network

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Abstract

This study evaluated the effect of different dynamic and static loadings and different storage periods on the firmness of pear fruit. Pear fruit was first segregated into three groups of 27 pear in order to undergo three loadings: static thin-edge compression loading, static wide-edge compression loading and dynamic loading. All loaded pears were stored in accordance with three storage period designs: 5-day storage, 10-day storage, and 15-day storage. Following each period, the variations of pear texture were scanned by using the CT-Scan technique as a non-destructive test. Then, the firmness of pear texture was measured using a penetrometer. Data were simulated and evaluated using MLP and RBF artificial neural networks. The results showed that with increasing storage time and loading force , the firmness significantly decreased (1% level) in all three types of loading, In addition, pear texture was destructed under dynamic compression loading in order to compare with other two loadings. Best value artificial neural network for wide edge loading (12 neuron-RBF) was (R^2 Wide edge= 0.9738– RMSE Wide edge=0.3419- MAE Wide edge =0.268) and for thin edge loading (4 neuron-RBF) was ($R^2_{\text{Thin edge}}$ = 0.9946– $\text{RMSE}_{\text{Thin edge}}$ =0.170977- $\text{MAE}_{\text{Thin edge}}$ =0.133), also for dynamic loading (8 neuron-RBF) was ($R^2_{\text{Dynamic loading}}$ = 0.9933– $\text{RMSE}_{\text{Dynamic loading}}$ =0.230- $\text{MAE}_{\text{Dynamic loading}}$= 0.187).

Keywords: Artificial Neural Network, Firmness, Loading Pear, Storage.

Introduction

Pear fruit is cultivated in more than 70 countries across the world. When pear matures, it becomes a fruit with a buttery texture. The dense texture of pear is dependent on the specifications of its cells and in turn, depend on different factors including cell size, cell wall thickness and strength and the water content. Generally, consumers assess a fruit texture by chewing and hand touching and these are important factors in buying fruits and evaluate the sweetness, freshness, and maturity of fruits. Therefore, the texture is a key factor of quality and is widely used as a measure to assess and accept the quality of products in fresh and recycled food industries (Yan *et al.*, 2018)(W. Zhang *et al.*, 2014). Today, fruits play an important role in human health as they carry a considerable content of biological compounds with physiological and biochemical functions (Tavarini *et al.*, 2008). On the other hand, the increased demand for high-quality fruits in developed countries is an important challenge. Therefore, different non-destructive techniques are used to assess fruits quality (Khalifa *et al.*, 2011). Fruit firmness is an important qualitative variable. It is the indirect measurement of maturity so that suitable storage intervals and ideal transportation conditions can be predicted by the accurate evaluation of this factor (Zhang *et al., 2018*, Mirzaee *et al.*, 2009). Today, many inspections are carried out in commercial markets to assess fruits quality in order to determine whether they are high-quality fruits. Firmness is an important factor. Fruit softness is the most prevalent drawback that wastes fruits (Moggia *et al.*, 2016). Mechanical and shear damages are among factors affecting the qualitative and chemical properties of fruits and result in bruised fruits. The measurement of fruit firmness is an important factor by which this drawback can be predicted and avoided (Mazidi *et al.*, 2016). The mathematical models of predictions face different limitations including the selection of parameters and applying defaults for solving equations.

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Artificial neural network addresses mathematical methods and can process software and hardware structures and models (Leśniak and Juszczyk, 2018) .Neural networks are used in different sectors such as prediction, approximation, control, communication, classification, pattern identification and data sorting (Leśniak and Juszczyk, 2018; Read *et al*., 2017). Different studies have been conducted by different researchers on the impact of loading and storage period on fruits firmness.

Moggia *et al*. (2017) studied the firmness and internal browning of blueberries induced by mechanical damages and compared them with undamaged blueberries. Their results showed that blueberries with lower firmness have higher internal damages. They concluded that during the storage period, the percent of total soluble solids, the acidity of the fruit and fruit firmness can be used to estimate the harvesting time and storage potential of fruits (Moggia *et al*., 2017). Mazidi *et al*. (2016) evaluated mechanical damages of oranges and their firmness variations during the packing process. They showed that damaged fruits showed a 9% change in firmness compared to control group. However, statistical analysis showed that this effect is not significant (Mazidi *et al*., 2016). Montero *et al*. (2009) evaluated the effect of impact loading on two orange types. They reported that impact has no effect on the appearance and firmness of oranges and the imposed damage reduced the sweetness as well as vitamin C content of the oranges (Montero *et al*., 2009). Afsharnia *et al*. (2017) studied the effect of dynamic loading on the scratch and tear of mulberry. They reported that dynamic damages and storage decrease the firmness of mulberry (Afsharnia *et al*., 2017). Jahangiri *et al*. (2016) conducted a test on the effect of storage on the mechanical properties of viola cucumber under compressive loading. Their results showed that the firmness of this fruit reduced by 49% during storage compared to its initial state (Jahangiri *et al*., 2016).

Since the different forces generated during transportation, handling, and harvesting processes, definitely affected the texture of fruits and decrease their firmness. The aim of this study was to evaluate the firmness and texture of pear fruit under static and dynamic loadings in order to assess the variations of firmness and texture during storage as both factors affecting storage period and result in the degradation and wastage of fruits. In addition, this study evaluated and simulated data and calculated the sensitivity factor for different loadings.

Materials and Methods

Sample Preparation

Pears (Spadana variety) was prepared from the markets of Gorgan, Golestan province, Iran.. They were placed in an oven at 103℃ for 16 hours and their moisture content was measured. The moisture content of the pears was calculated to be 77.92% (w.b %). Environmental conditions for testing were conducted at a temperature of 18°C and relative humidity of 72%.

Quasi-Static test

To perform the wide and thin edge compression mechanical test, a pressure-deformation device (the Santam Inestrone - STM5-Made in Iran) with a load cell of 500 N was used. The compression test was performed at a speed of 5 mm/s with three forces of 70, 100, and 130 N and three replications. In this experiment, the pear was horizontally placed between the two plates and pressed, with the duration of the measurement recorded. Concerning thin edge compression test, we designed a double-jaw of plastic with a rectangular cross-section dimension of 0.3× 1.5 cm. The test was performed at a speed of 5 mm/s with three forces of 15, 20, and 25 N and three replications (Fig. 1).

Impact Test

First, the pendulum and the required masses were made in a workshop in Gorgan Biosystem Mechanics Group (Fig. 2). The fruits were placed in the desired position and then the device arm was raised to the desired angle (90°), and in the controlled state of the arm impact the pear. The pendulum had a 200 g arm and three different attachment masses of 100,

150, and 200 g for knocking. It should be noted that air resistance and friction were neglected through this procedure.

Imaging via CT scan method

After the quasi-static and dynamic loading and 5, 10, and 15 days storage, each pear was scanned with the Siemens Compute Tomography (CT) Scans of the SOMATOM Emotion 16-slice model, made in Germany. This device is a third-generation CT device in which the tube and detector are placed opposite to each other 360° around the pears in a series of turns to create the image. Also, the pitch was locked for the test; i.e., pitch 1. Images were recorded at 80 kV and 120 mAh current, and 1 mm slices were used to create full images. The images created by the Syngo CT 2012 software were recorded and extracted in the form of two-dimensional and grayscale images. Convolution kernel, which shows image resolution level, was B31Smooth and the images were created by 512× 512 matrixes. The interval between bruises and imaging was applied to allow bruises to reduce their moisture content and be better fixed on the fruit. Such a difference in moisture can increase the absorption of X-rays between healthy and unhealthy texture (Diels *et al*., 2017).

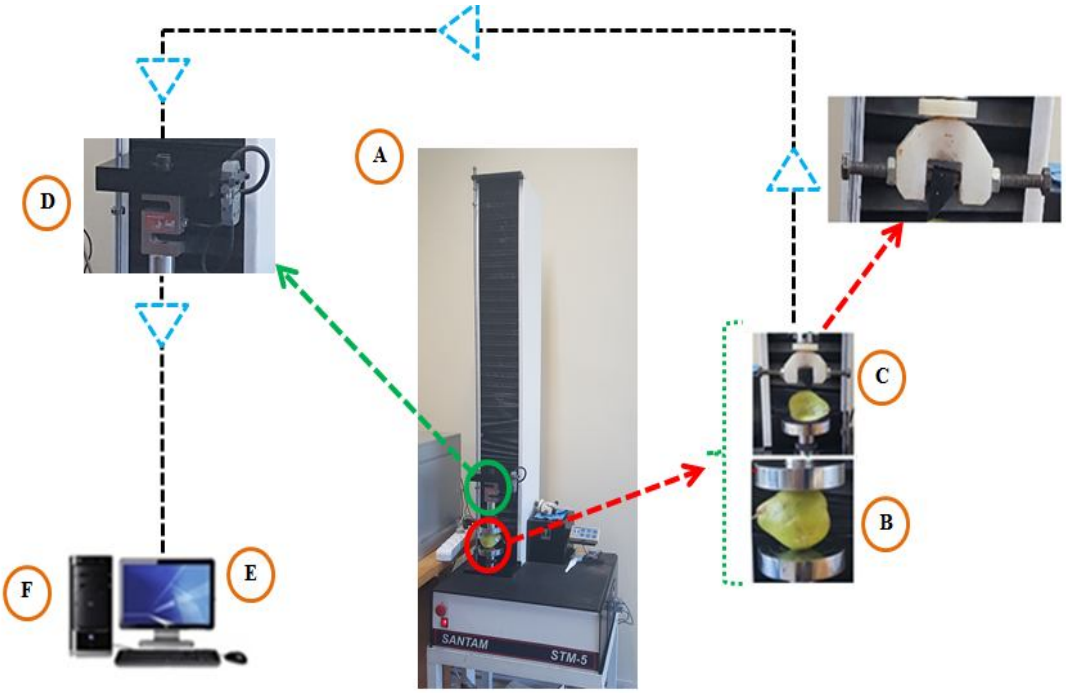

Fig. 1. Static quasi-load diagram of pear
A: The force-deformation device (Inestrone), B: Jaw wide edges, C: Jaw thin edges D: Load Cell, E: Computer, F: Information Extract.

Evidence (Control) Treatments

Four evidence (Control) pear groups were firstly taken into account for the investigation and comparison of the experimental data obtained for pear firmness. The firmness of pear texture was then measured using a penetrometer (Effeg FT327- Made in Italy with a diameter of 8 mm). The first evidence group was called zeroth day and its pear firmness was measured on the first day, on the day before being subjected to the specified loads and on the day before being stored. Then, besides measuring the firmness of each of the pears subjected to loads, the firmness of the evidence pears that had only been stored was evaluated during each specified periods. The evidence pears were

labeled 5-day, 10-day, and 15-day evidence pears, respectively.

Statistical Analysis

Samples were stored for 5, 10, and 15 days after quasi-static and dynamic loading, pear firmness was measured. All experiments were performed in three replications and the results were analyzed using a factorial experiment in a completely randomized design with SAS statistical software.

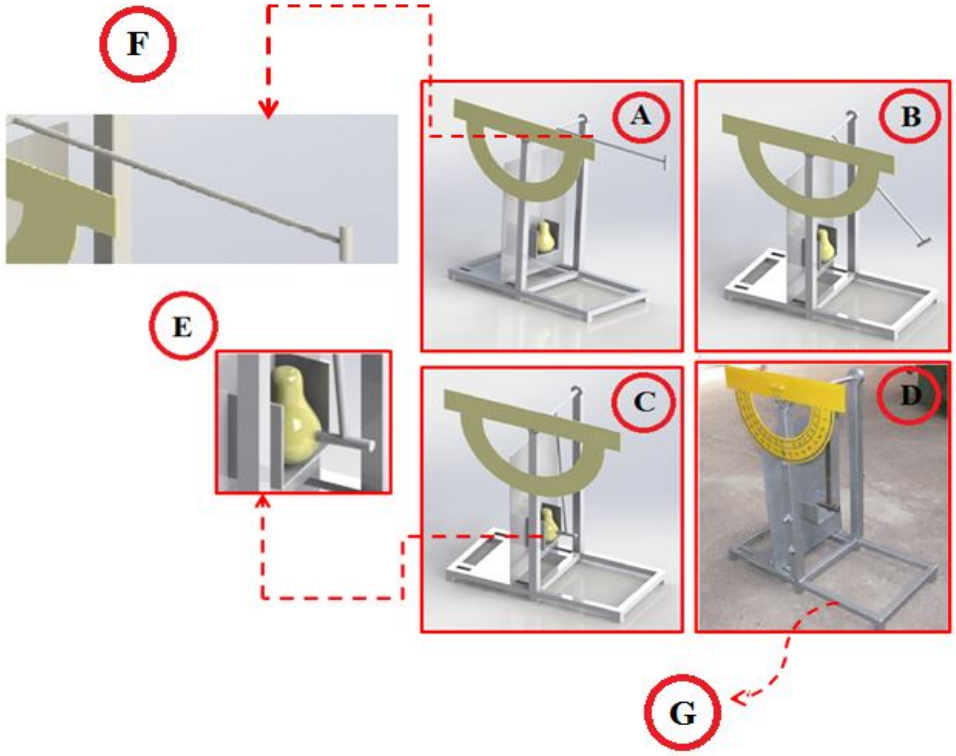

Fig. 2. Schematic of the impact machine.
A: Pendulum at a 90-degree angle, B: Walking along the path, C: Collapse pendulum to pear, D: Main device profile, E: Place the pear, F: Pendulum blow, G: the base of the device.

Artificial Neural Network Modeling

In this research, the artificial multilayer perceptron (MLP) and radial basis function (RBF) neural network were used for modeling the examined pear firmness during storage and different loading by two hidden layer and 4, 8, and 12 neurons using the Neuro Solution 5 software. Hyperbolic tangent activation functions (Eq. 1), which are the most common type of activation functions, were used in the hidden input and output layer. In this study, the Levenberg- Marquardt algorithm was used to learn the network(Taheri Garavand *et al*., 2018). Additionally, 80% of the data were used for training, and 20% of the data were used for testing the network (Testing data) (Table 2). The loading value and storage time as network inputs and firmness was the considered network outputs. Five replications were considered to achieve the minimum error rate and maximum network stability as a mean of 4000 Epoch for the network. The error was estimated using an algorithm with back propagation error. Statistical parameters including, Root Mean Square Error (RMSE), R^2, and Mean Absolute Error (MAE) were calculated for inputs and relationships were calculated using the formulas shown in Table 1.

Table 1. Neural Network Relationships

Formula	Formula Number	Reference

$$\text{Tanh}= \frac{e^{x}-e^{-x}}{e^{x}+e^{-x}} \quad (1) \quad \text{(Soleimanzadeh } et\ al.\text{, 2015)}$$

$$R^2=1-\frac{\sum_{i=1}^{n}(P_i-O_i)^2}{(P_i-O)^2} \quad (2) \quad \text{(Azadbakht } et\ al.\text{, 2016)}$$

$$r=\sqrt{1-\frac{\sum_{i=1}^{n}(P_i-O_i)^2}{(P_i-O)^2}} \quad (3) \quad \text{(Salehi and Razavi 2012)}$$

$$RMSE=\sqrt{\sum_{i=1}^{n}\frac{(P_i-O_i)^2}{n}} \quad (4) \quad \text{(Khoshnevisan } et\ al.\text{, 2013)}$$

$$MAE=\frac{\sum_{i=1}^{n}|P_i-O_i|}{n} \quad (5) \quad \text{(Azadbakht } et\ al.\text{, 2017)}$$

Equations 2, 3, 4 and 5include the predicted values (Pi) and the actual values (Oi) and the mean value of data (O).

Table2. Optimization values for artificial neural network parameters

	Number of hidden layers	Learning rule	Type of activation function	The number of hidden layer neurons	Testing data %	Training data %
MLP	2	Levenberg Marquardt	Hyperbolic tangent	4 , 8 , 12	20%	80%
RBF	2	Levenberg Marquardt	Hyperbolic tangent	4 , 8 , 12	20%	80%

Results and Discussion

Table 3 presents an analysis of variance (ANOVA) results for the effect of loading force (Wide and Thin edge– dynamic Loading) and storage period on pear firmness. According to the Table (3), all loading forces and storage periods had significant effects at 1% level on pear firmness. Moreover, considering the results, the interaction of loading force (thin edge and dynamic loading) and storage period on the pear firmness at 5% level and the insignificance of interaction was evidenced for wide edge loading. In addition, considering the significance of the interaction of pear firmness for thin-edge and dynamic loading, mean comparisons were made using the least significant difference (LSD) test the results of which have been illustrated in Figures (3) and (9).

Table 3. Analysis of variance (ANOVA) results for the effect of loading force (Wide and Thin edge-dynamic loading) and storage period on pear firmness

	Variables	DF	Mean Squares	F value
Thin edge	**Storage**	2	32.7600	563.39**
	Loading	2	5.0011	86.01**
	Storage× Loading	4	0.1877	3.23*
	Error	18	0.0581	
Wide edge Loading	**Storage**	2	18.1417	97.62**
	Loading	2	7.1095	38.26**
	Storage× Loading	4	0.1678	0.90ns
	Error	18	0.185	
Dynamic Loading	**Storage**	2	38.6233	327.93**
	Loading	2	13.9511	118.45**
	Storage× Loading	4	0.4194	3.56*
	Error	18	0.117	

** Significant at level 1%, * Significant at 5% level, ns insignificant

Static Loading

Thin-edge compression loading

Fig. 3 shows the interaction of storage× loading on the firmness of fruits during thin-edge compression loading. According to the obtained results, firmness decreases as the loading and storage period increase. Fig. 3 shows that within 5 day storage, this effect was not significant compared to the 5 day storage of the control group but, as the storage period increases, the firmness of pear fruits decreases. The maximum firmness was obtained in pears underwent 15 N compression loading and stored for 5 days (11.03 N) while the minimum

one was obtained in pears underwent 25 N compression loading and stored for 15 days (5.8 N). Fig. 4 shows the destruction of the texture of the studied pear during storage. According to Figure 4, the density of texture decreases during storage. One reason may be the degradation of the healthy texture of fruit whereas loading force increases, the internal damages increase and this, in turn, decreases the firmness of pears and changes their texture. Our results are comparable with the results of Moggial *et al.* (2017) for blueberries (Moggia *et al*., 2017).

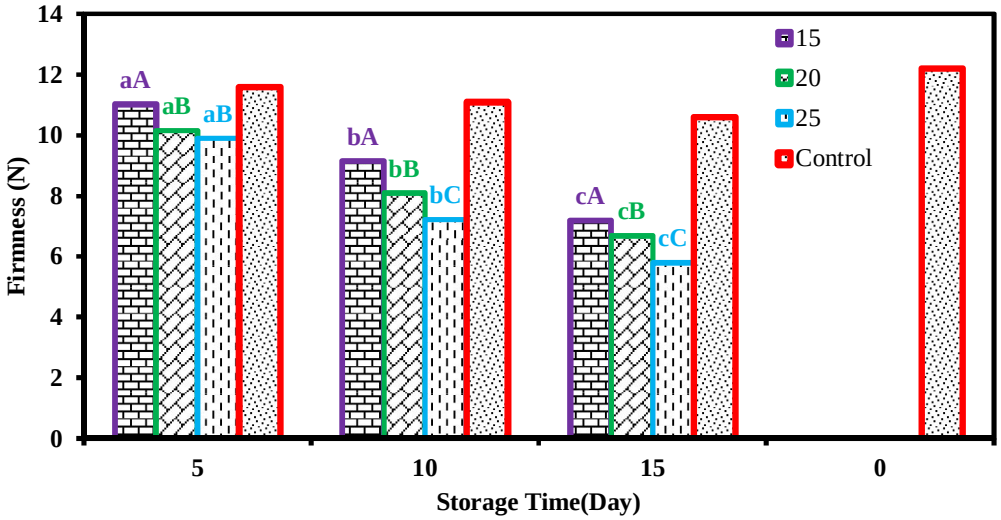

Fig. 3. Interaction effect of loading force during storage period on pear firmness at thin edge pressure Lower cases stand for the no significance of the loading force while capital letters stand for the significance of storage period

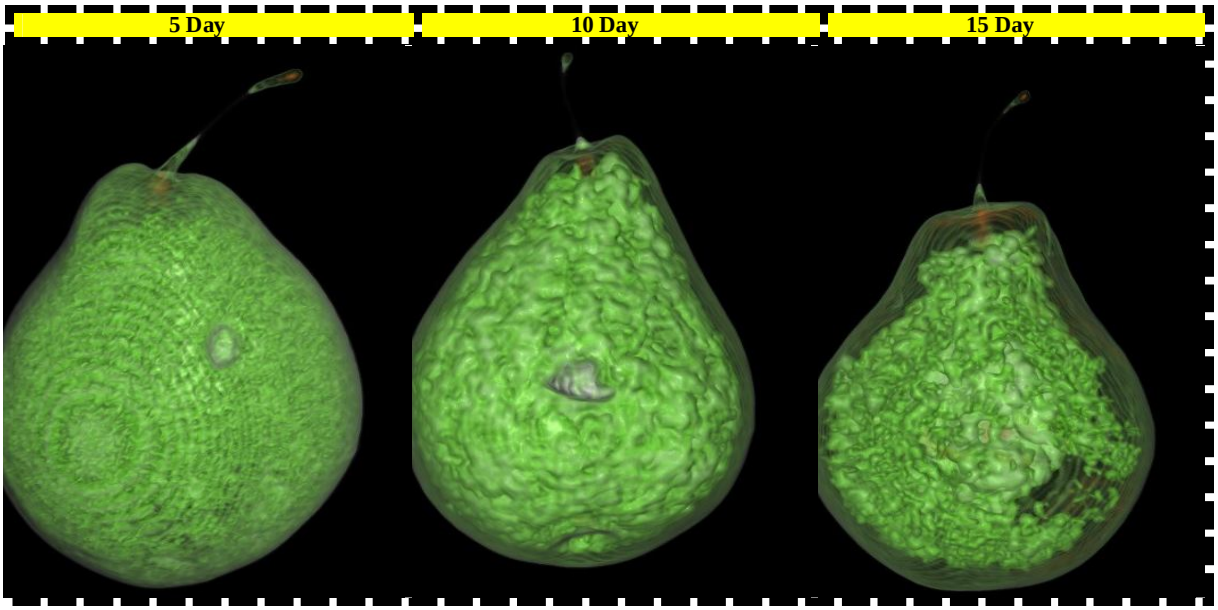

Fig. 4. Extraction images of fruit tissue, by CT in Thin edge Loading

Figures 5 and 6 show firmness reduction in pear fruits under the loading force and storage periods compared to the pears of the control group on day 0 and in each storage period,

respectively. Both comparisons show that firmness reduction (compared to control pears in each period and control pears of day 0) increases in each storage period. The maximum reduction compared to control pears of day 0 is 60.37%, which corresponds to the 15-day storage and loading force 25N. For the same storage period and loading force, this reduction was 52.45% compared to control group pears stored for 15 days. On the other hand, the minimum firmness reduction belongs to loading force 15N and 5-day storage period where the firmness reduced by 9.59% compared to the control pears of day 0 while for the same period and loading force, firmness reduction compared to the control pears stored for 5 days was only 0.63%. This indicates that in negligible loading forces and in short-term storage periods, firmness reduction was not significant compared to control.

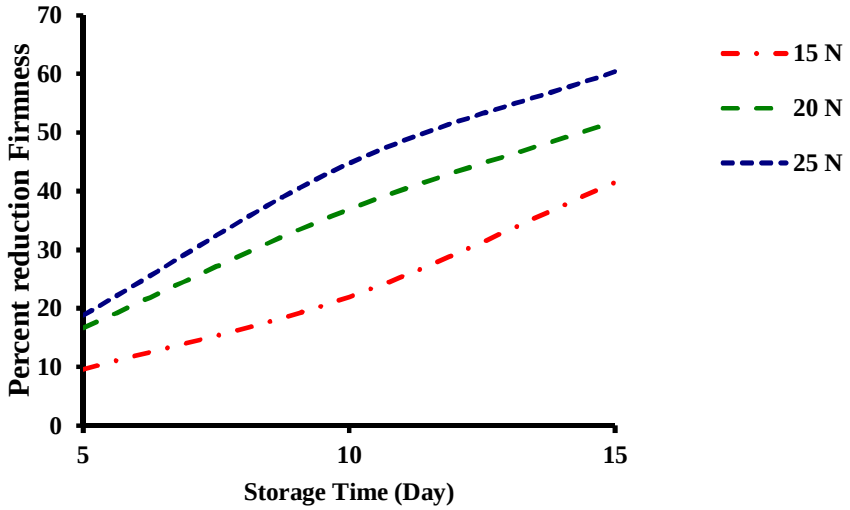

Fig. 5. Percent reduction Firmness proportion to the control sample(zeroth day)

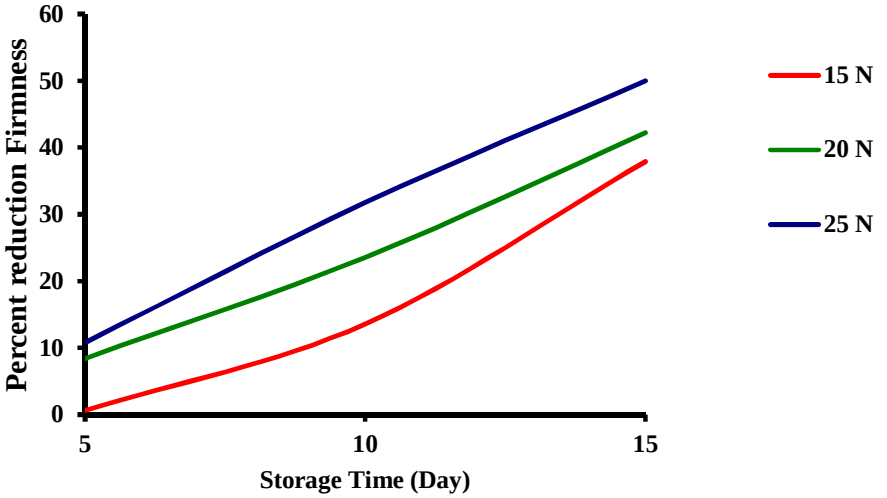

Fig. 6. Percent reduction Firmness proportion to the control sample for each storage period

Wide-edge compression loading

Table 1 shows the effect of storage and wide-edge compressive force on pear firmness. Fig. 7 shows firmness reduction during the

storage period and under loading forces compared to control pears in each storage period. According to fig. 7, as storage period and loading force increase, firmness decreases. Within the 5-day storage period, the effect of storage on firmness reduction was higher than that of the loading force. However, by the lapse of time, firmness was more affected by loading force than storage. Fig. 8 shows the variations of pear texture during wide-edge compression loading at different storage periods. Storage matures pears. This, in turn, changes the type of cell wall texture and breaks its beneficial enzymes. Moreover, the increased loading force increases the enzyme activities of cell walls. This, in turn, reduces firmness during the storage period. Our results are comparable with the reports of Jahangiri *et al.* (2016) about the viola cucumber under loading (Jahangiri *et al.*, 2016).

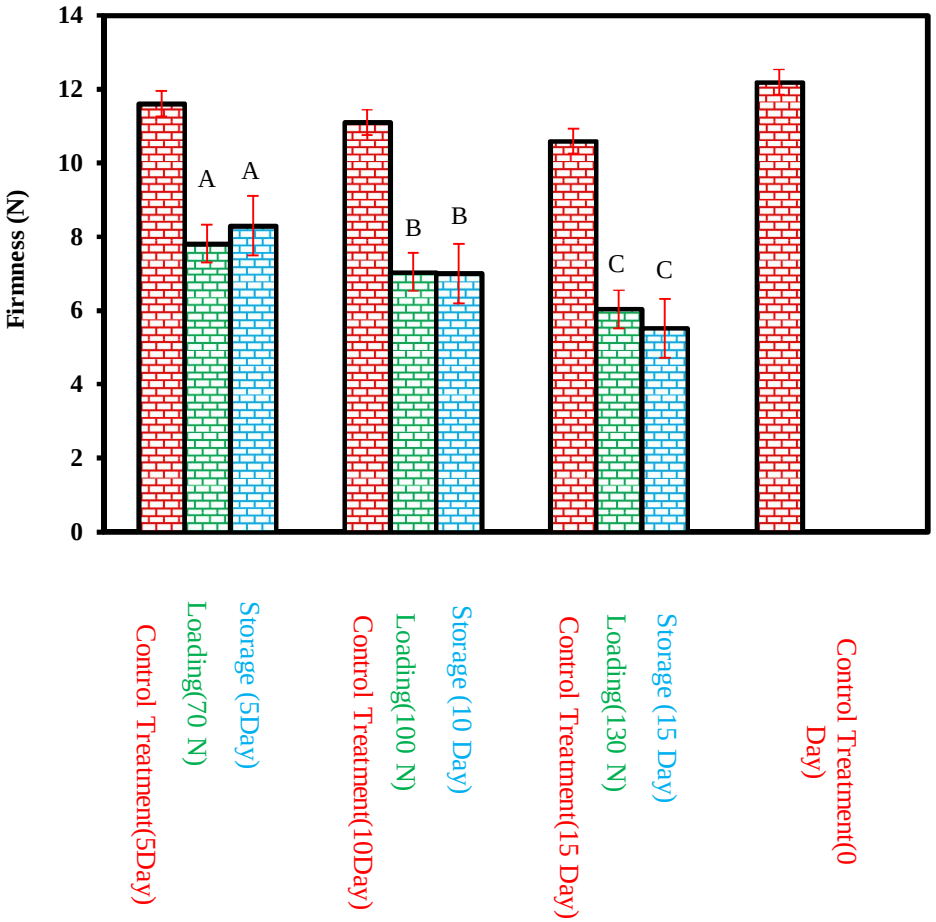

Fig. 7. Effect of loading force in storage period on pear firmness at wide edge pressure

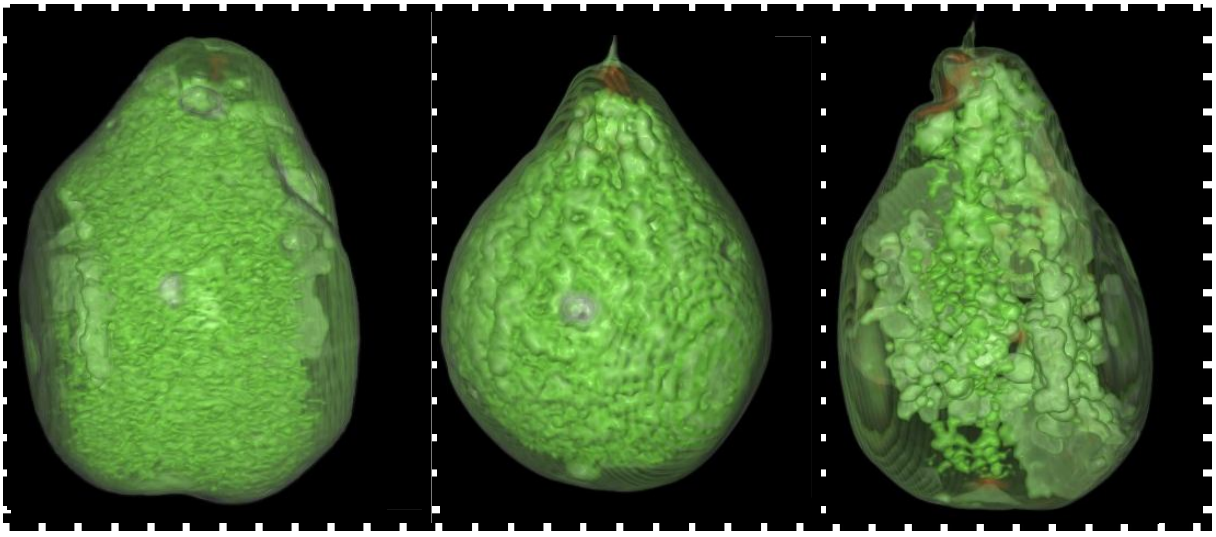

Fig. 8. Extraction images of fruit tissue, by CT in Wide edge loading

Dynamic Loading

Fig. 9 shows the interaction of storage × loading on the firmness of fruits during dynamic loading. According to the obtained results, firmness decreases as the loading and storage period increase. The maximum firmness was obtained in pears underwent Weight of 300 grams and stored for 5 days (9.2 N) while the minimum one was obtained in pears underwent Weight of 400 grams and stored for 15 days (2.43 N). According to the results, as the number of loading weights increases, the magnitude of impact increases. This increases the energy absorption of pear fruits and, in turn, damages cell wall and scratches the external texture of fruits. On the other hand, this impact increases the phenolic activity of fruits and, in turn, bruises pears disrupts the functions of the structural cells and reduces firmness. Fig. 10 shows the variation of pears texture during storage where the effect of impact after storing for 15 days is shown. Our results are comparable with the results of Afsharnia *et al.* (2017) for mulberry (Afsharnia *et al*., 2017).

Figures 11 and 12 show firmness reduction in pear fruits under the impacts of loading force and storage periods compared to the pears of the control group on day 0 and in each storage period, respectively. Both comparisons show that firmness reduction (compared to control pears in each period and control pears of day 0) increases in each storage period. The maximum reduction compared to control pears of day 0 was 92.17%, which corresponds to the 15-day storage and Weight of 400 grams. For the same storage period and loading force, this reduction was 79.02% compared to control group pears stored for 15 days. On the other hand, the minimum firmness reduction belongs to loading force with Weight of 300 grams and 5-day storage period where the firmness reduced by 24.54% compared to the control pears of day 0, while for the same period and loading force, firmness reduction compared to the control pears stored for 5 days was only 17.11%. This indicates that in negligible loading forces and in short-term storage periods, firmness reduction was not significant compared to control pears. Percent reduction Firmness indicated high destruction of the internal tissue and decreased firmness of the fruit due to loading weights and storage period.

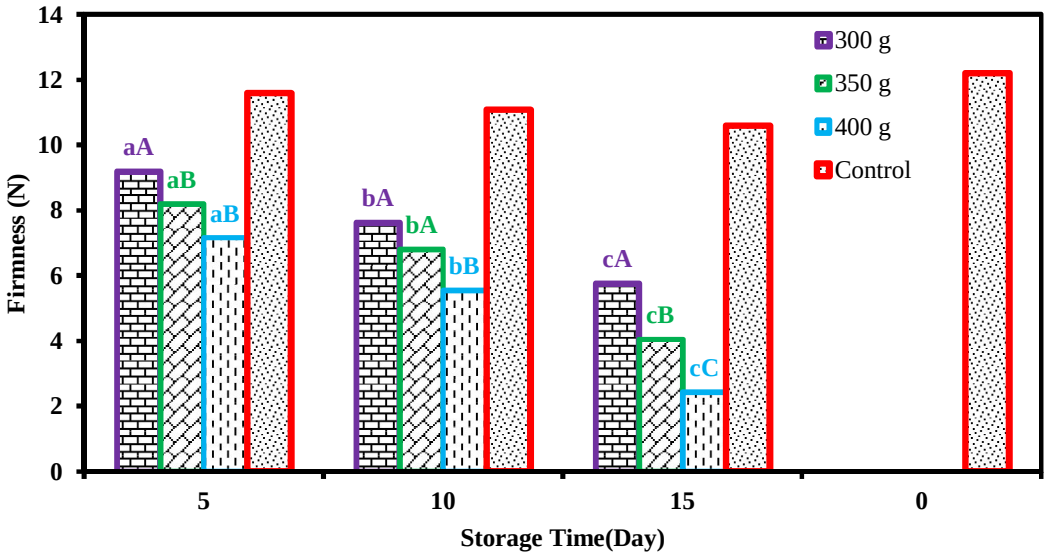

Fig. 9. Interaction effect of loading force during the storage period on pear firmness at dynamic loading Lower cases stand for the no significance of the loading force while capital letters stand for the significance of storage period

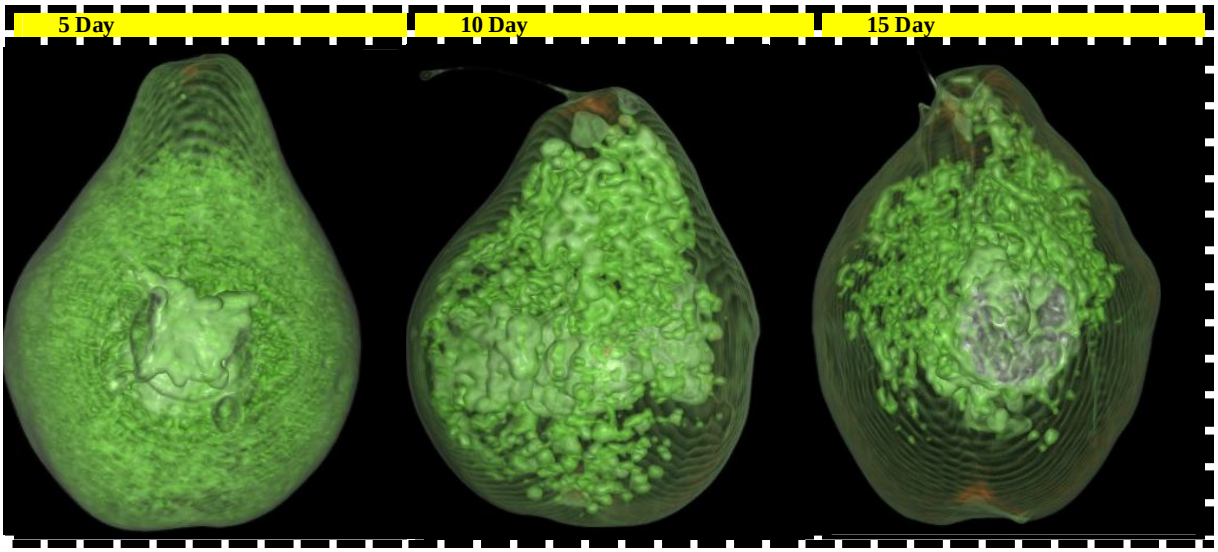

Fig. 10. Extraction images of fruit tissue, by CT in dynamic loading

Artificial Neural Network

The quasi-static results showed for error values for the quasi-static (thin and wide edge) and dynamic loading in predicting experimental data using the optimal artificial neural network in Table 4. Also some of the best MLP and RBF neural network topologies to predict training values presented in Table 4.

Table 5 shows the best network between input data and data simulated by the network for each of the neurons in the hidden layer. The lower value of Epoch indicates that the number of neurons in the layer has been able to have learned from the neural network compared to another number of neurons.

The results for thin edge Loading showed that neural network has 4 neurons in the hidden layer and RBF network for firmness ($R^2_{\text{Thin edge}}$ = 0.9946– RMSE $_{\text{Thin edge}}$ =0.170977- MAE $_{\text{Thin edge}}$ =0.133) can predict firmness in different loading and storage time (Table 4). In addition, the neural network with 12 neurons in the hidden layer and RBF network has the best neural network topologies to predict training (Run= 1, Epoch= 11). Also, according to the results, the RBF network is faster than the MLP (Table 5).

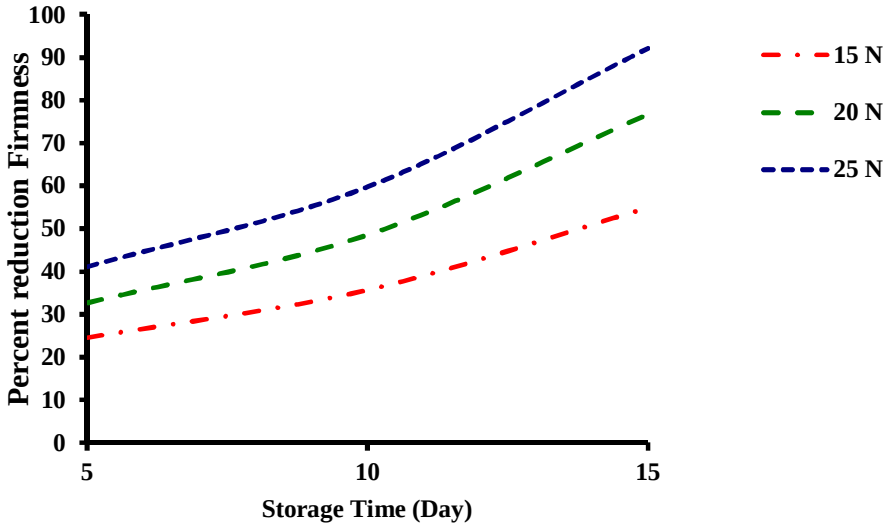

Fig. 11. Percent reduction Firmness proportion to the control sample(zeroth day)

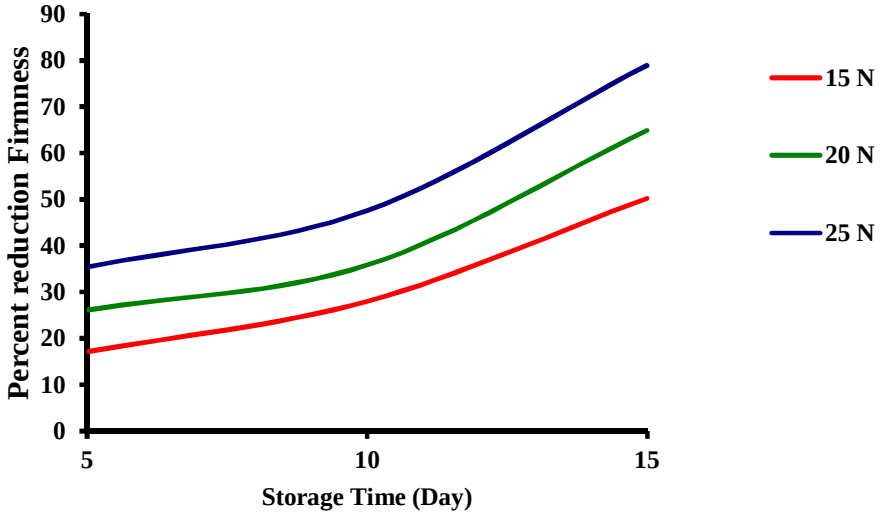

Fig. 12. Percent reduction Firmness proportion to the control sample for each storage period

Table 4. Error values for the quasi-static (thin and wide edge) and dynamic loading in predicting experimental data using optimal artificial neural network

	Network	Neuron number	MSE		RMSE		MAE		R^2	
			Training	Test	Training	Test	Training	Test	Training	Test
Thin edge Loading	MLP	4	0.120	0.115	0.3464	0.3391	0.30658	0.273	0.9907	0.987
		8	0.02931	0.134	0.17129	0.3660	0.13786	0.317	0.9942	0.986
		12	0.0335	0.0765	0.18303	0.2765	0.13787	0.23	0.9937	0.990
	RBF	4	0.02924	0.133	0.17099	0.3646	0.1333	0.3	0.9946	0.977
		8	0.0399	0.0485	0.19975	0.2202	0.15909	0.18	0.99305	0.997
		12	0.036	0.058	0.18973	0.2408	0.1575	0.19	0.99307	0.993
Wide edge Loading	MLP	4	0.1177	0.2786	0.34307	0.5278	0.2717	0.472	0.9675	0.9618
		8	0.12	0.231	0.34641	0.4806	0.2810	0.365	0.9732	0.9873
		12	0.440	0.421	0.66332	0.6488	0.515	0.59	0.874	0.986
	RBF	4	0.1363	0.103	0.36918	0.3209	0.306	0.23	0.967	0.9872
		8	0.1380	0.0926	0.37148	0.3043	0.297	0.245	0.9621	0.9948
		12	0.1169	0.3800	0.34190	0.6164	0.268	0.480	0.9738	0.04
Dynamic Loading	MLP	4	0.085	0.375	0.29154	0.6123	0.253	0.21	0.98953	0.958
		8	0.0687	0.1645	0.26210	0.4055	0.2166	0.37	0.99100	0.8771
		12	0.0554	0.3820	0.23537	0.6180	0.1939	0.199	0.9919	0.9821
	RBF	4	0.0771	0.134	0.27766	0.3660	0.225	0.269	0.9912	0.9744
		8	0.05318	0.071	0.23060	0.2664	0.187	0.18	0.9933	0.996
		12	0.085	0.074	0.29154	0.2720	0.251	0.58	0.9887	0.9911

Table 5. Some of the best MLP and RBF neural network topologies to predict training values

	Activation function	Neuron Numbers	Run	Epoch
Thin edge Loading	MLP Network	4	2	26
		8	1	13
		12	1	16
	RBF Network	4	1	17
		8	1	15
		12	1	11
Wide edge Loading	MLP Network	4	1	16
		8	1	18
		12	1	19
	RBF Network	4	2	15
		8	1	10
		12	1	9
Dynamic Loading	MLP Network	4	2	14
		8	1	10
		12	1	11
	RBF Network	4	1	15
		8	1	11
		12	1	11

For wide edge showed best values in neural network is 12 neuron in the hidden layer and RBF network for firmness (R^2 $_{\text{Wide edge}}$ = 0.9738– RMSE $_{\text{Wide edge}}$ =0.3419- MAE $_{\text{Wide edge}}$ = 0.268) and the neural network with 12 neurons in the hidden layer and RBF network has best neural network topologies to predict training (Run= 1, Epoch= 9)

For dynamic loading best value was shown in the hidden layer by 8 neurons and RBF

network (R^2 $_{Dynamic\ loading}$ = 0.9933– RMSE $_{Dynamic\ loading}$= 0.230- MAE $_{Dynamic\ loading}$= 0.187). Best neural network topologies to predict training was in hidden layer with 8 neurons and MLP network

Also, Figures (13, 14 and 15) illustrate the output amounts between the real and predicted data. Based on the figures, it can be observed that the neural network well capable of predicting and comparing the given numbers and it can be stated considering the closeness and similarity of the numbers outputted from the ANN to the real data that the neural network possesses an appropriate competency for data prediction. Moreover, considering the R^2 value, the RBF network has the best overlap with the experimental data for all loading. For thin edge loading was showed the best overlap in a hidden layer by 4 neurons for training data (Fig. 13).

Figure 14 showed the best overlap for wide edge loading and the best overlap between experiments data with network output is in the hidden layer by 12 neurons.

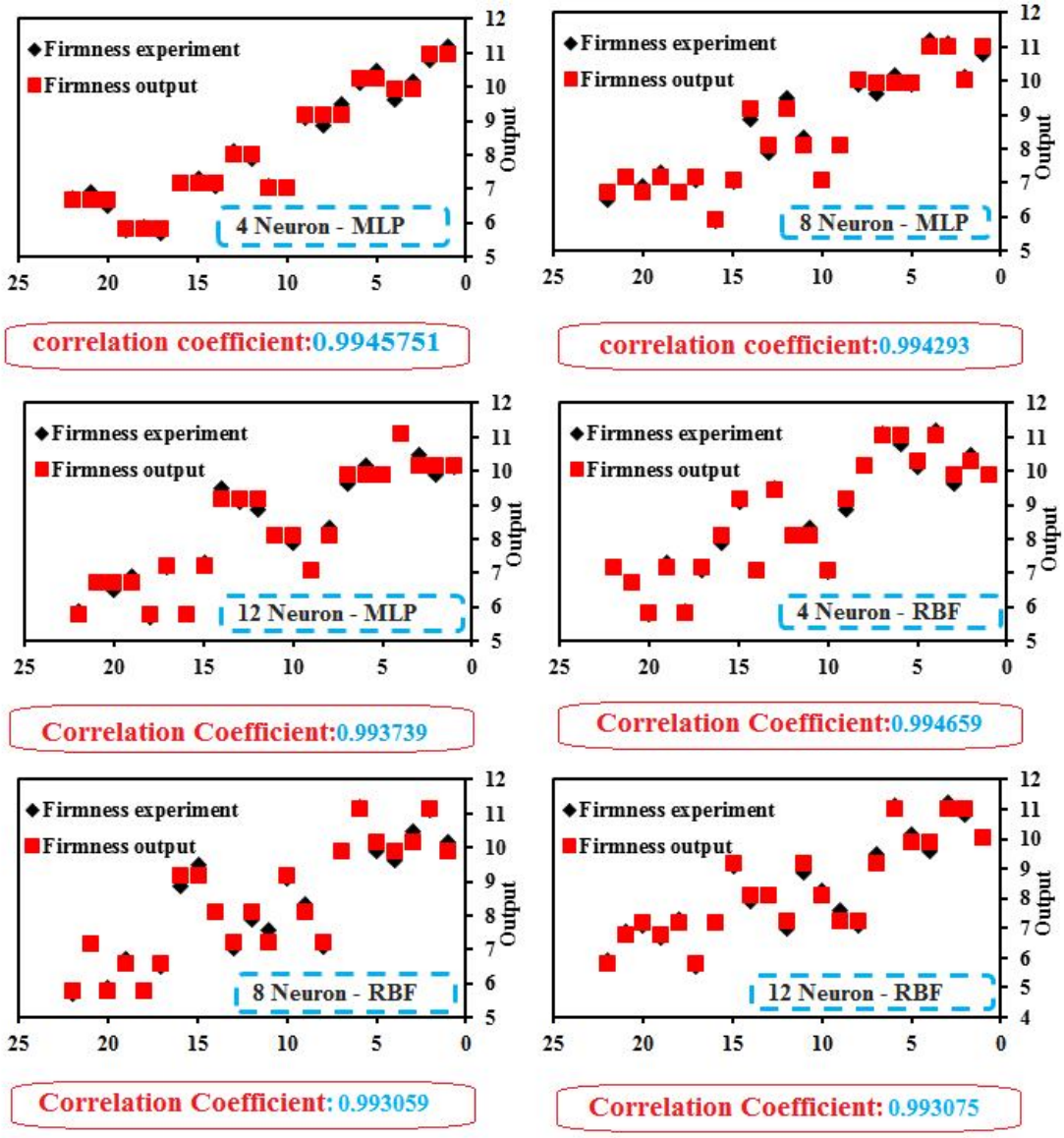

Fig. 13. Compare experiment data with network output data for thin edge loading

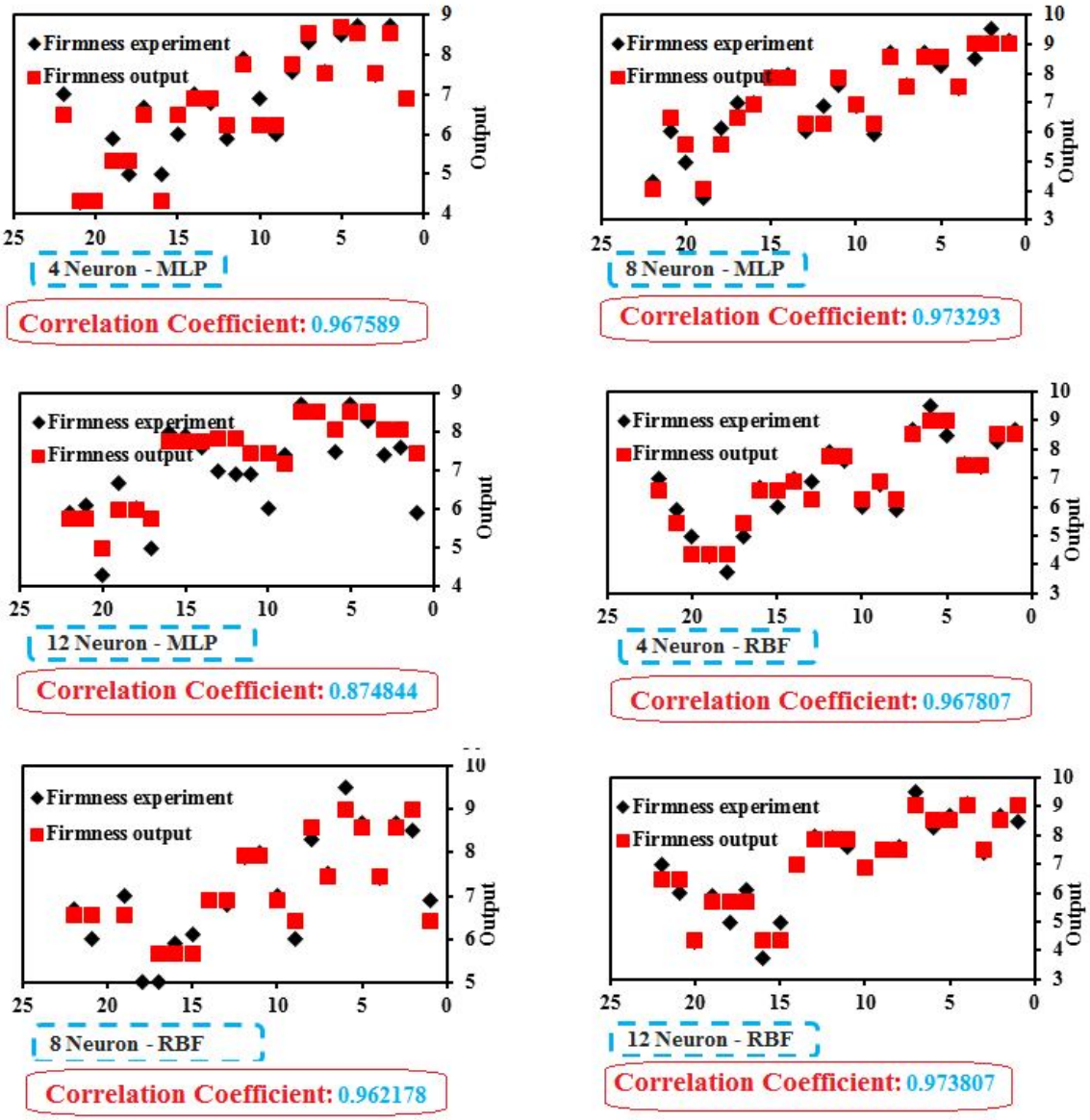

Fig. 14. Compare experiment data with network output data for wide edge loading

Figure 15 showed the best overlap for dynamic loading and the best overlap between experiment data with network output is in the hidden layer by 8 neurons.

Sensitivity Coefficient for quasi-static (Wide and thin edge)

The results of the sensitivity analysis for firmness (Wide and thin edge) are shown in Figure 16. Based on this figure, the highest sensitivity for training data was obtained for the loading in hidden layer by 4 neuron (Thin edge loading) and 8 neuron (Wide edge loading) with RBF Network and for storage time in the hidden layers with 4 neuron and RBF Network (Thin edge loading) and 8 neuron in hidden layer and MLP Network (Wide edge loading) (Figure 16).

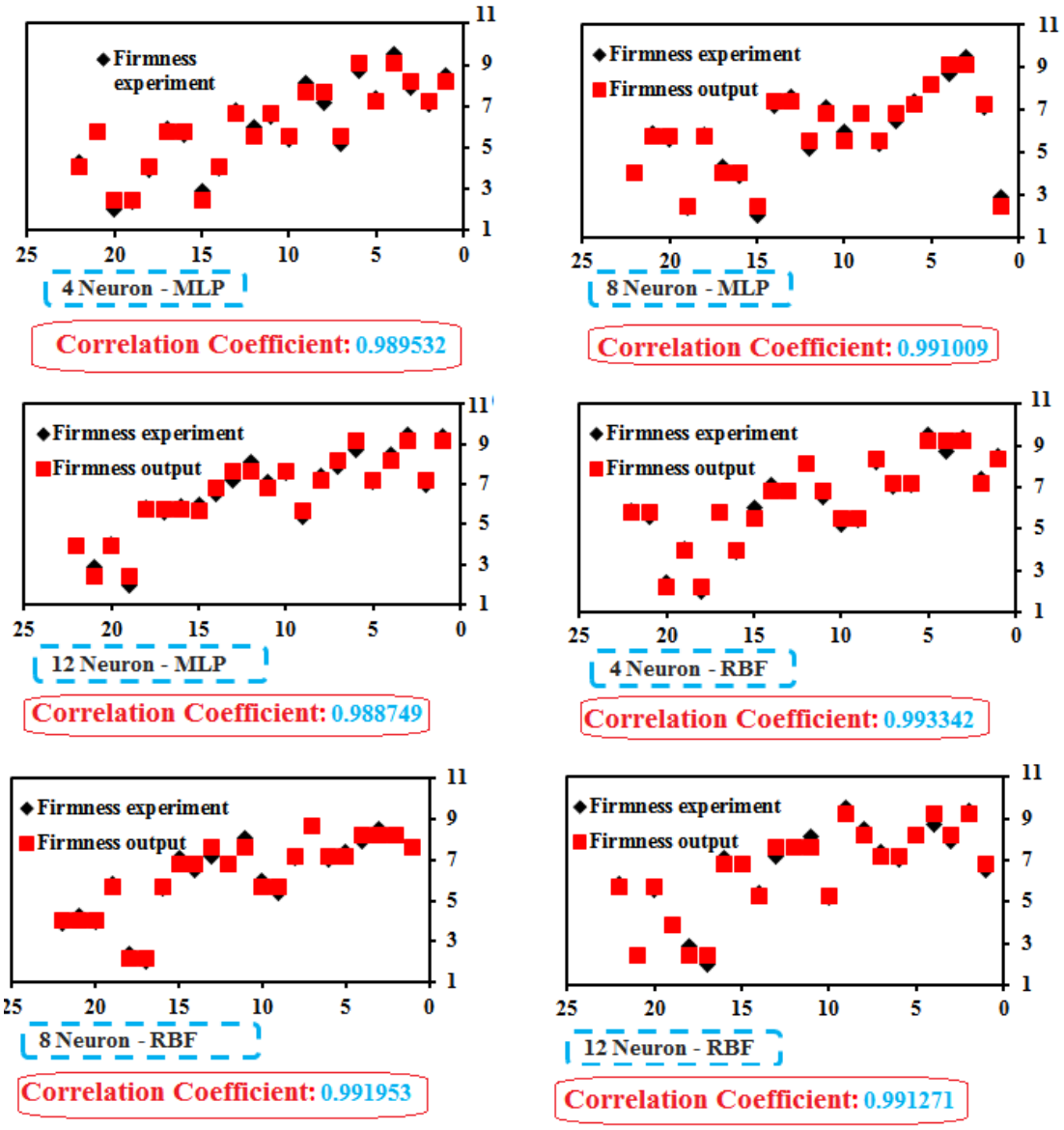

Fig. 15. Compare experiment data with network output data for dynamic loading

The lowest sensitivity analysis for firmness (Wide and thin edge) are shownin Figure 16 . According to the figure for a thin edge, loading had lowest value for firmness in a hidden layer by 8neuron and RBF network for loading and for storage in a hidden layer by 4 neurons and MLP Network. For the wide edge, loading was obtained for loading and storage in the hidden layers with 12 neurons by MLP Network.

The result for Test Data showed that highest sensitivity for a thin and wide edge in hidden layer 12 neuron and RBF and MLP Network for loading respectively and in storage time for thin and wide edge loading had lowest firmness in a hidden layer by 4 neurons and MLP Network and 12 neurons and RBF Network.

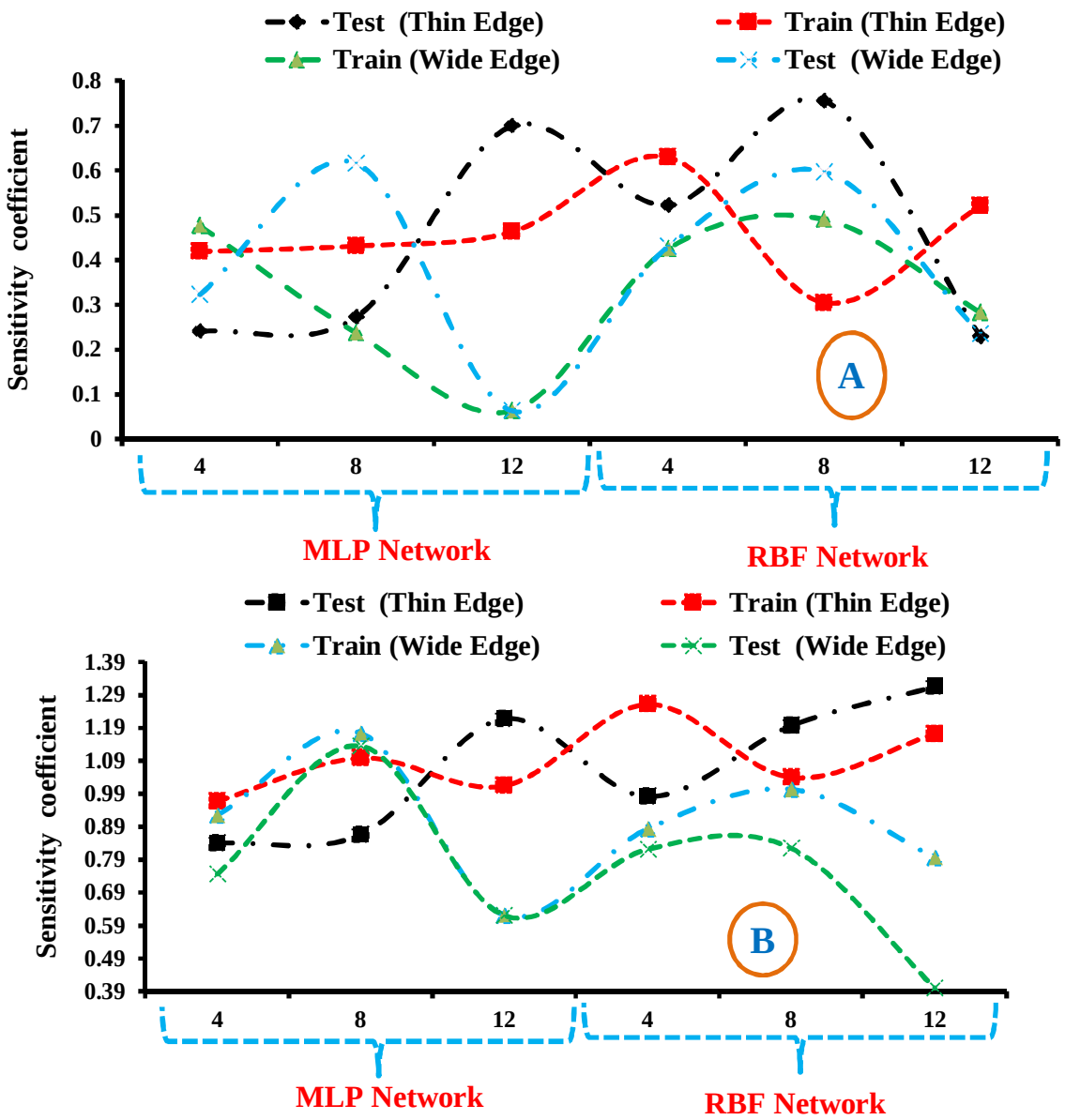

Fig. 16. Sensitivity coefficient (Thin and Wide edge) for firmness A: Loading B: Storage time

Sensitivity Coefficient for quasi-static (Dynamic Loading)

The results of the sensitivity analysis for firmness (Wide and thin edge) are shown in Figure 17. The highest sensitivity for training and Test data was obtained for the loading in a hidden layer by 4 Neuron with MLP Network and 12 Neuron in hidden layer with RBF network respectively. for storage time in the hidden layers with 12 neurons and MLP Network (Training Data) and 12 neurons in the hidden layer and MLP Network (Test data) (Figure 17). The lowest sensitivity analysis for firmness (Dynamic Loading) is also shown in Figure 17 . According to the Figure, for loading lowest value was for firmness in hidden layer by 4neuron and RBF network (Training Data) and 12 neuron in hidden layer by MLP Network (Test Date) and for storage, Lowest firmness was in hidden layer by 4 neurons and RBF Network (Training Date) and 8 neurons in hidden layer by MLP network (Test Data).

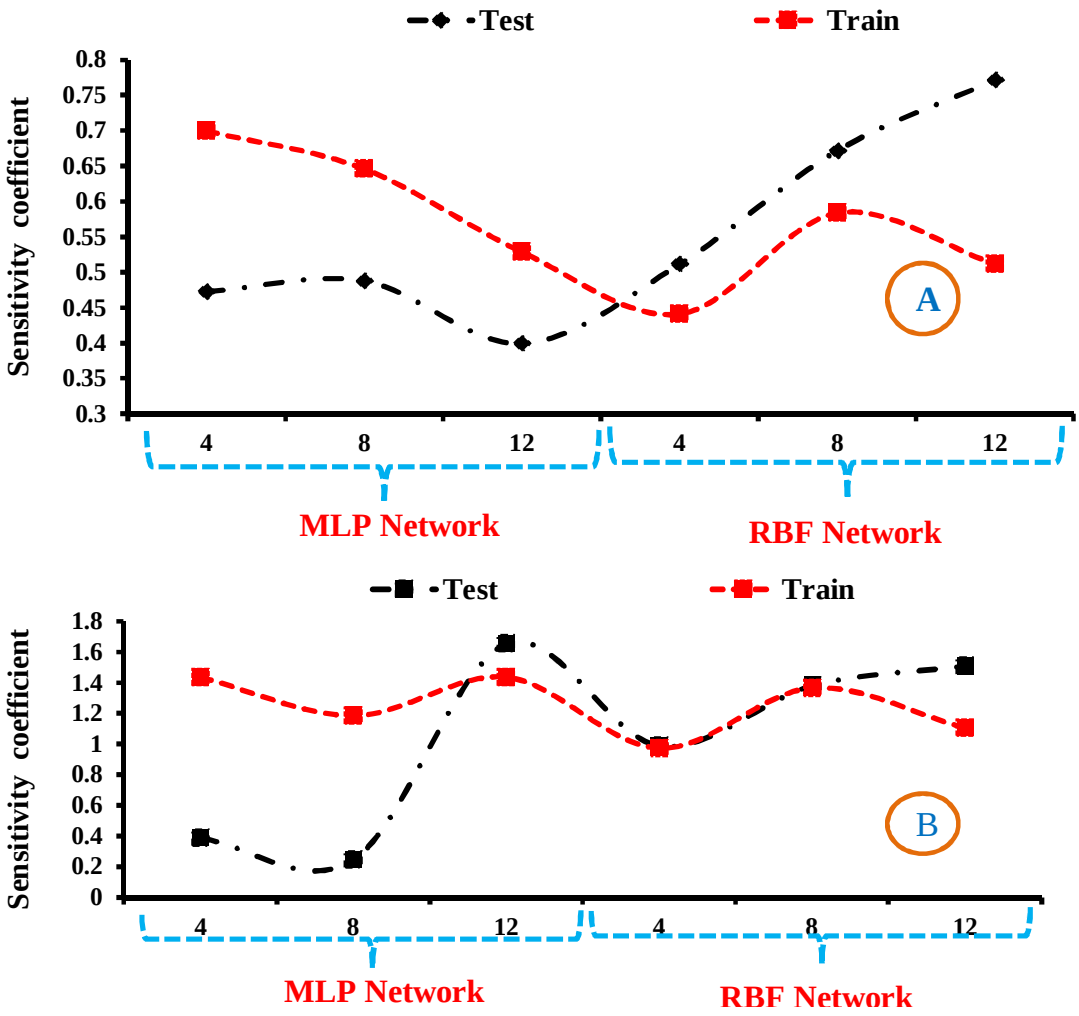

Fig. 17. Sensitivity coefficient (Dynamic Loading) for firmness A: Loading B: Storage time

Conclusion

- The maximum firmness reduction was obtained in dynamic loading where firmness reduced by 92% compared to the firmness of pears on day 1. Moreover, the minimum firmness reduction was obtained in wide-edge compression loading where firmness reduced by 60.37% compared to the firmness of pears on day 1. This reduction was obtained in the 15-day storage period.

- The effect of loading and storage on firmness reduction in static and dynamic loading states was significant.

- During the storage period, dynamic loading more destructs pear texture than static loading.

- Considering R^2 value obtained for training and test, RBF is the most accurate neural network. However, R^2 is acceptable for MLP too.

- In static loading, RBF is the fastest network for learning neural network.

- In dynamic loading, MLP is the fastest network for learning neural network. The epoch of MLP and RBF is 10 and 11, respectively which is negligible considering the high accuracy of RBF.

- Considering experimental and simulated data, there is an acceptable overlap between data. This implies the capability of the employed network in predicting pear firmness.

- In static loading, the highest sensitivity factor was obtained in thin and wide-edge compression loading in an RBF network with 4 and 8 neurons in a hidden layer respectively. Moreover, considering the storage period of both loadings, the maximum sensitivity factor was obtained in an RBF network with 4 neurons in the hidden layer and in an MLP network with 8 neurons in a hidden layer respectively.

- Considering the storage period and loading, the maximum sensitivity factor was obtained in an MLP network with 4 and 12 neurons in a hidden layer respectively.

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بررسی تغییرات سفتی و بافت میوه گلابی در بارگذاری نیروهای مختلف و دوره‌های متفاوت انبارداری با شبکه عصبی مصنوعی

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تاریخ دریافت: 1397/06/08
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چکیده

در این تحقیق به بررسی اثر نوع بارگذاری‌های دینامیکی و استاتیکی و دوره انبارداری بر میزان سفتی گلابی پرداخته شد. برای این کار ابتدا گلابی‌ها به سه گروه 27 تایی برای سه بارگذاری استاتیکی لبه نازک، استاتیکی لبه پهن و دینامیکی دسته‌بندی شده و بارگذاری شدند. هر یک از گروه‌های بارگذاری شده در سه دوره 5، 10 و 15 روزه انبار دار شده و بعد از هر دوره انبارداری با استفاده از آزمون غیرمخرب CT-Scan از تغییر بافت گلابی‌ها عکس‌برداری شد و سپس میزان سفتی بافت گلابی با استفاده از سفتی‌سنج اندازه‌گیری شد. همچنین داده‌ها با استفاده از دو شبکه مصنوعی MLP و RBF شبیه‌سازی و مورد بررسی قرارگرفت. نتایج نشان داد که با افزایش دوره انبارداری و میزان نیروی بارگذاری در هر سه نوع بارگذاری میزان سفتی به‌طور معنی داری (سطح 1%) کاهش یافت. همچنین بافت گلابی در بارگذاری دینامیکی به شدت نسبت به دوبارگذاری دیگر تخریب شده است. بهترین مقادیر شبکه عصبی مصنوعی برای فشار لبه پهن (12 نرون- RBF) (R^2 Wide edge= 0.9738– RMSE Wide edge= 0.3419- MAE Wide edge= 0.268) و برای فشار لبه نازک(4 نرون -RBF) (R^2Thin edge= 0.9946– RMSE Thin edge=0.170977- MAE Thin edge= 0.133) و در نهایت برای بارگذاری دینامیکی (8 نرون- RBF) (R^2 Dynamic loading = 0.9933– RMSE Dynamic loading =0.230- MAE Dynamic loading=0.187) بوده است.

واژه‌های کلیدی: انبارداری، بارگذاری گلابی، سفتی، شبکه عصبی.

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Optimization of polysaccharide extraction from olive leaves and evaluation of its antioxidant and rheological properties

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Abstract

Central composite design response surface methodology was used to optimize polysaccharide extraction from olive leaves. Effect of three independent variables [extraction time (3- 7 hours), extraction temperature (60- 100°C) and water-to-raw material ratio (5-25 mL/g)] on extraction yield were studied. Extracted polysaccharide was evaluated for antioxidant properties, total phenolic and flavonoid content and its structure and functional groups were studied using FTIR. Rheological properties and flow behavior of polysaccharide were determined by fitting to power law model. The most important parameter in experimental ranges was temperature and the lowest effect was seen in extraction time. Highest extraction yield was obtained at extraction time of 2 hours, extraction temperature of 80.96°C and water-to-raw material ratio of 17.94 mL/g. Antioxidant properties of extracted polysaccharide were measured using DPPH radical at 517 nm that showed notable antioxidant properties. Rheological property of extracted polysaccharide was studied at 1, 2.5 and 5% concentration. Results showed that at high concentration, polysaccharide shows shear thinning behavior. One of the most important obstacles in native polysaccharide applications is their extraction yield. Extract of olive leaf polysaccharide is highly affected by extraction temperature. Extracted polysaccharide showed good antioxidant properties comparing to BHT and phenolic extract of olive leaf. Moreover it could be used for increasing solution viscosity at higher concentrations.

Keywords: Olive leaf polysaccharide, optimization, response surface methodology, FTIR, antioxidant properties

Introduction

Bioactive compounds have gained more attention especially in the last decade due to their health benefits and functional properties. Bioactive compound extraction usually is done using maceration techniques. Recently some supplementary techniques such as ultrasonic assisted, microwave assisted, supercritical extraction and superheated liquid are used to reduce extraction time and increase bioactive substance extraction yield (Ahmad-Qasem *et al*., 2013).

Bioactive polysaccharides from natural sources are recently gained attention in biochemistry and pharmacology due to their beneficial activities including antioxidant, anti-tumor/ anticancer, anticoagulant and immune stimulating properties (Tadayoni *et al*., 2015; Tahmouzi and Ghodsi, 2014). They may have several applications in food industry including, increasing viscosity in different concentrations or synergistic effect with other native biopolymers.

Olive (*Olea europaea* L.) is an evergreen tree which is native to Mediterranean area. Olive fruit is mostly used for preparing oil and table olives (Galanakis, 2011). Olive oil due to presence of functional bioactive compounds like tocopherols, carotenoids and phenolic compounds is considered as health promoting compound. Phenolic compounds of olive leaves have showed antioxidant, anti-hypertensive and anti-inflammatory properties (Ahmad-Qasem *et al*., 2016). Olive leaf extract is traditionally used for treating fever, malaria, colic, alopecia, paralysis, rheumatism, gout, sciatica, hypertension, arrhythmia, diabetes and cancer (Kamran *et al.*, 2015).

Water soluble polysaccharide extraction usually is done by extracting soluble parts using warm or hot water and finally precipitating extracted polysaccharide using alcohol.

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Response surface methodology (RSM) is a collection of mathematical and statistical techniques that used for understanding a complex system performance and optimization of process output (Mazarei *et al.*, 2017). In addition to mathematical optimization, RSM reduces number of experimental runs and consequently decreases time and costs of experiments. In RSM, central composite rotatable design, central composite design and Box- Behnken design using a least square technique, fit a second order polynomial equation to estimate effect of independent variables on output (Samavati and Manoochehrizade, 2013).

In this study at first stage, the effects of three independent variables of temperature, time and water to raw material ratio on extraction yield of olive leaf soluble polysaccharides were evaluated. At the second stage, antioxidant properties of the extracted polysaccharide and its rheological properties were determined.

Materials and Methods

Olive leaves were collected from Khuzestan Agricultural sciences and Natural Resources University garden (Mollasani, Iran). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from *Sigma* Chemical Company (St. Louis, MO, USA). Analytical grade ethanol and methanol were used for decolorization, polysaccharide sedimentation and DPPH test. All other chemicals including Folin Ciocalteau reagent, Aluminum Chloride, Gallic Acid and Quercetin were purchased from Merck Chemical Co (Darmstadt, Germany)

Polysaccharide extraction

Olive leaves were cleaned and dried in a drying oven at 105℃ for 3 hours and after cooling to room temperature, the dried leaves were kept in plastic bags at -18°C until the day of experiments. Dried leaves were ground to 0.05 mm particles. 80% ethanol was added to dried leaf and the mixture was stirred for 8 hours at room temperature to remove fat and color. The mixture was then filtered through nylon filters and residues on filter were washed three times using ethanol and the filtrate was dried at 50°C.

Defatted powder was used for polysaccharide extraction by hot water at different temperatures, times and water to dried matter ratios based on RSM design. To remove suspended particles, the extract was centrifuged at 6000 rpm for 15 minute. Extracted soluble polysaccharide was sedimented by adding 3 volume 96% ethanol and keeping for 48 hours at 4℃. Precipitated polysaccharide was removed by centrifugion at 6000 rpm for 20 minutes and the pellet was dried at 50℃. Polysaccharide Extraction yield (%w/w) was calculated using following formula:

$$\text{Yield Extraction \%} = (W_{DP} / W_{DR}) \times 10 \quad (1)$$

W_{DP} is dried extracted polysaccharide and W_{DR} is dried raw material.

Experimental design

After pretests the range of three extraction variables including X_1, extraction temperature; X_2 extraction time and X_3, water-to-raw material ratio were introduced to the software (Design Expert 9) based on central composite design (Table 1). Variables were coded according to following equation:

$$x_i = (X_i - X_o / \Delta X_i) \quad (2)$$

Where x_i is coded value of variable; X_i actual value of variable; X_0 actual value at the center point and ΔX_i is the step change of actual value

20 experiments were designed using the software that based on non-linear quadratic model as given by equation 3

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 - b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (3)$$

While (Y) is extraction yield (b_0) is polynomial coefficient represent by constant term, (b1, b2 and b_3) are coefficient of linear effects, (b11, b22 and b_{33}) are coefficient of quadratic effect and interactions (b_{12}, b_{13} and b_{23}).

Regression coefficient (R^2), adjusted-R^2

(R2adj), adequate precision (AP), the prediction error sum of squares (PRESS) and coefficient of variation (CV) were used to evaluate adequacy of model.

Table 1. Independent variables and their levels

Independent variable	Factor levels				
	-2 (-α)	**-1**	**0**	**1**	**2 (+α)**
Extraction temperature	60	70	80	90	100
Extraction time	3	4	5	6	7
Water ratio	5	10	15	20	25

Olive leaf composition

Fresh leaves of olive were used to determine moisture, ash, protein, fat and total carbohydrate.

Antioxidant activity

Radical scavenging of extracted polysaccharide was measured based on its ability to scavenge DPPH radicals. Briefly 3 mL of different concentrations of polysaccharide was added to 1 mL methanolic solution of DPPH (0.004%) and kept 30 minute in dark place at room temperature. Aliqout absorbance was measured at 517 nm and radical scavenging activity was obtained as follow (Shen *et al.*, 2014):

$$RSA\% = \frac{Abs\ of\ blank - Abs\ of\ sample}{Abs\ of\ blank} \times 100 \quad (4)$$

Results were compared with ethanolic extract of olive leaf and BHT as synthetic antioxidant.

Total phenolic content

Total phenolic content was measured using Folin Ciocalteu colorimetric method. One mL of 0.1 mg/mL of polysaccharide solution was mixed with 2.5 mL Folin Ciocalteu reagent (1:10 dilution) and kept at room temperature for 2 minute. Two mL of sodium carbonate solution (7.5% in deionized water) was then added to the mixture and after 1 hour incubation at room temperature, absorbance was measured at 725 nm using UV-Vis spectrophotometer and result expressed as Gallic acid equivalent (GAE) (Scherer *et al.*, 2013).

Flavonoid content

Total flavonoid content was measured based on formation of flavonoid compounds and aluminum complex. One mL of 0.1 mg/mL polysaccharide solution diluted with 2.5 mL distilled water and 75 µl of 5% sodium nitrite was then added to diluted solution. After 6 minute, 150 µl Aluminum chloride (10%, pH=5.8) was added and after 5 minute, 1 mL NaOH (1M) added to the mixture and the absorbance was measured at 510 nm. Results expressed as Quercetin Equivalent (QE) (Hossain and Rahman, 2011).

Fourier transform infrared spectroscopy (FTIR)

FTIR spectrometer was used to perform infrared scanning on extracted polysaccharide to determine functional groups. Infrared scan preformed in range of 4000-400 cm^{-1} using KBr pellet method with sample to KBr ratio of 1:100.

Rheological properties of polysaccharide

To evaluate rheological properties of extracted polysaccharide, its flow properties was measured. Rheological properties of three concentrations of 1, 2.5 and 5% were measured at room temperature using rheometer equipped with spindle no. 18 (LVDV Pro II, Brookfield Engineering Laboratories, USA)

Statistical analysis

All measurements were done in three replicates and their mean values were compared using Duncan test at 5% significant levels. Statistical analysis was performed using SPSS 16.

Results and discussion

Chemical composition olive leaf

Results of chemical composition of olive leaf are shown in Table 2.

The average moisture content of olive leaves was 55% and it could be categorized as an intermediate moisture product. In other

researches olive leaf moisture content was reported 39-64%. Total carbohydrate was measured by subtracting other chemical constituents from leaves weight. Reported total carbohydrate for different varieties was 11-43% (Boudhrioua *et al.*, 2009; Cavalheiro *et al.*, 2015; Kamran *et al.*, 2015).

Table 2. Average composition of olive leaf

Constituent	composition (g/100g)
Moisture	55.17± 0.99
Ash	6.12± 0.11
Fat	3.18± 0.075
Protein	8.21± 0.32
Water soluble carbohydrate	8.58± 0.397
Insoluble carbohydrate	18.74± 0. 53
Total carbohydrate	27.32± 0.86

Single factor effect on olive leaf polysaccharide extraction

To evaluate the effect of single factors of temperature, time and water-to-raw material ratio on polysaccharide extraction yield, two factors were kept constant and at the middle point and the effect of one factor was determined.

Results (Fig. 1) showed that temperature and water-to-raw material ratio had the highest influence on polysaccharide extraction and the lowest effect belonged to extraction time. By increasing temperature (Fig. 1A) from 60-100°C, at fixed time of 5 hours and water-to-raw material ratio of 15, extraction yield increased 59%.

In water-to-raw material range of 5 to 25 (Fig. 1C), at fixed point of 80°C and extraction time of 5 hours, extraction yield increased 50% but in 3-7 hours extraction (Fig. 1B) at fixed temperature of 80 °C and water-to-raw material ratio of 15, extraction yield just increased 11.8%. Increasing polysaccharide extraction by increasing temperature may be due to higher solvation and increasing diffusion coefficient. For industrial use it is important to find a balance between high temperature causing higher cost for extraction and other extraction parameters. Highly positive effect of water-to-raw material ratio may be due to higher concentration gradient of polysaccharide which improves polysaccharide extraction. The most important point is the weak effect of extraction time on polysaccharide extraction that could be very precious in industrial extraction of polysaccharide.

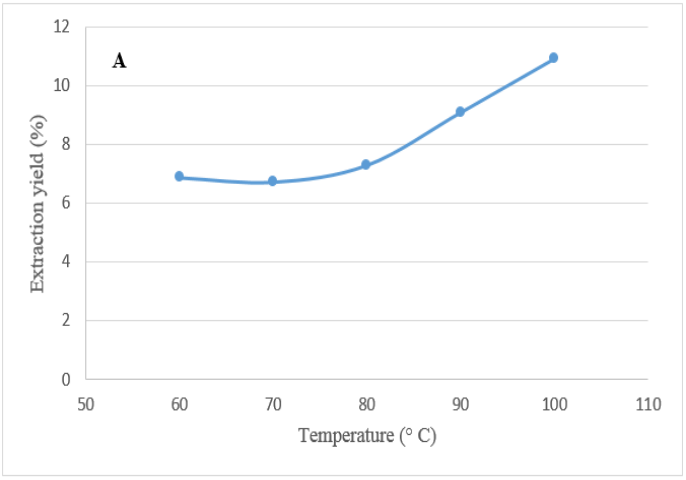
Predicted model and statistical analysis

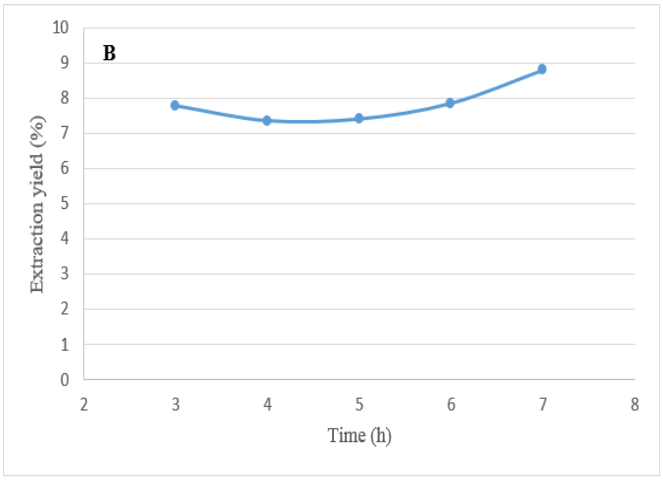
Response surface methodology advantageous over single parameters or factorial design is saving time and costs. Twenty runs containing 5 central point were designed by software in a central composite rotatable design. Experiment variables and final data of extraction yield are showed Table 3.

Software using experimental results introduced a quadratic equation based on multiple regression analysis.

$$Y = +18.56 - 0.4\,X_1 + 0.64\,X_2 - 0.33X_3 - 0.039X_1X_2 + 0.004\,X_1X_3 + 0.04\,X_2X_3 + 0.003\,X_1^2 + 0.21X_2^2 - 0.003X_3^2 \quad (5)$$

To evaluate model adequacy and determine significant factors, analysis of variance (ANOVA) was performed (Table 4). The important thing is that lack of fit model was significant which may be due to some noises but models R^2 and adjusted R^2 was 0.94 and 0.88 respectively that means it reasonably fits on data. Adequate precision shows signal to noise ratio and ratio greater than 4 is desirable. Adequate precision for the model was 16.53. CV of model was 5.91 that indicates high degree of model precision. All statistical parameters except lack of fit show reliability of model to predict data trend





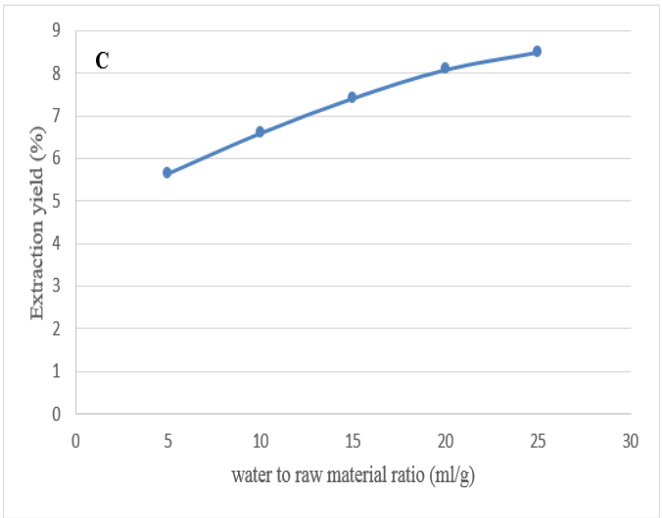


Fig. 1. Effects of temperature, time and water-to-raw material ratios on extraction yield

Table 3. Response surface central composite design and extraction yield

Std order	Run order	X1 (°C)	X2 (h)	X3 (ratio)	Extraction yield (%)		
					Actual	predicted	Residual
8	1	90	6	20	10.32	10.00	0.32
11	2	80	3	15	7.28	7.80	-0.52
18	3	80	5	15	7.32	7.42	-0.10
12	4	80	7	15	8.58	8.75	-0.17
7	5	70	6	20	8.25	8.23	0.019
14	6	80	5	25	8.37	8.55	-0.18
20	7	80	5	15	7.41	7.42	-0.014
1	8	70	4	10	6.36	6.00	0.36
17	9	80	5	15	7.29	7.42	-0.13
15	10	80	5	15	7.1	7.42	-0.32
2	11	90	4	10	9.09	8.42	0.67
3	12	70	6	10	7.2	6.85	0.35
10	13	100	5	15	10.63	11.11	-0.48
5	14	70	4	20	6.92	6.56	0.36
4	15	90	6	10	8.02	7.70	0.32
16	16	80	5	15	7.32	7.42	-0.10
19	17	80	5	16	7.42	7.42	-4.091E-003
6	18	90	4	20	10.23	9.90	0.33
9	19	60	5	15	6.71	6.92	-0.21
13	20	80	5	5	5.18	5.69	-0.51

Table 4. Analysis of variance of fitted model

Source	Sum of square	df	Mean Square	F- Value	Probability>F
Model	33.95	9	3.77	17.54	< 0.0001
A-temp	17.58	1	17.58	81.75	< 0.0001
B-time	0.90	1	0.9	4.18	0.0683
C-ratio	8.17	1	8.17	37.97	0.0001
AB	1.24	1	1.24	5.77	0.0372
AC	0.42	1	0.42	1.95	0.1931
BC	0.34	1	0.34	1.58	0.2370
A^2	3.96	1	3.96	18.43	0.0016
B^2	1.13	1	1.13	5.26	0.0448
C^2	0.15	1	0.15	0.69	0.4262
Residual	2.15	10	0.22		
Lack of Fit	2.08	5	0.42	31.19	0.0009
Pure Error	0.067	5	0.013		
Core total	36.10	19			

An important part of optimization is deleting insignificant parameters from model which can lower time and cost of the method. P-value is used to evaluate significance of model terms. In model proposed for extraction yield of polysaccharide linear terms of temperature and ratio were significant and time of extraction with p-value of 0.0683 was insignificant that shows its slight effect on extraction yield. For quadratic terms temperature and time highly influenced extraction model and were significant, but ratio was insignificant. For interaction terms just temperature × time was significant.

Contour plots shows combination effect of independent variables on extraction yield. It is clear that extraction time of 2- 7 hour has the lowest effect on extraction yield and extraction temperature has the highest effect. Low effect of extraction time may be due to high solubility of olive leaf polysaccharide and consequently exiting major parts within 2 hours.

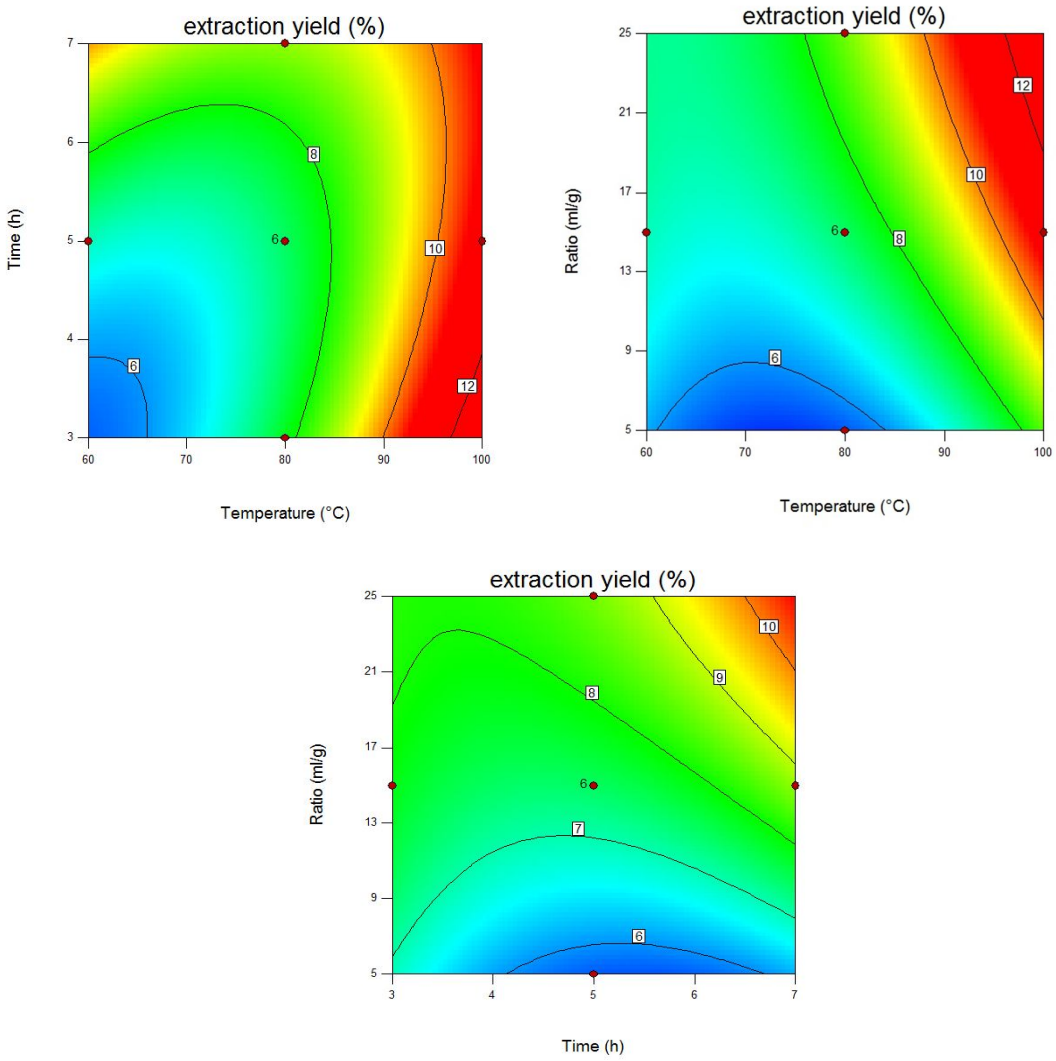


Fig. 2. Contour plots of independent variables interaction

Verification of model

To verify model ability to maximize the response, in optimization section of software two conditions of temperature and water-to-raw material ratio were introduced to evaluate their effect and importance (Table 5). In first scenario, temperature and ratio were defined in range, time set on minimum and our goal was defined for maximizing the yield with highest importance.

In second scenario ratio was defined in range and temperature and time were set on minimum and the goal was defined to be minimized with highest importance.

Table 5. Optimization conditions and predicted extraction yield

	Optimized conditions			Extraction yield (%)	
	Temperature (°C)	**Time (h)**	**ratio**	**predicted**	**actual**
1st scenario	89.87	2	19.37	11.43	9.74± 0.27
2nd scenario	80.96	2	17.94	8.88	8.27± 0.15

Results showed that based on optimization conditions, proposed model can predict extraction parameters to maximize extractions. At high temperature and water-to-raw material ratio predicted extraction was 11.43%, but in experimental conditions extraction yield was 9.74%.

Antioxidant properties of extracted polysaccharide

DPPH radical was used to monitor antioxidant of extracted polysaccharide. Ability of polysaccharide for donating electron to free radical of DPPH and decreasing violet color of DPPH solution to yellow color was compared to synthetic antioxidant of BHT and ethanolic extract of olive leaf (Figure 3).

Results showed good antioxidant properties and concentration dependent activity of extracted polysaccharide. IC_{50} for extracted polysaccharide was 250 µg/mL, comparing to 130 and 53 µg/mL for ethanolic extract and BHT respectively. Acceptable scavenging activity of polysaccharide is due to high proton donating activity that may be attributed to polyphenol or tannin extraction with polysaccharide (Tadayoni *et al.*, 2015).

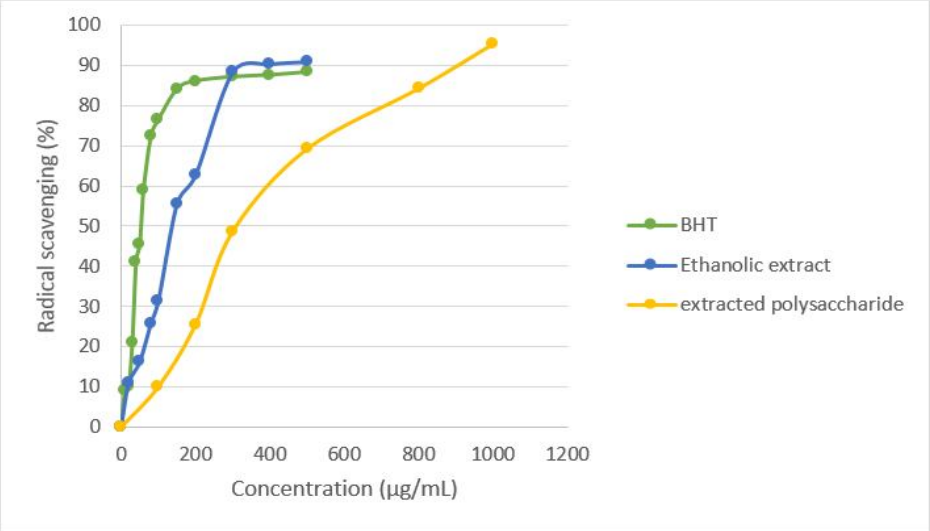


Fig. 3. Radical scavenging activity of extracted polysaccharide

Total phenol and flavonoid content

Total phenolic content of extracted polysaccharide and ethanolic extract of olive leaves are in compatible with their antioxidant properties. Total phenol and flavonoid contents were 57.9 and 36.66 mg GAE/mL and 1127 and 926 µg QE/mL for ethanolic extract and extracted polysaccharide respectively. Results showed that in extraction process of polysaccharide, some parts of phenolic compounds were entered in extracted polysaccharide.

Olive leaf polysaccharide structure by FTIR

Extracted polysaccharide structure and functional group was studied by FTIR spectroscopy based on vibrations at molecular state. In FTIR spectrum (Fig. 4) of polysaccharides C-H stretching was seen at 2925 cm^{-1} band. Observed band at 3376 cm^{-1} attributed to O-H group that usually is seen at 3200- 3500 cm^{-1}. Absorption peak at 890 cm^{-1} could be attributed to β-D-Glucose or β-D-Galactose (Yang and Zhang, 2009). Broad peaks in the 950-1200 cm^{-1} range indicate polysaccharide as major component in the extract (Azmi *et al.*, 2012). Bands at 1415 and 1272 cm^{-1} belong to COH bending groups and Absorption at 1077 cm^{-1} indicate pyranose form of sugar (Luo *et al.*, 2010).

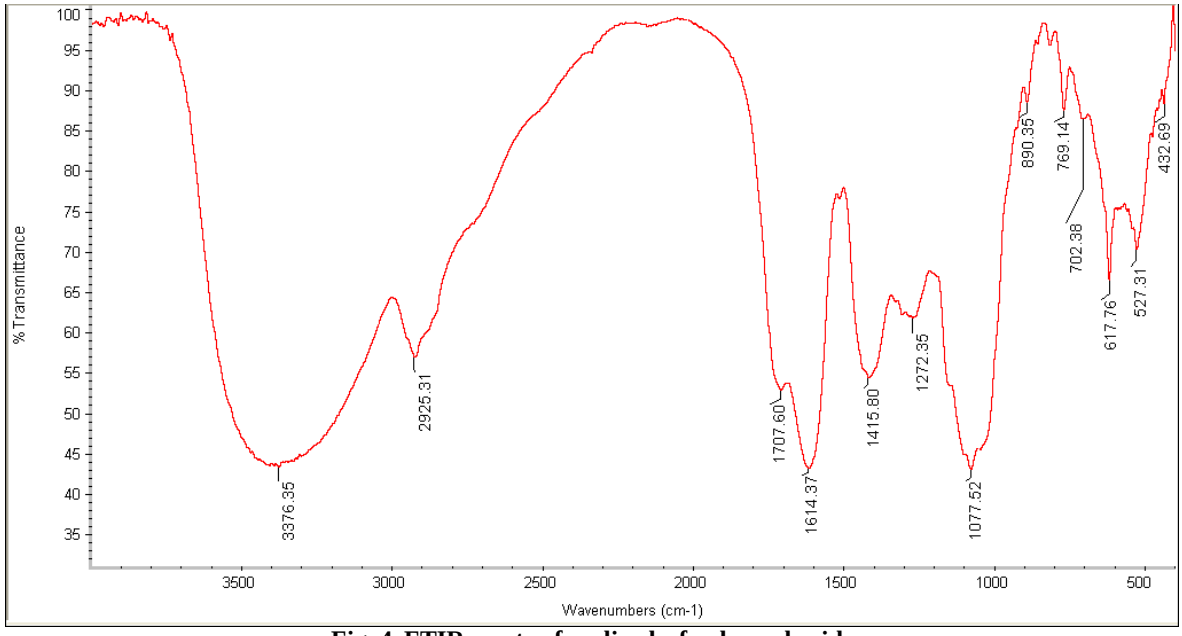


Fig. 4. FTIR spectra for olive leaf polysaccharide

Rheological properties

One of the most used applications of extracted polysaccharides is its usage in increasing solutions viscosity. At low concentrations of polysaccharide, it showed mostly Newtonian behavior (Fig 5) but at higher concentration it showed slightly shear thinning behavior. Flow behavior of extracted polysaccharide was evaluated in 1, 2.5 and 5% concentrations by fitting power law model on shear rate, shear stress data. Results showed that consistency coefficients were 1.44, 2.52 and 3.11 Pa.s and flow behavior indices were 0.985, 0.898 and 0.89 for 1, 2.5 and 5% concentrations respectively. Flow behavior index less than 1 in all concentrations shows shear thinning behavior of polymer solutions (Mehrnia *et al.*, 2017).

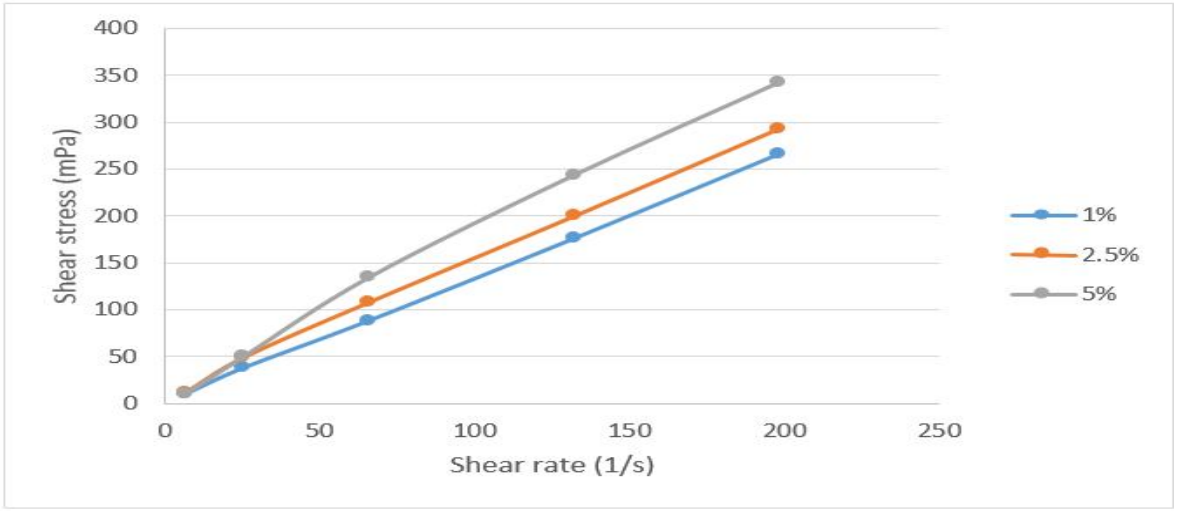


Fig. 5. Rheological properties of olive leaf polysaccharide

Conclusions

Native and extracted polysaccharides could be used as good substitutes for commercial polysaccharide. In this research the effects of temperature, time and water-to-raw material ratio on extraction yield were evaluated. Results showed that temperature has the highest and extraction time the lowest effect on extraction yield. Antioxidant properties of polysaccharide were notable comparing synthetic antioxidant of BHT and compatible with total phenol and flavonoid content of polysaccharide. Rheological properties and flow behavior of extracted polysaccharide showed shear thinning behavior at high concentration. It is concluded that extracted polysaccharide of olive leaf could be used as low cost biopolymer with high antioxidant and good rheological properties.

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بهینه‌سازی استخراج پلی‌ساکارید از برگ زیتون و ارزیابی ویژگی‌های رئولوژیکی و آنتی‌اکسیدانی آن

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تاریخ دریافت: 1398/01/26
تاریخ پذیرش: 1398/03/21

چکیده

طرح مرکب مرکزی روش سطح پاسخ برای بهینه‌سازی استخراج پلی‌ساکاریدهای برگ زیتون مورد استفاده قرار گرفت. اثر سه متغیر مستقل زمان استخراج (3-7 ساعت)، دمای استخراج (60- 100 درجه سانتی‌گراد) و نسبت آب به ماده خشک (5- 25 ml/g) برر روی بازده استخراج مورد بررسی قرار گرفت. پلی‌ساکارید استخراج شده برای ویژگی‌های آنتی‌اکسیدانی، میزان کل ترکیبات فنلی و فلاوونوئیدی و ساختار و گروه‌های فعال با FTIR بررسی شد. ویژگی‌های رئولوژیکی و رفتار جریان با برازش بر روی مدل توان تعیین شد. مهمترین پارامتر در دامنه‌های مورد آزمایش دما و کم اثرترین در مورد زمان استخراج مشاهده شد. بالاترین بازده استخراج در شرایط 2 ساعت، دمای 80/96 درجه سانتی‌گراد و نسبت آب به ماده خشک 94/17 ml/g مشاهده شد. ویژگی‌های آنتی‌اکسیدانی پلی‌ساکارید استخراج با استفاده از رادیکال DPPH در طول موج 517 نانومتر بررسی شده که نشان‌دهنده ویژگی‌های آنتی‌اکسیدانی قابل توجه نمونه بود. ویژگی‌های رئولوژیکی پلی‌ساکارید استخراجی در غلظت‌های 1، 2/5 و 5% بررسی شد. نتایج نشان داد که در غلظت‌های بالا رفتار از نوع شل‌شونده می‌باشد. یکی از مهم‌ترین مشکلات در ارتباط استخراج پلی‌ساکاریدهای بومی بازده استخراج آنها می‌باشد. پلی‌ساکارید استخراجی ویژگی‌های آنتی‌اکسیدانی خوبی در مقایسه با BHT و عصاره فنلی برگ زیتون نشان داد. علاوه بر این پلی‌ساکارید استخراجی می‌تواند برای افزایش ویسکوزیته محلول در غلظت‌های بالا مورد استفاده قرار گیرد.

واژه‌های کلیدی: پلی‌ساکارید برگ زیتون، بهینه‌سازی، روش سطح پاسخ، FTIR، ویژگی‌های آنتی‌اکسیدانی

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Discerning expiration status of edible vegetable oils based on color changes during oxidation process: Using digital image and linear discriminant analysis in both primary and secondary oxidations

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Abstract

Discerning the expiration status (non-rejected and rejected) of edible vegetable oils is very significant because of the hazardous primary and secondary oxidation products. Therefore, it is of outmost importance to monitor the quality and safety of these oils. Based on previous literature, reports and experimental observation, the oil color changes during oxidation. Thus, the present study investigates the use of image processing and linear discriminant analysis (LDA) for the classification of non-rejected and rejected edible vegetable oils during oxidation process at 85°C, with respect to the induced period in both primary and secondary oxidation of four oil type (Olive, Sunflower, Palm and Soybean). The purpose of this study was to find less costly and quicker methods with environmental protection, by using the color spaces (RGB, HSI, L*a*b* with Grayscale) instead of chemical analyses to determine the expiration status of edible vegetable oils. Results of this study indicated that the best classification for expiration status of known oils according to induced period of peroxide value in each color space, was achieved with LDA model were for palm with 100% (HSI and Grayscale), olive with 84.61% (L*a*b* and RGB), soybean with 95% (Grayscale) and sunflower with 100% (RGB and HSI), also in induced period of carbonyl value test, the best classification performance was achieved in palm with 100% (L*a*b*), olive with 100% (L*a*b*), soybean with 89.47% and sunflower with 95% (HSI).

Keywords: Edible Vegetable Oil; Oxidation; Peroxide Value; Carbonyl Value; Linear Discriminant Analysis; Imaging.

Introduction

Edible oils bring essential nutrient components for human beings such as vitamins, fatty acids, and micronutrients, which are necessary for daily life [1]. However, the use of expired edible oils leads to a decrease in the nutritive value and an increase in potential hazards to people's health [2]. Therefore, the authentication and identification of edible oils are of great importance in the field of food safety and quality monitoring.

A reliable, fast and non-destructive detection method to identify various types of edible oils is essential. Recently, different methods have been employed to identify the types of edible oils [3]. Several works have been reported in literature exploring the use of liquid chromatography [4], fluorescence spectroscopy [5], fourier transform infrared [6], differential scanning calorimeter [7], supercritical liquid chromatography (SFC) and gas chromatography [8], for the quality control of vegetable oils. However, most of these methods are highly sophisticated, expensive and involve laborious analysis.

Lipid oxidation in vegetable oils is associated with unsaturation of the oils. This reaction leads to the formation of a series of intermediate compounds named hydro peroxides. Hydro peroxides are the primary oxidation products of lipid oxidation. This is due to the unstable nature of these products which leads to their decomposition and turning into secondary oxidation products, such as carbonyl compounds. These products are generally unstable and decompose into a variety of secondary oxidation products, including carbonyl compounds [9].

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This condition causes flavor and odor change; Hydro peroxides do not play an important role in flavor deterioration, whereas carbonyl compounds are mainly responsible for the typical rancid off-flavors [10].

Two of the best analytical indicators of oxidative changes in fats and oils are peroxide (PV) and Carbonyl (CV) values [11]. Determination of these values provides valuable information in regard to primary and secondary oxidations [12]. However, due to the use of chemical materials (solvents such as chloroform, n- Hexane etc.), determination of these values is time- consuming, financially costly and costly in terms of damage to environment. Therefore, finding a quicker, less expensive and more environmentally friendly method is crucial.

Nowadays, digital imaging is becoming more important because of its ability to perform fast and non- invasive, low- cost analysis on foods. In fact, a wide variety of digital cameras and digitalization equipment has contributed to an increase in the number of papers published exploring the use of webcam [13], scanner [14], cell phones [15] and digital camera [16] to monitor the quality of several food samples. A positive feature of using digital image to monitor the quality of foods is that it replaces the human visual system, often employed in these types of analyses [17].

Therefore, the use of digital image eliminates the subjective character of analyses as well as the dependence of the human visual system, which is substantially influenced by ambient conditions and subject to inconsistencies [18].

Given this, techniques based on digital images are a promising alternative for the analysis of food and other products. Some studies have shown the availability of using the technique for quality control in various matrices, including shrimp [19], cereal grains [20], Kiwifruit [21], castor seeds [22], olive oils [23-25] and other edible vegetable oils [26]. Most of these studies use information from color models associated with pattern recognition techniques to cluster, or classifythe samples into categories, according to similarity standards.

The red, green and blue additive color added together to form RGB color space that was designed to match an intuitive human perception of the colors. In HSI color space, parameters are the intensity I, chromaticity hue H and saturation S. HSI approximates the way in which humans perceive and interpret colors.

At present, usually, the color of foods has been measured in L*a*b*. The L*a*b*, or CIE L*a*b*, color space is an international standard for color measurements, adopted by the Commission Internationale d'Eclairage (CIE) in 1976 [27].

However, at present available commercial colorimeters, measure L*a*b* only over a very few square centimeters, and thus their measurements are not very representative in heterogeneous materials such as most food items [27]. Some of the instruments most frequently used in the measurement of color are colorimeters (e.g Minolta chroma meter, Hunter Lab colorimeter and Dr. Lange colorimeters). There is, however, a disadvantage in using them [26, 28-29].

Using a computational technique with a combination of a digital camera, and image processing software has already been used to provide a less costly and more adaptable way to measure the color of many food products and foodstuffs instead of traditional color measuring instruments [27].

Although there have been many studies in the field of image processing and classification of foods, studies on the classification of edible vegetable oils based on image processing have been very limited especially in the field of determination of expiration status (rejected and non-rejected).

As was pointed before, understanding the first oxidation based on odor and flavor is not possible since the off flavor occurs in the second oxidation, therefore, it is critical to find a new technique for discerning oxidation. This is possible based on the color of oil, however, discerning the condition of rancid is impossible by the naked eye, therefore, it is necessary to use pattern recognition and image processing.

According to no significant color change just before and after rejection, the aim of this study was to determine rejected and non-rejected status of edible vegetable oils during oxidation process in specified intervals of two and six hours according to the induce period of peroxide and carbonyl value by using only the oil color changes instead of expensive and laborious current recognition and quality control methods of oils. To the best of our knowledge, this is the first study to investigate the expiration status of edible vegetable oils according to exact rejection point by using the color changes.

Materials and Methods

Four vegetable oil samples including Soybean, Sunflower, Palm, and Olive oils were purchased from the Segol Co. (Nishaboor, Iran). They were stored at -18°C until the time of analysis. All chemicals and solvents were purchased from Sigma-Aldrich (St. Louis, MO) and Merck (Darmstadt, Germany). Images were taken in every two (Soybean, Sunflower oils) and six hours (Palm, and Olive oils), during the heating process (at 85°C in an oven). Also, the chemical (PV and CV) analysis was performed at the same time of imaging.

Peroxide value

The spectrophotometric method of Shantha and Decker [30] was used to determine the peroxide value (PV). The oil samples were mixed with chloroform: methanol in a glass tube. Ammonium thiocyanate and iron (II) chloride was then added. After that the sample was placed for 5-min at room temperature, the absorbance of the sample was read at 500 nm against a blank by using a spectrophotometer. Results were expressed in milliequivalents of oxygen per kilogram of oil.

Carbonyl value

The carbonyl value (CV) was measured by using 2-propanol and 2, 4-decadienal as solvent and standard, respectively. Any trace of carbonyl compounds which may present in the solvent was removed with mixed solution of sodium borohydride and 2-propanol. Standard aldehyde (2, 4-decadienal) dissolved in the solvent. The oil was mixed with 2, 4-Dinitrophenylhydrazine (DNPH) solution in a test tube. Then the test tube was stoppered and heated. After that, the sample was cooled in water, and 2% KOH solution was added. After centrifugation, the absorbance of the upper layer was measured at 420 nm by using a spectrophotometer against a blank. The results were expressed in micromols of 2, 4 decadienal per gram of oil [31].

Apparatus and digital image acquisition

Apparatus

The size of system that was built for imaging was 120 cm× 90 cm× 90 cm with dark walls to isolate the samples from external light. The compartment has camera canon model, EOS 1000D, which was connected to computer by a USB port. The illumination of the compartment was performed by using eight fluorescent lamps with 8 W (white color), the lamps were placed at a distance of 20 cm and 40 cm from the sample and with 45 degree angle for preventing the reflection of light.

The Illustration was performed by Zoombrower EX 0.5, the other characteristics of camera for imaging were as follow: flash (off), zoom (on), Iso speed (100), Aperture priority (F / 20) and Shutter speed (0.6 Sec). The illumination condition at compartment for each sample was the same.

Image color analysis

Image color analysis was performed using the MATLAB (R2013) software to convert images from R*G*B color space to L*a*b, HSI, Grayscale.

The recorded images contained 24-bit (16.7 million colors) and 3888 pixels× 2592 pixels spatial resolution, were stored in JPEG format (jpg). A circular region with a radius of 2.75 mm at the center of each image was selected for converting R*G*B to L*a*b, HSI and Grayscale. The extracted color values were then used for linear discriminant analysis (LDA) classification (Fig. 1).

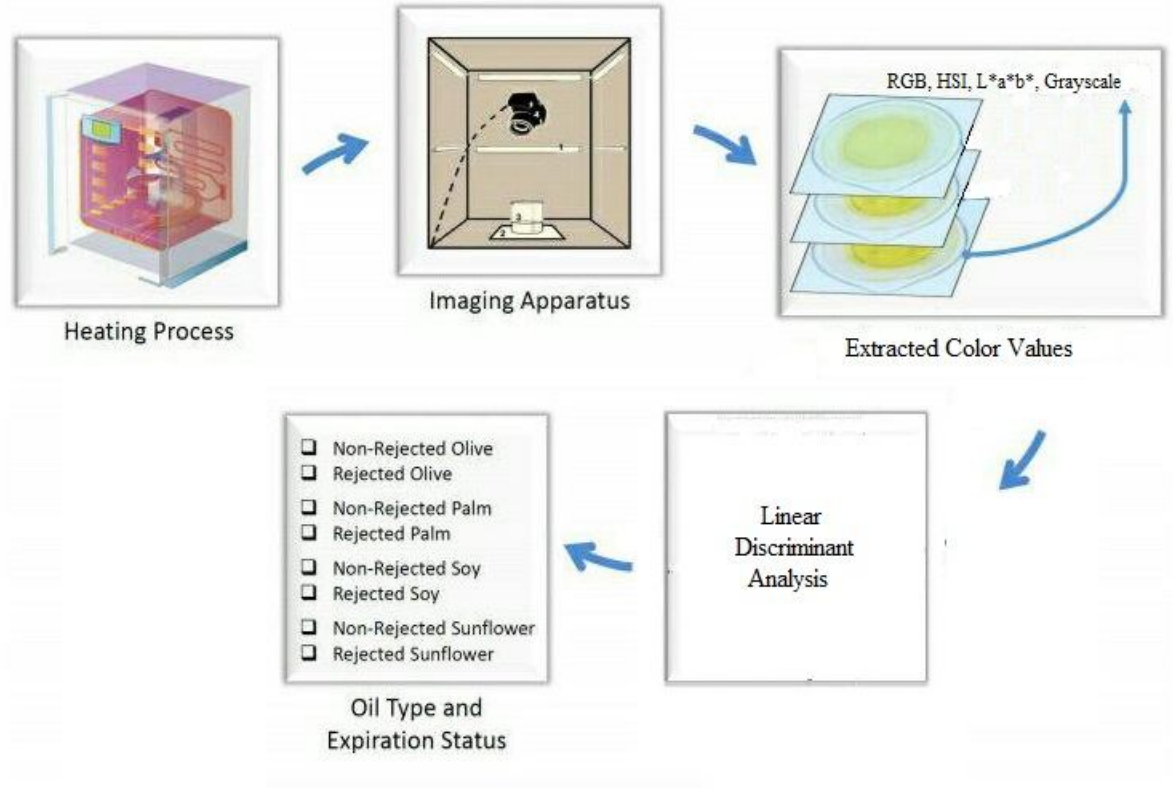

Fig. 1. System used in the acquisition of images and classification of oils samples.

Chemometric procedures

The extracted color features were analyzed based on two approaches. The first approach involved the classification of non-rejected and rejected status of each oil samples with respect to induced period of peroxide value, and the second classification involved the classification with respect to induced period of carbonyl value, to distinguish non-rejected and rejected status of edible oils according to primary and secondary oxidation. A total of 99 images of oils data were collected during the heating process. Data analysis was performed by classical multivariate procedures including LDA with MATLAB (R2013). The RGB, HSI, L*a*b* and Grayscale color space features were extracted from images of oils samples then they were used in LDA classification for both color changes during primary (90 images) and secondary (99 images) oxidation (Table 1).

Table 1. The total number of oil samples in both PV and CV tests.

		Palm	Olive	Soybean	Sunflower
PV	Non-rejected	20	16	7	7
	Rejected	5	10	12	13
	Total	25	26	19	20
CV	Non-rejected	24	20	11	11
	Rejected	10	6	8	9
	Total	34	26	19	20

To find the effect of oxidation process on the color of edible vegetable oils during time, the color changes in each oil type at each color spaces RGB, HSI, L*a*b* and Grayscale was considered.

Linear Discriminant Analysis (LDA) is a classification method that is commonly used as dimensionality reduction technique for pattern-classification and machine learning to find a linear combination of features that characterizes or separates two or more classes of objects which was developed by Fisher [32]. Multi-class LDA is a generalization of standard two-class LDA that can handle arbitrary number of classes. We are seeking p projections (*i.e.*$\boldsymbol{y}_1, \boldsymbol{y}_2 \ldots \boldsymbol{y}_p$) of the input vector $\boldsymbol{x}$ by means of p projection vectors $\boldsymbol{w}_i$ as follows:

$$y_i = \boldsymbol{w}_i^T \boldsymbol{x}, \qquad i = 1,2, \ldots, \quad (1)$$

If we arrange all $\boldsymbol{w}_i$ in a projection matrix as $W=[\boldsymbol{w}_1,\boldsymbol{w}_2,\ldots,\boldsymbol{w}_p]$, it can be written:

$$\boldsymbol{y} = W^T \boldsymbol{x} \quad (2)$$

where

$$\boldsymbol{x} = [x_1 \quad \cdots \quad x_m]^T$$

and

$$\boldsymbol{y} = [y_1 \quad \cdots \quad y_n]^T.$$

By stacking all feature vectors in one matrix, we can write:

$$Y = W^T X \quad (3)$$

where

$$X = [\boldsymbol{x}^1, \boldsymbol{x}^2, \ldots, \boldsymbol{x}^n] = \begin{bmatrix} x_1^1 & x_1^2 & . & x_1^n \\ . & . & . & . \\ x_m^1 & x_m^2 & . & x_m^n \end{bmatrix} \quad (4)$$

$$Y = [\boldsymbol{y}^1, \boldsymbol{y}^2, \ldots, \boldsymbol{y}^n] = \begin{bmatrix} y_1^1 & y_1^2 & . & y_1^n \\ . & . & . & . \\ y_q^1 & y_q^2 & . & y_q^n \end{bmatrix} \quad (5)$$

For p-classes case, we will measure the within-class and between-class scatters with respect to the mean of all classes, respectively, as follows:

$$S_W = \sum_{i=1}^{p} \sum_{\boldsymbol{x} \in c_i} (\boldsymbol{x} - \boldsymbol{\mu}_i)(\boldsymbol{x} - \boldsymbol{\mu}_i)^T \quad (6)$$

$$S_B = \sum_{i=1}^{p} N_i (\boldsymbol{\mu}_i - \boldsymbol{\mu})(\boldsymbol{\mu}_i - \boldsymbol{\mu})^T \quad (7)$$

Where

$$\boldsymbol{\mu} = \frac{1}{N} \sum_{\forall_x} \boldsymbol{x} = \frac{1}{N} \sum_{i=1}^{p} N_i \boldsymbol{\mu}_i \quad (8)$$

$$\boldsymbol{\mu}_i = \frac{1}{N_i} \sum_{\boldsymbol{x} \in C_i} \boldsymbol{x} \quad (9)$$

Such that C_i represents the i-th class. Similarly, we can define the mean vectors for the projected samples as follows:

$$\tilde{\boldsymbol{\mu}}_i = \frac{1}{N_i} \sum_{\boldsymbol{y} \in C_i} \boldsymbol{y} \quad (10)$$

$$\tilde{\boldsymbol{\mu}} = \frac{1}{N_i} \sum_{\forall \boldsymbol{y}} \boldsymbol{y} \quad (11)$$

Similarly, the within-class and between-class scatter matrices of the projected samples can be given, respectively, by:

$$\tilde{S}_W = \sum_{i=1}^{p} \sum_{\boldsymbol{y} \in C_i} (\boldsymbol{y} - \tilde{\boldsymbol{\mu}}_i)(\boldsymbol{y} - \tilde{\boldsymbol{\mu}}_i)^T \quad (12)$$

$$\tilde{S}_B = \sum_{i=1}^{p} N_i (\tilde{\boldsymbol{\mu}}_i - \tilde{\boldsymbol{\mu}})(\tilde{\boldsymbol{\mu}}_i - \tilde{\boldsymbol{\mu}})^T \quad (13)$$

By using Eqns. (6) and (7) and some algebraic manipulations, we can obtain:

$$\tilde{S}_W = W^T S_W W \quad (14)$$

$$\tilde{S}_B = W^T S_B W \quad (15)$$

To obtaine an appropriate discrimination between all classes, the coefficients of W should be optimally adjusted such that the between-class scatter increases while simultaneously, the within-class scatter decreases. Thus, the optimal W^* can be obtained by maximizing the following objective function:

$$J(W) = \frac{|\tilde{S}_B|}{|\tilde{S}_W|} = \frac{|W^T S_B W|}{|W^T S_W W|} \quad (16)$$

$$W^* = index(\max_W J) \quad (17)$$

Where|.| computes the determinant of a matrix. For W^*, it is sufficient to set the differential of J (W) (with respect to W) equal to zero. It can be shown that the columns of W^* are the eigenvectors corresponding to the

largest eigenvalues of the following generalized eigenvalue problem:

$$S_W^{-1} S_B w_i^* = \lambda_i w_i^* \quad (18)$$

Where

$$W_i^* = [w_1^*, \ w_2^*, \dots, w_{p-1}^*].$$

Evaluation Measure

This feature was studied by using the accuracy (ACR) analysis as follow:

$$\mathrm{ACR} = \frac{TP+TN}{TP+FP+TN+FN} \quad (19)$$

Where TP, TN, FP, and FN are the true positive, true negative, false positive and false negative, respectively, TP (FP) or true positive (false positive) means all samples correctly (incorrectly) identified. Similarly FN or false negative means that those samples are incorrectly identified.

Results and Discussion

Table 2 illustrates the Chemical indicators, Peroxide values (PV) and Carbonyl values (CV) of the oil samples. The PV and CV values of the soybean, sunflower, olive and palm oils after heating process are shown in Table 2. The PV and CV of the non-rejected oils were lower than 2 meq O_2/kg oil and 3.1 µmol/g for all samples and their levels after heating did differ significantly to reach their rejection points.

Table 2. Chemical indicates and Peroxide value (PV), and carbonyl value (CV) of the oils before and after heating process at 85°C.

	PV[b]	IP_{pv}Time (hour)	CV[c]	IP_{cv}Time (hour)
Palm R	0.69±0.021		3.06±0.012	
Olive R	1.99±0.46		3.047±0.072	
Sunflower R	0.28±0.034		0.26±0.005	
Soybean R	0.82±0.057		0.136±0.001	
Palm NR	62.74±0.11	119.09	8.74±0.21	140.68
Olive NR	32.053±0.90	93.64	10.96±0.15	114.66
Sunflower NR	2.23±0.13	13.70	4.61±0.03	21.42
Soybean N	1.89±0.19	12.94	4.52±0.04	21.39

a Mean value ± standard deviation, All values are means of three determinations.
b Peroxide value (meq O_2/kg oil).
c Carbonyl value (µmol/g).

During the heating process, a wide range of PV and CV was observed among the oil samples every 2 and 6 hour (from 0.82 to 1.89 meq O_2/kg oil for Soybean, 0.28 to 2.23 meq O_2/kg oil for sunflower, 1.99 to 32.053 meq O_2/kg oil for olive and from 0.69 to 62.74 meq O_2/kg oil for palm and also from 0.136 to 4.52 µmol/ g for Soybean, 0.26 to 4.61 µmol/ g for sunflower, 3.047 to 10.96 µmol/ g for olive and from 3.06 to 8.74 µmol/ g for palm, PV and CV values ,respectively), indicating that the oils had no similar rejection points. Table 2 also demonstrates the IPPV and IPCV for each oil type. The peroxide value (PV) for soybean was 12.94, sunflower 13.70, olive 93.64 and palm 119.09 hour And similarly carbonyl value (CV) for soybean was 21.39 sunflower 21.42, olive 114.66 and palm 140.68 hour.

These chemical analyses were carried out in order to find the exact rejection point of primary and secondary oxidation of these oils, so that their classification could be done accurately in all their different rancidities (Fig.2).

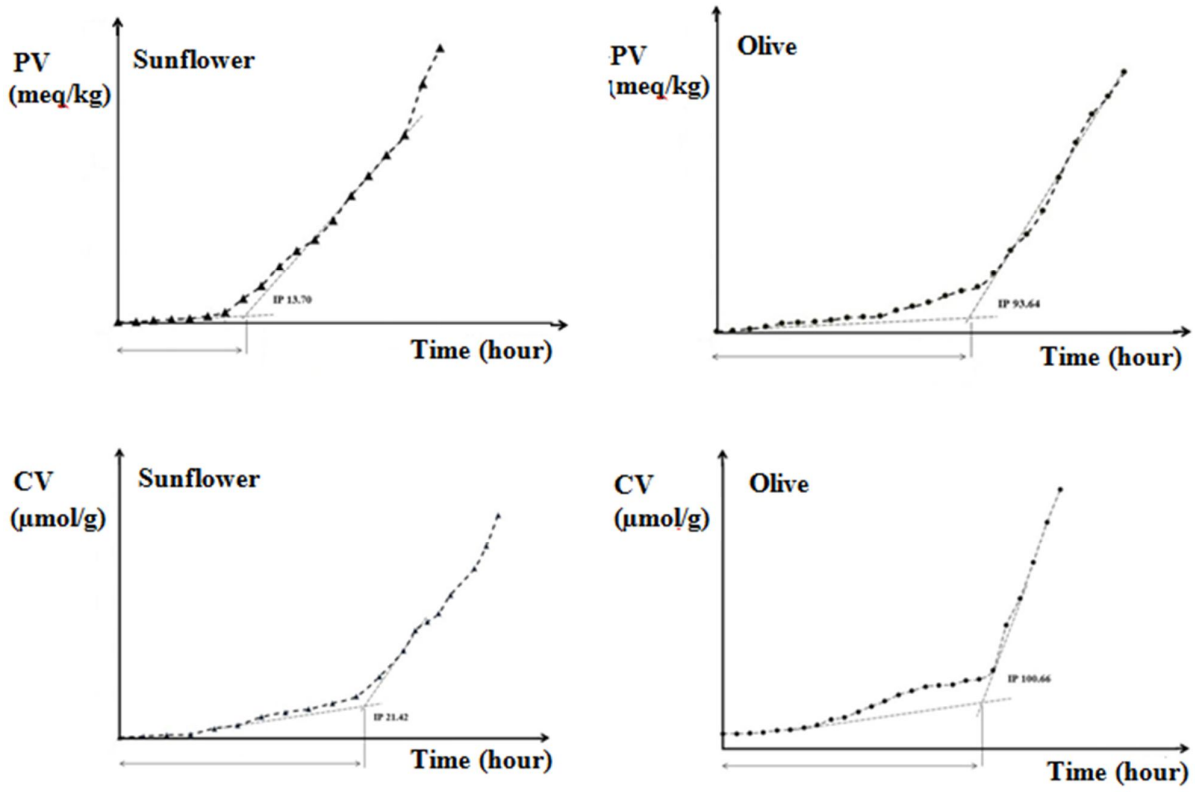

Fig. 2. A schematic kinetic curve of peroxide and carbonyl accumulation during oxidation of lipid systems in Sunflower and Olive oil.

By increasing the amount of PV and CV during time of heating, the color of the oils changed in each stage. According to the difference in values and color feature extraction of images, it may be possible to make a model for classification of expiration status of these oils without the necessity to perform high cost chemical experiments. Therefore, it is suggested that the oxidation can significantly affect the color.

In classification part, Table 3 illustrates the classification accuracy of non-rejected and rejected of each known oil type during the heating time at each color space separately to identify the expiration status according to carbonyl and peroxide values.

Table 3. Accuracy of each known oil type during the heating time at each color space in both PV and CV tests.

		Olive	Palm	Soybean	Sunflower
PV	**L*a*b***	84.61%	100%	89.48%	90%
	RGB	84.61%	96%	78.95%	100%
	HSI	80.77%	100%	84.21%	100%
	Grayscale	80.77%	96%	95%	75%
CV	**L*a*b***	100%	100%	84.21%	90%
	RGB	96.15%	91.18%	78.95%	90%
	HSI	84.62%	88.25%	89.47%	95%
	Grayscale	88.46%	100%	78.95%	70%

As it can be seen in Table 3, all rejected and non-rejected oils according to peroxide induced period in each color space and Grayscale were identified appropriately. It is apparent from the Table, that the highest accuracy among studied color spaces were 100% at L*a*b* and HSI for palm, 84.61% at L*a*b*, RGB color spaces for olive, 95% at Grayscale color space for soybean and finally 100% at RGB and HSI color spaces for sunflower.

Similarly, Table 3 shows the accuracy of edible oils according the carbonyl induced period. This indicated that, the best results for palm, olive, soybean and sunflower oils were achieved with 100% at L*a*b* and Grayscale, 100% at L*a*b*, 89.47% at HSI and lastly 95% at HSI color space respectively.

Table 4 illustrated the classification accuracy of four oils (Palm, Olive, Soybean and Sunflower) in non-rejected and rejected status in both oxidations. At first all non-rejected oils were classified in each color space (L*a*b*, RGB, HIS and Grayscale). As shown in Table 4 the best result belongs to HSI with 88% in primary oxidation and with regard to secondary oxidation L*a*b* with 91.04% have the best accuracy. For rejected oils classification the best color space are HSI and L*a*b* with 90% and 87.5% in primary and secondary oxidation, respectively.

Table 4. Accuracy of Non-rejected and Rejected four oils (Palm, Olive, Soybean and Sunflower) during the heating time at each color space in both PV and CV tests.

		L*a*b*	RGB	HSI	Grayscale
PV	**Non-rejected oils**	86%	80%	88%	68%
	Rejected oils	80%	77.5%	90%	75%
CV	**Non-rejected oils**	91.04%	68.66%	82.09%	58.21%
	Rejected oils	87.5%	81.25%	71.88%	56.25%

Figs. 3A and 3B show the score plots of the two discriminant functions (F2 * F1) obtained by the LDA classifier for the R and NR oils samples. It is possible to observe that Soybean-R, Sunflower-R, Olive-R and Palm-R oil samples are separated along the F1 direction. It is obvious that the F1 separated Soy-R and Palm-R groups from other groups.

As can be seen, the Olive-R and Sunflower-R sample has overlap with each other (Fig. 4A). Fig. 4B illustrated that Sunflower-NR, Soybean-NR, Olive-NR and Palm-NR oil samples presented a tendency of separation along the F1 direction. Although Sunflower-NR and Olive-NR samples well discriminated but Palm-NR and Soybean-NR have a slight overlap.

As it can be seen in Table 5, all rejected and non-rejected oils according to both oxidations in all color spaces and Grayscale were classified. It is apparent from Table 5 that the highest accuracy belongs to the classes with more sample numbers (according to Table 2). For example, Olive NR with 100% in both oxidations has best accuracy in comparison with the Olive R (30% and 50%). Because of different rate of expiration in each oil-type, the number of samples is quite limited in some classes. However, total accuracy of LDA classifier in primary and secondary oxidation relatively well performs with 74.44% and 75.76%, respectively.

The classification performance of LDA classifier is demonstrated by Fig. 4A and B, which presents the score plots of the first two discriminant functions (F2 * F1) for all Non-rejected and Rejected oils during the heating time at all color spaces in each oxidation. In case of carbonyl test (Fig. 4a), it is obvious Olive oil samples in both status (NR and R) are separated along the F2 direction with slightly overlap. F1 clearly distinguishes Olive oil samples from other oils classes.

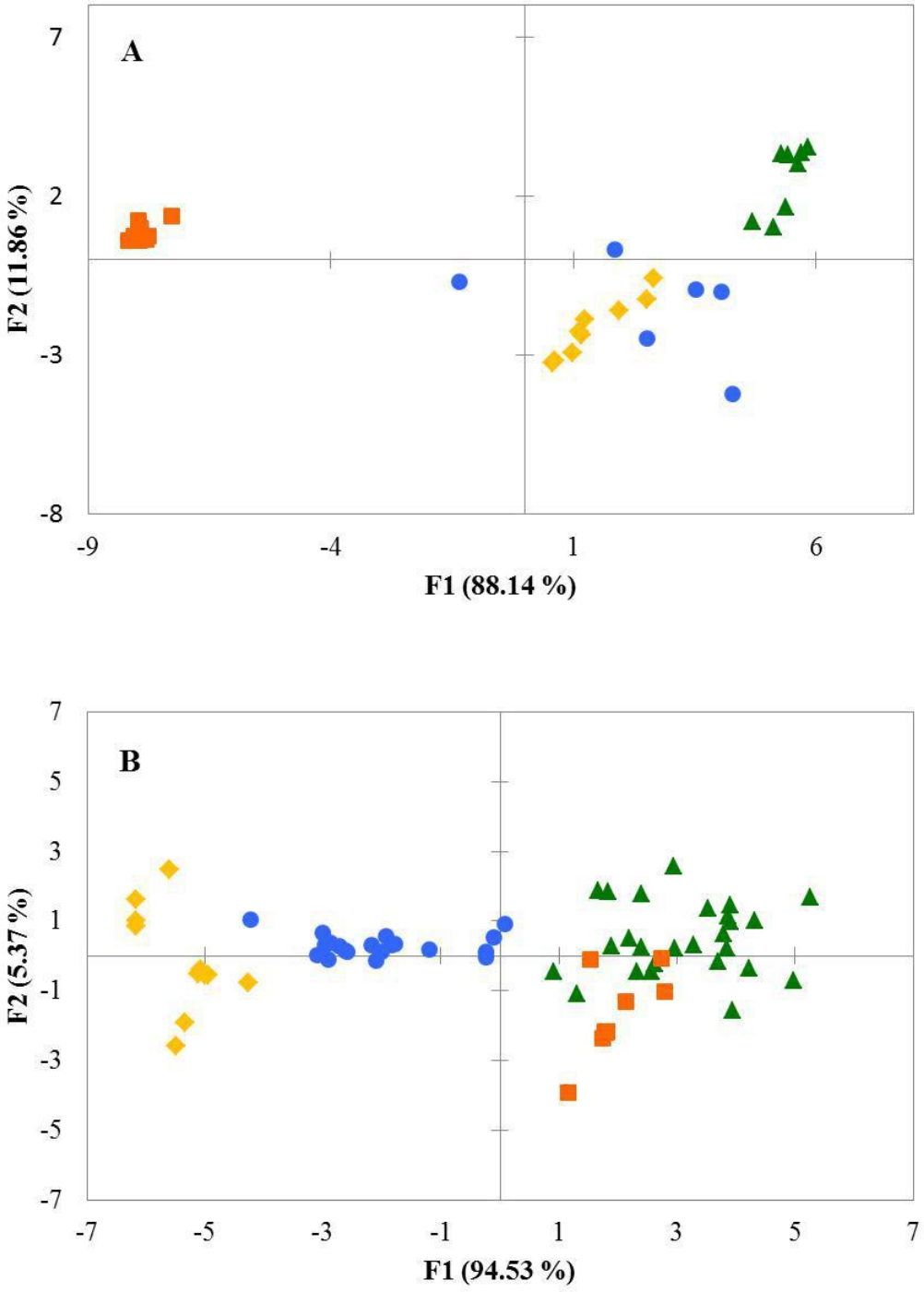

Fig. 3. F2× F1 score plots for Non-rejected and Rejected oils during the heating time atL*a*b*color space in carbonyl test (a) All R oilsand (b) All NR oils. Olive; ●: Palm; ▲: Soybean; ■and Sunflower; ◆.

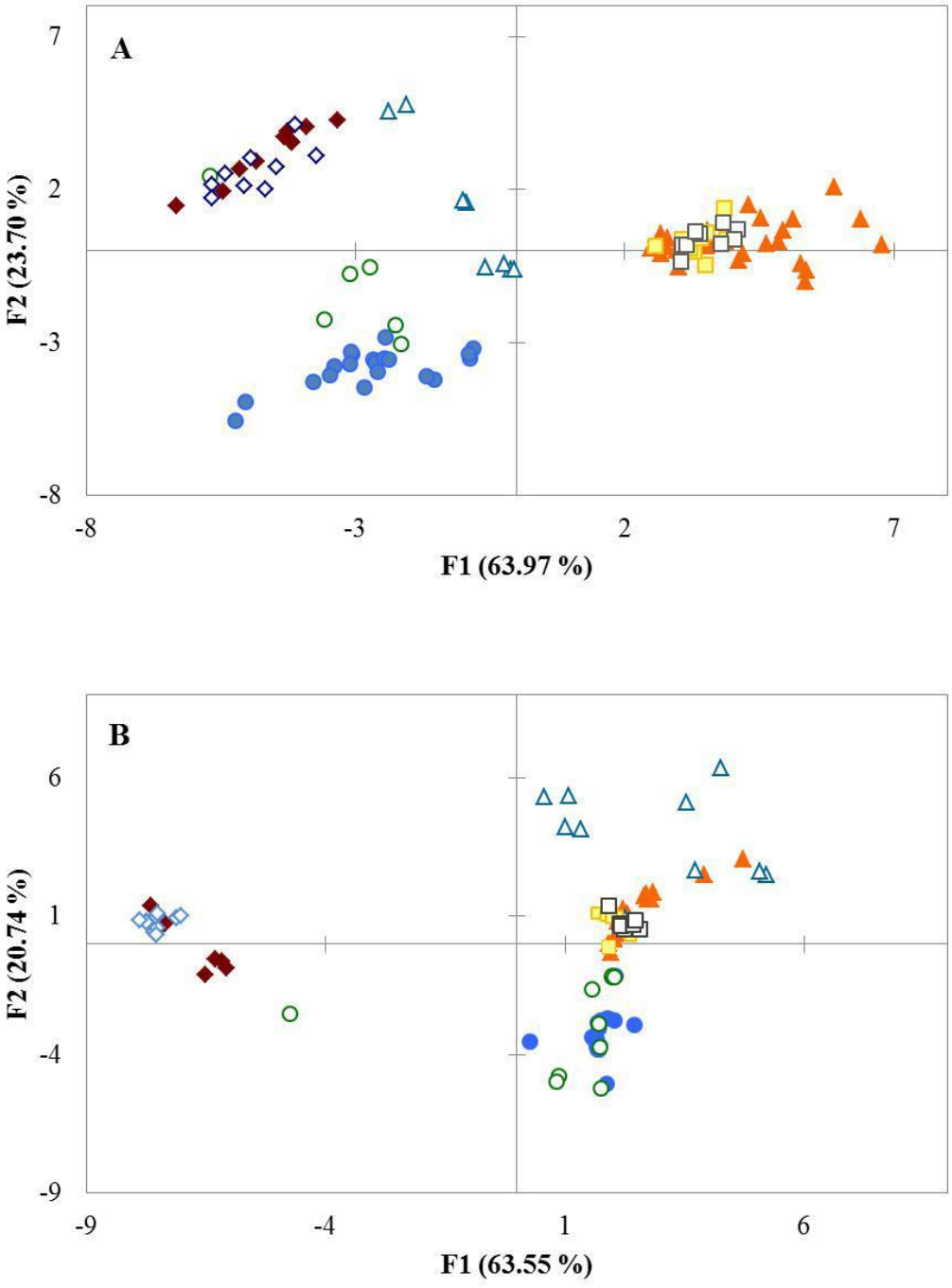

Fig.4. F2× F1 score plots for all Non-rejected and Rejected oils (Palm, Olive, Soybean and Sunflower) during the heating time at all color spaces (a) carbonyl test and (b) peroxide test. Olive NR; ●: Olive R; ○: Palm NR; ▲: Palm R; △: Soybean NR; ■: Soybean R; □: Sunflower NR; ◆and Sunflower R; ◊.

In the case of Sunflower oil samples, this class was well discriminated, but the NR and R status are intense overlap in both directions. The Palm oil samples overlap with other oil classes but the NR and R status were separated well in F1 direction. Finally, the worst classification performance was presented by Soybean oil samples in both directions which had severe overlap with Palm-NR.

Milanez and Pontes [25] tried to distinguish the type and conservation state of four different edible oils based on color image processing and linear discriminant analysis (LDA). They only attempted in classification of different type of oil, and also classification expired and non-expired oils without considering the oxidation process of oils during time with analytical experiments.

It could be inferred from our study that, it is appropriate to use color space to identify the expiration status of each oil type during heating process. As much as the oil color changes with different rates during oxidation, it could be possible to detect the exact rejection state (based on both the primary and secondary oxidation) of known oil sample.

It seems that while classification of edible oils from color features can be applicable, selection of the suitable color space and classifier are significant steps to develop new simple and inexpensive method for detection of the non-rejected and rejected status of unknown oil.

Table 5. Accuracy (%) of all Non-rejected and Rejected oils (Palm, Olive, Soybean and Sunflower) during the heating time at all color spaces in both PV and CV tests.

	Olive NR	Olive R	Palm NR	Palm R	Soybean NR	Soybean R	Sunflower NR	Sunflower R	Sample Number	Accuracy
PV	100	30	93.33	90	63.64	62.50	44.44	81.82	90	74.44
CV	100	50	92.31	37.50	40	44.44	81.82	88.89	99	75.76

Conclusions

The purpose of this paper was to find less costly and quicker method with environmental protection, by using the color spaces (RGB, HSI, L*a*b* with Grayscale) instead of chemical analysis to determine the expiration status of edible vegetable oils in both PV and CV induced period.

The results obtained from this study indicated that the best classification result of expiration status of known oils according to induced period of peroxide value at each color space, was achieved with LDA model were in palm with 100% (L*a*b* and HSI), olive with 84.61% (L*a*b* and RGB), soybean with 95% (Grayscale) and sunflower with 100% (RGB and HSI), also in induced period of Carbonyl value test, the best classification performance was achieved in palm with 100% (L*a*b* and Grayscale), olive with 100% (L*a*b*), soybean with 89.47% and sunflower with 95% (HSI).

As pointed out in the result and discussion part, it is recommended that by using each color feature it would be possible to identify expiration status (reject or non-reject) of known edible oils and classification unknown oils. Although there is no significant color change just before and after rejection, but also this study enables to classify reject and non-reject status of each oil type according to IP value. This methodology can be applied for automated control in identification expiration status of edible vegetable oils. It is worth mentioning that distinguish the expiration status (non-rejected and rejected) of edible vegetable oils is more applicable and will lead to high accuracy of the applied procedure.

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تعیین تاریخ انقضاء روغن‌های خوراکی مبنی بر تغییر رنگ طی فرآیند اکسایش: کاربرد تحلیل تصویر رقمی و آنالیز افتراقی خطی طی اکسایش اولیه و ثانویه

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چکیده

تشخیص وضعیت انقضا (سالم و تند شده) روغن‌های گیاهی خوراکی به‌خاطر محصولات اولیه و ثانویه اکسیداسیون حائز اهمیت است. بنابراین بررسی کیفیت و سلامت روغن‌های خوراکی بسیار مهم است. بر اساس گزارشات و آزمایشات تجربی رنگ روغن طی اکسیداسیون تغییر می‌کند. پژوهش حاضر به شرح بررسی انجام شده توسط پردازش تصویر و تحلیل تفکیک خطی (LDA) برای طبقه‌بندی روغن‌های گیاهی خوراکی سالم و تند شده در طی اکسیداسیون در دمای 85 درجه سانتی‌گراد با توجه به اکسیداسیون اولیه و ثانویه در چهار نوع روغن (پالم اولئین، زیتون، سویا و آفتابگردان) پرداخته است. هدف از این پژوهش یافتن روش‌های ارزان و سریع‌ترو همچنین حافظ محیط زیست به جای آزمون‌های شیمیایی به کمک فضاهای رنگی (RGB، HSI، L*a*b* با Grayscale) برای تعیین وضعیت انقضا روغن‌های خوراکی است. این مطالعه نشان داد که بهترین نتیجه برای تشخیص وضعیت انقضا در روغن‌های معلوم با توجه به دوره القا عدد پراکسید در هر فضای رنگی توسط طبقه‌بند LDA برای پالم 100% (HSI و سیاه وسفید)، زیتون 84/61% (L*a*b* و RGB)، سویا 95% (سیاه و سفید) و آفتابگردان 100% (RGB و HSI) می‌باشد. همچنین با توجه به دوره القا آزمون عدد کربونیل بهترین عملکرد طبقه‌بند در پالم 100% (L*a*b*)، زیتون 100% (L*a*b*)، سویا 89/47% و آفتابگردان 95% (HSI) بدست آمد.

واژه‌های کلیدی: روغن گیاهی خوراکی، اکسیداسیون، عدد پراکسید، عدد کربونیل، تحلیل تفکیک خطی، تصویرگیری.

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