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Extraction Efficiency of β -D-glucan from Waste Part of Bottom Mushroom (*Agaricus bisporus*) and its Ability to Adsorb Aflatoxin B₁

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Abstract

β -Glucans which are found in a variety of natural sources such as yeast, mushrooms, bacteria, algae, barley and oat show different biological effects. They are composed of D-glucose units linked by β -glycosidic bonds to each other. Adsorption of fungal toxins such as aflatoxin by β -glucan has been widely considered in recent years. Aflatoxins are a group of naturally-occurring carcinogens that are known to contaminate different human and animal foodstuffs. Aflatoxin B₁ is the most genotoxic hepatocarcinogenic compound among all types of the aflatoxins. The efficiency of adsorption of fungal toxins is directly related to the molecular structure, extraction method and source of β -glucan. Fungal derived β -glucan consists of β (1-3) bonds in main and β (1-6) at lateral branching point, with the specification of short shoulder length, has high ability to adsorb fungal toxins. In this study, for the first time, the efficiency of various extraction methods of β -glucan from stem cell wall of bottom mushroom (*Agaricus bisporus*) was measured and the ability to adsorb aflatoxin B₁ was evaluated. The results showed that although the yield of β -glucan from acid based extraction was higher than other methods (20.5%), the hot alkaline extracted β -glucan could adsorb and discard 90.2% of aflatoxin B₁ from contaminated samples based on HPLC analysis.

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Keywords

Aflatoxin B₁

Agaricus bisporus

β -D-glucan

Extraction method

Waste stem

Introduction

Today, fungal toxins have become a global problem based on the records that mention around 25% of the world cereal are contaminated with mycotoxins (Dhand, Joshi, & Jand, 1998). Among mycotoxins, aflatoxins are the most important because the diseases caused by ingesting aflatoxin

contaminated materials put forth a high risk for humans, livestock and poultry (Resnik *et al.*, 1996; Whitaker, Horwitz, Albert, & Nesheim, 1996). Aflatoxins belong to a group of compounds called furanocoumarin, which are secondary metabolites produced by certain fungi such as *Aspergillus flavus*, *Aspergillus*

parasiticus and *A. numius*. These toxins are found in various types of human foods and animal feeds including milk, cheese, peanut butter, corn, cottonseed, almonds, seasonings, figs, sorghum, and dry bread. Furthermore, eggs and meat products are sometimes contaminated with aflatoxins (Lizárraga-Paulín, Moreno-Martínez, & Miranda-Castro, 2011). Among various types of aflatoxins that have been identified so far, 4 different types of them namely B₁, B₂, G₁ and G₂ are more common, in which, B₁ has the highest toxicity in humans and animals (McLean & Dutton, 1995). Aflatoxin B₁ (AFB₁) is classified in class I as carcinogenic compounds in mammals; means exposure to this toxin leads to the development of tumors, especially in the liver (International Agency for Research on Cancer, 1992). The best and most effective way to prevent the formation of mycotoxins is to prevent the growth of fungi during planting, harvesting, storage, and transfer of plants (Doyle, Applebaum, Brackett, & Marth, 1982; Huwig, Freimund, Käppeli, & Dutler, 2001; Ramos & Hernandez, 1997). Despite the control of fungal growth in all of these stages, the incidence of infection with various fungi is unavoidable. As mycotoxin contaminations in foods are rising, it has led to an increased demand for methods to remove and eliminate toxicity (Kumar, Mahato, Kamle, Mohanta, & Kang, 2017). Among various physical and chemical methods for reduction of mycotoxins, the utilization of adsorbent compounds, which can adsorb toxins and excrete them from the gastrointestinal tract, is gaining much attention (Dixon, Kannewischer, Arvide, & Velazquez, 2008). Adsorbent compounds are mainly divided into the two groups, inorganic and organic compounds. Inorganic adsorbents include clay compounds such as aluminum silicate, sodium bentonite, potassium bentonite and zeolite which are generally called the hydrated sodium calcium alumina silicate (HSCAS) group (Dixon *et al.*, 2008). Organic adsorbents mainly include cell wall derived β -D-glucan from a wide

range of microorganisms (yeasts, molds, bacteria, fungi) as well as plants (Rahar, Swami, Nagpal, Nagpal, & Singh, 2011; Zhu, Du, Bian, & Xu, 2015). The main chain of β -D-glucan consists of β -D-glucopyranosyl units which are composed of glucose units bound by β (1-3) glycosidic bonds in the main chain and β (1-6) bonds at lateral chain points. This compound is widely distributed in the cell walls of plants (most cereals, oats and barley), yeasts (*Saccharomyces cerevisiae*, *S. fragilis*, *Candida tropicalis* and *C. utilis*), some bacteria (especially *Alcaligenes faecalis*, *Cellulomonas flavigen* and *bacillus*), algae and, in particular, fungi (Rahar *et al.*, 2011; Zhu *et al.*, 2015). The presence of β -glucan has been proven in the cell wall of some vegetables (carrots, soybeans and radishes) and fruits (banana) (Peumans *et al.*, 2000), it is also reported that β -D-glucan from different sources somehow show different biological effects (Ishibashi *et al.*, 2004) which are directly related to β -D-glucan chemical structures (types of bonds in the main backbone and branches as well as the number and length of lateral branches), solubility in water, extraction method and source of β -D-glucan. For instance Bueno, Casale, Pizzolitto, Salvano, & Oliver (2007) reported that AFB₁ was adsorbed by *S. cerevisiae* (Bueno *et al.*, 2007), but results of other studies demonstrated that the reduction efficiency of AFB₁ by various species of *S. cerevisiae* can be varied between 10 to 60% (Shetty & Jespersen, 2006). Some researchers believe that the adsorption ability of fungal toxins, such as AFB₁, is related to the β -glucan chemical structure (El-Naggar & Thabit, 2014). Accordingly, the best bioactive β -glucan is the one with specification of β (1-6) bonds in the lateral short chains, and β (1-3) bonds in the main chain (Synytsya *et al.*, 2009). Carbonero *et al.* (2012) believes that structurally, there is a similarity between β -glucan extracted from yeast and fungi, especially edible fungi whereas the chemical bounds of β -glucan extracted from these two sources in the main and lateral

branches are as β (1-3) and β (1-6) respectively. Edible fungi contain numerous compounds, including phenolic compounds, sterols, terpenoids etc. Among them, polysaccharides, and especially β -glucans, are known as a major group with medicinal properties (Ishibashi *et al.*, 2005; Kumar, Joo, Choi, Koo, & Chang, 2004). One of the most common types of edible macro-fungi is *Agaricus bisporus*, which is a white fungus commonly known as button mushroom. This species comprises one of the most economically important fungi in terms of world production, global market and consumption. This fungus is a valuable, healthy food containing polyphenols, vitamins, minerals and polysaccharides (Dubost, Ou, & Beelman, 2007; Tian *et al.*, 2012). Considering the worldwide production of this mushroom, their waste materials including, stem part, can be a valuable and inexpensive source for extraction of compounds with medicinal applications, including β -glucan. Achieving higher physicochemical properties of β -glucan is due to the chemical structure which is somehow related to the extraction methods. Therefore, an optimal extraction process is required to achieve higher efficiency and purity of β -glucan accompanying suitable functional properties (Vilkhu, Mawson, Simons, & Bates, 2008). Given the mentioned above points, this study was conducted to determine the best extraction method of β -glucan from fungal waste part which is stem cell walls of bottom mushroom (*A. bisporus*). It was also planned to find out the efficiency of each of the methods in the adsorption of aflatoxin based on high performance liquid chromatography (HPLC) analysis.

Materials and methods

The waste part of button mushroom whose stem was collected from SAYE RAS Farm (Mashhad, Iran). First they were washed and dried using a freeze dryer (Model Operon CO. FDO-8606, South Korea), then powdered with an electric mill (Model Pars Khazar, Iran). AFB₁ content was

determined by Waters 1525 HPLC (USA). All reagents and chemicals such as methanol, phosphate buffer and acetonitrile were provided from Merck (USA). Standard powder of AFB₁ and dialysis bag was obtained from Sigma (USA). Ethanol (96%) from Pars Alcohol (Iran) and, trichloroacetic acid (Carlo Erbai, Italy) was purchased.

Extraction methods of β -glucan

Hot water

In order to extract β -glucan with hot water, 2 g of dried powder were weighed and then stirred in 100 mL of distilled water for 3 h at 100 °C. After cooling, the solution was centrifuged at 5000 rpm for 20 min. Ethanol was added to the supernatant in a ratio of 4:1 (v/v), and then left to settle down for an overnight at 4 °C in order to precipitate polysaccharides including β -glucan. The precipitate was collected by centrifugation at 7000 rpm for 20 min. It was then dissolved in distilled water, dialyzed for 48 h at 4 °C in a dialysis bag (12 kDa meshes) in phosphate buffer in order to remove other small molecules such as monosaccharide. The purified precipitate was then lyophilized, weighed, and subsequently stored in moisture resistant containers (Jantaramanant, Sermwittayawong, Noipha, Hutadilok-Towatana, & Wititsuwannakul, 2014).

Acid-based

Acid-based extraction was accomplished according to the method of Szwengiel & Stachowiak (2016) with slightly modification. First, 100 mL of pure methanol was added to 2 g of mushroom powder; the mixture was stirred at 500 rpm and 60 °C for 18 h. After cooling, the solution was centrifuged at 5000 rpm for 15 min. The precipitate was dried in an oven at 46 °C to remove alcohol. The dried precipitates were then poured into a container containing 50 mL of hydrochloric acid (3.8%); the mixture was stirred at 1000 rpm and 30 °C for 5 h. The pH of sample was adjusted to 7 using

NaOH (5N); again centrifugation at 5000 rpm was done for 15 min. Next, the supernatant was mixed with ethanol in a ratio of 1:4 (v/v), and the final solution was allowed to settle down for an overnight at 4 °C. The precipitate was collected after centrifugation at 7000 rpm for 20 min. It was then dissolved in distilled water, lyophilized, weighed, and finally stored in moisture resistant containers (Szwengiel & Stachowiak, 2016).

Hot alkaline

In this method, 2 g of mushroom powder was added to the 100 mL of pure methanol; the mixture was stirred at 500 rpm and 60 °C for 2 h. The obtained solution was filtered through a whatman No. 1 filter paper. The separated material then left to dry at room temperature for 24 h. After drying, the sediment was added into 100 mL of distilled water; the mixture was then stirred at 400 rpm and 25 °C for 24 h. After separation of solid by using filter paper, it was added again into a container containing 100 mL of distilled water; this mixture was stirred at 150 rpm and 100 °C for 24 h. The filter paper separated sediments, were dissolved in 1 M NaOH by stirring at 200 rpm and 100 °C for 24 h. Centrifugation was then carried out at 7000 rpm for 15 min in order to precipitation of sediments. Next, 100 mL of the supernatant solution was dissolved in 10 g of trichloroacetic acid (10% w/v) by stirring at 100 rpm and 4 °C for 4 h. The solution was then centrifuged at 5000 rpm for 15 min, and the supernatant was mixed with the same volume of 96% ethanol. The final solution was allowed to stand overnight at 4 °C. Finally, centrifugation was carried out at 7000 rpm for 15 min, and the pellet were isolated and dried using freeze-drier afterward (Palacios, García-Lafuente, Guillamón, & Villares, 2012).

Measurement of extraction efficiency

The efficiency of extraction for each method was calculated according to the

following equation:

$$\text{Extraction efficiency} = \frac{\text{dried weight of extracted } \beta\text{-glucan (g)}}{\text{consumed powder of mushroom (g)}} \times 100 \quad (1)$$

Sample treatment

Standard solution of AFB₁ was prepared at the concentration of 1 ng/mL. It was prepared by dilution of the stock solution of AFB₁ with 80% methanol. In order to prepare aflatoxin solution with 1 ng/mL concentration, 250 µL of standard AFB₁ with 100 ng/mL concentration, was diluted to 25 mL with 80% methanol (HPLC grade). In this study to prepare contaminated samples, the protocol from Di Natale, Gallo, & Nigro (2009) was followed. To determine adsorption efficiency of extracted β-glucan, 3 samples were prepared. Each sample contained 0.05 g β-glucan from each extraction method contaminated with aflatoxin B₁ in a final volume of 5 mL solution. The concentration of AFB₁ was 1 ng/mL in each sample. The samples were then incubated at room temperature for 30 min on a shaker at 80 rpm and then centrifuged for 5 min at 3500 rpm (Di Natale *et al.*, 2009).

HPLC analysis

The supernatant was injected on HPLC to determine the remaining AFB₁ in the sample and then compared with standard. An isocratic elution containing water: methanol: acetonitrile (60:20:20% v/v) was used as the mobile phase. Fluorescence detector was also used at the wavelength of 365 and 445 nm for excitation and emission wavelength respectively. The chromatographic column was C₁₈ (250×4.6 mm, 5µm). During the analysis, the flow rate was kept at 1 mL/min, and the column temperature was maintained at 40 °C. For the derivatization of aflatoxin, a photochemical derivatization was used. The injection volume was 20 µL, and all procedures were performed in 3 replications (Iranian National

Standardization Organization [ISIRI], No. 6872, 2012).

Statistical analysis

In this study all the experiments were carried out in 3 replicates. The data were statistically analyzed based on randomized complete design (RCD) and then to determine the superior treatment, the Duncan mean comparison was used.

Results and discussion

To date, there is no scientific report that shows the optimum type of extraction of β -glucan from waste part of button mushroom and also its ability to adsorb AFB₁ in liquid conditions. The results of this study can clarify, which β -glucan extraction method has the higher efficiency among evaluated methods and also determine which method has higher ability to adsorb and discard AFB₁ from contaminated samples.

Calculation of extracted β -glucan

The efficiency of β -glucan production in each extraction method is presented in Table (1).

Table 1. Average β -glucan yield efficiency based on extraction method

Extraction method	Efficiency (%)
Hot water	3.0 ^b
Acid-based	20.5 ^a
Hot alkaline	7.0 ^b

Numbers with the same letters indicate insignificance ($P < 0.05$).

According to the obtained results shown in Table (1), the highest percentage of β -glucan yield was related to the acid-based extraction method (20.5%), which is significantly higher than hot water and hot alkaline extraction methods which are 3 and 7% respectively. Although there is no

report to compare all 3 extraction methods in a specific source, in some studies at least two methods have been compared together. Synytsya *et al.* (2009) had a comparison between acid-based and hot water for extraction of β -glucan from *A. bisporus*. They found that the yield of extraction in acid-based was 7 times higher than hot water (Synytsya *et al.*, 2009). This result confirms the findings in the present study. Despite the fact that hot water for extraction of β -glucan is stated as the most common, easier and cost-effective method (Ahmad, Anjum, Zahoor, Nawaz, & Din, 2009; Jantaramanant *et al.*, 2014), based on the results of this study, it is concluded that hot water has the lowest efficiency in β -glucan production (3%).

Calculation of the amount of aflatoxin B₁ adsorption

The amount of aflatoxin adsorption by extracted β -glucan from each method is presented in Table (2).

HPLC chromatograms of Aflatoxin B₁ for standard and contaminated sample treated with hot alkaline extracted β -glucan, was shown in Figs. (1) and (2) respectively. The obtained results in this study demonstrated that although β -glucan extracted from all methods could adsorb AFB₁, the adsorption efficiency are significantly ($P < 0.05$) different among these extracted methods, so that the lowest one, related to acid-based (5%), then after hot water (14%) and the highest attributed to the hot alkaline with 90.2% adsorption and elimination of aflatoxin B₁ in a liquid condition (Table 2). Accordingly, the optimal extraction method in terms of yield and aflatoxin adsorption can be attributed to the acid-based and hot alkaline method with the efficiency of 25 and 90.2% respectively.

Table 2. Percentage of adsorbed aflatoxin in different extraction methods

Extraction Method	Amount of AFB ₁ added to the sample (ng/mL)	Remaining amount of AFB ₁ after treatment (ng/mL)	Percentage of adsorbed AFB ₁
Hot water	1	0.860 ^b ±0.013	14
Acid-based	1	0.950 ^a ±0.010	5
Hot alkaline	1	0.098 ^c ±0.000	90.2

Numbers with the same letters indicate insignificance ($P < 0.05$).

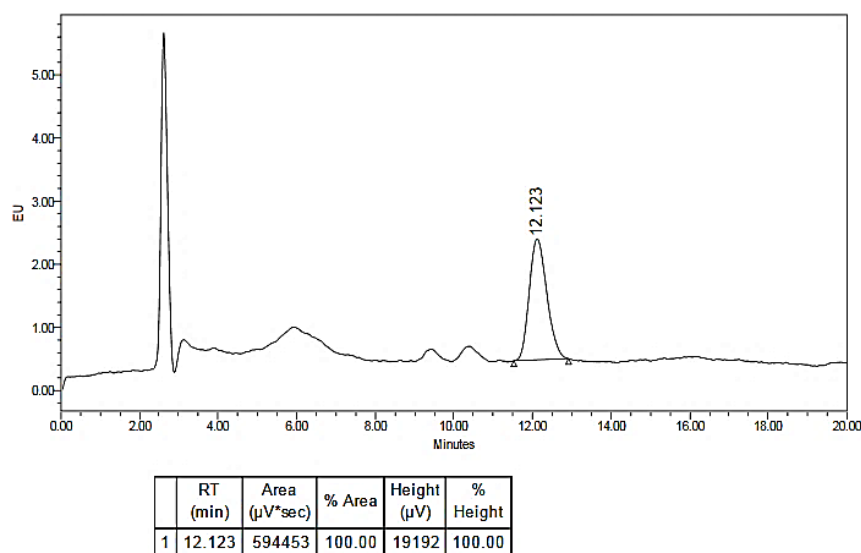


Fig. 1. HPLC chromatogram of standard solution of AFB₁ with at a concentration of 1 ng/mL

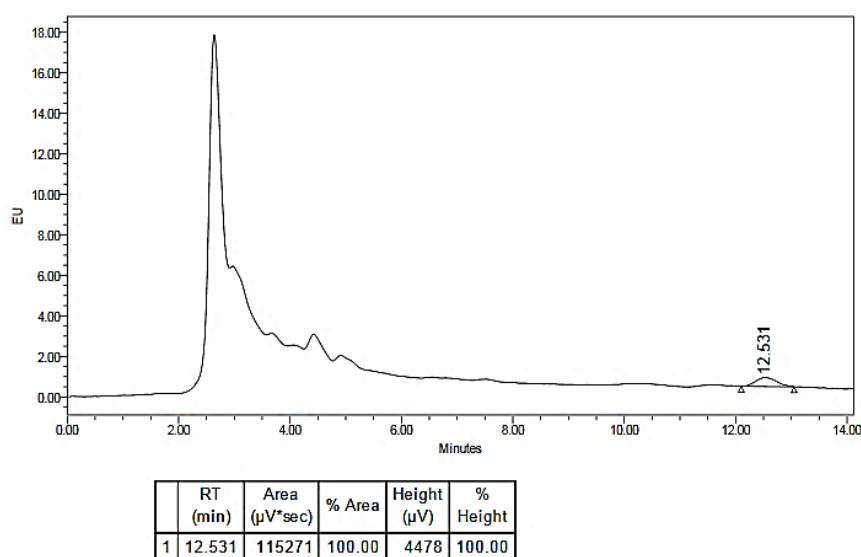


Fig. 2. HPLC chromatogram of aflatoxin B₁ in contaminated sample after treatment with hot alkaline extracted β -glucan

The results of this study also revealed that the highest ability to adsorb AFB₁ was achieved by β -glucan extracted through hot alkaline method (90.2%), which followed the protocol described by Palacios *et al.* (2012). As above mentioned the source and method of β -glucan extraction can affect chemical structure and, consequently, biological activity of β -glucan such as adsorption of aflatoxin B₁. For example, Palacios *et al.* (2012) used hot alkaline and hot water extraction methods to extract β -glucan from edible fungi. In that study, the GC-MS and H-NMR results showed that the sample extracted with hot water,

contained linear glucose units linked β (1-4) bonds with no branches, while the sample extracted through hot alkaline, contained glucose units linked by β (1-3) bonds in the main chain and β (1-6) bonds at lateral branching point. In case of acid-based extraction method, Szwengiel & Stachowiak (2016) demonstrated that the obtained β -glucan from edible fungi has higher yield efficiency as compared to the hot water, but contains protein impurities which can have effects on β -glucan functionalities. According to the explanations given earlier, it is clear that the ability of AFB₁ adsorption is related to

β -glucan that contains glucose units with the β (1-3) bonds in the main chain and β (1-6) bonds at the branching point. In this study the priority of hot alkaline derived β -glucan to adsorb AFB₁, can be attributed to the specific chemical structure of β -glucan, in which the length of lateral branches is shorter and the number is higher as compared to the other extracted β -glucans (Elaine R Carbonero *et al.*, 2012).

Conclusions

In this research, for the first time, β -glucan was extracted from waste materials produced from button mushroom (*Agaricus bisporus*). *A. bisporus* is a fungal source with the worldwide production and consequently huge amount of stem parts as waste materials. The results of this study demonstrated that although the highest extraction efficiency was related to acid extraction (25%), the highest rate of toxin adsorption belongs to

the sample extracted through the hot alkaline method (90.2%). The present study reconfirmed that method of extraction has a major effect on the molecular structure and biological properties of β -glucan. On another note, β -glucan from edible fungi should be evaluated to be used as an AFB₁ adsorption agent, instead of yeast β -glucan. Fungal β -glucan is suitable for use in the food industry as it has additional benefits including antiviral, anti-bacterial and anti-allergic properties, besides having the ability to stimulate and modulate the immune system. As above mentioned, the highest percentage of toxin adsorption by *S. cerevisiae* yeast had previously been reported to be 60%, while a rate of 90.2% was obtained in the present study. On the other hand, the use of live yeast in food products as an adsorption agent may lead to undesirable reactions.

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بازدهی استخراج بتا-دی-گلوکان از ضایعات قارچ دکمه‌ای (*آگاریکوس بیسپوروس*) و توانایی آن در جذب آفلاتوکسین B₁

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چکیده

بتاگلوکان‌ها در گستره وسیعی از موجودات مختلف از جمله مخمر، قارچ، باکتری، جلبک، جو و جو دوسر یافت می‌شود و اثرات بیولوژیکی متنوعی را از خود نشان می‌دهند. بتاگلوکان‌ها از پیوند بتاگلوکوزیدی واحدهای D-گلوکز به یکدیگر تشکیل شده‌اند. توانایی جذب سموم قارچی از جمله آفلاتوکسین‌ها توسط بتاگلوکان در سال‌های اخیر بسیار موردتوجه قرار گرفته است. آفلاتوکسین‌ها گروهی از ترکیبات سمی با قدرت سرطان‌زایی بالا هستند که به‌عنوان یک عامل آلوده‌کننده مواد غذایی برای انسان و حیوان، شناخته می‌شوند. آفلاتوکسین B₁ سمی‌ترین ترکیب در ایجاد سرطان در بین انواع آفلاتوکسین‌هاست. توانایی جذب سموم قارچی توسط بتاگلوکان به‌طور مستقیم به عواملی مانند ساختمان مولکولی، روش استخراج و منبع حاوی بتاگلوکان بستگی دارد. بتاگلوکان مستخرج از منبع قارچی دارای اتصالات بتا (۱-۳) در رشته اصلی و بتا (۱-۶) در رشته جانبی و در محل انشعاب‌هاست. وجود انشعاب‌های زیاد با طول کوتاه با ساختاری شانه‌مانند، سبب افزایش جذب سموم قارچی توسط این ترکیب می‌شود. در تحقیق حاضر، برای اولین بار میزان بازدهی چندین روش استخراج بتاگلوکان از دیواره سلولی ساقه‌های دورریز قارچ دکمه‌ای (*آگاریکوس بیسپوروس*) اندازه‌گیری و توانایی جذب آفلاتوکسین B₁ نیز سنجیده شد. نتایج نشان داد علی‌رغم اینکه میزان بازدهی استخراج بتاگلوکان به روش اسیدی بیشتر از سایر روش‌هاست (۲۰/۵ درصد)، اما براساس سنجش به روش HPLC، بتاگلوکان استخراج‌شده با استفاده از قلیای داغ، توانایی جذب و خارج کردن ۹۰/۲ درصد آفلاتوکسین B₁ را از نمونه آلوده‌شده را دارد.

واژه‌های کلیدی: آفلاتوکسین B₁، *آگاریکوس بیسپوروس*، بتا-دی-گلوکان، روش استخراج، ساقه‌های دورریز

Determination of Optimum Osmotic Dehydration As a Pretreatment in Hot Air Drying of Turnip Slices By Response Surface Methodology (RSM)

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Abstract

The objective of the present study was to investigate the effect of concentration of osmotic solution (30, 45 and 60%, w/w), temperature osmotic solution (30, 40 and 50°C), immersion time (4, 5 and 6 h) on water loss (WL), solids gain (SG), weight reduction (WR), vitamin C content, shrinkage, rehydration ratio (RR), and color indexes (L, a, b) during osmotic dehydration-drying of turnip slices. Response surface methodology (RSM) was also used to find out the optimum condition. The results showed that during osmotic dehydration of turnip samples, the variables of temperature of osmotic solution, solution concentration and time of immersion had significant effects on mass transfer parameters (WL, SG, and WR), vitamin C content, shrinkage, RR, and color index (L). Optimal conditions of osmotic dehydration for turnip were found to be: solution temperature of 45 °C, osmotic solution concentration of 58.29, and immersion time of 20 min. Under these conditions, the amounts of WL, SG, WR, shrinkage, rehydration ratio (RR), vitamin C content, and color indexes (L, a, b) were 83.10, 12.91 and 70.19%, 27.76, 4.19 and 11.64 (mg/100 g solids), 33.85, 25.49 and 15.91, respectively. The results of this study can be used in the minimal processing of turnips slices using osmotic dehydration and subsequent drying of samples.

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Keywords

Optimum condition
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Introduction

Turnip (*Brassica Napus L.*) has active biological compounds such as flavonoids, indole alkaloids, and sterol glycosides (Alizadeh, Ghiamirad, & Ebrahimiasl, 2014). Also, it is a valuable source of calcium and magnesium that prevents dangerous diseases such as cancer (Gharehbeglou *et al.*, 2014). According to

the limited shelf life of this product in the agricultural products depots as well as wastes after cultivation, to increase shelf life, this product can be exposed to osmotic dehydration or drying processes. Over the last decade, food production with moderate humidity using osmotic dehydration has been taken into consideration due to minimal processing

(low temperature) (Ahmed, Qazi, & Jamal, 2016). Osmotic dehydration is a process to decrease food humidity through immersion in osmotic solutions (usually with 30 to 70% concentrations) (Yadav & Singh, 2014). This process is used as a pretreatment for many food preservation processes, especially hot air drying (Shahidi, Mohebbi, Noshad, Ehtiat, & Fathi, 2012). Osmotic dehydration has disadvantages over other methods such as use of lower temperatures, better color, taste, and aroma preservation, and nutritional value (Yadav & Singh, 2014).

Different studies on osmotic dehydration have shown that parameters such as temperature and osmotic solution concentration, dehydration time, shape and size of the food product, and the ratio of the osmotic solution to the sample have considerable effects on mass transfer phenomenon during the osmotic dehydration (Chandra & Kumari, 2015). It has been reported that the use of high osmotic concentrations as pretreatment to dry with hot air leads to increased water loss (WL) and solids gain (SG), (Shahidi *et al.*, 2012). Moreover, in another study, it is shown that increased osmotic solution temperature leads to increased WL and SG over the osmotic dehydration process of pineapple slices (Ramallo & Mascheroni, 2005). However, studies show that in a certain osmotic concentration, WL and SG may decrease due to various factors such as structural changes or stiffness of surface layers and barriers against water loss by solid layers (Giraldo, Talens, Fito, & Chiralt, 2003; Teles *et al.*, 2006; Yadav & Singh, 2014).

The response surface methodology (RSM) is a set of statistical methods that have ability to analyze multi-parameter equations and expand mathematical models that predict simultaneous assessment of the effect of independent variables on dependent variables and optimization of different processes (Rafigh, Yazdi, Vossoughi, Safekordi, & Ardjmand, 2014). In recent years, RSM has been widely used to promote and optimize different processes in food

industry such as osmotic dehydration (Derossi, Severini, Del Mastro, & De Pilli, 2015). Indeed, by using RSM, it is possible to determine optimal conditions of parameters that affect osmotic dehydration and as a result, increase the product quality and dehydration process outcome. According to previous studies, no study has been conducted on combined treatment of osmotic dehydration-turnip drying to reduce moisture content and increase its shelf life as optimize process conditions. Therefore, the objective of the present study is to investigate the simultaneous effect of osmotic process conditions including concentration and osmotic solution temperature and immersion time in osmotic solution on mass transfer parameters such as WL, SG, and weight reduction (WR) on turnip slices as well as physical and nutritional properties (vitamin C, the ratio of reabsorption and shrinkage) and color indicators (b, a, L). Finally, optimal osmotic dehydration process conditions were determined using RSM.

Materials and methods

Turnip and sugar with nutritional grade were bought from the market. The main equipment included oven, (Memert, China), bain-marie (Memert, China), digital scale with an accuracy of 0.0001 g (AND HR200, Japan), and desiccator and caliper (Mhar Company, Germany, with an accuracy of 0.01 mm).

Methods

Sample preparation

To remove surface soil, fresh turnip samples were washed. After separating the wasters with catheter, slices (5 mm) were prepared and immediately treated with osmotic dehydration process.

Drying and osmotic dehydration

The osmotic dehydration process was carried out in a beaker containing sucrose osmotic solution. To prepare sucrose osmotic solution with brixes 30, 45, and 60, a specific amount of sugar or sucrose was poured into the beaker. Then, using distilled water, the beaker containing sucrose reached the desirable level and by

stirring, sucrose was dissolved in water and the osmotic solution was prepared. For osmotic dehydration, samples were transferred to the beaker containing osmotic solution and this process was carried out for 4, 5, and 6 h at different temperatures (30, 40, and 50 °C). Immersion concentrations in osmotic solutions and temperatures were determined according to the primary tests. To control osmotic solution temperature, bain-marie was used. In the end of the osmotic dehydration process, samples were removed from the solution and washed with distilled water for 30 seconds and the surface moisture was filtered. In the next step, the samples were distributed and parameters including WL, SG, and WR were calculated using Eq. (1), (2) and (3), (Kek, Chin, & Yusof, 2013).

(1)

$$WL = \frac{W_i \times X_i - W_f \times X_f}{W_i} \times 100$$

(2)

$$SG = \frac{W_f(1 - X_f) - W_i(1 - X_i)}{W_i} \times 100$$

(3)

$$WR = WL - SG$$

W_i : initial sample mass (g); W_f : sample mass after osmotic dehydration (g); X_i : initial moisture (percent, based on moisture); X_f : sample moisture after osmotic dehydration (percent, based on moisture); WL: WL (percent, g for 100 g of the initial sample); SG: solid gravity absorption (percent, g for 100 g of the initial sample); WR: weight reduction (percent, g for 100 g of the initial sample).

By the end of the osmotic dehydration pretreatment, samples were dried under the temperature of 70 °C to reach the dry weight.

Measuring the moisture content and the solid gravity

Measurement of moisture and solid content of fresh samples and osmotic standards was according to AOAC (2000) standard, No. 931.04.

Measurement of vitamin C

Vitamin C of turnip slices was measured to

investigate the effect of osmotic pretreatments on vitamin C content in the final product according to the Iranian national standard of 5609 after drying with hot air (Iranian National Standardization Organization [ISIRI], 2000).

Rehydration

To measure rehydration of samples, a specific weight of the dried samples was exposed to a temperature of 95 °C for 20 min based on osmosis-hot air method. Then, rehydration capacity was calculated using Eq. (4):

(4)

$$RR = \frac{W_r}{W_d}$$

W_r : sample weight (g); W_d : the dried weight of the sample used in rehydration test (g).

Shrinkage

Shrinkage percentage was measured according to fluid transfer (toluene) method (Alam, Amarjit, & Sawhney, 2010; Noshad, Mohebbi, Shahidi, & Ali Mortazavi, 2012). To calculate the shrinkage, Eq. (5) was used:

(5)

$$S = \frac{V_0 - V}{V_0} \times 100$$

V_0 : fresh sample volume (mL); V : final sample volume (dried) (mL).

Color indicators

To measure color, image processing technique was used. A small amount of each sample was placed on a uniform surface in a color recognition device. To take image with a digital camera, the distance between the camera and the sample was 20 cm. Then, in the Photoshop software, L, a, and b rates were obtained (Sutar & Gupta, 2007).

The experimental design and statistical analysis

To investigate the effect of osmosis dehydration on WL, WR, SG, vitamin C content, shrinkage, rehydration ratio (RR), and L, a, and b indicators, RSM was used.

The Box-Behnken design including osmotic solution temperature (30, 40, and 50 °C), osmotic solution concentration (30, 45, and 60%, w/w), and immersion time (4, 5, and 6 h) with 17 treatments and 5 replications at the central points was used. The coded and real rates of independent variables are presented in Table (1). Data were analyzed using Design Expert 6.0.2. The empirical data were fitted by the second-order polynomial model.

$$Y = b_0 + b_1 A + b_2 B + b_3 C + b_{11} A^2 + b_{22} B^2 + b_{33} C^2 + b_{12} AB + b_{13} AC + b_{23} BC \quad (6)$$

B_n : regression coefficients for constant coefficient factors (b_0), linear effect coefficient (b_1 , b_2 , and b_3), second order effect coefficient (b_{11} , b_{22} , and b_{33}), and the interaction effect (b_{23} , b_{13} , and b_{12}).

Y: dependent variables or desirable responses including WL, SG, and WR.

Table 1. Real and coded levels of the independent variables of osmotic dehydration process of turnip slices

Independent variables	Coded and real values		
	-1	0	+1
Temperature (A)	30	40	50
Osmotic solution concentration (B, %)	30	45	60
Process time (C, hour)	4	5	6

Results and discussion

Mass transfer parameters rates (WL, SG, and WR) in osmotic dehydration of turnip slices are shown in Table (2). According to Table (3), the results of the analysis of variance showed that the second order model was significant for the responses (except b, $P > 0.05$) that indicates the appropriateness of the model used to predict the effect of the independent variables on the responses. In response surface optimization, the suitable model is

selected according to the significance of F test ($P < 0.05$), insignificance of lack of goodness of fit ($P > 0.05$), high explanatory coefficient (R^2), and lower variation coefficient (CV). In the current study, high explanatory coefficient for responses ($R^2 > 0.94$) and variation coefficient (CV) lower than 4% for the fitted model, indicate acceptable consistency between the used regression model and their high accuracy in predicting the dependent variables rates (Table 3).

Table 2. Experimental conditions and response rates in osmotic dehydration process of turnip slices

Treatment	Independent variables			The dependent variables								
	A	B	C	WL	SG	WR	S	RR	Vit C	L	a	b
1	30	30	5	66.92	10.59	56.33	28.42	3.67	13.49	31.16	27.89	17.01
2	30	45	4	69.54	11.72	57.82	28.26	4.05	12.35	32.78	26.91	16.93
3	40	30	6	73.69	11.06	62.09	28.54	3.88	13.06	33.93	26.20	18.53
4	30	60	5	79.70	12.66	67.04	27.86	4.14	11.53	30.28	23.81	17.49
5	50	60	5	84.93	13.04	71.89	27.47	4.32	10.34	34.03	25.18	16.82
6	40	45	5	78.05	12.87	65.18	28.58	4.15	11.18	32.29	24.59	18.53
7	40	30	4	66.18	10.33	55.85	28.82	3.41	13.75	32.38	24.12	17.68
8	40	60	4	75.51	12.23	63.28	28.35	4.41	11.86	31.75	23.69	19.26
9	40	45	5	78.60	12.82	65.78	28.57	4.53	11.51	34.39	24.58	18.36
10	40	45	5	78.15	12.88	65.27	28.54	4.54	11.44	33.84	22.19	18.39
11	40	45	5	78.27	12.79	65.48	28.34	4.53	11.48	32.53	24.63	17.39
12	50	45	6	84.82	12.92	71.90	27.81	4.61	10.18	37.08	23.12	17.52
13	30	45	6	77.09	12.34	64.75	28.31	4.39	11.74	32.26	26.82	16.69
14	40	60	6	86.09	13.44	72.65	27.83	4.49	11.05	38.35	25.06	16.41
15	50	45	4	74.23	12.01	62.22	27.88	4.07	11.84	34.49	25.36	16.83
16	50	30	5	76.48	11.03	65.45	27.84	3.29	13.04	30.47	23.41	17.29
17	40	45	5	78.44	12.64	65.80	28.46	4.42	10.96	33.70	22.16	18.33

A: osmotic solution temperature; B: osmotic solution concentration (%); C: process time (hour); WL (%); SG (%); WR (%); S: shrinkage (%); RR: rehydration ratio (%); Vitamin C (mg/100 g of the dry matter).

Table 3. Analysis of variance of the second-order polynomial model

Sources Change	Degrees of freedom	Value F								
		WL	SG	WR	S	RR	Vit C	L	a	b
Model	9	516.84 ^{**}	13.79 ^{**}	385.66 ^{**}	2.10 ^{**}	2.44 ^{**}	16.49 ^{**}	62.17 [*]	29.81 ^{ns}	8.49 [*]
A	1	92.54 ^{**}	0.35 ^{**}	81.40 ^{**}	0.42 ^{**}	0.0002 ^{ns}	1.72 ^{**}	11.49 [*]	8.73 ^{ns}	0.01 ^{ns}
B	1	230.69 ^{**}	8.73 ^{**}	154.35 ^{**}	0.55 ^{**}	1.20 ^{**}	9.15 ^{**}	5.23 ^{ns}	1.88 ^{ns}	0.02 ^{ns}
C	1	164.07 ^{**}	1.50 ^{**}	129.76 ^{**}	0.08 ^{ns}	0.25 [*]	1.77 ^{**}	13.05 [*]	0.15 ^{ns}	0.34 ^{ns}
A ²	1	0.061 ^{ns}	0.25 ^{**}	0.15 ^{ns}	0.89 ^{**}	0.12 [*]	0.01 ^{ns}	3.46 ^{ns}	5.22 ^{ns}	4.12 ^{**}
B ²	1	5.79 ^{**}	2.22 ^{**}	1.11 [*]	0.08 ^{ns}	0.69 ^{**}	3.00 ^{**}	3.86 ^{ns}	0.45 ^{ns}	0.005 ^{ns}
C ²	1	13.05 ^{**}	0.40 ^{**}	9.72 ^{**}	0.003 ^{ns}	0.001 ^{ns}	0.31 ^{ns}	12.31 [*]	2.75 ^{ns}	0.16 ^{ns}
AB	1	4.68 ^{**}	0.0008 ^{ns}	4.55 ^{**}	0.009 ^{ns}	0.07 ^{ns}	0.13 ^{ns}	4.92 ^{ns}	8.55 ^{ns}	0.22 ^{ns}
AC	1	2.31 ^{**}	0.02 ^{ns}	1.89 [*]	0.003 ^{ns}	0.009 ^{ns}	0.27 ^{ns}	2.41 ^{ns}	1.15 ^{ns}	0.21 ^{ns}
BC	1	2.35 ^{**}	0.05 [*]	2.44 ^{**}	0.01 ^{ns}	0.03 ^{ns}	0.003 ^{ns}	6.37 ^{ns}	0.12 ^{ns}	3.24 ^{**}
left over	7	-	-	-	-	-	-	-	-	-
Lack of fit	3	0.73 ^{ns}	0.02 ^{ns}	0.94 ^{ns}	0.08 ^{ns}	0.039 ^{ns}	0.16 ^{ns}	6.27 ^{ns}	4.81 ^{ns}	0.99 ^{ns}
Net error	4	-	-	-	-	-	-	-	-	-
Total	16	-	-	-	-	-	-	-	-	-
Coefficient of explanation (R ²)	-	0.9982	0.9954	0.9967	0.9438	0.9422	0.9766	0.8672	0.7151	0.8267
Coefficient of variation (CV)	-	0.47	0.78	0.66	0.47	3.51	2.01	3.50	5.28	2.87

** Significance at a probability level of 1%, significant at a probability level of 5%.

* Significance at a probability level of 1%, significant at a probability level of 1%.

ns: no significance

Water Loss (WL)

According to Table (3), concentration (B), time (C), and temperature (A) have the largest positive effects on WL. All linear effects, second-order (except the effect of the osmotic solution temperature A²) and the effects of independent variables on WL were positive. Eq. (7) shows the second-order polynomial model to predict WL rate according to the coded rates after removing the insignificant factors.

(7)

$$WL = +78.30 + 3.40 A + 5.37 B + 4.53 C - 1.17 B^2 - 1.76 C^2 - 1.08 AB + 0.76 AC + 0.77 BC$$

The effect of independent variables of osmotic solution temperature-osmotic solution concentration, osmotic solution temperature-immersion time, and osmotic solution concentration-immersion time on WL response is shown as a response surface chart in Fig. (1). About WL, temperature (A) only had a positive linear effect ($P < 0.01$) and the existence of low upward curvature in interaction curves of the interaction effect of temperature-concentration, temperature-time, and concentration-time shows this fact.

Fig. (1) shows that with increased concentration, dehydration time, and osmotic solution temperature, WL increases but in higher concentrations and dehydration time, WL tone decreases compared with the initial times of the process. Generally, higher temperatures lead to swallowing and plasticity of cellular membrane and rapid release of moisture. Also, at higher temperatures, osmotic solution viscosity decreases. Therefore, due to reduced viscosity, moisture release is performed better (Sutar & Gupta, 2007). The positive effect of increased concentration can be related to the increased osmotic pressure due to increased intracellular concentration and osmotic solution difference at higher concentrations (Lazarides, Katsanidis, & Nickolaidis, 1995). The above results are consistent with the results of other studies (Ebrahim Rezagah, Kashaninezhad, Mirzaei, & Khomeiri, 2009; Falade, Igbeka, & Ayanwuyi, 2007; Singh, Panesar, Nanda, & Kennedy, 2010). High water outflow rate in the early osmosis stages and its reduction over time are reported by (Eren & Kaymak-Ertekin, 2007).

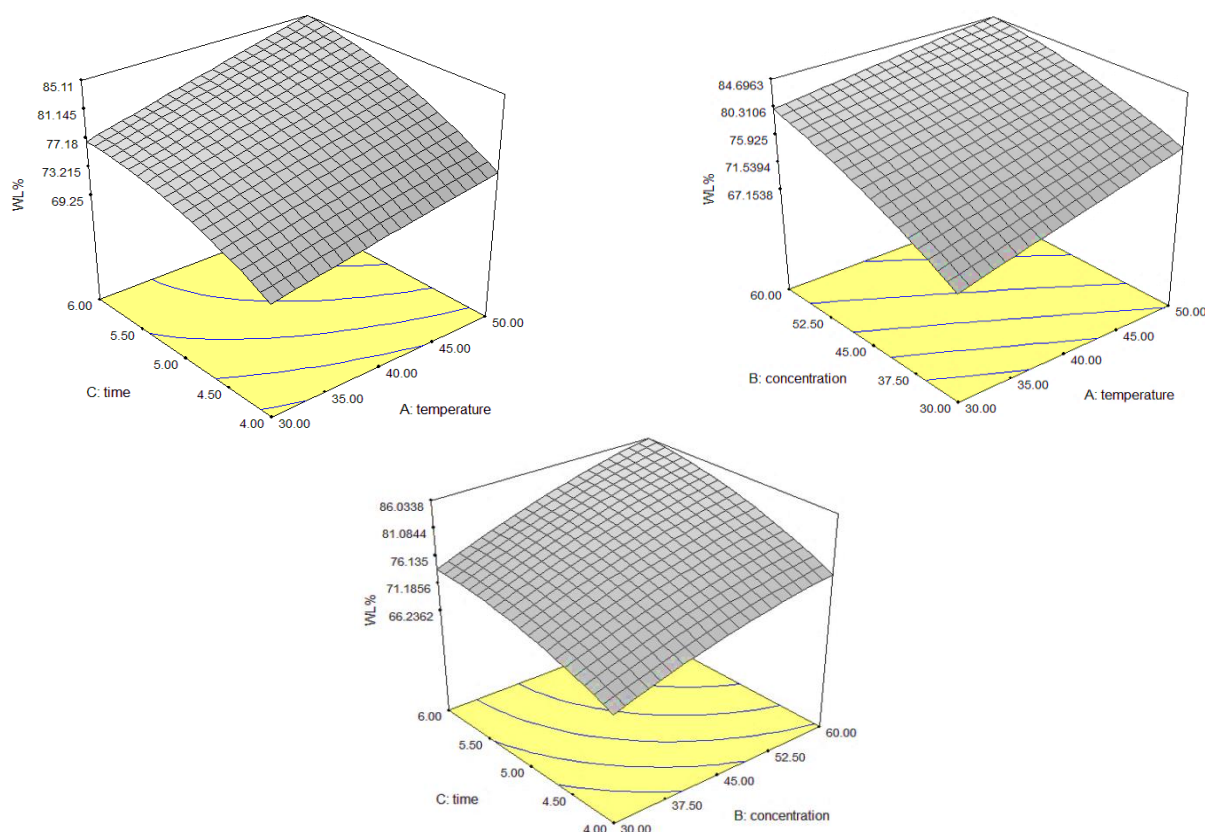


Fig. 1. The interaction effect of independent variables of osmotic solution temperature - osmotic solution concentration - osmotic solution temperature - immersion time - osmotic solution concentration - immersion time on WL (%) of turnip slices

As the osmotic dehydration process continues, due to the loss of moisture from the product tissue and entrance of sucrose, osmotic pressure difference and concentration difference between turnip tissue and osmotic solution reduce. Therefore, WL tone reduces gradually. On the other hand, rapid moisture removal and SG lead to structural changes and stiffness of surface layers. Therefore, resistance against mass transfer increases (Eren & Kaymak-Ertekin, 2007; Vieira, Pereira, & Hubinger, 2012). Giraldo *et al.* (2003) reported that excessive increase in common medlar osmotic solution concentration leads to lower WL.

Solid Gravity (SG)

SG rate during osmotic dehydration is dependent on all three parameters of osmotic solution temperature, osmotic solution concentration, and immersion time. Table (3) shows that all linear

effects and second-order effects of the independent variables have a significant effect on SG. However, according to the numerical value of the coefficient, its effect was smaller than other significant sentences. But the negative coefficients about the second-order sentences show that excessive increase in this parameter leads to reduced SG rate. These effects can be observed in Fig. (2).

Fig. (2) shows the effects of independent variables on SG response. As can be observed, with increased concentration, dehydration time and osmotic solution temperature, SG rate increases rapidly but gradually decreases. Also, the observed curvature for the independent variables in Fig. (2) is consistent with the second-order the effects of all three independent variables in Table (3).

The driving force concentration difference is mass transfer to absorb sucrose (Rastogi & Raghavarao, 2004).

Enhancement of mass transfer properties due to increased temperature and concentration which may affect increased SG of turnip. As stated about WL, increased temperature affects cell membrane permeability and can lead to SG penetration into fruit or vegetable tissues. Other researchers reported that increased temperature leads to simultaneous increase in WL and SG (İspir & Toğrul, 2009). In the current study, increased osmotic concentration leading to increased SG but then, SG rate decreased. Increased SG and increased concentration can be due to increased osmotic pressure gradient (Phisut, 2012).

Teles *et al.* (2006) reported that mass transfer reduction in high osmotic solution concentrations is due to barrier against WL by layers of SG on the melon

surface. Observation of the negative effect of increased temperature on SG and WL can be due to cell wall permeability destruction (Yadav & Singh, 2014). In another study, it was reported that if the dehydration process continues, mass transfer gradient decreases and equilibrium rates are obtained (Ebrahim Rezagah *et al.*, 2009). Therefore, according to the negative coefficient of the second-order sentence of time (C^2), reduced SG during long osmotic dehydration is not expected. About the effect of process time on SG (Fig. 2), in maximum concentration in use, with increased osmotic dehydration time around 5 to 6 h, SG rate reaches to the maximum level and with further increase in time up to 6 h, no significant change is resulted in SG rate.

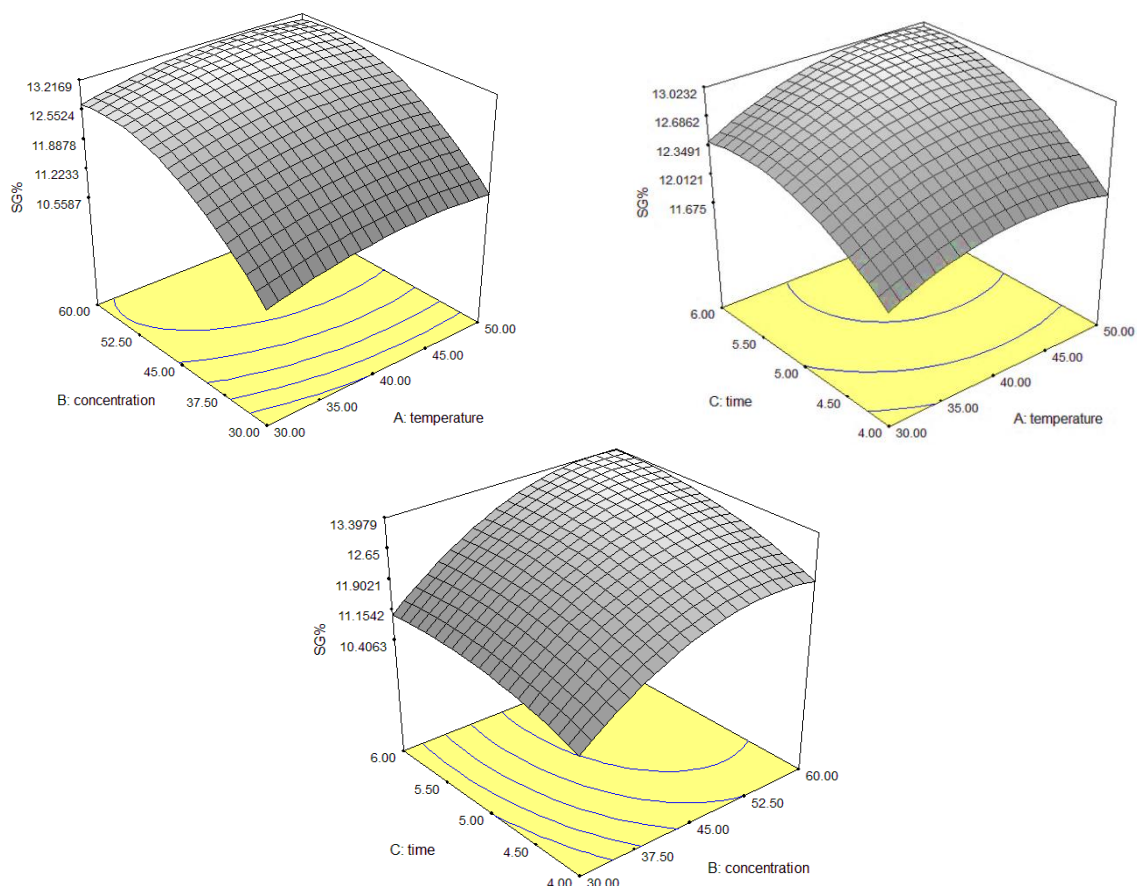


Fig. 2. The interaction effect of osmotic solution temperature - osmotic solution concentration - osmotic solution temperature - immersion time - osmotic solution concentration - immersion time on SG (%) of turnip slices

Weight Reduction (WR)

According to Table (3), all linear, second-order, and interaction effects of independent variables have a significant effect on WR. The first-order sentences of time (C), concentration (B), and temperature (A) have the largest positive effect on WR during osmotic dehydration process. The second-order sentences of time (C^2) and osmotic solution concentration (B^2) had the largest negative effect on WR rate. This finding shows that during long osmotic dehydration process or with excessive increased solution concentration, WR rate decreases. However, according to the numerical coefficients of the second-order sentences, increased concentration has a slight negative effect compared with the second-order effect of time (Fig. 3). Moreover, the interaction effect of temperature-concentration (AB) has a negative effect and the interaction effects of temperature-time (AC) and concentration-time (BC)

have a positive effect on WR. Eq. (9) shows the polynomial model used according to the coded rates to predict WR rates after removing sentences with insignificant effects:

$$\text{WR} = +65.50 + 3.19 A + 4.39 B + 4.03 C - 0.51 B^2 - 1.52 C^2 - 1.07 AB + 0.69 AC + 0.78 BC \quad (9)$$

In the osmotic dehydration process, WR is defined as WL and SG difference. Therefore, similar to the effects observed for linear sentences of concentration, temperature, and time in increasing WL, the trend observed in WR is justifiable. Vieira *et al.* (2012) reported that with increased osmotic solution concentration and temperature, WL and WR increase. Fig. (3) shows that with simultaneous increase in temperature-time and concentration-time, WR rate increases during turnip osmotic dehydration. However, in longer processes, increasing time in WR decreases.

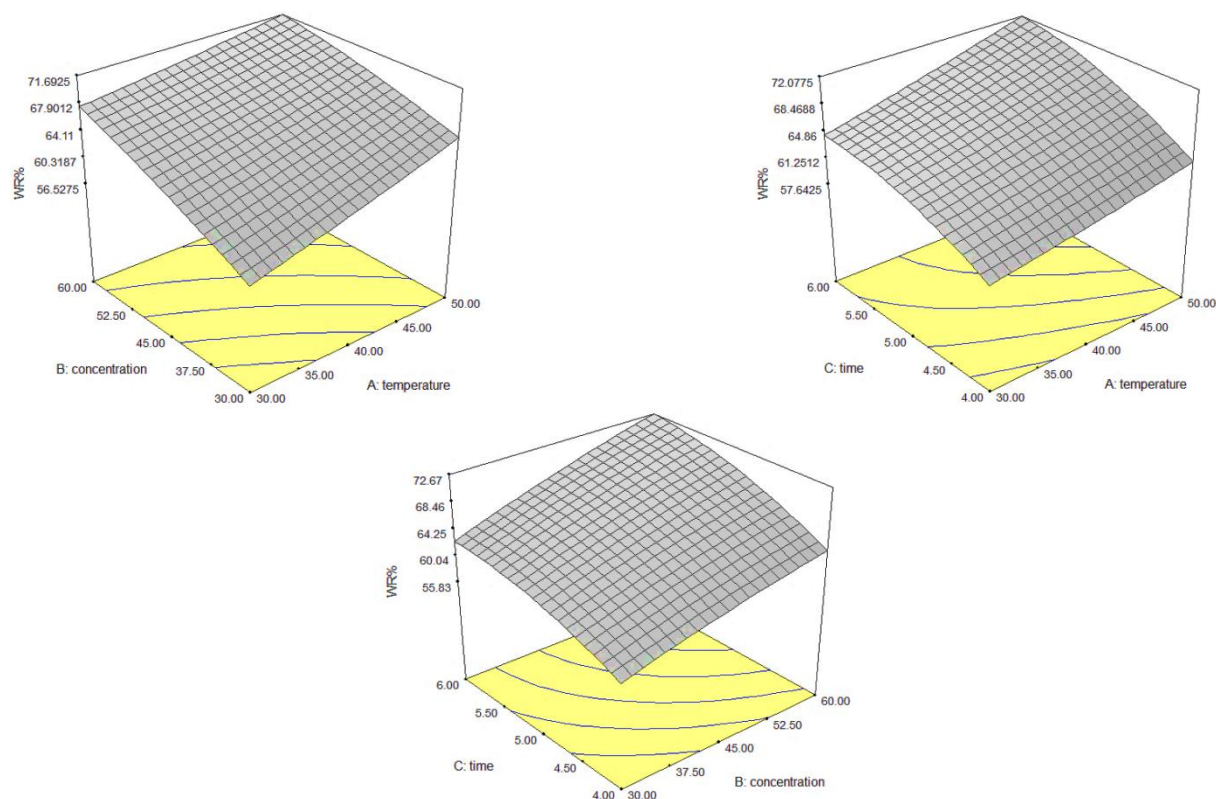


Fig. 3. The interaction effect of the independent variables of osmotic solution temperature - osmotic solution concentration - osmotic solution temperature - immersion time and osmotic solution concentration - immersion time on WR (%) of turnip slices

Shrinkage

Shrinkage is one of the important parameters that affects structural properties of the food product. According to Table (3), only the linear effect of temperature (A) and concentration (B) and the second-order effect of temperature (A^2) have a significant effect of shrinkage rate (A^2) and osmotic solution concentration (B^2) has a negative effect on shrinkage. The negative effects of the significant sentences show that with increased temperature and concentration of the osmotic solution, shrinkage increased and then decreases and this can be deduced from Fig. (4). Also, the curvature observed in the graph shows the interaction effect of temperature-concentration and temperature-time consistent with the significance of the second-order effect of temperature (A^2) in Table (3), (Fig. 4). Eq. (10) shows the polynomial model used according to the coded rates to predict shrinkage rates after removing sentences with insignificant effects.

(10)

$$S = +28.50 - 0.23 A - 0.26 B - 0.46 A^2$$

Shahidi *et al.* (2012) in investigating shrinkage of banana leaves during osmotic-drying dehydration reported that with increased osmotic solution concentration, SG increased and shrinkage decreased.

According to the positive effect of increased concentration and temperature on increased SG, decreased shrinkage at higher osmotic concentrations can be due to increased turnip tissue resistance against deformation as a result of sugar penetration. Indeed, solid matters that penetrate into the interstitial space, fill the gaps and prevent shrinkage during hot air drying. Moreover, Shahidi *et al.* (2012) stated that with increased osmotic solution concentration, a hard crystal layer is formed on banana. These researchers believed that this layer is the result of hot air drying and decreased shrinkage. It seems that the above assumption about turnip osmotic drying is true in the current study.

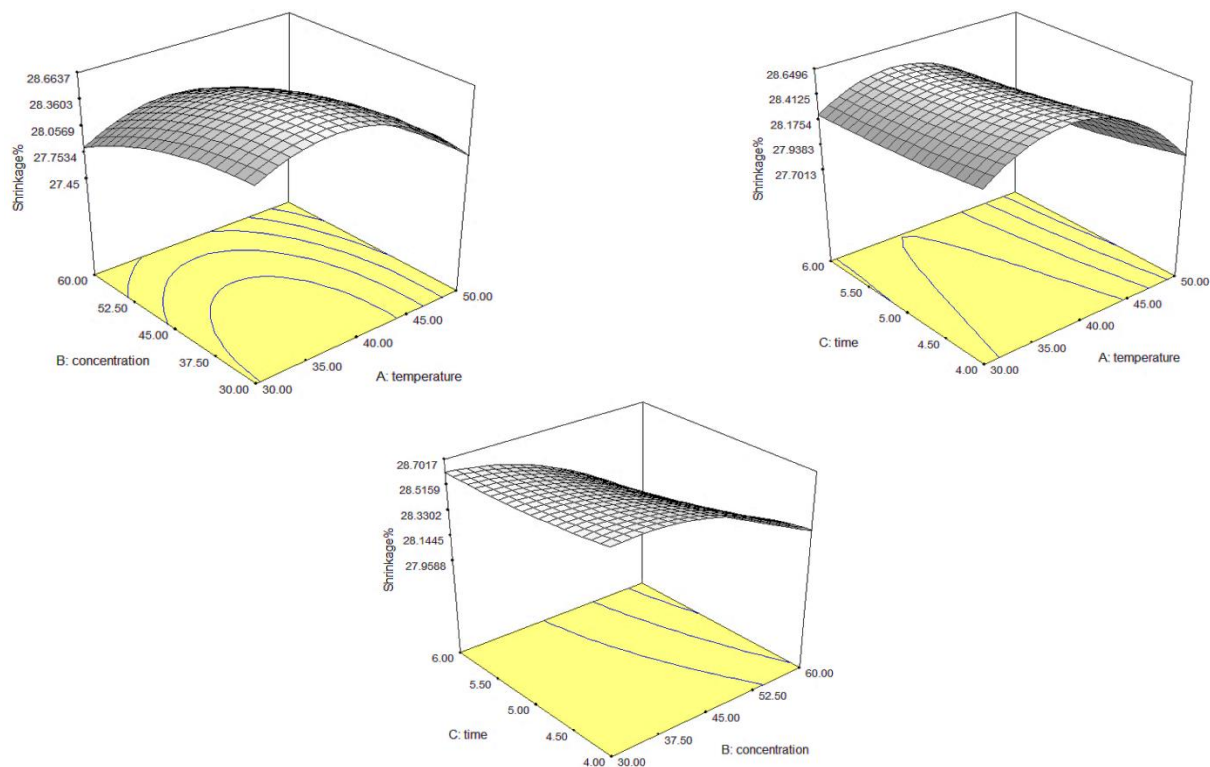


Fig. 4. The interaction effect of the independent variables of osmotic solution temperature - osmotic solution concentration - osmotic solution temperature - immersion time and osmotic solution concentration - immersion time on SR (%) of turnip slices

Rehydration

Rehydration is one of the qualitative parameters in the dehydrated product. The results of Table 3 show that only the linear effects of concentration (B) and time (C) as well as the second-order effects of temperature (A^2) and concentration (B^2) have a significant effect on RR. The first-order sentences of concentration and time had a positive effect and the second-order sentences of temperature (A^2) and concentration (B^2) has a negative effect on RR. These observations showed that with increased concentration and dehydration temperature, RR increases, but for higher concentrations and temperatures, RR decreases. These findings can be seen in Fig. (5). Also, lack of curvature for time in temperature-time and concentration-time chart (Fig. 5) is consistent with the insignificant effect of time (C^2) in Table

(3). Eq. (11) shows a polynomial model according to the coded rates to predict RR after removing sentences with insignificant effects.

(11)

$$RR = +4.43 + 0.39 B + 0.18 C - 0.17 A^2 - 0.41 B^2$$

During osmotic dehydration, SG affected cell penetration and reduced rehydration (Singh *et al.*, 2010).

The above results are consistent with the findings of other researchers (Bakalis & Karathanos, 2005; Lewicki, 1998; Rastogi & Raghavarao, 2004). Previous studies show that osmotic dehydration has a negative effect on RR (Shahidi *et al.*, 2012). This is due to the saturation of the bottom layer or lower dehydration of the sugar layer compared with the natural tissue of the food product.

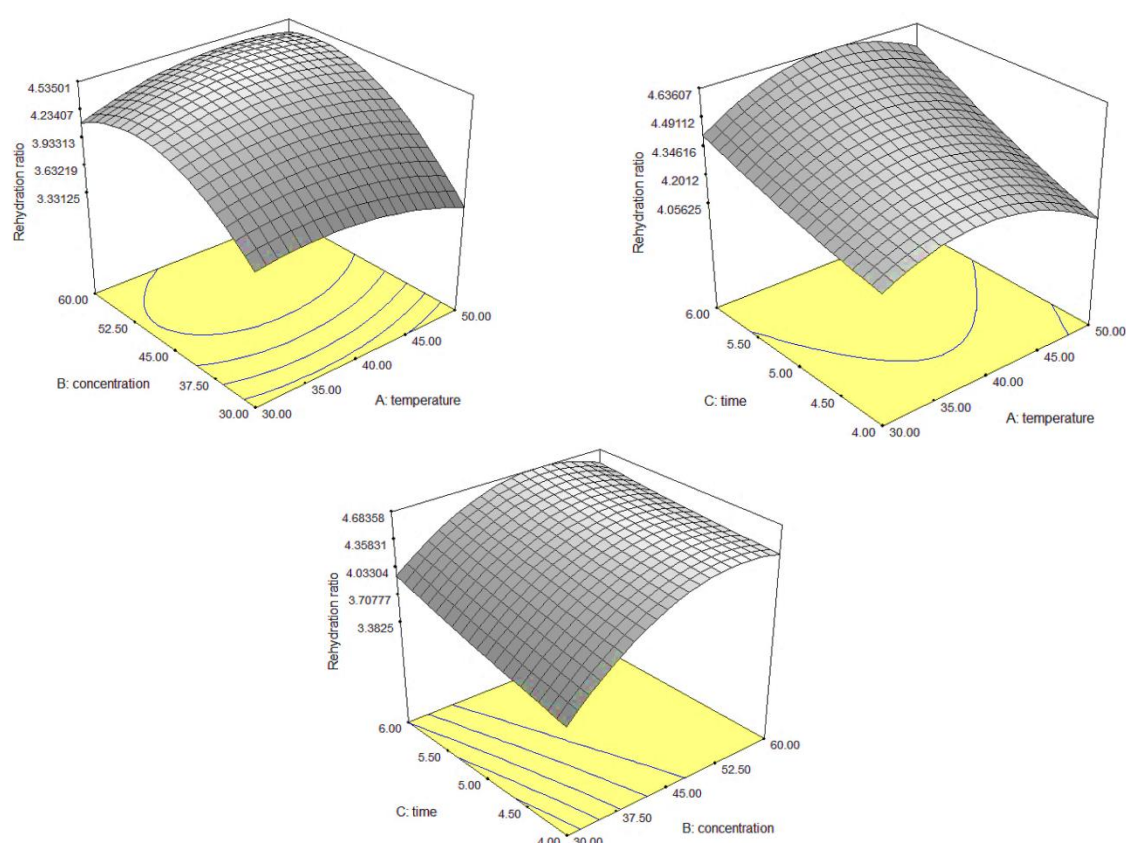


Fig. 5. The interaction effect of the independent variables of osmotic solution temperature-osmotic solution concentration-osmotic solution temperature-immersion time and osmotic solution concentration-immersion time on RR (%) of turnip slices

Vitamin C

Vitamin C content is one of the important qualitative parameters that affects the nutritional value of the processed product. The results of Table (3) about vitamin C show that only linear sentences of three variables (i.e. concentration, temperature, and time) and the second-order effect of concentration (B^2) were significant while other sentences had insignificant effects on vitamin C content and removed from the model. According to the results, the first-order effects of temperature, concentration, and time as well as the second-order effect of temperature (A^2) had a negative effect and the second-order sentences of concentration (B^2) and time (C^2) had a positive effect on vitamin C content. Eq. (12) shows the polynomial

model used according to the coded rates to predict vitamin C content after removing sentences with insignificant effects.

$$\text{Vit C} = +11.31 - 0.46 A - 1.07 B - 0.47 C + 0.84 B^2 + 0.27 C^2 \quad (12)$$

Among significant sentences, temperature (A) and time (C) or concentration (B) have the largest negative effect on vitamin C content. The findings show that with increase in these parameters, especially concentration, vitamin C content in turnip decreases (Fig. 6). Azoubel *et al.* (2009) reported vitamin C drop about apple osmotic dehydration. According to high solubility of vitamin C in water, its drop during osmotic dehydration with WL is not unexpected.

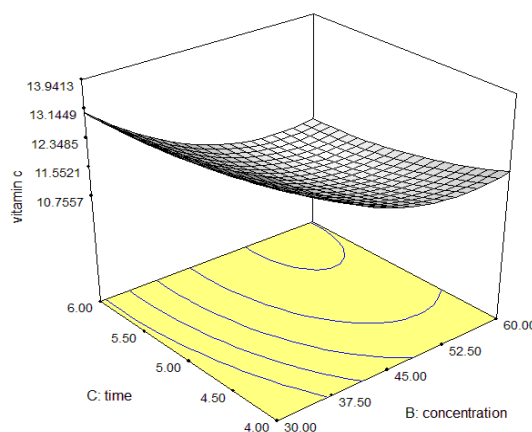


Fig. 6. The interaction effect of the independent variables of osmotic solution temperature-osmotic solution concentration-osmotic solution temperature-immersion time and osmotic solution concentration-immersion time on vitamin C content (mL/100 g of dry matter) of turnip slices

Color indicators

According to Table (3), the fitted model was significant for b indicators. About light (L), only linear sentences of temperature (A), time (C) and the second-order sentence of time (C^2) showed a significant effect. Eq. (13) shows the polynomial model used according to the coded rates to predict L after removing the sentences with insignificant effects:

$$L = +33.35 + 1.20 A + 1.28 C + 1.71 C^2 \quad (13)$$

The positive coefficients of the significant sentences indicate increased L indicators with increased temperature (A) and time (C). The above effects can be observed in Fig. (7). According to Fig. (7), with increased concentration and temperature simultaneously, L rate increases and then decreases gradually.

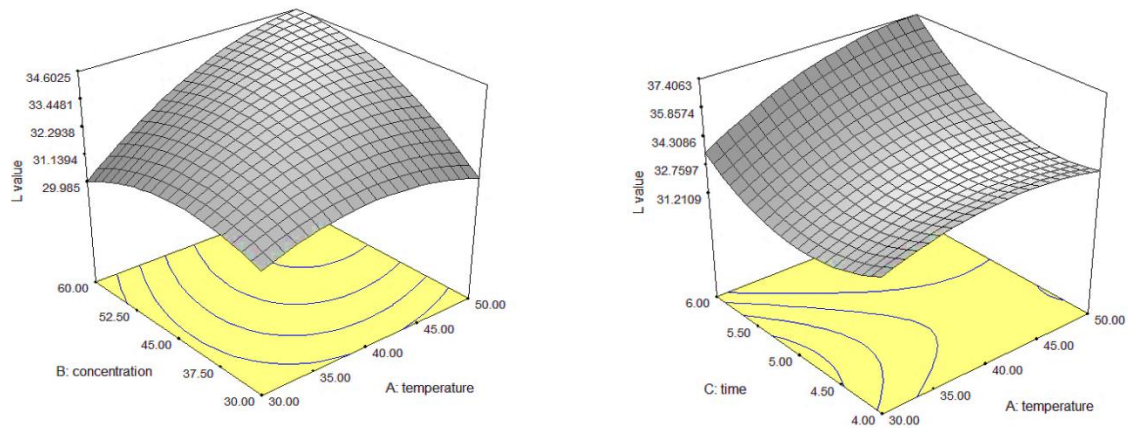


Fig. 7. The interaction effect of the independent variables of osmotic solution temperature-osmotic solution concentration-osmotic solution temperature-immersion time on L indicator of turnip slices

Also, the observed curvature for dehydration time (C) in temperature-time chart (Fig. 7) is consistent with the significance of the second-order sentence of time (C^2) in Table (3).

About parameter b, only the second-order sentence of temperature (A^2) and the interaction effect of concentration-time (BC) had a significant effect. Eq. (14) shows the polynomial model used according to the coded rates to predict b indicator after removing sentences with insignificant effects.

$$b = +18.18 - 0.99 A^2 - 0.90 BC \quad (14)$$

According to Fig. (8), the lowest b rate was obtained under maximum concentration-time condition. The negative effect of the interaction effect of concentration-time (BC) confirms this finding. Singh *et al.* (2010) reported similar results in optimizing osmotic dehydration. Also, the observed curvature for temperature (A) in temperature-concentration (Fig. 8) chart is consistent with the significance of the second-order sentence (A^2) in Table (3). According to Fig. (8), with increased temperature, b parameter increased and then decreased, so that maximum b was resulted in average rates of temperature and concentration.

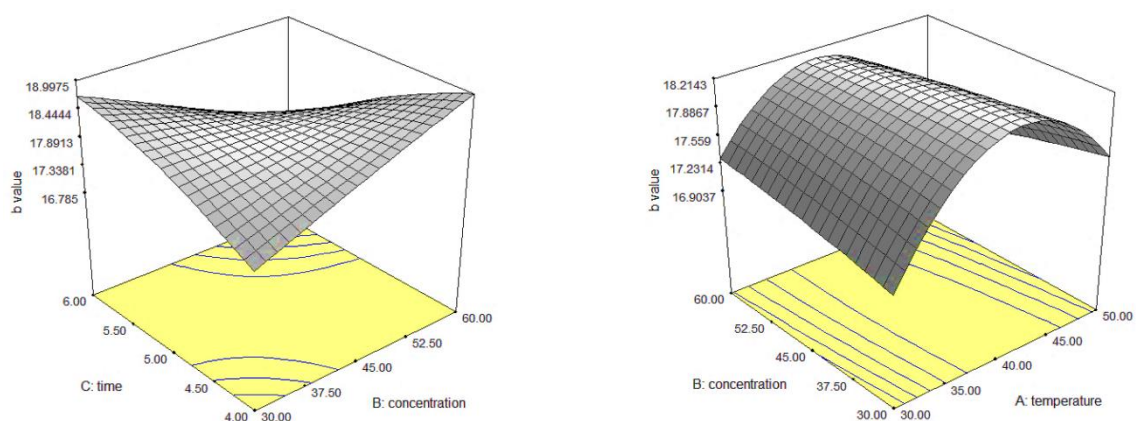


Fig. 8. The interaction effect of the independent variables of osmotic solution temperature-osmotic solution concentration-osmotic solution temperature-immersion time on b indicator of turnip slices

Optimization of the osmotic dehydration process

To achieve optimal conditions for turnip osmotic dehydration process, numerical optimization technique was used (Table 4).

According to different studies on osmotic dehydration of fruits and vegetables, this study aimed to maximize WL, WR, RR, vitamin C, and L parameters and minimize SG, shrinkage, and a and b parameters (Eren & Kaymak-Ertekin, 2007; Noshad *et al.*, 2012; Vieira *et al.*, 2012). Finally, using desirability function method, the optimal conditions were determined as 30.81 °C, sucrose concentration of 60%, and 6 h. The predicted rates for the responses were predicted as 83.10, 12.91, and 70.19%, 27.76, 4.19, and 11.64 (mg/100 g of dry matter), 33.85, 25.49, and 15.91 for WL, SG, WR, shrinkage, RR, vitamin C, and L, a, and C parameters. To check the accuracy of the predicted points by the second-order polynomial sentence,

validation test was used. The turnip samples resulted under optimal conditions were dehydrated and dried at 70 °C. Osmotic dehydration parameters and the qualitative properties of the dried samples based on osmosis-hot air method (in three replications) were compared with the predicted conditions. The predicted error percentage for each response was calculated using Eq. (15):

$$Error(\%) = \frac{R_t - R_p}{R_p} \times 100 \quad (15)$$

R_t : real data resulted from validation test; R_p : the predicted data by the model. The results of this comparison are presented in Table (4). Table (4) shows that the presented model can predict the responses very well. The prediction error about all responses, except parameter a, was led than 10%. Therefore, the above model can be used to optimize turnip osmotic dehydration.

Table 4. The predicted and experimental results for responses in turnip osmotic dehydration

Response	The predicted results	The experimental results*	The prediction error percentage
WL	83.10	83.47±0.97	0.44
SG	12.91	12.66±0.38	-1.88
WR	70.19	70.80±1.33	0.87
Shrinkage	27.76	28.27±0.38	3.72
RR	4.19	3.97±0.14	-5.25
Vitamin C	11.64	11.66±0.70	0.17
Parameter L	33.85	33.98±0.87	0.40
Parameter a	25.49	23.26±0.82	-8.74
Parameter b	15.91	15.20±0.42	-4.42

Conclusions

In the current study, the effect of osmotic dehydration process conditions (i.e. concentration, temperature, and time)-hot air drying (70 °C) on mass transfer phenomena and physical and nutritional properties of turnip (shrinkage, RR, vitamin C, and L, a, and b parameters) were investigated. To determine optimal osmotic dehydration conditions, RSM was used. The results showed that the second-order polynomial models to predict all responses were statistically significant (except a). During osmotic dehydration of turnip samples, temperature, concentration, and

time had significant effects on WR, SG, WL, vitamin C, shrinkage, RR, and L. The optimal osmotic dehydration conditions for turnip, we had osmotic solution temperature of 30.81 °C, osmotic solution concentration of 60%, and time of 6 h. Under these conditions, WL, SG, WR, shrinkage, RR, vitamin C, and L, a, and b parameters were 83.10, 12.91, and 70.19%, 27.76, 4.19, and 11.64 (mg/100 g solids), 33.85, 25.49, and 15.91. Therefore, the results of the current study can be used in turnip processing method using osmotic dehydration and drying the next samples.

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تعیین شرایط بهینه آب‌گیری اسمزی به‌عنوان پیش‌تیمار در خشک‌کردن با هوای داغ برش‌های شلغم به روش سطح

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چکیده

هدف از مطالعه حاضر، بررسی اثر غلظت محلول اسمزی (۳۰، ۴۵ و ۶۰ درصد وزنی/وزنی)، دمای محلول اسمزی (۳۰، ۴۰ و ۵۰ درجه سانتی‌گراد) و زمان غوطه‌وری (۴، ۵ و ۶ ساعت)، بر خروج آب (WL)، جذب ماده جامد (SG)، کاهش وزن (WR)، محتوی ویتامین C، چروکیدگی، نسبت بازآپوشی (RR) و شاخص‌های رنگ (L، a و b) طی آب‌گیری اسمزی- خشک‌کردن ورقه‌های شلغم بود. روش سطح پاسخ (RSM) نیز برای یافتن شرایط بهینه مورد استفاده قرار گرفت. نتایج نشان داد که طی آب‌گیری اسمزی نمونه‌های شلغم، متغیرهای دمای محلول اسمزی، غلظت محلول و زمان غوطه‌وری تأثیر معنی‌داری بر پارامترهای انتقال جرم (WL، SG و WR)، محتوی ویتامین C، چروکیدگی، RR و شاخص رنگ (L) داشتند. شرایط بهینه آب‌گیری اسمزی برای شلغم، دمای محلول اسمزی ۳۰/۸۱ درجه سانتی‌گراد، غلظت محلول اسمزی ۶۰ درصد و زمان غوطه‌وری ۶ ساعت بود. تحت این شرایط، مقادیر پاسخ‌های WL، SG، WR، چروکیدگی، نسبت بازآپوشی (RR)، محتوی ویتامین C و شاخص‌های رنگی L، a و b به ترتیب ۸۳/۱۰، ۱۲/۹۱ و ۷۰/۱۹ درصد، ۲۷/۷۶، ۴/۱۹ و ۱۱/۶۴ (میلی‌گرم در ۱۰۰ گرم ماده خشک) و ۳۳/۸۵، ۲۵/۴۹ و ۱۵/۹۱ بود. نتایج پژوهش حاضر می‌تواند جهت فراوری حداقلی برش‌های شلغم با استفاده از آب‌گیری اسمزی و خشک‌کردن بعدی نمونه‌ها مورد استفاده قرار گیرد.

واژه‌های کلیدی: آب‌گیری اسمزی، بهینه‌یابی، روش سطح پاسخ، شلغم

The Effect of Reduction Rolls During the Milling Process on the Physicochemical Properties of Flour and Rheological Characteristics of Dough and Barbari Bread

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Abstract

Reduction rolls play an important role in the process of flour production and increasing the quality of flour used in baking industry is considered as the most important factor to improve the qualitative and nutritional qualities of bread. So the aim of this study was to evaluate the effect of removing C1A, C1B, C2, C3 and C5 reduction rolls on the physicochemical properties of the flour and rheological properties of dough and the selection of the most effective rolls in milling process in a completely randomized design ($P \leq 0.05$). The results showed that two samples of flour that Also the sample, which C3 reduction rolls was removed, had the lowest gluten content (25%), gluten index (70), and zeleny sedimentation volume (17 mL). And the water absorption, development time, stability, and valorimeter number of the dough had the greatest reduction in this sample. Therefore, according to the results, it was found that by removing the C3 reduction roll in milling process, the physicochemical properties of the flour and the rheological properties of dough were strongly weakened. In the next step, the Barbari bread was produced by the flour obtained from this sample and control and its characteristics were examined. The results showed that the hardness of the sample by removing C3 reduction roll was higher (more than 35%) than the control sample during 2 and 72 h after baking. Also, the control had the highest score of overall acceptance in sensory evaluation. According to the results of this study, removing the C3 reduction roll, had the most effect on the quality of flour, dough and final product than the other roll in milling process.

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Keywords

Barbari bread
Milling process
Reduction roll
Rheological properties
Wheat

Introduction

One of the most important cases in producing good quality bread is using good quality flour. The milling process is of particular significance in obtaining flour with the desired characteristics. In the

milling process, the most significant purpose is to separate the shell (bran) and germ from the central part of the wheat (endosperm) and to reach the highest value of flour extraction with the lowest amount of bran and germ, since these compounds

increase flour ash. Ever since the process of milling the grain has been recognized by humans, simplification and making the milling process efficient using existing facilities and solutions, has always been the focus. New concepts and ideas will only succeed if the quality of the finished product is not affected and the reduction in capital required for operating and maintenance costs is reached (Baltensperger, 1993). The common technical knowledge in the milling process is that after each milling step, the compounds must be screened and the smaller material must be removed before re-milling (Owens, 2001).

Over years, the main equipment used in the milling process has been designed to increase efficiency, yet the flour production technology has not changed significantly since the introduction of the roll mill (Walz), purifier and sieve (Baltensperger, 2001). In order for the wheat to become conditioned flour, it is passed through two series of rolls (1-crushing or grooving roll and 2- reduction or softening roll). In the first series rolls (crushing or grooving) made of stainless steel, the wheat shell is opened and the endosperm is separated from the shell in successive and gradual stages (Ahmadi Nodoushan, 2017; Fistes, 2015).

The first series is often made up of 4 (or more) crushing rolls, which are serially interconnected and each feeds its own feed from the previous one. Each machine has two rolls that rotate in parallel with each other at opposite directions and different speeds and the wheat is shed and crushed between them (Ahmadi Nodoushan, 2017; Ali Akbarnia & Azarbad, 2010). After each crushing to separate the endosperm, bud, shell, and the shell to which the endosperm is still attached, the mixture is passed through a sieve system to separate the above compounds. The sieves are usually positioned horizontally, so that the larger sieve are higher the smaller ones are placed lower. The sieves have a circular motion and are parallel to the floor. Each device

may be composed of 12 sieves. Ultimately, after crushing rolls and sieves, the shattered particles enter the second series of rolls, i.e. the section of the reduction rolls. The number of rolls in this section may be 12 or more. In this section, as in the previous section, each device consists of two rolls (which rotate in parallel and in opposite directions at different speeds) and feeds from the previous machine (Ahmadi Nodoushan, 2017; Fišteš & Vukmirović, 2009; Gilbert, 2002).

Ultimately, the endosperm particles are converted into flour and the last shell and germ particles are removed. The rolls in this section are divided into two types of flat and rough, which is the criterion for the division between the rolls. If the distance between the two rolls is low, the soft roll is produced and the name of the roll is soft. If the distance between the two rolls is large, the coarser roll is called the rough roll device. The order of entry of the endosperm particles is such that the particles first enter the rough roll and then the soft roll. In this section, like the crushing roll part, sieves are used to separate the particles by size. Finally, the final output of the sieves is flour with uniform particles (Ahmadi Nodoushan, 2017; Posner, 2000). The roll mill compared to older mills has advantages like lower power consumption, precise adjustment of roll pressure, uniform feeding throughout the rolls, less energy wastage, longer life, easier roll replacement, occupying less space, targeting the crushing operation, and being healthy (Ali Akbarnia & Azarbad, 2010).

This machine is of the main and most significant machines in the production line of flour mills. The significance of rolling mills is undeniable, especially when producing industrial flours, which sometimes have a flour content of up to 85% below 125 μm . It is clear that the task of softening the flour can only be reached by rolls with the proper design and construction characteristics. In line with this, Fistes (2015) stated that under the same conditions in terms of the distance

between the rolls and the sifting process, in the 8 roll milling process, increasing the extraction efficiency followed by decreasing the distance between the rolls and increasing the size of the soft flour particles and then energy consumption is reduced without reducing flour quality. Moreover, Opáth (2014) examined the technical parameters of rolling mill flour extraction. The purpose of the study was to examine the power consumption of roll mill during flour grinding. The results indicated that the milling process has 15 stages and the hourly efficiency of the roll mill in the first stage is 3006.72 kg/h. The highest crushing effect was seen in the first milling stage, so that the particle size after the first milling stage was 12% of the initial grain size.

Scanlon, Dexter, & Biliaderis (1988) examined the relationship between particle size and the physical properties of hard red spring wheat flour produced by a reduction roll and stated that starch damage increased with increase in heterogeneity and decreasing distance between rolls. These researchers screened the flour produced by two sieves with 53 and 91 μm aperture sizes and observed that particles smaller than 53 μm had greater starch damage than coarse particles. He stated that starch damage is most likely due to the more fragmentation of flour particles than the change in their stress state. The shear stress applied to the bond between the starch and the protein causes the state to change and break the flour particles.

Hence, given the points stated regarding reduction rolls in flour production process, as well as increasing the quality of flour used in the baking industry is considered as the most important factor in improving the quality and nutritional properties of bread. In the present study, the effect of reduction rolls during grinding process on physicochemical properties of flour and rheological properties of Barbari dough and bread were examined.

Nowadays, turning to a shorter production line to reduce energy

consumption and costs is one of the weaknesses of flour mills in the country that in the short line of the product should be removed as soon as possible from the flour mesh and roll pressure to accelerate this issue. Moreover, the flour meshes are selected in larger size and by increasing the volume of the inlet load to the roll and the overpressure, the rolls are heated by friction that the roll heating has adverse effects on gluten and pressure and starch damage highly increases. It is worth noting that damage to starch in a good range that maximizes water uptake is desirable, but excessive damage to the crop. Here, reduction rolls have the greatest effects on the quality of flour produced. Thus, the purpose of the study was to evaluate the quality of flour produced and to compare flour production in a long line mill and a short line mill (the shortness and length of a mill line are the number of rolls in a mill). There are significant disagreements between the line makers and the product designers. Furthermore, given the variety of wheat inputs to the factories, it was impossible to import a variety of wheat into two factories because the wheat was distributed in quota by government agents and could be intercepted and seized. Thus, only one roll was removed from the circuit for 2 h each to produce a shorter production line and the flour produced was evaluated.

Materials and methods

Materials

The study was done at the Iranian flour factory (Tehran, Iran) and a traditional bread unit in Tehran was used for the second stage of the present study (Barbari bread preparation). The incoming wheat was thoroughly mixed with the plant and ready to be conditioned and subsequently milled to produce quality flour.

Other chemicals required for chemical tests were the Merck brand (Germany). Materials used in the production of Barbari bread such as yeast (*Saccharomyces cerevisiae*) were prepared from Razavi Yeast Factory (Mashhad, Iran) and stored in

a refrigerator (4 °C).

Wheat conditioning

The time needed for wheat sleep is at least 20 h during the warm seasons of the year (the time the present study was conducted) and moisture was added to the wheat and then entered storage. The temperature of incoming dry wheat was 26 °C. The moisture content was added to the samples at a ratio of two-thirds at first conditioning (wheat temperature about 29 °C) and one-third at second conditioning (wheat temperature about 32 °C).

Sampling from the input wheat to production line

Given the variety of wheat cultivation, which is more than a hundred native and modified varieties cultivated in the country, in the field of flour production in factories we do not encounter a specific wheat type and we tried our best to mix it. Then, it was cleaned using a laboratory winnowing machine (a/s Rationel Kornservice, Denmark) and during this phase dust, straw, stone, other herbaceous seeds and broken wheat seeds were separated. Wheat samples were milled to full flour using a laboratory hammer mill (Laboratory Mill 3100, Germany).

Evaluation of physicochemical properties of incoming wheat flour

Measuring moisture was done according to Iranian National Standard No. 2705 (Iranian National Standardization Organization [ISIRI], 2011a), method for measuring the moisture content of cereals and its products and measuring the amount of ash, wet gluten and falling number edited by the AACC (2000), 08-01, 38-11 and 81-B56, respectively.

Sampling by removing reduction rolls

The first stage of the present study was done at the Iran flour factory. In this industrial unit, wheat flour was produced with 15 rolls (MIAG, Germany) including 7 rolls are crushers and 8 rolls are reduction. It has to be noted that the reduction rolls are

shown on the German and Turkish production line with the letter C and on the American production line with the letter M. Thus, according to the studies conducted and the purpose of this study that was to examine the effect of reduction rolls on the quality of flour produced, at each stage one of C1A, C1B, C2, C3 and C5 rolls are taken out from the production circuit and the wheat enters the sieve without going through the removed roll and goes through the other milling steps. Indeed, the roll numbers or alphabetical order is the coarse-grained display of the particles that slide forward, so that C2 roll load goes from C1A, C1B, B2 and B1 and the C2 load goes to C3 and C4. Moreover, C1A roll load comes from B1 and load goes to C2. C1B roll load comes from B2 and its load goes to C2 and C4. C3 roll load comes from C2 and the load goes to C4 and C5 and finally C5 reduction roll load comes from C3 and C4 and the load goes to C6 and B4F. Sampling was done at the end of the line to examine the physicochemical properties of wheat flour and rheological properties of the dough. It has to be noted that as the distance between the rolls is not measurable and in the grinding process the rolls are adjusted based on the load output and the output load shattering rather than measuring the distance to change the type of wheat to always constant distance. This is because our testing and sampling took at least 2 h to maintain constant distance between the rolls at all sampling steps.

Evaluation of physicochemical properties of wheat flour

Moisture content was measured according to Iranian National Standard No. 2705 (Iranian National Standardization Organization [ISIRI], 2011a), and ash content, gluten index and Zeleny sedimentation volume were measured using AACC (2000) 08-01, 38-12A, and 54-11, respectively.

Wet gluten content was measured according to AACC (2000) 11-38. A two-step wash of gluten (Perten, Sweden) was used to measure the gluten in whole flour

samples containing bran particles. Thus, in the first step of washing, the starch is washed and a gluten structure is formed, and in the second step, the bran particles easily pass through a 700 μm sieve.

The damaged starch content in the flour particles was measured according to the Amperometric principle and in accordance with Iranian National Standard No. 16933 (Iranian National Standardization Organization [ISIRI], 2013). This machine (Chopin, France) reports the damaged starch in less than 10 min with a UCD unit with a numerical range from 12 to 28.

Flour particle aggregation was measured using a sieve shaker (BADI, Iran) with a rotational speed of 200 rpm and sieves with 125, 180 and 475 μm apertures according to Iranian National Standard No. 103 (Iranian National Standardization Organization [ISIRI], 2011b). In doing so, the sieve was first weighed and placed in the upstream of the vibrating device from 125 to 475 μm in size, respectively.

Evaluation of rheological properties of the dough

Farinography test was done according to AACC Standard No. 21-54 (AACC, 2000) by Farinograph machine (Yucebas machine, Turkey). Farinograph measures and records dough resistance to mixing. This experiment was used to evaluate flour water absorption and specify the strength and other properties of dough during mixing.

The effect of reduction roll on the quality of Barbari bread

At the end of the first step, the most significant reduction roll, with the most effect on the physicochemical properties of the flour and the rheological properties of the dough, was identified and the flour sample was prepared in the absence of this roll, with the control sample with all rolls active in its production steps to bake Barbari bread. To prepare Barbari bread dough, two wheat flour samples, selected after reviewing the results of the above tests, were used with water (according to

Farinograph test water absorption rate), 2% yeast and 1% NaCl (Shekholeslami & Karimi, 2012).

Evaluation of qualitative and sensory characteristics of Barbari bread

Texture firmness evaluation of Barbari bread samples was done using a TTS (QTS, UK) during 2 and 72 h after baking. The maximum force needed to perform a penetration test by a cylindrical end probe (2 cm in diameter at 2.3 cm in height) at a speed of 30 mm/min from the center of the bread was calculated as an index of firmness (Pourfarzad *et al.*, 2011).

Moreover, the sensory characteristics of Barbari bread produced in terms of taste (nasty taste, salty and alkaline taste, raw or sour taste or natural aroma of bread), texture (doughiness or unusual softness, bread firmness, crunchiness and fragility, chewing capability, dryness and hardness of bread, pellets and paste in the mouth and adhesion to teeth) and general acceptance (overall acceptability of the sample) were evaluated by 10 panellist. The coefficient of evaluation of traits ranged from very bad (1) to very good (5) (Stone & Sidel, 2004).

Statistical analysis

The results were evaluated using SPSS18. A completely randomized design with 6 treatments was used in doing so. The samples were prepared in three replications and the means were compared by Duncan test at the significance level ($P < 0.05$). Finally, Microsoft Excel 2013 was used to plot the graphs and the most important roll with the most effect on the flour quality and rheological properties of the dough was introduced. Additionally, the texture and sensory properties of two samples of Barbari bread made from control flour and flour with the most important reduction roll removed from the circuit were compared using T-test.

Results and discussion

Physicochemical properties of whole wheat flour

The physicochemical properties of whole wheat flour used in the study are described in Table (1).

Table 1. Physicochemical properties of whole wheat flour

Physicochemical properties	Value
Moisture (%)	8.48±0.36
Wet gluten (%)	21.3±0.08
Ash (%)	1.22±0.02
Falling number (s)	385.00±15.00

Evaluation of the physicochemical properties of flour

Ash

The results of removing C1A, C1B, C2, C3 and C5 reduction rolls and control samples (without roll removal) on the ash content of the flours produced are shown in Fig. (1). As the results show, with the removal of C1A and C5 rolls, the highest and lowest ashes were observed in the flour samples, respectively. Ash value is one of the main characteristics of flour, according to which the flour is classified into different types. Wheat bran has the highest value of ash so if the ash content in the flour increases, it indicates an increase in the amount of bran in the flour. According to the Iranian National Standard No. 103 (Iranian National Standardization Organization [ISIRI], 2011b), the permissible range for ash for Barbari flour is 0.70 to 0.85, with the amount of ash in all samples being within the permissible range. It seems that as at the beginning of the mill the flour particles are obtained from the farthest point from the bran (wheat brains), they have at least minerals. Thus, by adding flour, this portion is reduced to the final flour of the finished product ash and if the primary rolls (especially C1A) are removed, the sample ash may increase.

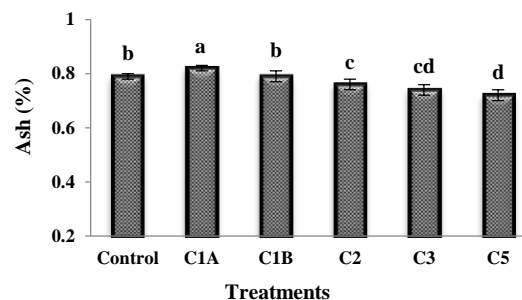


Fig. 1. The effect of removing C1A, C1B, C2, C3 and C5 reduction rolls and control on the ash content of the flour samples. Similar letters were not statistically significant at ($P < 0.05$).

Damaged starch

The results of the effect of removal of C1A, C1B, C2, C3 and C5 reduction rolls and control (without roll removal) on damaged starch in flour samples are seen in Fig. (2). As the results show, with the removal of C1A roll, the highest damaged starch was observed in the flour produced in the control sample. Starch shapes the main component of all cereal grains and their products. In evaluating the quality of wheat flour, the physical conditions and the mechanical damage to the starch granules is very important during the mechanical grinding operations. The semi-crystalline structure of starch granules can be damaged by the mechanical operation of the grinding process. Limited damage to starch granules has a positive effect on wheat flour quality. However, over-damaged starch will have a significant negative impact on product quality (Kent & Evers, 1994). Limited starch damage makes it easier to release internal granule components like amylose and better penetration of water and enzymes into the granule, which in turn affects the dough properties and product properties of such flours (Peighambardoust, 2017). The degree of physical damage to the starch largely depends on the conditions of the grinding process. From the beginning to the end of the mill path, the damaged starch increases with the progress of mechanical operations. Using mechanical forces and pressures (reducing the gap

between crushing rolls, using sharp groove rolls and increasing the number of crushing rolls) significantly increases the damaged starch during the crushing process of wheat grains (Cochet, 2012; Peighambardoust, 2017). During the grinding process, when the flour is softened by the reduction rolls, the particle size becomes smaller at each step, resulting in a smaller gap between the rolls. Thus, it seems that when C1A roll is removed, particles larger than normal suddenly (i.e., when C1A worked and the particles were slightly softened) enter C1B roll. Thus, at this stage, as these particles bear a lot of pressure, the starch damage increases with the removal of other rolls. It is normal in the control sample because no roll is removed and the particles enter the next roll at the appropriate size at each step, thus experiencing proper and predictable pressure and the damaged starch is minimized.

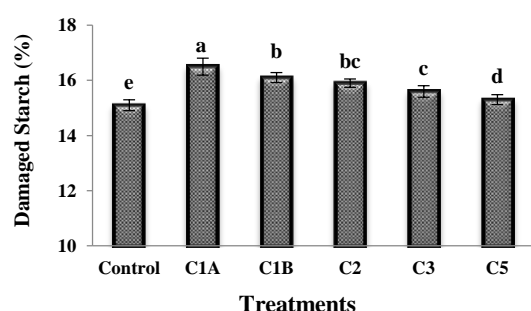


Fig. 2. The effect of removing C1A, C1B, C2, C3 and C5 reduction rolls and the control sample on the damaged starch in the flour samples

Similar letters were not statistically significant at ($P < 0.05$)

Wet gluten and gluten index

The results of the effect of reduction roll removal on wet gluten and gluten index in flour samples are shown in Table (2). As the results show, after the control sample, the highest wet gluten and gluten index were in the samples that had C1A and C5 rolls removed. However, the sample with the lowest C3 roll was removed when it was produced. According to Iranian National Standard No. 103 (Iranian National Standardization Organization

[ISIRI], 2011b), the minimum gluten content for Barbari flour is 27%, which is lower than the standard reference in flour samples produced by the removal of C2 and C3 rolls. Wheat protein contents and quality are known as indices with determinant effects on the physicochemical properties of wheat flour dough and consequently on its functional properties and baking potential (MacRitchie, 1980). As these prominent properties of the protein are primarily related to gluten-forming proteins, measuring protein in wheat or wheat flour is usually related to the determination of wet and dry gluten (Peighambardoust, 2017). This production unit seems to have the highest value of soft endosperm (this portion of the endosperm contains the highest amount of gluten protein) as the input to C2 and C3 rolls. Thus, when the rolls are removed because the flour particles enter the subsequent rolls and the ability to extract flour with appropriate gluten is reduced, wet gluten in the final flour will reduce. Concerning the control sample, as no roll was removed; the endosperm was gradually separated from the bran at each step, resulting in higher wet gluten and final flour content compared to the other samples.

Table 2. The effect of removing C1A, C1B, C2, C3 and C5 reduction rolls and control on wet gluten and gluten index in flour samples

Treatment	Wet gluten (%)	Gluten Index (-)
Control	28.30±0.69 ^a	90.00±2.20 ^a
C1A	27.50±0.51 ^a	83.00±1.90 ^c
C1B	26.50±0.53 ^a	79.00±1.70 ^d
C2	26.03±0.38 ^{cd}	75.00±2.00 ^e
C3	25.00±0.22 ^d	70.00±1.80 ^f
C5	27.50±0.61 ^b	86.00±1.90 ^b

Similar letters were not statistically significant at ($P < 0.05$).

Zeleny sedimentation volume

The results of the effect of reduction roll removal on Zeleny sedimentation volume in the flour samples are given in Fig. (3). As the results show, after the control sample, the highest volume of Zeleny sedimentation was produced in the flours after the removal of C1A and C5 rolls.

However, in the sample with C3 roll removed during production had the lowest Zeleny sedimentation volume. In the past, wheat was used to determine the quality of total protein, but since not all wheat proteins are suitable for baking quality and only gluten proteins are bakery properties, the method for measuring total protein content is another. Outdated and quality determination of wheat and flour bakeries is done using specialized tests such as the Gluten Index and the Zeleny sedimentation Test (Peighambaroust, 2017). As clarified in the measurement for wet gluten, the amount of sediment deposited by Zeleny sedimentation likely decreased when C2 and C3 rolls were removed. It should be noted that according to the results of Shahedi, Kabir, & Bahrami (2005), who determined the flour quality and rheological properties of dough for production of Taftoon bread using Iranian wheat, it was stated that Zeleny number showed the highest correlation with bread quality and accounted for 68.8% of the variation in bread quality. Moreover, Baniasadi, Azizi, & Sahari (2005) showed that the Zeleny number has a 95% positive effect on bakery quality.

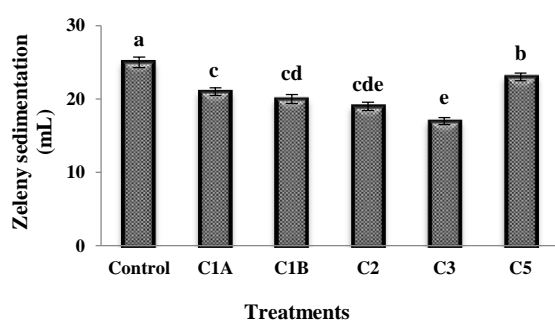


Fig. 3. Effect of removal of C1A, C1B, C2, C3 and C5 reduction rolls and control sample on the Zeleny sedimentation in the flour samples

Similar letters were not statistically significant at ($P < 0.05$).

Flour particle aggregation

The results of the effect of reduction roll removal on particle aggregation of flour samples are shown in Table (3). As the results show, the control sample and the samples in which C1A and C1B rolls were

removed had the highest particle size smaller than $125 \mu\text{m}$. However, roll removal had no significant effect on particle size greater than $475 \mu\text{m}$. Similar results were seen for particle aggregates with sizes greater than $125 \mu\text{m}$ and more than $180 \mu\text{m}$, such that with the removal of C5 roll, more aggregates with sizes greater than 125 and $180 \mu\text{m}$ were obtained.

Concerning grain particle size, one has to note that uniformity of flour particles and maximum particle size of less than $125 \mu\text{m}$ are desirable. At least 50% of flour particles must be less than $125 \mu\text{m}$ in size, according to Iranian National Standard No. 103 (Iranian National Standardization Organization [ISIRI], 2011b). As the distance between the rolls is reduced to the end rolls (such as the C5 roll) and subsequently the flour particle size becomes smaller, it is natural that when these rolls are removed, the probability is greater flour particle size increases.

Moreover, it has to be noted that in the short line, the product should be removed as soon as possible from the flour mesh, which will increase the pressure of the rolls to accelerate this issue, and this increase in pressure will cause the rolls to warm up. As a result of the friction that the roller bearings have a gluten-free effect, the high pressure disrupts the starch damage process, which is too much damage to the starch. On the other hand, increase in the size of the mesh as a result of coarse-grained crop production reduces water absorption due to the large size of the particles because, as mentioned, the best grain size is $125 \mu\text{m}$. As only the endosperm breaks into smaller particles by this size and passes through the mesh, but the bran becomes elastic due to the absorption of moisture (in conditioned operations) and rarely crosses the roll, it reaches below $125 \mu\text{m}$.

One can state that with the removal of C5 roll the particle size is smaller than $125 \mu\text{m}$ less than the other samples and as a result the particle size of more than $125 \mu\text{m}$ increases with the removal of C5 roll.

Table 3. The effect of removal of C1A, C1B, C2, C3 and C5 reduction rolls and control on particle size in flour samples

Treatment	Particle graining *			
	On a 475 μm sieve	On a 180 μm sieve	On a 125 μm sieve	Under a 125 μm sieve
Control	0.10 \pm 0.01 ^{ns}	0.70 \pm 0.05 ^c	21.20 \pm 0.80 ^d	78.20 \pm 1.20 ^a
C1A	0.20 \pm 0.01 ^{ns}	0.70 \pm 0.04 ^c	21.10 \pm 0.70 ^d	78.10 \pm 1.00 ^a
C1B	0.10 \pm 0.02 ^{ns}	0.70 \pm 0.05 ^c	21.20 \pm 0.80 ^d	78.30 \pm 1.20 ^a
C2	0.10 \pm 0.01 ^{ns}	0.90 \pm 0.03 ^{bc}	23.70 \pm 0.50 ^c	75.30 \pm 1.10 ^b
C3	0.20 \pm 0.02 ^{ns}	1.00 \pm 0.04 ^b	24.50 \pm 0.40 ^b	74.30 \pm 1.30 ^c
C5	0.10 \pm 0.01 ^{ns}	1.20 \pm 0.05 ^a	25.80 \pm 0.50 ^a	72.90 \pm 1.20 ^d

* Mean numbers are three replication.

Similar letters in each column were not statistically significant at ($P < 0.05$).

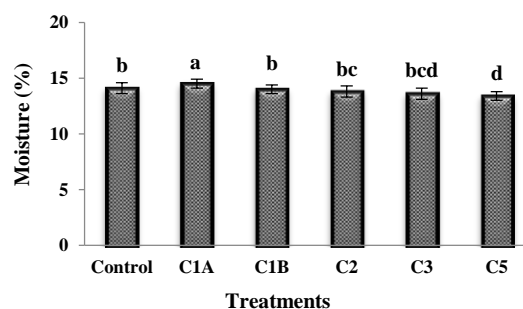
ns: No significant difference was seen at ($P < 0.05$) level.

Moisture

The results of the effect of removing the reduction rolls on the moisture contents of the flour samples are shown in Fig. (4). As the results show, by removal of C1A and C5 rolls, the highest and lowest moisture contents were observed in the flour samples, respectively. Moisture is one of the most important factors in preventing microbial, chemical and enzymatic spoilage during storage of a food material (Fatemi, 2004; Jafari, Poormohammadi, & Asadpou, 2011). Wheat grain behavior in both the storage and the grinding stages depends largely on their moisture contents. Wheat with inadequate moisture will not be technologically appropriate at the milling stage. Wheat and flour moisture levels correlate with the economic benefits of milling units. Wheat flour is sold by weight, and any change in its moisture contents can have a benefit or disadvantage to the production unit, and unfortunately can be a source of profit for a limited number of flour producers. Thus, it is important to control the moisture contents and accuracy of its test methods from a technological, maintenance and economic point of view (Ali Akbarnia & Azarbad, 2010; Peighambaroust, 2017).

According to the Iranian National Standard No. 103 (Iranian National Standardization Organization [ISIRI], 2011b), the maximum moisture content of Barbari flour is 14.2%. According to the results, the sample in which the C1A roll was removed has moisture contents of 14.5% which is higher than the standard

limit. Various factors are involved in determining flour moisture like particle size, ash or bran content, damaged starch, and gluten flour content. The sample that produced C1A roll seems to have been removed because it had more ash (or in other words bran) and damaged starch as well as a particle size of less than 125 μm . Hence, it is more capable of absorbing and retaining moisture. In the case where C5 roll was removed, the conditions are quite the opposite of the one in which the C1A roll was removed and so it is normal to have the lowest moisture content.

**Fig. 4.** The effect of removal of C1A, C1B, C2, C3 and C5 reduction rolls and control on the moisture contents of the flour samples

Similar letters were not statistically significant at ($P < 0.05$).

Evaluation of rheological properties

Water absorption

The results of the effect of reduction roll removal on farinograph properties are presented in Table (4). As the results show, the highest water uptake was observed in the control and sample with C1A roll removal, whereas the lowest water uptake was observed in the sample with C2 roll

removal and with C3 roll removal. Overall, strong flours with high gluten contents and quality, high extraction flours, soft flours (fine particle size), flours with damaged starch percentage and high pentosan content and mature flours (aged) have high water absorption percentages (Peighambardoust, 2017). As the sample had the highest value of these parameters by removing C1A roll in the damaged starch measurement section, gluten content, particle size less than 125 μm and ash content, it was expected that this sample would have higher water absorption. In the case of the control sample, it was expected that the amount of gluten, ash, and particle size below 125 μm would increase as in the case of C1A roll

removal. It is natural that the lower the samples tested, the lower the effective parameters in increasing flour water uptake, so the amount of flour absorbed will decrease. Concerning the effect of ash content, Moradi, Ghiassi Tarzi, Seyyedain Ardebili, & Azizinejad (2010) stated that higher ash contents show higher value of bran that increase water absorption. Concerning the effect of gluten on water absorption of flour, Moradi *et al.* (2010) and Mohtarami, Esmaili, Alizadeh Khaledabad, & Seyedain Ardabili (2014) stated that strong flour has more water absorption than weak flour and this phenomenon is because of high protein quality of flour that can retain and absorb more moisture.

Table 4. The effect of reduction roll removal on dough farinograph properties

Treatment	Farinographic characteristics of dough					Valorimetric value (-)
	Water absorption (%) *	Dough development time (min)	Dough stability time (min)	Degree of dough softening (Brabender Unit)		
				10 min	20 min	
Control	56.50±1.20 ^a	3.31±0.07 ^a	7.14±0.07 ^a	65.30±2.50 ^e	96.10±3.40 ^d	67.00±1.07 ^a
C1A	56.00±1.40 ^a	3.00±0.05 ^b	6.78±0.05 ^b	74.10±1.50 ^d	108.20±2.50 ^c	63.30±0.05 ^b
C1B	56.30±1.30 ^a	2.30±0.08 ^c	6.20±0.08 ^c	85.30±2.20 ^c	114.50±2.80 ^{bc}	59.10±0.08 ^c
C2	53.00±1.00 ^c	1.80±0.06 ^d	5.53±0.06 ^d	94.20±1.80 ^b	121.70±2.80 ^b	57.60±0.12 ^{cd}
C3	53.00±1.10 ^c	1.24±0.07 ^e	4.10±0.07 ^e	102.70±3.00 ^a	134.30±2.20 ^a	54.00±0.90 ^d
C5	54.80±1.40 ^b	3.08±0.09 ^b	6.82±0.09 ^b	74.60±2.10 ^d	108.80±1.90 ^c	63.20±1.33 ^b

* Mean numbers have three replication.

Similar letters in each column were not statistically significant at ($P < 0.05$).

Dough development and stability time

The results indicate that the control sample showed the highest dough development time and dough stability time, whereas the lowest value of these parameters was observed in the sample with C3 roll removal. Indeed, the time it takes (in minutes) from the start of mixing to reaching the first curve to the maximum peak is called the development time or optimal mixing time. During the development of the dough, one can state that the hydration of the flour is complete and the dough's gluten network is fully formed due to the mechanical forces involved in the mixing process and encapsulates the starch granules (Peighambardoust, 2006). Concerning the significance of gluten in this test, it is

normal for the control sample to have more development time because of the gluten contents and gluten index than other samples (William, El-Haramein, Nakkoul, & Rihawi, 1986). Moreover, the sample has less development time by C3 roll removal as it has the lowest gluten value.

On the other hand, the time (minutes) that the upper point of the Farinogram curve reaches 500 line (time of arrival) until the upper point of the curve crosses the 500 line (exit time) is termed the dough resistance or durability. Dough stability is used more than other Farinogram indices to compare the strengths or weaknesses of different flours. However, as long as Farinogram curve remains on line 500, the gluten network acquires and maintains its viscoelastic properties and has good

performance properties (formability, mechanical strength tolerance and gas retention) (Peighambardoust, Van Brenk, Van der Goot, Hamer, & Boom, 2007). Given the significance and quality of gluten in this test, it is normal for the control sample to have a longer retention time than other samples (William *et al.*, 1986) because of the gluten content and gluten index. Moreover, the specimen with lower C3 roll removal has less stability time because it has the lowest gluten contents.

Dough softening degree

As the results indicate in the sample with C3 roll removal, the highest dough softening was observed at 10 and 20 min after the test, whereas the lowest was observed at the control sample at 10 and 20 min after the start of the test. Regarding this, Akbari Rad, Najafian, Esmailzadeh Moghadam, & Khodarahmi (2010) claimed that increasing the degree of softening indicates that with increase in gluten content, the dough strength increased and became looser. The later the dough loosens, the longer the dough evolves and the longer it stays. Considering the significance and quality of gluten in this test, it is normal for the control sample to have a lower degree of softening at 10 and 20 min after onset because of gluten content and gluten content than the other samples. Furthermore, the sample with C3 roll removal has a higher degree of softening of the dough at 10 and 20 min after the start of the test as it has the lowest gluten content.

Valorimetric value

The results showed the highest valorimetric value was in the control sample, whereas the lowest valorimetric value was observed in the sample with C3 roll removal. A value called the valorimeter is obtained using a special Farinograph ruler on a chart recorded by mechanical Farinographs to show the strength of the flour as a single number.

Given the importance and quality of gluten in this test, it is normal for the control sample to have a higher valorimetric value than other samples because of the gluten content and gluten index. Moreover, the sample with C3 roll removal has lower valorimetric value because it has lower gluten content.

Quantitative and qualitative evaluation of Barbari bread

According to the results of the previous tests, it was found that the flour sample prepared by removing the C3 reduction roll had the highest contrast with the control sample (without roll removal). By eliminating this roll, the physical and chemical properties of the flour and the rheological properties of the resulting dough are greatly weakened. Hence, as the texture and sensory evaluation are key parameters for baking products, especially traditional breads, Barbari bread samples obtained from the removal of C3 reduction roll and bread made with control flour (where no roll was removed) were compared.

Texture firmness

Comparing the firmness of the sample and control texture with removal of the C3 roll is presented in Table (5). As stated in the previous step, removing C3 roll drastically reduces the quality of flour, whereas the control sample where all rolls are in orbit has a higher overall quality. Hence, by comparing the firmness of Barbari bread with control flour and Barbari bread with C3 reduction roll removal at 2 and 72 h after baking, there was a significant difference at 5% level. It was seen that Barbari bread made from control flour had less firmness at both intervals. Previous studies clearly showed that flour prepared by removing C3 roll had higher starch content, coarse particle size and weaker mechanical rheological properties (Farinography) than control flour. Thus, given the significance of starch in the staling process, it is natural that the greater

the damage to this compound, the more amylopectin retrograde (starch back) occurs, the faster the staling will occur. Moreover, as the particle size of the flour in the sample is larger with the removal of C3 roll and thus with the ability to absorb less moisture, and according to the results of the Farinography section (water absorption part), expect the reduction of water absorption power in the sample by roll removal C3 is not far-fetched.

Table 5. Textural and sensory characteristics of Barbari bread prepared from control sample flour (without roll removal) and flour with C3 roll removal

Textural and sensory characteristics	Flour sample	
	Control (without roll removal)*	C3 sample with roll removal
Firmness (2 h after baking)	25.09±0.15 ^b	33.82±1.08 ^a
Firmness (72 h after baking)	42.21±1.26 ^b	58.67±1.43 ^a
Taste	4.18±0.12 ^a	3.87±0.16 ^b
Texture	4.56±0.08 ^a	3.42±0.12 ^b
Overall acceptance	4.63±0.16 ^a	3.80±0.21 ^b

* Mean numbers are three replications.

Similar letters in each row were not statistically significant at ($P < 0.05$).

Sensory properties

Tasting judges showed significant differences at 5% level when examining texture, taste and overall acceptance parameters between Barbari bread made from control flour and from C3 softener roll removal. Barbari bread produced from control flour had the highest scores in all three parameters (Table 5).

In sensory test to score a product texture, doughiness or abnormal softness, firmness, brittleness and excessive fragility cause the fracture points. Regarding the texture characteristics of the sensory evaluators, the control sample was smoother and uniform, which was expected with respect to the previous section (bread texture evaluation). When it comes to examining the taste of the product, it is critical to understand that taste is a combination of the two senses of

smell and taste. Hence, the taste depends on two main components, volatile combinations (aroma) and non-volatile, which are sensed by the taste buds on the tongue. Various factors in the nutrient may affect the release of volatile components plus taste. Most scholars argue that understanding the taste intensity and release of flavoring agents depends on the texture of the finished product (Baines & Morris, 1987). Boland, Buhr, Giannouli, & Van Ruth (2004) justify the cause of this event by various interactions between flavorings and texture structure. Thus, according to the studies and the results obtained from the textural evaluation, the control sample with a softer texture was predicted to have a better taste.

Conclusions

One of the weaknesses clear in mill factories in Iran is turning to the short line by eliminating a number of rolls to reduce energy consumption and costs. However, in a long line as there is more opportunity for production operations, the roll pressure is proportional to the input product and the output can be proportional to the sieve with arbitrary aggregation and conventional damaged starch and compensate for some of the weakness of incoming wheat. Some of the rolls have more effect on the quality, and the results of this study showed that the sample that had been removed in the grinding process of the C3 softener roll had the lowest gluten content, gluten index, Zeleny sedimentation volume, important qualitative in the baking process, was enjoyed. The rheological properties evaluation showed that by removing C3 roll, the water absorption, development time, stability time and valorimetric value of the dough reduced the most. Thus, the flour removed in the milling process, C3 roll, is most in contrast to the control sample. Additionally, the firmness of the sample of Barbari bread prepared from the control sample at both 2 and 72 h after baking was lower than that of C3 roll removal. Moreover, in the sensory

evaluation section, the control sample received a greater overall acceptance score. Thus, given the results of this study, it was found that by removing C3 reduction roll the physicochemical properties of flour, rheological properties of the dough and baking properties of the bread significantly weakened and this roll was superior to other soft rolls with the greatest effect on

the quality of flour, dough and finished product. Indeed, as the layered endosperm is converted into flour in the rolls, one can state that the best endosperm layer is separated by C3 reduction roll and this type of roll failure and being faulty has great effects on the quality of flour and finished product.

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تأثیر غلتک‌های نرم‌کننده طی فرایند آسیابانی بر خصوصیات فیزیکوشیمیایی آرد و ویژگی‌های رئولوژیکی خمیر و نان بربری

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چکیده

غلتک‌های نرم‌کننده در فرایند تولید آرد نقش مهمی دارند و از آنجایی که افزایش کیفیت آرد مورد استفاده در صنایع پخت به عنوان مهم‌ترین فاکتور در بهبود خصوصیات کیفی و تغذیه‌ای نان بشمار می‌آید، هدف از انجام این پژوهش ارزیابی تأثیر حذف غلتک‌های نرم‌کننده C1A، C1B، C2، C3 و C5 بر ویژگی‌های فیزیکوشیمیایی آرد و خصوصیات رئولوژیکی خمیر و انتخاب مؤثرترین غلتک در فرایند آسیابانی در یک طرح کاملاً تصادفی بود ($P \leq 0.05$). نتایج نشان داد که دو نمونه آرد که در فرایند آسیابانی آنها غلتک‌های C5 و C3 حذف شده بودند، از میزان رطوبت، خاکستر، اندازه ذرات کوچک‌تر از ۱۲۵ میکرون و نشاسته آسیب‌دیده کمتری برخوردار می‌باشند. همچنین نمونه‌ای که در فرایند آسیابانی آن غلتک C3 حذف شده بود از کمترین میزان گلوتن (۲۵ درصد)، اندیس گلوتن (۷۰)، حجم رسوب زلنی (۱۷ میلی‌متر) برخوردار بود. همچنین با حذف غلتک C3 جذب آب آرد، زمان توسعه، زمان پایداری و ارزش والوریمتری بیشترین کاهش را نسبت به نمونه شاهد نشان داد. بنابراین مشخص شد آردی که در فرایند آسیابانی آن، غلتک C3 حذف شده است، ویژگی‌های فیزیکوشیمیایی آرد و خمیر به شدت تضعیف گردید. در ادامه این پژوهش با آرد حاصل از این نمونه و نمونه شاهد نان بربری تولید شد و نتایج نشان داد که میزان سفتی بافت نمونه با حذف غلتک C3 نسبت به نمونه شاهد در هر دو بازه زمانی (۲ و ۷۲ ساعت پس از پخت) بیش از ۳۵ درصد افزایش یافت. همچنین در بخش ارزیابی حسی، نمونه شاهد امتیاز پذیرش کلی بیشتری را به خود اختصاص داد. بنابراین باتوجه به نتایج این تحقیق مشخص شد که حذف غلتک C3 نسبت به سایر غلتک‌های نرم‌کننده بیشترین تأثیر را بر کیفیت آرد، خمیر و محصول نهایی دارد.

واژه‌های کلیدی: خصوصیات رئولوژیکی، غلتک نرم‌کننده، فرایند آسیابانی، گندم، نان بربری

The Effect of Modified Atmosphere Packaging and Packaging Material on Walnut Kernel Shelf-life

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Abstract

Walnuts with essential and unsaturated fatty acids from omega-3 group, are considered as one of the most important dried fruits with nutritional value. Genotypes 25 and 29 (superior walnut genotypes) were selected to investigate the effect of modified atmosphere packaging on the beneficial compounds of their kernels oil. Walnut kernels were packaged under modified atmospheric conditions into a 90- μ m metalized plastic. After the oil cold extraction and purification of walnut kernels, the quantitative and qualitative composition of fatty acids was measured. The percent of fatty acids, aflatoxin content, peroxide index, iodine value, acidity and sensory evaluation of samples were determined in the 0-day and during one year storage. The results showed that the unsaturated fatty acids content are predominant in walnut kernels oil, and the linoleic acid is the dominating fatty acid. The saturated fatty acids content was less than 10%. The aflatoxin content of genotypes 25 and 29 were measured 0 and 5 ppb, respectively. The moisture content and the peroxide value were determined 1.55-4.32% and 0.48-4.65 meqO₂/kg, respectively. The highest level of acidity was observed in the 25 genotypes. The peroxide index, titratable acidity, and weight loss increased with the increasing of storage time, while the iodine value decreased. Totally, the packaged genotype 25 in the PA/PE/PA/PE/Aluminum foil films under modified atmospheric containing 5-6% O₂, 15% CO₂ and 79-80% N₂ is recommended due to the high quality of chemical and organoleptic properties and the lack of aflatoxin in the walnuts kernel.

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Keywords

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Packaging
Walnut kernel

Introduction

Given its climate, Iran is one of the most significant walnut planting centers in the world. Average annual production of walnuts in the world is 1.14 million tons and with more than 379000 tons Iran is the largest producer of walnuts in the world. The major walnut production centers in

Iran are Kerman and then Isfahan, Hamedan, and Khorasan Razavi. According to the Agricultural Census of 2017, the area under walnut cultivation in Kerman amounted to 18993 out of 195280 hectares and has the first position. Kerman walnut is high in quality dating back 5000 years. The average crop yield per hectare is

3.5 tons per hectare using mechanized methods (Ahmadi *et al.*, 2018).

Walnut, with scientific name *Juglans regia* L., is from Juglanaceae family: a multifunctional plant with various uses, such as walnut kernels. The high fat content (over 60%) of good quality protein, minerals, and vitamins in walnut kernels (Table 1) has made it fresh or dried for commercial value (Bayat & Mahjub, 2017). Most plant proteins are incomplete given the lack of essential amino acids, but walnut kernel proteins contain essential amino acids and are therefore classified as valuable animal proteins such as meat or eggs (Golzari, Rahemi, Hassani, Vahdati, & Mohammadi, 2013).

Table 1. Compounds in 100 g of raw walnut kernels

Composition		Value
Water		3.50 g
Protein		14.80 g
Fat		65.00 g
Starch		13.00 g
Minerals	Calcium	100.00 mg
	Phosphorus	380.00 mg
	Iron	3.00 mg
	Sodium	2.00 mg
	potassium	50.00 mg
Vitamins	Vitamin A	30.00 units
	Vitamin B ₁	0.35 mg
	Vitamin B ₂	0.12 mg
	Vitamin B ₃	1.00 mg
	Vitamin C	2.00 mg

Walnuts kernel has compounds called sterols naturally produced in plant compounds and are chemically similar to cholesterol. Some plant sterols are not absorbed along the digestive tract during the digestive process and block the cholesterol absorption pathway in the bloodstream. 100 g of walnut contains 150 mg of plant sterol. This substance has a significant role in the chemical and vascular protection of the body. Moreover, walnut has a flavonoid called ellagic acid that can block the growth of cancer cells (Stampar, Solar, Hudina, Veberic, & Colaric, 2006).

Walnut kernel is one of the dried fruits that is rapidly decayed by chemical and

microbial agents and the cause of spoilage is the presence of significant values of fat (about 64 to 71%) with unsaturated fatty acids like oleic acid, linoleic acid, linolenic acid and arachidonic acid and the oxidation problem always threatens fat (Tajeddin, 2004). Usually, the moisture content of the walnut harvested varies between 34-40% that decreases to 5% after the peeling and drying process. In long-term storage of kernels, the oxidation of lipids and hydrolysis reactions in monolayer water is at its lowest (Maskan & Karatag, 1997). If the moisture of walnut and its kernel reaches below the layer of monolayer water, the lipase enzyme is activated on its lipid and increases the peroxide value (Hamed, 2015). Using suitable coatings and packaging in modified atmosphere and appropriate temperature is essential to avoid this process (Yaman, 2004).

Raei, Sedaghat, Pourazarang, & Hashemi (2007) packed the pistachio and its kernels in crude polypropylene film with nitrogen and vacuum and then stored at 5 and 10 °C and ambient temperature at 65% relative humidity. The results showed that packaging in metallized film and five-layer film was effective in maintaining the quality of pistachio. At the end of 28 months storage at two mentioned temperatures, the fatty acids were reported as 0.6, 0.8 and 0.4%. At the end of storage, all samples except for vacuum packing contained a large value of insects and larvae and vacuum packing did not increase aflatoxin levels.

Ghanei Zare, Tavakolipour, & Elhamirad (2012) conducted a study on the evaluation of different types of packaging materials including cellophane, nylon, and metal cans combined with vacuum conditions for raw pistachios. The results showed that nylon, especially under vacuum, was more privileged than other treatments.

Sattar, Mohammad, Saleem, Jan, & Ahmad (1990) examined the effect of fluorescent light, gamma rays, and the type

of packaging on the oxidation of walnuts, almonds and peanuts. They stored these products at 25-40 °C for 200 days. The results showed that the oxidation rate increased with the use of fluorescent light and gamma rays. Moreover, glass and polyethylene packaging materials protected the product against oxidation.

The purpose of modified atmosphere packaging (MAP) is to increase the shelf life of perishable foods so that they can maintain the quality of freshness or almost freshness. Hence, MAP is a natural way to extend the life of products without using preservatives. On the other hand, hybrid packaging materials like multilayers improve the barrier features of packaging. For instance, with the presence of cardboard and plastic on the top wall of the beverage packaging, the cardboard provides stability and product protection while the plastic material prevents water vapor they provide the optimum for liquid materials (Stiles & Ooraikul, 1991).

According to the above points, for examining the viability of walnut kernels as one of the nutritious and valuable agricultural products of Iran, this product uses PA/PE/PA/PE composite film and aluminum foil, and modified atmosphere method for packaging and some of its important physical and chemical properties were evaluated during storage.

Materials and methods

Forty kg of walnuts were purchased from two genotypes 25 and 29 from Rabar (Walnut planting area of Kerman) 180 km south of Kerman. After peeling, it was dried in a cabinet dryer at 42 °C to 12% moisture contents. Walnuts were then removed from the dryer and stored at room temperature until 4-6% moisture contents and the kernels were prepared. Two types of 90 µm thick packaging films: a) cellophane (purchased from Freeman Company) and b) five metallized layers (polypropylene + polyethylene+ polyamide +polyethylene with aluminum layer) (Fireplace) were used. For packaging under

modified atmospheric conditions, two gas mixtures (2-3% O₂, 5-5% CO₂, and 92% N₂; 5-6% O₂, 15% CO₂, and 80-79%) were applied.

It should be explained that walnut kernel samples were packed with modified atmosphere in the five-layer film, and control samples in the conventional atmosphere with both types of films using the Hankelman Model A200. Additionally, the approximate weight of walnut kernel in each package was 200±5 g and 12 similar samples were prepared from each treatment.

After counting and coding, the samples were stored in a refrigerator at 10±1 °C and relative humidity 60% for one year. Once every three months, three replications of each sample were removed from the fridge and chemical tests were performed as follows. Sensory evaluation was done performed every three months, and aflatoxin contamination and fatty acid profiles were measured on walnut kernels samples at harvest.

Extraction of oil

The walnut kernel was first mixed with manual grinding and then mixed with 1 to 4 volumes of normal hexane. Extraction was done for 48 h in the dark and at ambient temperature with vigorous shaking intensities. The solvent was separated in an oven under vacuum at 40 °C.

Analysis and identification of fatty acids

Fatty acid composition of the oil sample was determined using HP-5890 gas chromatograph (Hewlett-Packard, USA) equipped with CP-FIL silica glass capillary columns, 60 m in diameter 0.22 mm, and ionic flame detector. Nitrogen was used as carrier gas at a flow rate of 0.75 mL/min. The oven was maintained at 198 °C and the injector and indicator at 25 °C.

The esterified fatty acids were in line with the methyl esters of fatty acids, created by intensive shaking of oil solutions in hexane (0.3 g/7 mL) with 2 mL methanol potassium hydroxide at 50

°C for 10 min. Methyl esters of fatty acids were identified using the above model gas chromatograph.

Iodine number

The iodine absorbed by one gram of fat is called iodine number. There is a relationship between the oxidation effect of fat and the iodine number. Oils with more double bonding are more rapidly oxidized under identical conditions and are more susceptible to oxygen degradation. Iodine number was calculated according to the fatty acid analysis (AOAC, 2005b).

Peroxide number

Li method was used to measure peroxide index. Hence, at first about 5 g of the extracted oil samples were weighed into a 250 mL Erlenmeyer flask and added to a 30 cm³ acetic acid-chloroform ratio of three to one. About 0.5 cm³ of saturated potassium iodide solution was added and stirred after shaking Erlenmeyer flask to dissolve the oil in the solvent. After 2 min, 30 cm³ of distilled water was added and titrated in the presence of starch reagent with 0.1 N sodium hyposulphite solution. The peroxide index was obtained from the following equation (Iranian National Standardization Organization [ISIRI], 2018).

$$\text{Peroxide number} = \frac{(a - b) \times N \times 1000}{M} \quad (1)$$

a=mL of sodium thiosulfate consumed as a sample

b=mL of sodium thiosulfate used as control

N=normality of thiosulfate used

M=weight of walnut oil in grams

Acidity

In an Erlenmeyer flask, first 20 cm³ of alcohol and 20 cm³ of chloroform were poured and neutralized in the presence of phenolphthalein reagent to measure the acidity. Then it was added to another Erlenmeyer flask containing 10 g of walnut oil titrated with 0.1 N NaOH, and the

acidity was obtained from the following equation after stirring to dissolve the oil (Hosseini, 1990).

$$\text{Acidity} = \frac{N \times 0.0282 \times 100}{M} \quad (2)$$

N=mL of one-tenth normal Sodium hydroxide

M=walnut oil weight

Moisture

A weighing plate and 15 g sample were added to measure moisture percent. Then, it was incubated with the oven for 70 h at 70 °C. The percentage of moisture was calculated from the following equation after fixing the weight by desiccator (AOAC, 2005a).

$$\text{Moisture percentage} = \frac{\text{dry sample weight} - \text{fresh sample weight}}{\text{fresh weight}} \times 100 \quad (3)$$

Sensory evaluation

The sensory evaluation test was used to evaluate sensory properties. In doing so, 25 people in the age group 15-45 years were selected. Hedonic test was administered after sufficient explanation of the observers' adherence to the test points. The samples were incubated at ambient temperature for 12 h prior to the test to reach temperature equilibrium. The traits examined and their definitions for walnut color, taste or bitterness, firmness, appearance (including pest-infestation rate), and walnut kernel uniformity ranged from very poor (score 1) to very good (score of 100) (Piggott, Simpson, & Williams, 1998).

Aflatoxin

Aflatoxin content of walnut kernel packed of the samples was measured by HPLC device in three replications. In doing so, 75 g of each sample was mixed with a mixer (model Hobart VCM 40, USA) for a full three minutes and after several steps of separation with 5% sodium chloride solution, the mixture of acetonitrile and water (84:16), methanol and water ratio

(80:20) and trifluoroacetic acid, aflatoxin content using Chromspher C18 column reverse phase with 5 μ m particle size and 46×100 mm (Chrompack cat. No. 28264) injection rate 20 μ L was measured. Solvent flow rate of 0.5 mL/min and aflatoxin values were used as controls according to B1, B2, G1 and G2 standards (Cheraghali *et al.*, 2007).

Statistical analysis of data

Statistical analysis was done as split plot factorial based on randomized complete block with two factors of packing type in two levels and storage time in three levels with three replications.

Results and discussion

Walnut characteristics and its changes during storage were analyzed in SPSS17 and data were compared using Duncan's multiple range test (Table 2).

According to the results of variance analysis, the effect of treatment, storage time and their interaction on peroxide, moisture, iodine, and humidity were at 1%

significance level, but the effect of replication on these traits was insignificant. Table (2) shows the effect of treatments on the indices. As the shelf life of the peroxide number increased, the acidity of the titer and the flux increased, but the iodine number reduced significantly (Table 3).

As Table (3) shows, the rate of changes in the peroxide value of walnut kernel samples varied from 1.55 to 4.32 during one year of storage. Of the two genotypes used in the project, genotype 29 had less peroxide content with 92% CO₂ and 5-6% O₂ packaging, and the referees rated the genotype more favorable. The moisture content of the samples varied from 0.48 to 4.65%. The highest acidity was observed in walnut cultivar 25. Walnut kernel peroxide index increased during storage. This is in line with the results of Sattar *et al.* (1990). However, there were no changes in the peroxide index in the first six months. There was also a slight change in the second six months.

Table 2. Comparison of the average effect of using modified atmosphere on the walnut kernel indices of the two genotypes

Row	Applied treatments	Peroxide Index (mEq oxygen per kg)	Moisture (%)	Titration acidity (%)	Iodine number (g/100 g oil)	Weight loss (%)
1	Genotype 29 with 5-6% O ₂ and 15% CO ₂	1.55±1.24 ^{ab}	4.18±20 ^{bc}	0.69±0.83 ^a	54.00±2.70 ^{abc}	0.00±0.05 ^a
2	Genotype 29 with 2-2% O ₂ and 6-5% CO ₂	2.44±1.49 ^{ab}	3.29±1.97 ^{bc}	0.86±0.92 ^a	56.9±2.75 ^{bc}	0.00±0.05 ^a
3	Genotype 25 with 5-6% O ₂ and 15% CO ₂	2.82±1.68 ^{ab}	4.60±2.15 ^c	0.11±0.33 ^a	119.70±3.30 ^d	0.00±0.05 ^a
4	Genotype 25 with 2-3% O ₂ and 6-5% CO ₂	2.56±1.60 ^{ab}	4.10±2.00 ^{bc}	0.11±0.33 ^a	116.90±3.20 ^d	0.01±0.09 ^a
5	Control 29 in the five-layer film	4.32±2.00 ^b	4.10±2.00 ^{bc}	4.32±0.35 ^a	99.60±3.16 ^c	0.69±0.38 ^b
6	Control 29 in ordinary film	4.02±2.00 ^b	4.60±2.01 ^{bc}	0.12±0.34 ^a	35.70±2.44 ^a	1.69±1.30 ^{bc}
7	Control 25 in the five-layer film	4.20±2.05 ^b	3.00±1.75 ^{ab}	0.22±0.47 ^b	73.80±2.90 ^c	1.47±1.21 ^{bc}
8	Control 25 in ordinary film	4.26±2.06 ^b	2.50±1.57 ^a	0.28±0.35 ^b	47.90±2.63 ^{ab}	2.82±1.68 ^c

The non-similar letters in each column show the difference in the significance level for that trait.

Table 3. Comparison of the average effect of storage time on the indices of two genotypes of walnut

Row	Storage time	Peroxide Index (mEq oxygen per kg)	Moisture (%)	Titrate acidity (%)	Iodine number (g/100 g oil)	Weight loss (%)
1	Three months storage	1.47±1.21 ^a	4.00±2.00 ^b	0.08±.28 ^a	82.28±3 ^b	0.20±0.45 ^a
2	Six months storage	1.65±1.28 ^a	4.10±2.01 ^b	0.12±0.35 ^b	84.30±3.01 ^c	0.25±0.5 ^a
3	Nine months storage	4.79±2.19 ^b	4.10±2.01 ^b	0.11±0.33 ^b	68.00±0.33 ^a	1.50±1.22 ^b
4	12 months storage	5.10±2.26 ^b	3.04±1.74 ^a	0.25±215 ^c	67.70±0.50 ^a	1.40±1.18 ^b

The non-similar letters in each column show the difference in the significance level for that trait.

As shown in Fig. (1), walnut kernel of genotype 29 in five-layer film PA/PE/PA/PE and aluminum foil was superior over control sample in five-layer film. Genotype 25 had a higher peroxide value than genotype 29. Peroxide values were lower in the gas filled samples than in the control samples. This is in line with the results of (Mexis, Riganakos, & Kontominas, 2011). Control samples kept in PA/PE/PA/PE film and aluminum foil had the same number as those preserved in cellophane film.

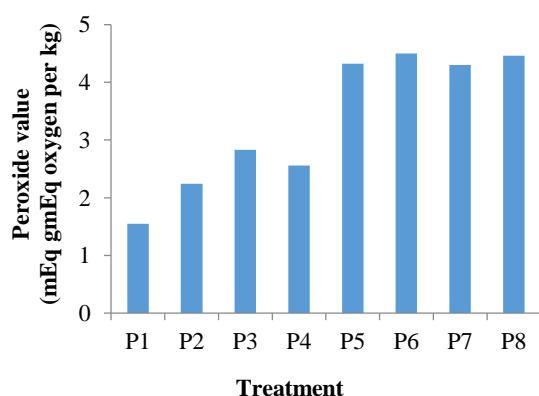


Fig. 1. Peroxide value of two walnut kernel genotypes with treatments applied during one year of storage

(P1=genotype 29 in five-layer film with 2-3% O₂, 6-5% CO₂ gas and 92% N₂ gas, P2=genotype 29 in five-layer film with 5-6% O₂, 15% CO₂ gas and 79-80% N₂ gas, P3=genotype 25 in five-layer film with 2-3% O₂, 5-6% CO₂ gas and 92% N₂ gas, P4=genotype 25 in five-layer film with 5-6% O₂, 15% CO₂ gas and 79-80% N₂ gas, P5=control, genotype 29 with five-layer film, P6=control, genotype 29 with cellophane film, P7=control, genotype 25 with five-layer film, P8=control, genotype 25 with cellophane film, P8=genotype 25 with cellophane cover)

Fig. (2) shows the iodine number changes. The lowest number of iodine

number was observed in the cellophane (conventional) film, and the highest in the walnut kernel genotype 25. In other words, this genotype has more unsaturated fatty acids. This is in line with the results of (Raee *et al.*, 2007) on cover under modified atmospheric conditions on pistachio kernels. The iodine number decreased during the shelf life, which is in line with the results of (Escobar, Estevez, & Guíñez, 2000).

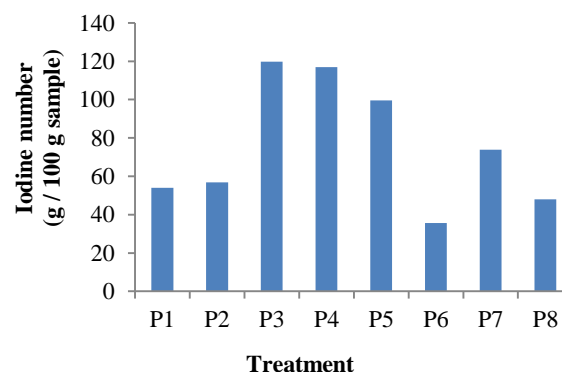


Fig. 2. Iodine number in walnut kernel genotype with treatments applied during one year of storage

(P1=genotype 29 in five-layer film with 2-3% O₂, 6-5% CO₂ gas and 92% N₂ gas, P2=genotype 29 in five-layer film with 5-6% O₂, 15% CO₂ gas and 79-80% N₂ gas, P3=genotype 25 in five-layer film with 2-3% O₂, 5-6% CO₂ gas and 92% N₂ gas, P4=genotype 25 in five-layer film with 5-6% O₂, 15% CO₂ gas and 79-80% N₂ gas, P5=control, genotype 29 with five-layer film, P6=control, genotype 29 with cellophane film, P7=control, genotype 25 with five-layer film, P8=control, genotype 25 with cellophane film, P8=genotype 25 with cellophane cover)

Edible fats, both animal and vegetable, have certain and minor values of free fatty acid, but may exceed the permissible limit due to spoilage and hydrolysis factors. Hence, measuring the acidity of oil is a way to show its

spoilage. As Fig. (3) shows, the lowest fat content was related to genotype 25 and the highest to genotype-29 spoilage in conventional film. As the storage life increased, the rate of spoilage increased as well.

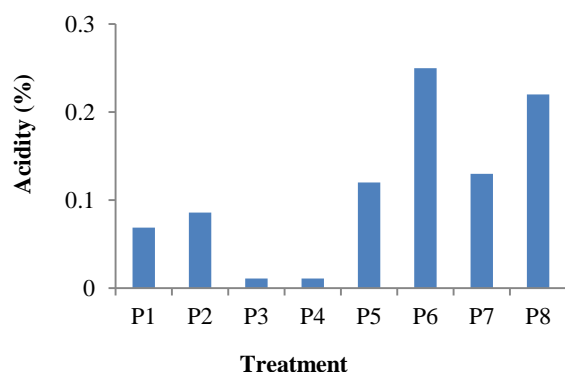


Fig. 3. Acidity percent of two walnut kernel genotypes with treatments applied during one year of storage

(P1=genotype 29 in five-layer film with 2-3% O₂, 6-5% CO₂ gas and 92% N₂ gas, P2=genotype 29 in five-layer film with 5-6% O₂, 15% CO₂ gas and 79-80% N₂ gas, P3=genotype 25 in five-layer film with 2-3% O₂, 5-6% CO₂ gas and 92% N₂ gas, P4=genotype 25 in five-layer film with 5-6% O₂, 15% CO₂ gas and 79-80% N₂ gas, P5=control, genotype 29 with five-layer film, P6=control, genotype 29 with cellophane film, P7=control, genotype 25 with five-layer film, P8=control, genotype 25 with cellophane film, P8=genotype 25 with cellophane cover)

During drying the fruits using hot and dry air, their moisture reduces and reaches about 4-6%. In this process, temperature and humidity must be controlled to prevent thermal damage to the fruit. The moisture percentage of walnut kernels should not exceed 6 and less than 2. If it is more than 6, conditions are provided for the growth of fungi and bacteria, and if it is lower than 2, oxidation of fats in walnuts will take place.

Based on the results, the composition of the inlet gas, the type of packaging and the storage time had a significant effect on moisture content, but the effect of repetition was not significant (Table 2). The moisture content of the samples over time can be due to the low range of relative humidity changes in the storage

location and the effectiveness of the impermeable plastic bags in controlling the moisture content during the storage period. On the other hand, walnut kernels contain a high amount of fat, proteins and peptides can react with lipid and affect membrane resistance and ultimately prevent water from leaving the product (Cline & Press, 1990). Fig. (4) shows the moisture changes.

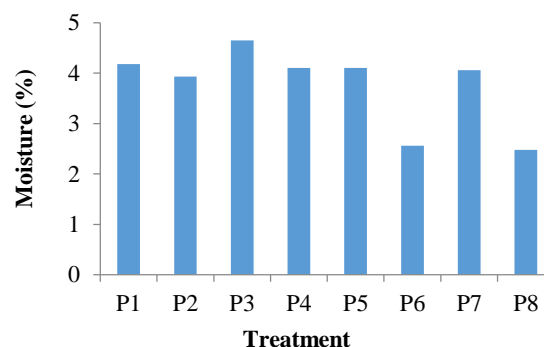


Fig. 4. Changes in moisture percentage of two walnut kernel genotypes with treatments applied during one year of storage

(P1=genotype 29 in five-layer film with 2-3% O₂, 6-5% CO₂ gas and 92% N₂ gas, P2=genotype 29 in five-layer film with 5-6% O₂, 15% CO₂ gas and 79-80% N₂ gas, P3=genotype 25 in five-layer film with 2-3% O₂, 5-6% CO₂ gas and 92% N₂ gas, P4=genotype 25 in five-layer film with 5-6% O₂, 15% CO₂ gas and 79-80% N₂ gas, P5=control, genotype 29 with five-layer film, P6=control, genotype 29 with cellophane film, P7=control, genotype 25 with five-layer film, P8=control, genotype 25 with cellophane film, P8=genotype 25 with cellophane cover)

According to the results, walnut kernel weight loss was insignificant (at zero level) until six months after storage. Within six months, it reached 1.4 and 1.6% in 12 months. Among the treatments applied, the highest weight loss was in the control sample kept in cellophane film (Fig. 5). Five-layer films with packaging under modified atmospheric conditions could reach zero weight loss. Weight loss was observed in the control samples kept in the five-layer film but it was lower than in the cellophane films.

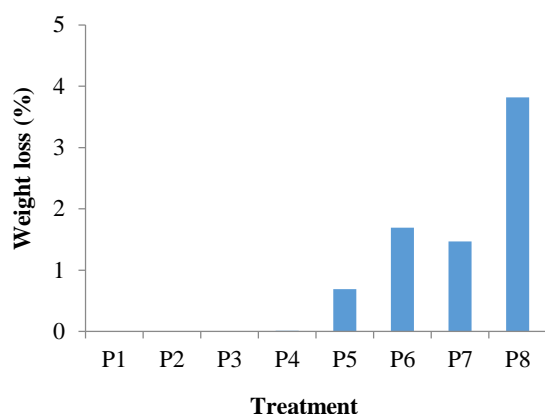


Fig. 5. Weight loss of two walnut kernel genotypes and the effect of treatments applied on it

(P1=genotype 29 in five-layer film with 2-3% O₂, 6-5% CO₂ gas and 92% N₂ gas, P2=genotype 29 in five-layer film with 5-6% O₂, 15% CO₂ gas and 79-80% N₂ gas, P3=genotype 25 in five-layer film with 2-3% O₂, 5-6% CO₂ gas and 92% N₂ gas, P4=genotype 25 in five-layer film with 5-6% O₂, 15% CO₂ gas and 79-80% N₂ gas, P5=control, genotype 29 with five-layer film, P6=control, genotype 29 with cellophane film, P7=control, genotype 25 with five-layer film, P8=control, genotype 25 with cellophane film, P8=genotype 25 with cellophane cover)

According to Table (4), it was clarified that linoleic acid is the dominant fatty acid in walnut oil. Oleic acid, linolenic acid, palmitic acid, and stearic acid were in the next ranks. Amaral, Casal, Pereira, Seabra, & Oliveira (2003) in Portugal found six genotypes of Mayette, Marbot, Franquette, Lara, Mellanaise, Parisenne, dominant fatty acid, linoleic acid. Genotype 25 had significantly higher linoleic acid content compared to sample 29. The unsaturated fatty acids in genotype 25 were lower. The average unsaturated fatty acids in the two mentioned genotypes were 90.85% and saturated fatty acids less than 10%. The average of unsaturated fatty acids with a double bond was 29.37% and the average of unsaturated fatty acids with multiple double bonds 30.73%. This was in line with the results of Ozkan & Koyuncu (2005).

Table 4. The results of fatty acids composing two fatty acid genotypes

Walnuts	Linoleic acid (%) C2: 18 ω6	Linolenic acid (%) C3: 18 ω3	Oleic acid (%) C1: 18 ω9	Palmitic Acid (%) C0: 16	Stearic acid (%) C0: 18	Extraction of total oil (%)
Genotype 29	47.55	11.82	30.91	5.72	2.48	65.40
Genotype 25	50.24	13.30	27.82	6.27	2.36	71.00

The sensory test results of walnut kernels are shown in Tables (5) and (6). According to the results of analysis of variance, the effect of repetition, treatment and holding time on color, taste, at 1% level, and the interaction effect of treatment and storage time at 5% level were significant. The effect of repetition on tissue firmness, appearance and uniformity of kernel was significant at 5% level and the interaction between treatment and storage time was insignificant. The best color score for the

control sample was obtained in a five-layer film and walnut kernel with modified atmosphere (5-6% O₂, 15% CO₂ and 79-80% N₂). The quality of these treatments was positive in measuring the chemical composition. In terms of taste, firmness, which control genotype 25 in conventional film, gained the lowest score. In terms of kernel uniformity, the genotype had 25 points lower because of the difficulty in getting the kernel out of the bony skin.

Table 5. Comparison of the average effect of modified atmosphere applied on kernel sensory properties of two walnut genotypes

Row	Applied treatments	Color	Taste	Tissue firmness	Appearance	Kernel uniformity
1	Genotype 29 with 5-6% O ₂ and 15% CO ₂	78.83±2.98 ^{bc}	79.44±2.00 ^{bc}	81.10±3.10 ^b	78.21±2.97 ^{bc}	79.6±2.97 ^b
2	Genotype 29 with 2-2% O ₂ and 6-5% CO ₂	78.34±2.97 ^{abc}	84.44±3.10 ^{bc}	82.10±3.15 ^b	79.70±2.98 ^b	78.45±2.97 ^b
3	Genotype 25 with 5-6% O ₂ and 15% CO ₂	79.60±2.98 ^{bc}	83.17±3.20 ^c	82.65±3.10 ^b	81.90±3.10 ^b	81.75±3.00 ^c
4	Genotype 25 with 2-3% O ₂ and 6-5% CO ₂	81.41±3.00 ^c	81.33±3.00 ^c	79.60±2.98 ^b	79.00±2.97 ^{bc}	80.60±2.99 ^b
5	Witness 29 in the five-layer film	82.14±3.10 ^c	80.42±3.10 ^{bc}	81.10±1.30 ^b	82.35±3.20 ^c	79.54±2.98 ^b
6	Witness 29 in ordinary film	81.47±3.20 ^c	80.00±2.99 ^{bc}	81.40±3.10 ^b	81.16±3.10 ^c	77.50±2.96 ^{ab}
7	Witness 25 in the five-layer film	74.98±2.98 ^{ab}	74.79±2.90 ^{ab}	76.98±2.96 ^a	74.10±2.39 ^a	75.85±2.95 ^a
8	Witness 25 in ordinary film	72.12±2.90 ^a	71.90±2.90 ^a	73.37±2.92 ^a	70.80±2.90 ^a	72.25±2.90 ^a

The non-similar letters in each column show the difference in the significance level for that trait.

Table 6. Comparison of the average effect of modified atmosphere use on kernel sensory properties of two walnut genotypes

Row	Storage time	Color	Taste	Tissue firmness	Appearance	Kernel uniformity
1	Three months after storage	84.15 ^c	85.35 ^b	85.52 ^b	84.89 ^b	84.83 ^a
2	Six months after storage	83.84 ^c	85.33 ^b	74.2 ^b	84.85 ^b	84.86 ^a
3	Nine months after storage	74.86 ^b	73.15 ^a	85.45 ^a	73.53 ^a	83.9 ^a
4	Twelve months after storage	69.97 ^a	70.82 ^a	85.52 ^a	69.51 ^a	84.22 ^a

The non-similar letters in each column show the difference in the significance level for that trait.

As the storage life of walnut kernels increased, color and taste decreased, but its tissue firmness increased. The reason is the decrease in walnut kernel moisture with increase in storage time. Appearance decreased and no changes were made to the kernel uniformity. Walnut kernels in this study had a significant difference with the taste of walnut kernels in the market and gained a higher score. Thus, it is desirable to use a five-layer film and a gas composition of 5% O₂ and 15% CO₂. Anyway, the storage time reduces the taste (Table 6).

According to Table (5), control genotypes 25 and 29 had the lowest score in conventional film. The highest score is for samples stored in the five-layer film with or without modified atmosphere. The best color for the control sample was in five-layer walnut film with modified atmosphere (5-6% O₂, 15% CO₂ and 79-80% N₂). At genotype 25, the samples had better color relative to genotype 29, with the main reason as the later harvest date of

genotype 29. These results show that care must be taken in the harvest date. When 85% of the walnut green skin has a cleft, it had to be harvested. Packed samples with 5-6% O₂, 15% CO₂, and 79-80% N₂ showed better appearance than other samples. Genotype 25 gained more scores. As the shelf life increased, the appearance of the properties decreased. Regarding the firmness of the tissue and the uniformity of the kernel, no changes were observed in the stored samples. No significant differences were seen between the treatments and the control samples for uniformity (Table 6).

Genotypes 29 were contaminated with aflatoxin and aflatoxin levels were determined at 5 parts per billion. No aflatoxin contamination was seen in genotype 25. This shows that besides controlling cracking of hard walnut skin, improper harvesting date also results in the growth of aflatoxin-producing fungi in walnut kernel samples.

Conclusions

Walnut is considered as one of the most important dried fruits given its nutritional value due to its essential and unsaturated fatty acids. Maintaining this nutritional value during maintenance was one of the goals of the project. In doing so, we examined the important physicochemical properties of two walnut kernel genotypes through its MAP, and by using five-layer

PA/PE/PA/PE composite packaging materials and aluminum foil. The results showed that unsaturated fatty acids in walnut oil was higher than saturated fatty acids and was the dominant fatty acid in linoleic acid. Overall, genotype 25 packaged in five-layer film containing 5-6% O₂, 15% CO₂ and 79-80% N₂ is recommended due to lack of aflatoxin and desirable chemical and sensory properties.

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تأثیر بسته‌بندی با اتمسفر اصلاح‌شده و نوع ماده بسته‌بندی بر ماندگاری مغز گردو

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چکیده

گردو با داشتن اسیدهای چرب ضروری و غیراشباع گروه امگا-۳، به‌عنوان یکی از مهم‌ترین خشکبار از نظر ارزش غذایی مطرح است. ژنوتیپ‌های ۲۵ و ۲۹ (ژنوتیپ‌های برتر گردو) با هدف بررسی تأثیر بسته‌بندی به روش اتمسفر اصلاح‌شده بر ترکیبات سودمند موجود در روغن گردوی آنها، انتخاب شدند. مغز گردوها تحت شرایط اتمسفر اصلاح‌شده داخل پلاستیک پنج‌لایه متالیزه با ضخامت ۹۰ میکرون بسته‌بندی گردیدند. بعد از استخراج روغن به روش سرد و خالص‌سازی آن، ترکیب کمی و کیفی اسیدهای چرب تشکیل‌دهنده اندازه‌گیری شد. میزان اسیدهای چرب، مقدار آفلاتوکسین، عدد پراکسید، عدد یدی، اسیدیته و ارزیابی حسی نیز در روز صفر و طی یک‌سال نگهداری تعیین گردید. نتایج نشان داد میزان اسیدهای چرب غیراشباع نسبت به اسیدهای چرب اشباع بیشتر بوده و اسید لینولئیک، اسید چرب غالب است. مقدار اسیدهای چرب اشباع نیز کمتر از ۱۰ درصد تعیین شد. میزان آفلاتوکسین در ژنوتیپ ۲۵ و ۲۹، به‌ترتیب صفر و ۵ قسمت در بلیون برآورد گردید. میزان تغییرات رطوبت و اندیس پراکسید نمونه‌های مغز گردو طی یک‌سال نگهداری، به‌ترتیب حدود ۴/۳۲-۱/۵۵ درصد و ۴/۶۵-۰/۴۸ میلی‌اکی‌والان اکسیژن بر کیلوگرم متغیر بود. بیشترین اسیدیته در مغز گردوی رقم ۲۵ مشاهده شد. با افزایش زمان نگهداری، عدد پراکسید، اسیدیته قابل تیتروفت و وزن افزایش، و عدد یدی کاهش یافت. به‌طورکلی، ژنوتیپ ۲۵ مغز گردوی بسته‌بندی‌شده در فیلم پنج‌لایه با اتمسفر اصلاح‌شده حاوی ۶-۵ درصد اکسیژن، ۱۵ درصد دی‌اکسیدکربن و ۸۰-۷۹ درصد ازت، به‌دلیل نداشتن آفلاتوکسین و خواص شیمیایی و حسی مطلوب، قابل توصیه است.

واژه‌های کلیدی: اتمسفر اصلاح‌شده (MAP)، بسته‌بندی، پلی‌پروپیلن متالیزه‌شده، خواص حسی و شیمیایی، مغز گردو

The Effect of Orange Peel Oil on Physicochemical, Microbiological and Sensory Properties of Plum Fruit Roll Ups

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Abstract

By increasing consumer awareness about food safety and quality, there is a high demand for the preservative (synthetic)-free foods and use of natural products as preservatives. Plants are the main source of antimicrobials and contain many essential oils that have preservation effect against different microorganisms. The effect of natural orange peel oil on the chemical and microbiological and sensory properties of plum fruit roll ups was investigated during 15 days. The result of the Tensile strength tests plum fruit roll ups showed that adding orange peel to plum fruit roll ups significantly ($P<0.05$) increased the tensile strength. At the end of storage of all samples, with orange peel oil the lowest pH content and highest acidity level were observed in the sample with 0.5% orange peel oil and no mold and yeast were observed in any of the samples. The sample with 0.5% had a maximum amount of lactic acid bacteria and the sample with 0.1% orange peel oil had a minimum amount of lactic acid bacteria. The results showed that sample with 0.5% orange peel oil had minimum number of total counts of bacteria and Control sample had a maximum amount of total counts of bacteria. The analysis of variance did not show any significant difference in plum fruit roll ups. According to the results of the analysis of variance, the highest texture, taste and aroma score belonged to the control sample. From the results, it can be deduced that due to the antimicrobial properties of orange peel essence and its positive effects on tissue characteristics, it can be used as a natural preservative in the plum fruit roll ups.

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Keywords

Antimicrobial properties
Fruit oil ups
Orange peel oil
Plum

Introduction

This specie belongs to *Rosaceae* family that comprises other plants that also produce edible fruits such as *peach* (*Prunus persica*), cherries (*Prunus cerasus* and *Prunus avium*) and apricot (*Prunus insitia*) (Stephen, 1983). Thought the original application of plums is for direct

utilization, there are many other applications for it in the culinary and industry, such as in the production of plum juice, plum puree, mixtures with cereal or even ground meat, and for the expansion of products that replace fat in baking (Stacewicz-Sapuntzakis, Bowen, Hussain, Damayanti-Wood, & Farnsworth, 2001).

Plums are also used as a therapy for diverse illnesses. Recent studies also showed clinical document of its activity in the remedy of constipation, osteoporosis, hypertension and dyslipidemia (Stacewicz-Sapuntzakis *et al.*, 2001). It was also shown that fibres from plums lowered plasma and liver lipids (Tinker, Davis, & Schneeman, 1994) and perhaps were the maximum important ingredients responsible for satiety boost by plums use (Farajian, Katsagani, & Zampelas, 2010). Carbohydrates are the principal macronutrient in plums, and include 62.7% of their whole weight (Stacewicz-Sapuntzakis *et al.*, 2001). Despite this numerous content and their importance for some biological functions attributed to plums, up to now, just the presence of a xyloglucan was reported in plums. Essential oils (EO's) and extracts taken from plenty plants have recently achieved a great amicability and scientific concern. Phenolic compounds present in essential oils have been identified as bioactive ingredients with antimicrobial activity. Maximum plant phenolic compounds are classified as generally identified as Safe (GRAS) substances, so they could be used to inhibit growth of multitude pathogenic and spoilage microorganisms in foods (Burt, 2004; Nedorostova, Kloucek, Kokoska, Stolcova, & Pulkrabek, 2009). However, EOs antimicrobial efficacy in foods is usually achieved at higher concentrations, which many times entail a sensory impact, caused by altering the natural taste and/or odor of the food by exceeding the passable flavor and/or odor thresholds (Nazer, Kobilinsky, Tholozan, & Dubois-Brissonnet, 2005). A small number studies have been published regarding prohibition of microorganisms by the vapor-phase generated by EO's (Inouye, Uchida, Maruyama, Yamaguchi, & Abe, 2006; López, Sánchez, Batlle, & Nerín, 2007; Nielsen & Rios, 2000; Suppakul, Miltz, Sonneveld, & Bigger, 2003), pointing out that EOs applied in

vapor phase could be impressive against foodborne pathogens and spoilage microorganisms at relatively lower concentrations than when applied in liquid phase, therewith causing less effect on sensory properties (Tyagi & Malik, 2011). López *et al.* (2007) established the antifungal activity of Mexican oregano EO by vapor contact on *Aspergillus flavus*. Farhat *et al.* (2011) reports that orange peel accounts for nearly 45% of the total bulk with considerable content of it available as a product after orange processing that make environmental problems, exclusively water pollution, due to the presence of biomaterials such as EO, pectin, and sugars. Citrus spp. EO's are present in plentiful quantities and it is known that can have an antimicrobial result against both bacteria and *fungi* (Chanthaphon, Chanthachum, & Hongpattarakere, 2008; Jafari *et al.*, 2011). Orange peel involves of epidermis covering the exocarp consisting of irregular parenchymatous cells, which are wholly enclosing many glands or oil sacs (Lin, Sheu, Hsu, & Tsai, 2010).

The present study was undertaken to determine the potential of various peel oil orange as an antimicrobial agent against microorganisms such as mold and yeast and lactic acid bacteria and total counts of bacteria; an attempt to formulate as natural food preservatives.

Materials and methods

Fruit materials

Fresh plums were collected from a garden in Shahmirzad, Semnan, Iran, and transported directly to the laboratory for tests. Fruits were selected according to their size, colour and appearance, discarding the ones with defects and physiological disorders. Then, the selected plums were sanitized with chlorinated water (200 ppm sodium hypochlorite) for 3 min and left to get dry at room temperature for about 1 h. Plums later got boiled and were filtered out. Further the peel and core were deleted. Later, according to the

formulations, the orange peel oil was added to the plum puree. This content, then, has been distributed on a tray and left to be dried in the sun.

Preparation of orange peel oil

Orange oil has been acquired from orange peels by the process of steam distillation (Harbone, 1998), using the Clevenger apparatus (Pyrex UK). The peels were placed in the round bottom flask and filled with water to about three quarter full. This flask was connected to distillation apparatus. Water was filled into the trap arm in order for the oil to condense on the surface of the water. The heating mantle supplied the necessary heat and as the water in the flask boiled, steam containing the volatile orange oil got into the neck of the flask and condensed on the layer of water in the graduated trap arm. The distillation procedure was continued up to point where there was no more diversity in continuous readings of the oil volume. The process then followed by draining off the oil which was successively dehydrated over anhydrous sodium sulphate (BDH). The density of the oil was distinguished according to the weight: volume ratio (Ayoola *et al.*, 2008)

Sampling

For all tested factors, determinations were performed on 1 and 15 days of storage at Laboratory temperature, on four separate samples in three replicates.

Formulations of plum fruit roll ups

Fruit roll ups (~100 g) were prepared with plum puree. Four samples were prepared according to following formulations:

A control sample, one with 0.1% peel oil orange, second with 0.2% peel oil orange, and the last one with 0.5% peel oil orange (Table 1). For all tested factors, determinations were performed on 1 and 15 days of Laboratory temperature storage on four separate samples in three replicates.

Table 1. Formulation for plum fruit roll ups made in the present study

Ingredients (g)	Fruit 1	Fruit 2	Fruit 3	Fruit 4
plum powder	100	100	100	100
Orange peel oil	0	10	20	50

Fruit 1: control fruit; Fruit 2: with 0.1% orange peel oil; Fruit 3: with 0.2% orange peel oil; Fruit 4: with 0.5% orange peel oil.

After mixing the material, the fruit roll ups were shaped into plates. These roll ups were placed in laminated bags and left to be used and investigated after 1, 15 days of storage at Laboratory temperature. From each sample three replicates were made.

Microbial tests

For microbial tests, a 10 g sample of fruit roll ups was aseptically weighted. Next samples were homogenized with 90 mL of a sterile solution of 0.1% (w/v) peptone water (Razi serum), for 2 min at 20-25 °C in a Masticator blender (Pause International, Iran), thus making a 1:10 dilution. Serial 10-fold dilutions were set by mixing 1 mL of the earlier dilution with 9 mL of 0.1% (w/v) sterile peptone water. Lactic acid bacteria were counted using duplicate 1 mL volumes of suitable dilutions in overlaid pour plates of MRS agar, incubated inverted at 30 °C for 3 days. For yeast and mold counts, duplicate 0.2 mL volumes of suitable dilutions were spread on to the dried surface of pre-poured plates of yeast glucose chloramphenicol agar (YGC, Quelab, Canada), which were incubated at 25 °C for 5 days. Eventually, total viable counts were specified with using 1 mL of suitable dilutions on pour-plates of plate count agar (Quelab, Canada) incubated at 32 °C for 3 days. After incubation, plates with 30-300 colonies were counted. The microbiological data were transformed into logarithms of the number of colony forming units (CFU/g).

pH determination

The pH of the samples was measured after homogenization with distilled water at a 2:8 ratio using a digital pH meter (3510 pH Meter, Jenway, England).

Ash determination

Ash percentage was computed by weight loss experimented by the sample (5 g) maintained in a muffle furnace (Carbolite RWF1200, Hope Valley, England) into a porcelain capsule at 700 °C until constant weight.

(1)

$$\text{Ash \%} = \frac{\text{Plant weight empty} - \text{Plant weight of ash}}{\text{Weight of sample}} \times 100$$

Tissue measurement

All texture measurements were undertaken using an Instron materials testing machine (Model Testometric, Rochdale, England) and 4 individual fruit per treatment were chosen for each of the three tests.

Brix measurement

10 g sample of the plum fruit roll ups that were homogenized in 50 mL of distilled water; the mixture was filtered and 50 mL of the filtered mixture were taken to Brix, using a Mettler automatic Tritator (Model KRUS, Optronic, Germany). TSS was measured directly from the filtered residue, using an Abbe digital refractometer (E-Inginst Electron Corp., USA) and expressed as Brix.

Sensory analysis

The sensory panel evaluation was conducted with 30 panelists selected between members of the students in the Department of Agriculture, Azad University of Damghan at day 15 of storage. The casing was removed and then, samples were cut in slices of approximately 4 mm thickness and finally samples were grilled at 170° C for 10 min and served on white plastic dishes. Samples were separately. A quantitative descriptive analysis (QDA) was used for evaluating aroma, taste, textural, overall acceptability. A seven-point hedonic

scoring scale (7: excellent; 6: very good; 5: good; 4: moderate; 3: slightly bad; 2: bad and 1: very much bad) was employed for evaluation of burgers. Water was used to clean the palates and remove residual flavours, at the beginning of the session and in between samples

Statistical analysis

All data were analyzed using the General Linear model of ANOVA with treatment and time as factors, all statistical analyses were conducted by use of the SPSS statistical package (SPSS 16.00), after normality and homogeneity of variances were confirmed. Differences between means were determined by the least remarkable difference test, and significance was well-defined at ($P < 0.05$) (with Duncan's Multiple Range Test).

Results and discussion

pH values

Changes of pH values during the 15-days storage period are presented in Fig. (1). In the total sample after 15 days of storage at laboratory temperature, pH values increased. This is associated mostly with increase of Gram-negative bacteria populations (Verma & Sahoo, 2000), such as *Enterobacteriaceae* and *Pseudomonads*, as well as yeasts and molds, which cause protein and amino acid degradation, resulting in formation of ammonia and consequent pH increase (Nychas, Drosinos, & Board, 1998). At the end of 15 days storage of samples in laboratory temperature, sample with the 0.5 orange peel oil (4.01) had the maximum and control sample (3.41) had the minimum pH values. Lower values of pH indicate that some fermentation occurs during storage of these products, although no sugars are usually added to our samples with orange peel oil. Carbohydrates contained in fruits, could be used as substrates for LAB metabolism, resulting in production of organic acids and lower values of pH (Papadima & Bloukas, 1999).

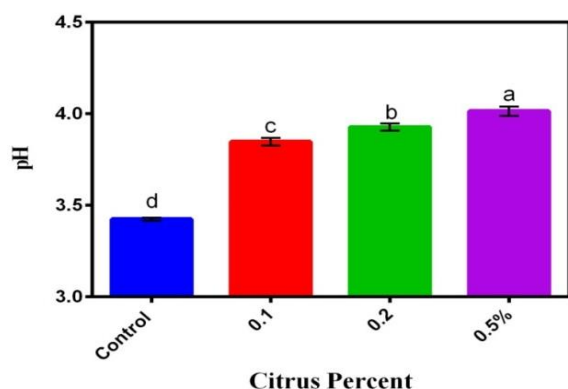


Fig. 1. Effect of orange peel oil on pH values of the experimental fruit roll ups

Acidity

Due to the fact that the acidity and pH levels have inverse correlation, results of variance analyses for acidity at the end of storage (15 days) showed that the sample with 0.5% orange peel oil had the minimum and control sample had the maximum level of acidity (Fig. 2).

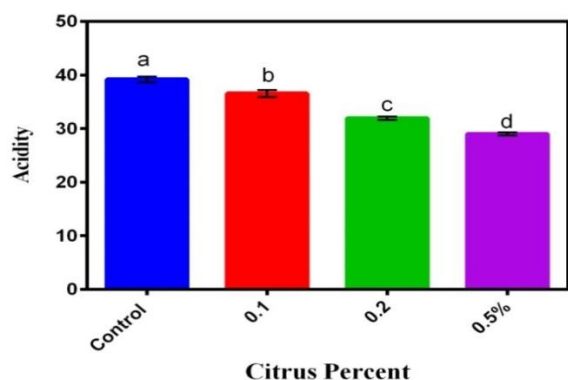


Fig. 2. Effect of orange peel oil on acidity values of the experimental fruit roll ups

Microbiological analysis

Lactic acid bacteria

Results related to the microbiological analyses of the samples of fruit roll ups with orange peel oil during the 15-days storage period are presented in Fig. (3). The counts of all determined microbiological indicators were significantly ($P<0.05$) affected by the addition of the natural antimicrobial and especially samples with 0.2% orange peel oil. All microbial groups increased in the control fruit roll ups. Increasing trends of different extents were also observed in

samples of the remaining treatments for total viable counts, lactic acid bacteria (LAB), yeasts and molds. According to the results of variance analysis for counts of lactic acid bacteria (LAB), values were significantly affected ($P<0.05$) by concentration and time of storage. Comparison of the data showed that sample with 0.1% orange peel oil (2.36) showed the lowest counts of lactic acid bacteria (LAB) and the control sample and sample with 0.5 orange peel oil showed the highest count of lactic acid bacteria (LAB). Subba, Soumithri, & Rao (1967) also showed inhibitory effect of essential oils of orange and lemon oil examined on bacteria and fungi in nutrient media. Orange oil was observed to be extra effective antimicrobial agent than lemon oil. All other conditions being identical, 2000 ppm of orange oil had result on all the Gram-positive cultures tested, containing the spores of *Bacillus subtilis*.

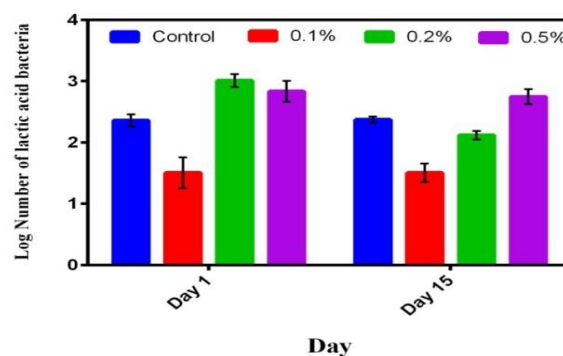


Fig3. Effect of orange peel oil on Log number of lactic acid bacteria of the experimental fruit roll ups

Total viable count

According to results of variance analyses for counts of total viable count, values were significantly affected ($P<0.05$) by kind, concentration and time of storage (Fig. 4). At the end of storage (15 days), sample with 0.5% orange peel oil showed the lowest and control sample highest total viable count of microbial load. Citrus essential oils have been reported to contain antibacterial activities against *Salmonella typhimurium*, *E. coli* O157:H7, *Listeria monocytogenes*, *Escherichia coli* and

Vibrio vulnificus in media (Kim, Marshall, & Wei, 1995) and to *S. typhimurium* on fish cubes (Kim *et al.*, 1995).

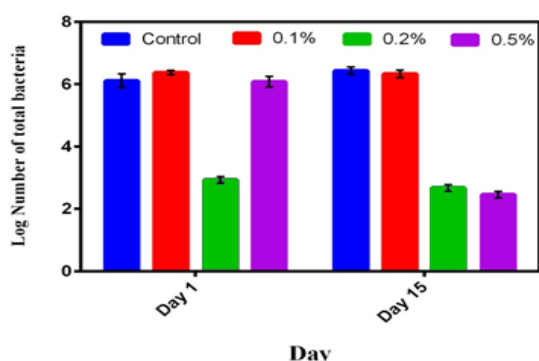


Fig.4. Effect of orange peel oil on Log number of total bacteria of the experimental fruit roll ups

Yeast and Molds

According to the results of variance analysis for count of yeast and mold, least number of yeast and molds was found in all of the samples with orange peel oil (Table 2). Most number of yeast and molds were found in control (6.41). Essential oils of lemon, orange, and bergamot were also illustrated to contain bactericidal effect against *Campylobacter jejuni*, *E. coli* O157:H7, *L. monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus* (Fisher & Phillips, 2006) and *Acrobacter butzlei* (Fisher, Rowe, & Phillips, 2007) in media and on foods. In addition, antifungal actions against *Penicillium digitatum*, *Penicillium italicum* and yeast, *Saccharomyces cerevisiae* have been reported (Caccioni, Guizzardi, Biondi, Renda, & Ruberto, 1998). Those reports also illustrated little water solubility of citrus essential oil could be overcome by mixing it with an emulsifier (Fisher & Phillips, 2006; Kim *et al.*, 1995; Kim & Shin, 2004).

Table 2. Effect of storage time on Log yeast and mold for fruit roll ups treatment

Sample	Day 1	Day 15
Control sample	1.33±0.75 ^a	1.33±0.75 ^a
Sample with 0.1% orange peel oil	N/A	N/A
Sample with 0.2% orange peel oil	N/A	N/A
Sample with 0.5% orange peel oil	N/A	N/A

Ash analysis

According to the results of variance analyses, values of ash were significantly not affected ($P<0.05$) by kind, concentration and time of storage. No significant change was observed in the fruit fly ash and the numbers are not much different. High levels of ash represents the material is inappropriate, in this experiment, the amount of ash is normal (Fig. 5).

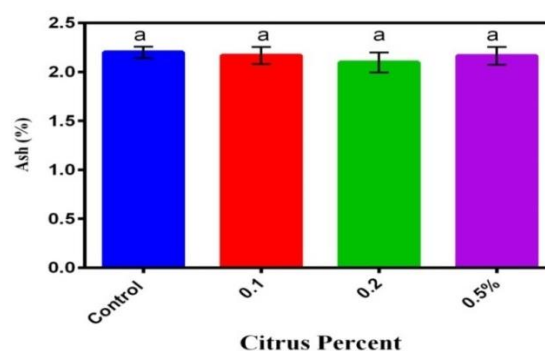


Fig. 5. Effect of orange peel oil on ash values of the experimental fruit roll ups

Mechanical properties of fruit roll ups

Quality evaluation of fruit roll ups

Textural properties may serve as an index of maturity or process ability to the food processor and of eating quality to the purchaser. Fruit roll ups produce. Thickness within the test sample: Control sample: 0.63; second sample: 0.52; third sample; 0.47 and the fourth sample was 0.37 mm. Method: The samples were cut in size 1 to 10 cm like tape, the samples were placed between two probes. Turn the machine, the probes were far apart. Expressed at high speed and until the sample is rupture, the amount of elasticity and the force required to rupture the Newton calculated.

Tensile strength (TS)

The results of measuring of the tensile strength of tissue for fruit roll ups acquired by measuring apparatus shown in Fig. (6). According to the results of variance analysis, added orange peel oil to plum fruit significantly ($P<0.05$) decreased the tensile strength (TS).

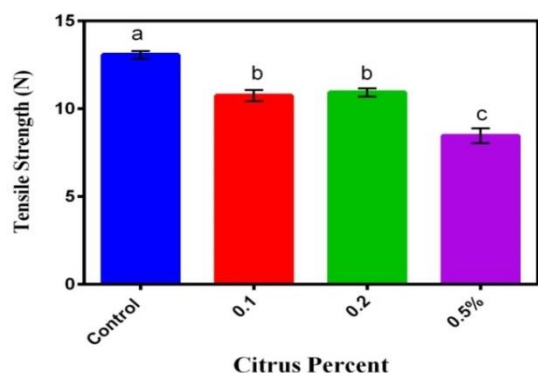


Fig. 6. Effect of orange peel oil on TS values of the experimental fruit roll ups

Elongation test (EB)

The results of measuring the elongation of tissue for fruit roll ups acquired by measuring apparatus shown in Fig. (7). According to the results of variance analysis, orange peel oil added to plum fruit roll ups significantly ($P>0.05$) increased the elongation. According to the statistics mentioned above, the maximum tensile strength of tissue belonged to the sample 1 (13.37) followed by sample 2 with 0.1% orange peel oil (10.92), sample 3 with 0.2% orange peel oil (10.77) and sample 4 with 0.5% orange peel oil (8.86). However, the results acquired for elongation of tissue changes reversely, which leaves us with the following results: sample 4 with 0.5% orange peel oil (26.17), sample 3 with 0.2% orange peel oil (23.12), sample 2 with 0.1% orange peel oil (22.47) and sample 1 which has no density of orange peel oil and is considered as control sample (19.10). Measured the elongation of tissue for kiwifruit roll up sample. According to the results this characteristic of the kiwifruit roll up was significantly lower than all the other samples. This is probably caused by the type, tissue and density of the fruit used in the formulation of the industrial fruit roll ups. Moreover, the collation of TS results showed that the tensile of tissue for the Kiwifruit sample is significantly higher

than other samples. This is perhaps a result of the tissue in this type of fruit roll ups, in which the texture of the Kiwifruit prevents the roll ups to be completely torn apart immediately after the rupture is applied. (Azeredo, Brito, Moreira, Farias, & Bruno, 2006) analyzed the elongation of tissue for the seedless pomegranate and pomegranate roll ups. The results showed that elongation of tissue is significantly lower for the seedless pomegranate sample than the normal pomegranate. This showed that normal pomegranate texture prevents the immediate rupture of the roll ups.

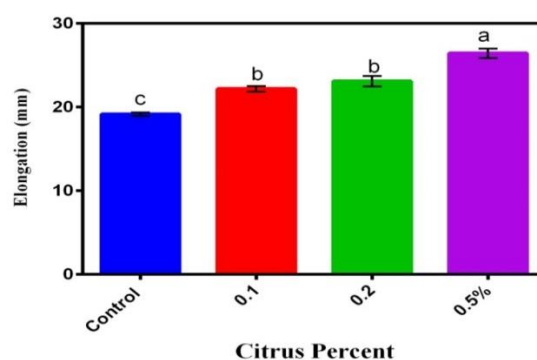


Fig. 7. Effect of orange peel oil on EB values of the experimental fruit roll ups

Sensory evaluation

You can see in Table (3), most tasting for the control sample (5.93) and sample with 0.2 orange peel oil (5.77). The highest rating was for the texture of the control sample (5.53). According panelist news, lowest score of the tissue belonged to the samples containing 0.5% orange peel oil. According to variance analysis, highest rating was related to the odour of the control sample (5.93) and the lowest scores belonged to samples with 0.5 orange peel oil. According to variance analysis, the highest rating was for the overall acceptance was for the control sample (5.69) and lowest score of the overall acceptance was for samples containing 0.5% orange peel oil (4.03).

Table 3. Sensory evaluation of various parameters fruit roll ups on the 15 day of temperature Laboratory.

	Control sample	Sample with 0.1% orange peel oil	Sample with 0.2% orange peel oil	Sample with 0.5% orange peel oil
Texture	5.53 ±0.78 ^a	5.33±0.06 ^b	5.37±0.68 ^b	5.17±0.26 ^c
Flavour	5.93 ±0.25 ^a	5.31±0.07 ^b	5.77±0.57 ^b	4.27±0.48 ^c
Adour	5.93 ±0.25 ^a	5.80±0.48 ^b	5.37±0.25 ^c	4.03±0.65 ^d
Overall acceptance	5.69 ±0.66 ^a	5.00±0.36 ^b	4.77±0.74 ^b	4.03±0.92 ^c

Conclusions

The results of the present study demonstrate the effectiveness of orange peel oil, added on microbial growth inhibition, and shelf life extension of fruit roll ups of the during temperature laboratory storage for 15 days. Samples

with orange oil peel, which showed the best results, could have a valuable potential for commercial use in order to improve preservation of these products without using other additives. Therefore, using orange peel oil as natural preservatives in plum fruit roll ups is suggested.

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تأثیر اسانس پوست پرتقال بر ویژگی‌های شیمیایی، میکروبی و حسی لواشک آلو

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چکیده

امروزه با افزایش آگاهی مصرف‌کنندگان از ایمنی و کیفیت مواد غذایی، تقاضا برای استفاده از مواد غذایی بدون نگهدارنده (مصنوعی) و حاوی افزودنی‌های طبیعی افزایش یافته است. گیاهان یکی از منابع اصلی ترکیبات ضد میکروبی و حاوی اسانس‌های روغنی می‌باشند که دارای تأثیرات محافظت‌کنندگی در برابر میکروارگانیسم‌ها هستند. در این پژوهش اثر اضافه کردن اسانس پوست پرتقال بر ویژگی‌های شیمیایی، میکروبی و حسی لواشک آلو طی دوره ۱۵ روزه مورد بررسی قرار گرفت. نتایج نشان داد که افزودن اسانس پوست پرتقال به‌طور معنی‌داری ($P < 0.05$) باعث کاهش استحکام کششی و افزایش مقاومت به کشش لواشک آلو گردید. در انتهای دوره نگهداری ۱۵ روزه لواشک آلو بالاترین مقدار pH و پایین‌ترین مقدار اسیدیته در نمونه حاوی ۰/۵ درصد اسانس پوست پرتقال مشاهده گردید. در هیچ کدام از نمونه‌های لواشک آلو حاوی اسانس پوست پرتقال کپک و مخمر مشاهده نگردید. نمونه حاوی ۰/۵ درصد اسانس پوست پرتقال دارای بالاترین میزان باکتری اسیدلاکتیک و نمونه حاوی ۰/۱ درصد اسانس پوست پرتقال کمترین میزان باکتری‌های اسیدلاکتیک را نشان دادند. نتایج حاصل از این پژوهش نشان داد که نمونه لواشک حاوی ۰/۵ درصد اسانس پوست پرتقال کمترین و نمونه شاهد بیشترین باکتری‌های کلی را دارا بودند. نتایج تجزیه واریانس، اختلاف معنی‌داری را در خاکستر لواشک‌ها نشان نداد. بیشترین امتیاز بافت، مزه و آروما مربوط به نمونه شاهد بود. پذیرش کلی نمونه شاهد و نمونه پوست پرتقال ۰/۱ درصد طعم آلو بیشترین بود. به ترتیب در این دو نمونه طعم پرتقال بیشتر حس می‌شد. باتوجه به خاصیت ضد میکروبی اسانس پوست پرتقال و تأثیرات مثبت آن در ویژگی‌های بافتی می‌توان از آن به عنوان یک نگهدارنده طبیعی در فراورده لواشک آلو استفاده کرد.

واژه‌های کلیدی: آلو، اسانس پوست پرتقال، خاصیت ضد میکروبی، لواشک

Predicting the Qualitative Changes of Silver Carp Packed in Vacuum with the Help of Response Surface Method

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Abstract

In this study, central composite design was used to predict microbial changes, volatile nitrogen basic (TVB-N), sensory analysis and also the freshness factor of silver carp (*Hypophthalmichthys molitrix*) fillet packed in vacuum at 0, 6 and 12 °C for 5, 10 and 15 days optimized for the state-Ease Design Expert software. There was a significant difference between the treatments at time and temperature in the fillets of silver carp ($P<0.05$). The two parameters of Inosine monophosphate (IMP) and inosine (HxR) showed a decreasing trend with increasing time and temperature ($P<0.05$). Freshness and sensory analyses were in optimal mode in short time (5 days). In this test, the predicted values were consistent with a satisfactory percentage of 78%. The observations showed a good correlation between the results obtained by the experimental method and the predicted values by the statistical method. The models studied had R^2 and R^2 -adj coefficients of approx. 1, indicating that the experimental model used was able to predict fillet quality changes with low error percentage. The success of this can improve kinetic models in the food industry, so its application can help sustain food products.

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Keywords

Hypophthalmichthys molitrix
Quality changes
Response surface methodology
Vacuum packaging

Introduction

Silver carp is one of the most important freshwater species (FAO, 2016) that its fillet, like other aquatic products, is susceptible to corruption due to enzymatic activities, microbial growth, and enzymatic reactions (Zhu, Luo, Hong, Feng, & Shen, 2013). Therefore, storage methods should increase shelf life of the food products. Vacuum packaging limits oxygen access that is the main factor for aerobic bacteria growth, keeps the moisture at an appropriate level, and prevents undesirable pollutants from the external environment (Genç, Esteves, Aníbal, & Diler, 2013).

A large body of information exists about the effect of vacuum packaging on many fish species such as Atlantic Herring (Özogul, Taylor, Quantick, & Özogul, 2000), carp (Křížek, Vácha, Vorlová, Lukášová, & Cupáková, 2004), and salmon (Dondero, Cisternas, Carvajal, & Simpson, 2004), but information about the prediction of the effect of packaging at refrigerator temperature on increasing the shelf life of fillet is very limited (Tsironi, Dermesonlouoglou, Giannakourou, & Taoukis, 2009; Wu, Wang, Luo, Hong, & Shen, 2014).

In addition to packaging, the use of kinetic models is an efficient managerial model that facilitates quality control of aquatic products over the storage period. On the other hand, many studies have shown that by using kinetic models, it is not possible to obtain satisfactory results to predict the quality of many species such as golden carp (Yao, Luo, Sun, & Shen, 2011) and ordinary carp (Bao, Zhou, Lu, Luo, & Shen, 2013), because these models have high deviations. Therefore, more studies are needed to promote the efficiency of the kinetic models (Liu, Qin, & Luo, 2016). The residual errors that indicate the difference between real and predictable values exist in these studies. However, most of these models cannot consider these errors in calculations (Tascikaraoglu & Uzunoglu, 2014). The difference between the logistic model and Arrhenius was studied by Bao *et al.* (2013) to investigate the qualitative changes in the ordinary carp in cold conditions. They concluded that both models are suitable for this purpose and the relative error between the observed values and the predictive values was 5%. The application of the hybrid model to predict qualitative changes and shelf life of the bighead carp was done by Liu *et al.* (2016). They evaluated K-value changes and microbial load at different temperatures and confirmed that this model is suitable to predict qualitative changes of fillet. Hong, Regenstein, & Luo (2017) investigated the predictive qualitative model to study the bighead carp shelf life at different temperatures and introduced it as an efficient tool to predict the freshness index in the temperature range of 3 to 15 °C.

To reduce the negative effects of errors, a hybrid model based on accuracy-error technique is proposed to generalize it in food models according to the mathematical models. Since this study is conducted recently and no similar study is conducted in Iran, the purpose of employing a hybrid model according to

error-accuracy technique was to predict some of qualitative changes in the fillet of the silver carp packaged under vacuum.

Materials and methods

Fish preparation

Several silver carps (*Hypophthalmichthys molitrix*) with the initial weight of 1200 ± 2.25 g were prepared from fish farms and transferred to the microbial and chemical laboratory of livestock and poultry in Sari. The fish were cleaned and their organs were removed. Three rectangular fillets with a dimensions of 5×15 cm were selected for each treatment and after vacuum packaging (NPR 200, Iran) at 0, 6, and 12 °C, they were stored for 5, 10, and 15 days, respectively. During storage in the ice, temperature was measured by using digital thermometer and temperature change was ± 1 °C (Liu *et al.*, 2016). To regulate temperature, ice was used and almost every day, ice was added to compensate for the melting ice and keep the temperature constant. In Table (1), the treatments used in this study are presented.

Table 1. Treatments to predict qualitative changes of the silver carp fillet packaged under vacuum

Treatment	Temperature	Day
1	0	5
2	6	5
3	12	5
4	0	10
5	6	10
6	12	10
7	0	15
8	6	15
9	12	15

Qualitative analysis of fillets

Sensory analysis

Sensory analysis was carried out by 20 trained men and women in the age groups of 25 to 27 years. Texture, taste, odor, and color of the samples were investigated with a 9-point Hedonic Scale with slight changes (Nirmal & Benjakul, 2011).

Microbial analysis

Total live bacteria count was done according to Standard 2325 (Iranian National Standardization Organization [ISIRI], 2016). For this purpose, 5 g of the sample with 45 mL of distilled water were transferred to the sterile stomacher bag and homogenized. Then, the solution was diluted to 10^5 mL. 1 mL of each dilution was poured into the pallet containing count agar medium. After several minutes, all pallets were placed in incubator for 48 h at a temperature of 37 °C. Bacteria were counted on days 5, 10, and 15.

Measurement of the freshness index and the related products

The freshness index and the related products were extracted by using the method proposed by Liu *et al.* (2016). 1 g of the muscle tissue was cut and solved with 2 mL of perchloric acid (10%) and mixed with 4000 rpm at a temperature of 4 °C for 5 min. After removing the supernatant, the precipitates were washed with 2 mL of perchloric acid and centrifuged with 4000 rpm at a temperature of 4 °C. This process was repeated for two times. All liquids were mixed and by using NaOH solution (1 mol/mL and 10 mol/mL) they reached 6.35-6.45 pH. Then, they were mixed with 3000 rpm for 3 min. The supernatant was removed and adjusted with 10 mL of perchloric acid (6.40 pH). The resulted solution was filtered by 0.22 μ m filter paper and stored at a temperature of -18 °C. Adenosine triphosphate (ATP), Inosine monophosphate (IMP), HxR (Inosine), and Hypoxanthine (Hx) values included adenosine triphosphate, inosine-5-monophosphate, inosine, and hypoxanthine, respectively (Liu *et al.*, 2016). The essential oil compounds were obtained by using chromatography machine (Agilent Technologies-7890A, USA) connected to mass spectrometry (Agilent Technologies-5975C, USA) with HP-5MS capillary column.

$$K_{value} (\%) = \frac{HxR + Hx \times 100}{ATP + ADP + AMP + IMP + HxR + Hx} \quad (1)$$

Measurement of TVB-N

Measurement of volatile nitrogen basic (TVB-N) was done by Automatic Kjeldahl Analyzer (K1100 Hanon, China). Then, 10 g of the sample was transferred to a 500 mL balloon. Also, 2 g of magnesium oxide was added as catalyzer and finally, 300 mL distilled water was added for dilution. In the next step, 25 mL of boric acid 2% was added to it. After system installation, the Kjeldahl machine was turned on and heated for 45 min until the solution inside the Erlenmeyer became yellow. Then, it was titrated with normal sulfuric acid 0.01 and became purple (AOCS, 1994).

$$TVB-N \text{ (mg/100 g)} = \text{Sample (mL)} - \text{Titrazol content of the control sample} \times 4.1 \times 100 \quad (2)$$

Data Analysis

The resulted data were analyzed by SPSS and State-Ease Design Expert V7 and Central Composite Design (Alpha = 1). The relationship between variables and the results of the tests was obtained as a linear approximate polynomial fitting model (i.e. linear equation, dual cross factor, second-order). Model appropriateness was investigated using analysis of variance and the quality was investigated by coefficient of explanation (R-Squared: R^2).

Results and discussion

Investigating the qualitative changes of silver carp fillet packaged under vacuum

Microbial load parameters, K-value, and its related parameters and TVB-N in the fillet of the silver carp packed under vacuum showed a significant difference in nine treatments ($P < 0.05$) and with increased time from 5 to 15 days and increased temperature from 0 to 12 °C, these parameters showed an increasing trend ($P < 0.05$). Accordingly, the highest microbial parameters value, VB-N, ATP,

and Hx were measured in day 15 and a temperature of 12 °C. It should be noted that adenosine-5'-diphosphate (ADP) and IMP values showed negligible values in all treatments and did not show any effect on the freshness index equation. 2 parameters of IMP and HxR showed a decreasing trend with increased time and temperature ($P<0.05$). Accordingly, in

both parameters, the lowest values were measured in day 15 and a temperature of 12 °C ($P<0.05$). Scores given to the sensory parameter showed a significant decrease over time and with increased storage temperature. Qualitative changes of the silver carp fillet packaged under vacuum are shown in Table (2).

Table 2. Investigating the qualitative changes of the silver carp filler packaged under vacuum

Treatment	Microbial load (Log CFU/g)	TVB-N (mg/100 g)	ATP ($\mu\text{mol/g}$)	IMP ($\mu\text{mol/g}$)	HxR ($\mu\text{mol/g}$)	Hx ($\mu\text{mol/g}$)	K (percentage)	Sensory index
1	3.58±0.10	9.43±0.25	0.16±0.007	7.18±0.09	4.46±0.14	0.13±0.02	54.42±2.77	9.23±0.16
2	4.51±0.27	10.59±0.07	0.23±0.01	6.13±0.02	3.18±0.09	0.16±0.01	52.04±1.95	8.80±0.78
3	4.87±0.29	18.33±1.09	0.29±0.02	3.23±0.17	2.30±0.07	1.30±0.07	89.64±3.46	6.05±0.56
4	4.44±0.26	11.73±0.15	0.20±0.007	6.31±0.04	4.15±0.06	0.41±0.00	65.03±1.42	5.88±0.18
5	8.38±0.04	18.00±0.78	0.25±0.007	4.28±0.04	2.61±0.05	0.73±0.02	66.33±1.03	5.49±0.89
6	8.70±0.11	31.47±1.11	0.43±0.02	1.45±0.24	1.59±0.01	2.71±0.04	95.62±0.41	5.21±0.13
7	5.44±0.11	13.10±0.66	0.23±0.007	5.35±0.16	3.58±0.12	0.70±0.10	70.46±2.02	4.35±0.21
8	9.56±0.06	22.25±0.14	0.27±0.007	1.18±0.04	2.21±0.05	2.58±0.03	77.47±3.13	4.23±0.17
9	10.56±0.12	42.07±0.62	0.59±0.02	0.65±0.13	1.48±0.05	3.33±0.31	98.04±0.86	3.91±0.33

Modeling

According to the results of analysis of variance of the effect of different factors including temperature and time on total microbial load and total volatile nitrogen of the fillets under vacuum, F-index is the ratio of model mean square to residual mean squares and its values for total microbial load and total volatile nitrogen

were 154.81 and 411.95, respectively (Fig. 1). These large values show that variance is larger than random error. According to Table (3), the fitted model is completely significant ($P<0.01$). Lack of goodness of fit test was not significant ($P>0.05$) that shows that employed model can show the data process very well.

Table 3. Analysis of variance of factors affecting the fillet quality of the silver carp

Source	df	Mean of squares							Sensor y index
		Microbial load	TVB-N	ATP	IMP	HxR	Hx	Freshness index	
Model	5	49.98**	975.09**	0.15**	44.86**	18.10**	11.98**	4637.30**	435.16**
A-Radiation	1	25.42**	562.60**	0.084**	29.97**	15.19**	8.48**	4242.03**	350.68**
B-Time	1	15.20**	262.28**	0.027**	14.88**	2.53**	1.76**	384.99**	77.62**
AB	1	1.31**	105.78**	0.13**	0.23	0.039	0.82**	9.83	5.44*
A ²	1	5.47**	43.97**	0.018*	0.19	0.33**	0.92**	0.044	0.70
B ²	1	0.35*	3.62*	1.847E-003	0.49	8.403E-003	1.469E-003	0.13	0.72
Residual	7	0.45*	3.31	0.13	3.32	0.23	0.46	88.33	11.19
Lack of Fit	3	0.37 ^{ns}	2.11 ^{ns}	9.086E-003 ^{ns}	2.90 ^{ns}	0.09 ^{ns}	0.40 ^{ns}	26.96 ^{ns}	7.86 ^{ns}
Pure Error	4	0.079	1.20	3.880E-003	0.42	0.14	0.06	61.37	3.33
Cor Total	12	50.43	978.41	0.17	48.17	18.33	12.44	4725.36	446.35

*Significance at 0.05% **significance at 0.01 ns: non-significance

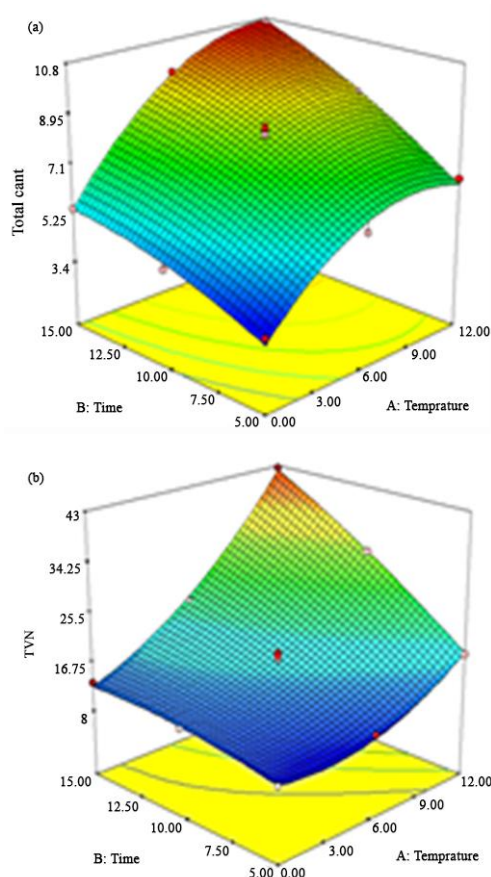


Fig. 1. The effect of input variables on changes a) total microbial load b) total volatile nitrogen

The results of analysis of variance of ATP production are presented in Table (3). The linear effect of temperature with high F-value (45.36) at a confidence level of 99% influenced ATP production. The linear parameter of time with lower curvature slope relative to temperature influenced ATP production ($P<0.01$). Also, the interaction effect of temperature and time and the power effect of temperature at a confidence level of 95% influenced ATP production ($P<0.05$). Fig. (2a) shows the 3D space of the effects of temperature and time on ATP production. According to the figure, increased temperature and time increased ATP production (Eq. 3).

(3)

$$Y_{ATP}=0.23+0.12A+0.067B+0.058AB+0.081 A^2$$

In adenosine monophosphate test, the linear parameter of temperature and time at a confidence level of 99% influenced

inosine monophosphate ($P<0.01$). Fig. (2b) indicates that temperature with higher curvature than time influenced IMP production, so that at lower temperatures, higher inosine monophosphate existed in fillets (Eq. 4).

(4)

$$Y_{IMP}=3.97-2.24A-1.58B$$

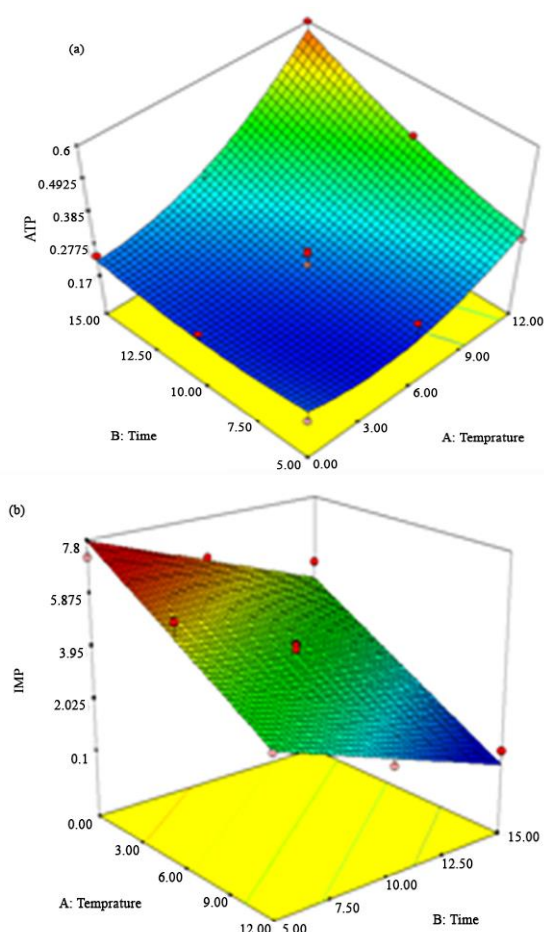


Fig. 2. The effect of input variables in a) ATP production b) IMP production in the fillet of the silver carp under vacuum

According to the response surface test output, the linear parameter of temperature and time significantly influenced inosine production in fillets ($P<0.01$) (Table 2). Also, temperature at a confidence level of 95% influenced inosine production that indicates the effective role of temperature in inosine production in fillets. In this response, the effect of time on inosine production was not significant ($P>0.05$). According to Fig. (3a), the temperature factor line was more effective in inosine

production with a higher slope. This figure indicates that fillets under vacuum at lower temperature had shorter time and higher inosine production (Eq. 5).

$$Y_{\text{HXR}} = 0.69 + 1.01A + 0.85B + 0.52A^2 \quad (5)$$

The results of the analysis of variance for inosine production in fillets under vacuum are presented in Table (3). In this test, the linear parameter of time at a confidence level of 99% ($P < 0.01$) with F-value of 30.85 had a significant effect on fillet hypoxanthine production. Also, the linear parameter of time with lower slope influenced fillet hypoxanthine production under vacuum. Fig. (3b) indicates that at higher temperature and time, the maximum hypoxanthine level existed in muscles (Eq. 6).

$$Y_{\text{HX}} = 0.69 + 1.01A + 0.85B + 0.52A^2 \quad (6)$$

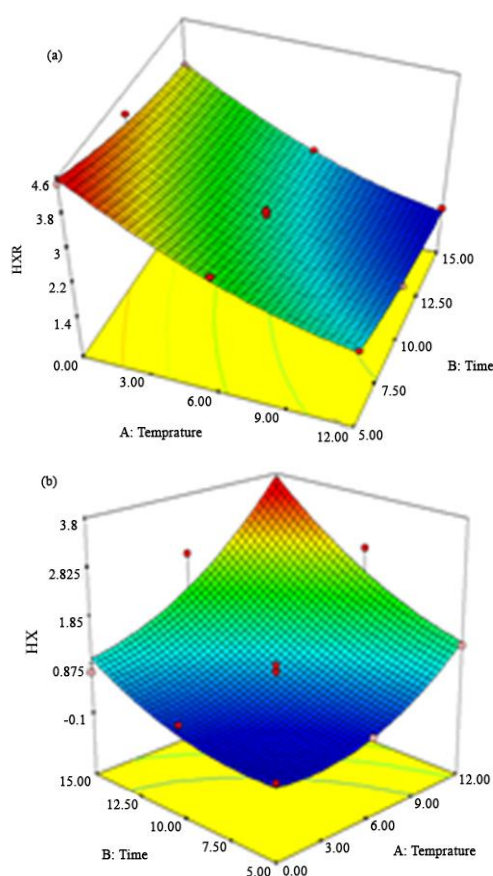


Fig. 3. The effect of input variables on a) HxR production and b) Hx production in fillets under vacuum

Changes in freshness index at high temperatures were considerable and this indicates the effect of temperature on this parameter ($P < 0.01$) (Table 2). According to the slope of the freshness index line, temperature (Fig. 4a) has a larger impact on this index (Eq. 7).

$$Y_{\text{Kvalue}} = 62.25 + 45.25A + 0.41B + 2.32B^2 \quad (7)$$

According to Table (3), the interactive effect of time and temperature indicates the dependency of these factors on sensory changes ($P < 0.05$). According to F-value, temperature factor (150.50) and temperature line slope in Fig. (4b) influenced sensory changes. In this test, the power effect of temperature and time on sensory changes was not significant. The fillets under vacuum at lower temperature and shorter time had larger sensory scores (Eq. 8).

$$Y_{\text{sensory}} = 11.10 - 5.01A - 3.60B \quad (8)$$

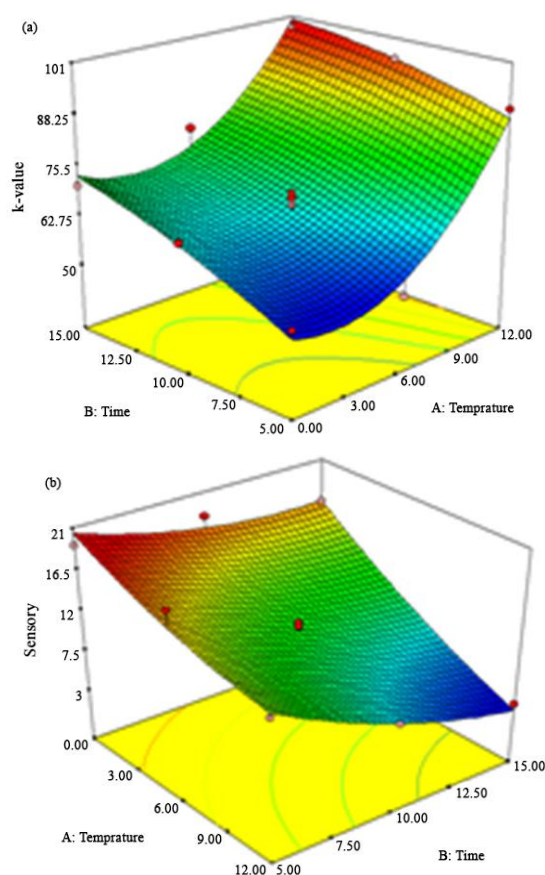


Fig. 4. The 3D diagram of the effect of input variables on a) freshness index b) sensory analysis of fillets under vacuum

In Table (4), the significance level of the fitted variables for fillets under vacuum is indicated. According to this table and R^2 level, the explained models are suitable for predictions.

Table 4. The fitted models for parameters for fillets under vacuum

Measured variable	R^2	R^2 -adj
ATP	0.9216	0.8655
IMP	0.9312	0.9174
HxR	0.9748	0.9569
Hx	0.9006	0.8297
Sensory index	0.9599	0.9312
Freshness index (K_{value})	0.9715	0.9512

In different studies, changes in ATP are used to evaluate freshness and corruption of fish and other aqueous products (Shi *et al.*, 2019). Since this study is an innovative study with high costs, it should be noted that its success is a potential model to improve efficiency of kinetic models and other models in food industry. In the current study, the qualitative changes of the silver carp packaged under vacuum were compared with the predicted conditions through surface response method. In experimental conditions, microbial load, TVB-N, ATP, and Hx with increased storage period from 5 to 15 days and increased temperature from 0 to 12 °C, showed a significant increase ($P < 0.05$). Özyurt, Polat, & Tokur (2007) reported different amounts of TVB-N for different fish as limit. According to this index, fishery products are divided into the following groups: 1) 25 g nitrogen/100 g meat; high quality 2) 30 mg nitrogen/100 g meat; good quality 3) 35 g nitrogen/100 g meat; consumption limitation 4) over 35 mg nitrogen/100 g meat; corrupted. According to Table (2), treatments 1, 0, 4, 5, 7, and 8 that were stored at 0 and 6 °C, showed high quality until day 15 and treatments 3, 6, and 9 that were stored at 12 °C, showed high quality until day 5, consumption limitation until days 10, and corrupted until days 15.

About microbial load, maximum acceptable total bacteria number for fish

and shrimp is proposed by the International Commission on Microbiological Specification for Foods as 7 Log cfu/g (ICMSF, 1986) that allows storage at 0 °C in days 5, 10, and 15 and for two temperatures of 6 and 12 °C, they were within the permitted range until days 5. According to these indexes, fish fillets under the temperatures of 6 and 12 °C in days 5 showed the best quality.

According to response surface methodology (RSM) analysis, the best treatment in terms of microbial load and volatile nitrogen was related to the silver carp fillets stored in 0 and 5 days and insignificance of the employed model confirmed the data process. Also, comparison of the empirical findings and RSM and the acceptance level (R^2 -adj and R^2) showed the similarity of the results and indicates that the presented model can predict the response of interest very well. Liu *et al.* (2016) studied the application of mathematical models to predict microbial load and TVB-N changes in the fillet of the bighead carp under vacuum and reported that the result of modeling is consistent with experimental findings. Bahramifar, Roomiani, & Askary Sary (2016) in a study on the effect of vacuum packaging on the storage of the grass carp (*Ctenopharyngodon idella*) reported increased microbial load and TVB-N that is due to the bacterial activities and autolytic enzymes available in fish meat. Rahmatipoor (2017) investigated the effect of the microbial load and TVB-N on the shelf life of the silver carp at a temperature of 4 °C with increased microbial load and TVB-N over the storage period. Increased volatile nitrogen loads is due to the aerobic bacteria (Hong *et al.*, 2017). This means that both parameters have similar processes and the parameters of temperature and time have equal effects on them. According to the proposed models and equations, the linear parameter of temperature and time influenced total microbial load and total volatile nitrogen of fillets under vacuum ($P < 0.01$). In a study by Ola & Oladipo

(2004), a strong relationship between total bacterial load and volatile nitrogen in filthy fillets was indicated ($r=0.98$). Moreover, Fazlara, Pourmahdi Brojeni, & Jaferi (2013) reported the consistency between bacterial load and volatile nitrogen. After the species death, ATP undergoes catabolization and becomes ADP, AMP, IMP, HxR and, Hx (Hong *et al.*, 2017). The sum of these parameters form K-value and high Hx values lead to low K-value.

K-value smaller than 20% indicates freshness, smaller than 50% indicates average quality, and over 60% indicates lack of freshness (Saito, Arai, & Matsuyoshi, 1959). According to Tables (2) and (3), with increased temperature and storage time, K-value index increased significantly and except days 5 and temperatures 0 and 12 °C, in other days, it indicates lack of freshness. Liu *et al.* (2016) investigated the effect of vacuum packaging in *Aristichthys nobilis* fillet quality and compared the mathematical model and the empirical model and reported increased K-value index over time as well as increased temperature that is consistent with the findings of the present study. They stated that storage in all times (days 0, 2, 4, 6, 8, 10, 12, 14, 16, and 18) and all temperatures (0, 3, 6, 9, and 12 °C) leads to a significant increase in K-value. Ehira & Uchiyama (1973) introduced K coefficient as the best freshness index that shows stronger relationship relative to other indexes. Mataragas, Bikouli, Korre, Sterioti, & Skandamis (2019) proved that time and temperature indexes are valid to recognize meat products corruption under isothermal and dynamic storage conditions.

About ATP and Hx, in experimental results as well as the production model, temperature and time are effective factors in production process that about ATP and Hx, the increasing trend was observable with temperature and time, so that in high temperature and long durations, ATP and Hx were at their maximum levels. Puchała & Pilarczyk (2005) investigated the effect of freezing on the quality of the ordinary

carp that ATP decomposition to inosine (HxR) in cold conditions and vacuum packaging was too slow, because the enzymes that convert ATP into IMP and then HxR consume oxygen and in cold conditions, their activities is influenced by the lack of oxygen. Moreover, in higher temperatures, ATP release from mitochondria increases (Saito *et al.*, 1959) and its deficiency is accompanied by the breakdown of IMP and HxR (Lougovois, Kyranas, & Kyranas, 2003) and this confirms reduced IMP and increased ATP (Hong *et al.*, 2017). IMP is one of the qualitative parameters of fillet (Lougovois *et al.*, 2003) and its reduction means decreased quality. They reported that hypoxanthine increases over the storage period of trout, Caspian kutum, and zander that is slow in +10 to +14 °C and very slow in +0 °C. On the other hand, this products causes bitterness in the texture (Kiesvaara, Heiniö, Mustranta, Hattula, & Hallikainen, 1992).

About IMP and HxR, temperature was more effective in IMP and HxR production and fillets under vacuum showed higher IMP and HxR in a short-term run. According to hypoxanthine production process from ATP production time, at high temperatures and long-term runs, ATP values of fillets increased and converted into inosine monophosphate, inosine, and hypoxanthine rapidly and inosine monophosphate and inosine values decreased. According to the explanations, fillets packaged under vacuum at 0 °C and days 5 showed the best consumption properties. Fillets under vacuum showed higher scores in terms of sensory analysis at low temperature and shorted time. Kumar, Dora, Sreekanta, Supratim, & Subha (2015) reported low sensory quality with increased storage time that is consistent with the findings of the present study, Yildiz (2017) investigated salty fillet changes using response surfaces and this modeling could predict changes in fillet. One of the reasons for reduced sensory properties is fat oxidation that

caused degradation and drop of sensory quality and reduced nutritional materials such as essential polyunsaturated fatty acids or PUFA and production of toxic oxidation products.

This result was observable both in model and empirical results. In sum, the fitted models as well as the predicted values for independent variables and dependent responses pointed to the consistency between the results and the empirical response and a temperature of 0 °C and time of 5 days constituted the best treatment for the storage of the silver carp fillet packaged under vacuum.

Conclusions

The current study showed that the silver carp fillets under vacuum and a temperature of 0 °C showed acceptable and desirable conditions for 5 days. According to the findings of this study, the real values of total microbial load and total nitrogen were similar. In investigating the freshness index and sensory analysis, it was observed that the amount of purine nucleotides and products of their decomposition, the silver carp fillet showed a desirable quality under vacuum and a temperature of 0 °C for 5 days.

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پیش‌بینی تغییرات کیفی کپور نقره‌ای بسته‌بندی‌شده در شرایط خلأ با کمک روش سطح پاسخ

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چکیده

هدف از مطالعه حاضر استفاده از مدل‌سازی مرکب برای پیش‌بینی تغییرات میکروبی، میزان بازهای نیتروژنی فرار (TVB-N)، آنالیز حسی و نیز فاکتور تازگی فیله کپور نقره‌ای (*Hypophthalmichthys molitrix*) بسته‌بندی‌شده در شرایط خلأ بود. ماهیان بسته‌بندی‌شده در خلأ در دماهای صفر، ۶ و ۱۲ درجه سانتی‌گراد که براساس خروجی نرم‌افزار State-Ease Design Expert بهینه‌شده بودند، به مدت ۵، ۱۰ و ۱۵ روز نگهداری شدند. درخصوص پارامترهای کیفی فیله کپور نقره‌ای بسته‌بندی‌شده، بین تمامی تیمارها در زمان و در درجه‌حرارت‌های موردبررسی اختلاف معنی‌داری مشاهده شد ($P < 0.05$). دو پارامتر اینوزین مونوفسفات (IMP) و اینوزین (HxR) با افزایش زمان و دما روند کاهشی را نشان دادند ($P < 0.05$). شاخص تازگی و آنالیز حسی در مدت زمان کوتاه (۵ روز) در حالت مجاز و بهینه قرار داشتند. در این آزمون مقادیر پیش‌بینی‌شده با درصد مقبولیت ۷۸ درصد با مقادیر برآورده‌شده همخوانی داشتند. مشاهدات بیانگر همبستگی خوب بین نتایج به‌دست‌آمده با روش تجربی و مقادیر پیش‌بینی‌شده با روش آماری بود. مدل‌های موردبررسی از ضریب تبیین (R^2) و ضریب تبیین اصلاح‌شده ($R^2\text{-adj}$) نزدیک به ۱ برخوردار بودند، که نشان می‌دهد مدل آزمایشی به‌کارگرفته‌شده، قادر به بررسی پیش‌بینی تغییرات کیفی فیله با درصد خطای پایین بود. موفقیت این کار می‌تواند سبب بهبود مدل‌های جنبشی در صنعت غذا شود، لذا کاربرد آن به ماندگاری محصولات غذایی کمک شایانی می‌کند.

واژه‌های کلیدی: بسته‌بندی خلأ، تغییرات کیفی، روش سطح پاسخ، کپور نقره‌ای

Evaluation of Chemical and Microbial Spoilage of Chicken Fillet Coated with Chitosan, Ginger Essential Oil (*Zingiber officinale*) and Medlar Concentrate (*Mespilus germanica* L.) During Refrigerated Storage

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Abstract

Lipid oxidation and microbial growth are the most important factors affecting the quality and Spoilage of the meat in refrigerated storage conditions. In this study, the effect of using chitosan, Medlar concentrate, ginger essential oil alone and in combination with each other on improving the quality and shelf life of chicken meat kept in the refrigerator was investigated. 8 groups in this study during 12 days were stored at refrigerator and Microbial (aerobic mesophilic & Psychotrophic Plate count) and chemical (PV, TBA, TVB-N) and sensory parameters were measured at days 0, 4, 8 and 12. Total phenol and reducing power tests were also performed to evaluate the antioxidant properties. Based on the results of GC/MS, the major compounds of ginger essential oil were α -Zingiberene (36.54%), β -Sesquiphellandrene (16.45%) and trans- γ -Cadinene (10.27%) were formed. The results of this study showed that the chitosan-coated treatment containing 2% ginger essential oil and medlar concentrate, decreased the microbial parameters significantly ($P < 0.05$) as compared to control group during the storage period ($P < 0.05$). The oxidation indices of chicken meat samples had significantly fewer changes ($P < 0.05$), had the strongest antioxidant and sensory effect on other groups during storage. The results of microbiological, chemical and sensory analysis of this study showed that the effect of chitosan coating containing 2% ginger essential oil and medlar concentrate was effective in increasing the shelf life and quality of chicken meat for 12 days during storage in the refrigerated condition.

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Keywords

Chicken fillet
Chitosan coating
Ginger essential oils
Medlar concentrate
Spoilage

Introduction

Long-term storage of meat in the refrigerator leads to undesirable changes such as oxidation and hydrolysis of fats. These changes are due to enzymatic, chemical, and microbial activities that lead to low quality and product corruption (Fan *et al.*, 2009). For this purpose, the use of

edible coatings with natural origins with or without antimicrobial and antioxidant compounds is an effective method to maintain quality of meats such as chicken, fish, etc. (Rahnemoon, Sarabi Jamab, Javanmard Dakheli, & Bostan, 2018; Vásconez, Flores, Campos, Alvarado, & Gerschenson, 2009). Chitosan is among

edible polysaccharide coatings resulted from chitin of hard shells such as crabs and shrimp that has antioxidant and antimicrobial and antifungal properties (Fan *et al.*, 2009; Sathivel, 2005) and is has many applications in food industry to cover fruits and vegetables (Jianglian & Shaoying, 2013), different kinds of meat (Gennadios, Hanna, & Kurth, 1997), egg (Kim, Daeschel, & Zhao, 2008), and cheese (Duan, Park, Daeschel, & Zhao, 2007; Kanatt, Chander, & Sharma, 2008). Bazargani-Gilani, Aliakbarlu, & Tajik (2015) showed that the use of pomegranate extract and chitosan coating enriched by thyme oil increases chicken mean shelf life, decreases peroxide index, TBARs, and protein oxidation in treatment samples (Bazargani-Gilani *et al.*, 2015).

Nowadays, the use of herbal ingredients and extracts instead of synthetic materials has been very popular. The use of natural materials such as extracts and concentrates that create desirable odor and taste in food that have antioxidant properties is increasing (Hosseini, Razavi, & Mousavi, 2009). Common medlar is found in Iran and is available in late autumn and is used in traditional medicine for different aspects such as neurons, mouth ulcers, bowel diseases, gastric ulcer, constipation treatment, etc. (Nabavi, Nabavi, Ebrahimzadeh, & Asgarirad, 2011). Also, it is reported that this fruit has antimicrobial and antioxidant properties (Qin, Kang, Zhang, Qi, & Wang, 2012). Cushnie & Lamb (2005) reported that common medlar has antimicrobial properties due to the existence of organic acids such as gallic acid as well as the presence of phenolic compounds (Cushnie & Lamb, 2005). Mamashloo, Sadeghi, Ghorbani, Alami, & Khomeiri (2012) reported antioxidant activity (80%) for common medlar (1 mg/mL) (Mamashloo *et al.*, 2012).

Zingiber (*Zingiberene officinale* Rosc) is mainly cultivated in Eastern Asia and tropical regions. Zingiber is used in bakery products, spices, pickles, and sauces to

create taste (Singh *et al.*, 2008). Also, it is used in traditional medicine to treat diseases such as cough, sinusitis, sore throat, fever, and influenza (Şener *et al.*, 2017). The antimicrobial and antifungal properties of Zingiber are reported in various studies (Şener *et al.*, 2017; Sharma, Singh, & Ali, 2016; Singh *et al.*, 2008). The objective of this study was to investigate the antioxidant and antimicrobial effects of chitosan, common medlar extract, and Zingiber extract (2%) on chicken meat in refrigerator to increase storage time.

Materials and methods

Preparation of concentrate and essential oil

Zingiber oil was prepared from Exir Gole Sorkh in Mashhad and common medlar concentrate was prepared from the local market in Amol. Identification and analysis of Zingiber compounds were performed by GC-MS (Thermoquest Trace GC 2000 Finnigan, England). All chemicals were bought from Sigma and Merck companies.

Preparation of coating and concentrate

To prepare chitosan solution, first, acetic acid solution 1% volume was prepared (Lungu & Johnson, 2005) and then, chitosan solution 2% w/v was prepared. After complete dissolution (for a night under room temperature on magnetic stirrer), glycerol 75% was added to the solution (Lungu & Johnson, 2005) and tween 80, 0.25% (v/v) was added as emulsifier and mixed on stirrer for 30 min under room temperature (pH around 5.8) and after solution homogenization, to ensure complete dissolution of chitosan and glycerol, the solution was mixed for 15 min under a temperature of 45 °C (Lungu & Johnson, 2005). Finally, Zingiber oil 2% was added to suspension and the final suspension was homogenized with stirrer for 10 min (Yingyuad *et al.*, 2006). Also, common medlar concentrate was diluted by adding distilled water and Brix 1.39 was prepared by Calze ophthalmic refract meter.

Preparation of chicken fillet and coating the samples

Fresh chicken meat was prepared the market and transferred to the laboratory and filled was prepared from it. All fillets were washed with distilled water and placed on sterile dewatering mesh and coated for treatment in the prepared solutions through immersion. After drying, samples were packed in a zip and stored in the refrigerator with a temperature of 4 °C. Eight treatment groups including the control sample, butylated hydroxyl toluene (BHT) sample (positive control), sample with chitosan coating, sample with Zingiber oil 02%, sample with chitosan coating and Zingiber oil 2%, sample with common medlar concentrate, chitosan coating sample with common medlar concentrate, and chitosan coating sample with Zingiber oil 2%, and common medlar concentrate were exposed to chemical and sensory tests in days 0, 4, 8, and 12.

Chemical tests

Measurement of total phenol concentration of common medlar

In order to examine phenolic compounds, the Folin-Ciocalteu method was used in which Folin-Ciocalteu is the reagent and gallic acid is the standard. Common medlar concentrate was dissolved in 2 mL (1400 μ L of ethanol+600 μ L of water) and then, distilled water, Folin-Ciocalteu reagent, and sodium carbonate were added to 500 μ L of concentrate and after 2 h, optical absorption was measured by Pharmacia (LKB, Novaspec, England) at the wavelength of 730 nm (Teets & Were, 2008).

Assessment of reducing power (RP)

To carry out this test, 2.5 mL of sodium phosphate buffer and 2.5 mL of potassium ferric cyanide 1% were added to 0.03 g of the sample and they were exposed to a temperature of 50 °C for 20 min. Then, 2.5 mL of trichloroacetic acid solution 10% was added to the pipes and it was

centrifuged. After that, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride 0.1% and after 10 min, it was read at the wavelength of 700 nm (Huang *et al.*, 2011).

Determination of peroxide value (PV)

For Peroxide Value (PV), fat extraction is carried out. For this purpose, 150 g of chicken fillet was homogenized adding 250 mL of chloroform with homogenizer (IKA, Germany) and was filtered and for dehydration, it was filtered by another filter that contained dry sodium sulfate. Finally, it was placed in Oven 105 °C to be dried (Pearson, 1976). To carry out peroxide test, 0.3 g of fat was mixed with 9.8 mL of chloroform-methanol; then, 0.05 mL of ammonium thio cyanate, 0.05 mL of iron chloride solution II was added to the tubes. After storage for 5 min under the room temperature, optical absorption 500 nm was read. By using the following relationship, peroxide as mEq peroxide in kg/oil was estimated.

(1)

$$\text{Peroxide Value} = (A_s - A_b) \times m / 55.84 \times m_0$$

Where A_s : sample absorption, A_b : blank absorption, m : calibration curve slop, m_0 : sample weight based on g, and iron atomic weight is 55.84 (Shantha & Decker, 1994).

Measurement of thiobarbituric acid (TBA)

In order to measure oxidation in samples, TBA index was carried out using (Wrolstad *et al.*, 2005). Here, 10 g of the sample was homogenized adding 1 mL of BHT 0.1% and 20 mL of trichloroacetic acid 5%. The mixture was filtered with Watman filter paper 42 and using trichloroacetic acid, it reached 50 mL. 5 mL of the filtered fluid was added to 5 mL of thiobarbituric acid 0.02 molar. Then, it was exposed to Bonnie Marie 100 °C for 1 h. After cooling, optical absorption was read at the wavelength of 532 nm using

spectrophotometer. Blank included 5 mL of distilled water and 5 mL of thiobarbituric acid (Wrolstad *et al.*, 2005).

Measurement of total volatile basic nitrogen (TVB-N)

To measure TVB-N, first, 10 g of chicken fillet was mixed with 10 mL of distilled water and poured into balloon containing 2 g of magnesium oxide and 300 mL of distilled water. At the steam outlet, a container containing 3% of boric acid and several drops of methyl red reagent was placed. In the end, titration was carried out with sulfuric acid 5%. Volatile bases are expressed based on mL nitrogen per 100 g of meat (Jeon, Kamil, & Shahidi, 2002).

Microbial tests

For microbial assessment, in days 1, 4, 8, and 12, 25 g of each treatment was selected for microbial test homogenized with 225 mL of peptone water 0.1% with 200 rpm for 1 min. Then, other dilutions were prepared. Aerobic mesophilic bacteria and cryogenic bacteria were counted in agar plate with incubation for 48 h/37 °C and for 10 days under 7 °C (Shavisi, Khanjari, Basti, Misaghi, & Shahbazi, 2017).

Sensory assessment

Examination of qualitative properties by performed by 10 trained experts and the samples were scored from 0 to 9. The results were expressed by 9-point hedonic scale. The assessment was based on total acceptability (color, odor and texture). Nine was the highest score and 0 was the lowest score and scores below 6 were unacceptable (Goulas & Kontominas, 2005).

Data analysis method

First, Kolmogorov-Smirnov test was used to examine data normality with three replications and Levene test was used to examine variance equality. Then, samples were analyzed by one way ANOVA and SPSS 20. A significant difference between samples was determined using Duncan test

and the significance level was ($P>0.05$). Kruskal-Wallis nonparametric test was carried out to determine the effect of storage time on the results. All tests were carried out with three replications.

Results and discussion

Zingiber essential oil compounds

According to the results of essential oil analysis, 26 compounds in 96.42% of Zingiber were specified. The major compounds of Zingiber essential oil are Alpha zinjiberm 36.54%, Beta-Sesquiphellandrene 16.45%, and trans gamakaddine 10.27% (Table 1).

Amiri, Mohamadi, Saadatmand, & Taheri (2016) analyzed chemical compounds of using GC-MS and the main compounds included alpha-Zingiberene 28.25%, Beta-sesquiphellandrene 15.65%, alpha-curcumine 15.23%, and trans gamma cadinene 11.88% and the main compounds of Indian Zingiber are alpha-Zingiberene 35.67%, Beta-sesquiphellandrene 15.27%, trans gamma cadinene 9.25%, and E-Citral 0.06% (Amiri *et al.*, 2016), consistent with the findings of this study, introduced alpha-Zingiberene, Camphene, Curcumene and Beta-sesquiphellandrene as the main compounds of the essential oil (Burt, 2004; Singh, Maurya, Catalan, & De Lampasona, 2005).

By comparing the main compounds of Zingiberene essential oil in various studies, it is observed that there are differences in rate and main compounds of Zingiberene constituents that can be due to geographical differences, plant type, harvesting time, environmental conditions, cooling method, and essential oil preparation that lead to difference in rate and type of compounds.

Table 1. The results of Zingiberene essential oil analysis

Row	Compound	Detention time	Rate	Detention index
1	Camphene	4.67	0.40	949
2	Linalool	8.11	1.20	1100
3	Borneol	9.91	4.49	1172
4	Alpha terpineol	10.52	0.11	1196
5	Z- citral	11.80	0.20	1244
6	E- citral	12.57	1.66	1273
7	2-Undecanone	13.12	0.51	1294
8	Beta caryophyllene	16.48	0.73	1425
9	Alpha Curcumene	18.10	4.40	1489
10	beta Selinene	18.16	1.70	1491
11	Alpha Zingiberene	18.37	36.54	1500
12	Cis gamma cadinene	18.40	3.30	1505
13	Trans gamma cadinene	18.70	10.27	1513
14	Zonarene	18.83	0.60	1519
15	Beta Sesquiphellandrene	19.13	16.45	1532
16	Trans gamma Bisabolene	19.24	0.17	1537
17	Zeta nerolidol	19.69	0.12	1556
18	Spatulenol	19.91	4.10	1557
19	Tumerol	20.30	0.33	1583
20	Alpha Cedrol	20.51	0.50	1594
21	10-epi gamma Eudesmol	21.10	1.03	1618
22	Gamma Eudesmol	21.31	1.43	1627
23	Hinesol	21.49	1.10	1635
24	Beta Eudesmol	22.40	2.90	1659
25	Alpha Bisabolol	22.36	0.60	1674
26	Nuciferal	22.97	1.50	1702
27	Cuparophenol	24.11	0.86	1775
28	Benzyl salicylate	26.20	0.15	1877
29	Shogaol	34.35	0.20	2298
Total			96.42	

Assessment of phenolic compounds of common medlar

Total phenol in common medlar concentrate is 1.220 ± 0.25 mg of gallic acid in 1 g of concentrate. In a study by (Mamashloo *et al.*, 2012), phenolic compounds of common medlar were investigated. Total phenol in Estonia extract (7.437), methanol extract (5.086), ethanol extract (4.106), and aqueous extract (1.240) are reported for 100 g of the dry matter (Mamashloo *et al.*, 2012).

RP results

Reducing Power (RP) test is according to the ability of phenol compounds in converting Fe^{3+} to Fe^{2+} that according to the results, the yellow color changes to green and blue and optical absorption increases (Roginsky & Lissi, 2005). In this test, RP power, from maximum to minimum level, is related to chitosan coating containing Zingiberene 2% and common medlar concentrate, chitosan coating with common medlar concentrate, chitosan coating containing Zingiber 2%,

Zingiber essential oil 2%, common medlar concentrate, chitosan coating, BHT, and the control sample (Fig. 1). RP of chitosan coating contains essential oil 2% and common medlar concentrate that is significantly higher than other samples and the control sample and BHT ($P < 0.05$). Chitosan coating RP with BHT is reported at a stable level and no significant difference was observed ($P > 0.05$). (Mamashloo *et al.*, 2012) assessed RP power of various concentrations of common medlar concentrations and the results showed that with increased concentration, RP power of the extracts increased (Mamashloo *et al.*, 2012). This is consistent with RP power assessment of various concentrations in our study and the desirable effect of employing chitosan in combination with the essential oil and concentrate of common medlar. The desirable effect of employing chitosan is consistent in combination with the essential oil and concentrate of common medlar.

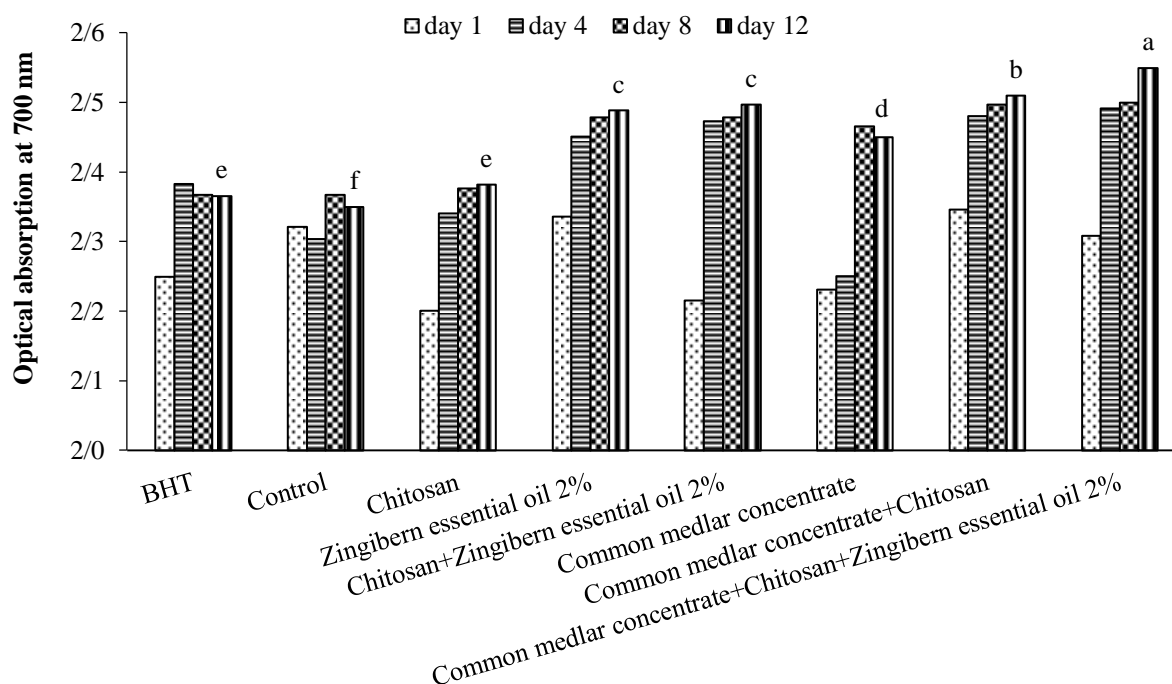


Fig. 1. The RP power of various treatments in chicken breast fillet stored at 4 °C

*Different lowercase letters (a, b, c) in each chart indicate a significant difference ($P<0.05$) in various treatments.

Investigating PV index changes

Fat oxidation is a major problem in meat that leads to undesirable odor and taste. Peroxides in the first oxidation level are formed through binding of oxygen to the double bond of unsaturated fatty acids. For this reason, primary fat oxidation is assessed measuring peroxide rate (Kanatt *et al.*, 2008). The measured rates of peroxide index are shown in Fig. (2). The control sample has the highest peroxide index and shows a significant difference from other treatments ($P<0.05$). In the last day of storage, the highest rate of peroxide index was observed in control samples and reached to 2.4 mEq/kg and the lowest peroxide rate was reported for the sample coated with chitosan containing Zingiber essential oil 2% and common medlar concentrate and this rate is due to this condition that the treatment has high amounts of antioxidant materials, especially phenolic materials in common medlar concentrate. The difference

between the measured peroxide index for chitosan coated samples and essential oil and samples with concentrate and essential oil 2% was statistically justifiable ($P<0.05$). Molaei Aghaee, Kamkar, Akhondzadeh Basti, Khanjari, & Kontominas (2015) investigated the effect of packaging with biodegradable chitosan films and formulated with *Silium sativum* L. on chemical properties of chicken fillet in 14 days and the results showed that PV values in control samples were higher and this difference was significant ($P<0.05$). Also, in the 10th day, PV values in all samples increased significantly and continued until the last day. However, in samples containing the highest extract level of 2% this process advanced with slightest slop. In the end, the lowest peroxide value was observed for samples with film 1% (Molaei Aghaee *et al.*, 2015).

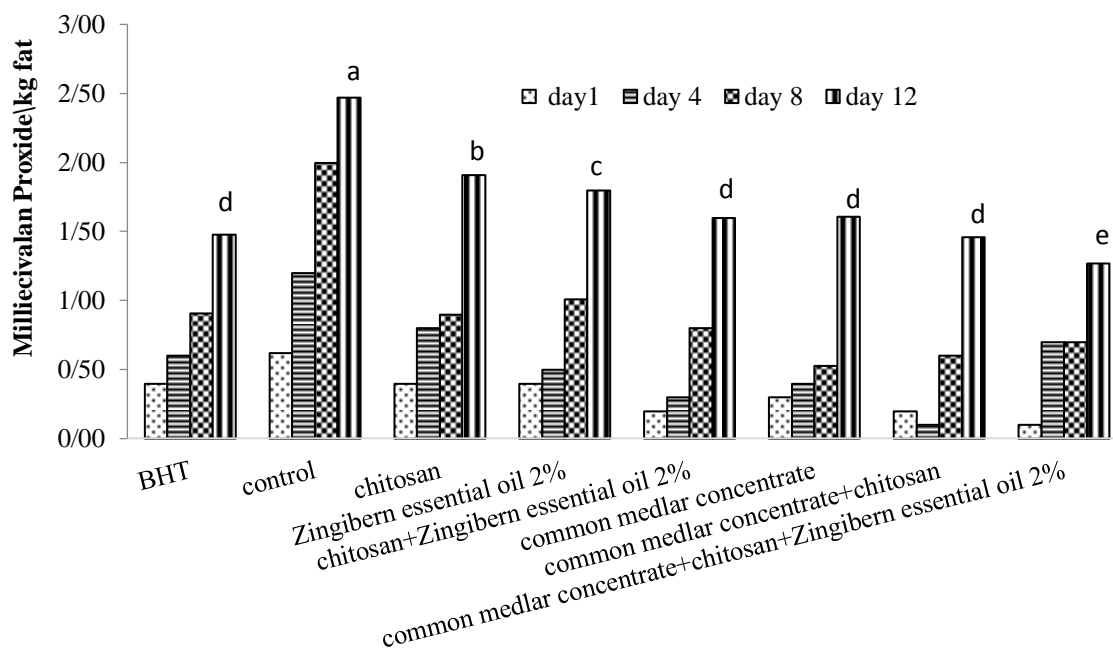


Fig. 2. Peroxide changes (PV) in various treatments of chicken breast fillet stored at 4 °C

*Different lowercase letters (a, b, c) in each chart indicate a significant difference ($P < 0.05$) in various treatments.

Investigating TBA index changes

Fat oxidation in meat causes compounds such as aldehyde and ketones that lead to change in taste and decreased nutritional value. Thiobarbituric acid is used to indicate secondary fat oxidation (Radha krishnan *et al.*, 2014). According to the results, with increased storage time, TBA in various samples increases that the highest level is observed in 12 days for the control group while this index in the coated samples was significantly lower than the control samples ($P < 0.05$) and the lowest level was related to chicken fillet coated with chitosan containing Zingiber 2% and common medlar concentrate that can be related to the antioxidant property and PV ability of Zingiber and common medlar concentrate and chitosan coating in reducing meat oxidation (Fig. 3). TBA index difference in the coated samples was not significant ($P > 0.05$) and this difference was observed between chitosan, extract/chitosan, and concentrate and no significant difference was observed between other treatments

($P > 0.05$). TBA index has a wide use to assess fat oxidation degree. With this index, Malondialdehyde is measured. The allowed rate for TBA index is 2 mg of Malondialdehyde/g (Byun *et al.*, 2003; Teets & Were, 2008). In the current study, this index did not go beyond the determined range. Radha krishnan *et al.* (2014) investigated the effect of different extracts on the storage of chicken meat and reported that increased thiobarbituric acid in samples containing various extracts was significantly weaker than the control group (Radha krishnan *et al.*, 2014) and this is consistent with our studies. Various studies such as Fazlara, Pourmahdi, Zarei, & Karimi (2017) and Petrou, Tsiraki, Giatrikou, & Savvaidis (2012) investigated the effect of chitosan coating, essential oil, and extracts on decreased TBA relative to the control group (Fazlara *et al.*, 2017; Petrou *et al.*, 2012).

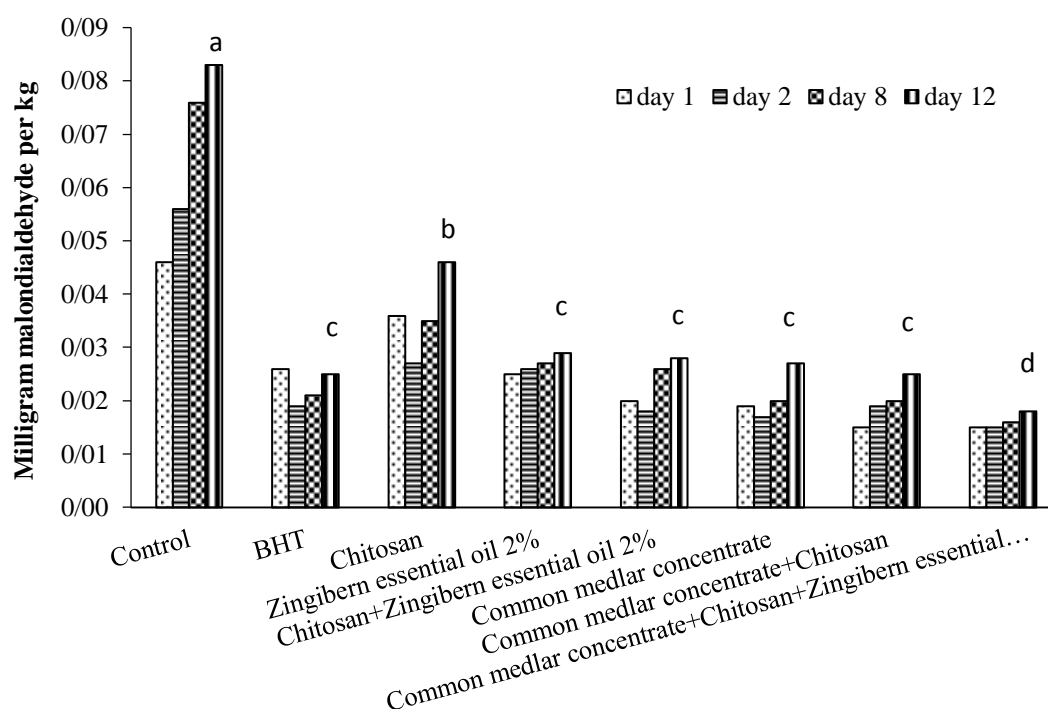


Fig. 3. Thiobarbituric acid changes in various treatments of chicken breast fillet stored at 4 °C
 *Different lowercase letters (a, b, c) in each chart indicate a significant difference ($P<0.05$) in various treatments.

Investigating TV-N index changes

In terms of TVB-N changes over the storage period, the highest TVN rate was observed in the control samples and the obtained difference with other treatments was significant ($P<0.05$), so that the control sample after 8 days became inconsumable in terms of TVB-N index. However, the coated samples were consumable until the end of the storage period (Fig. 4).

As can be seen in the figure, after storage in refrigerator for 12 days, the measured values for all treatments except the control sample were lower than 20 mg/100 g that are acceptable. Also, chitosan is lower than 25 mg/100 g and still is within the acceptable range. The acceptance range for chicken meat is 25 mg/100 g. The lowest TVB-N value was

observed in chitosan coated samples containing Zingibern 2% and common medlar that is lower than other samples that is surely due to the antioxidant effect of the extracts and the protective effect of chitosan coating. TVB-N is used to assess bacterial corruption and enzymatic activity and usually includes materials resulted from bacterial corruption and is used as an index to assess the products. Ranjbaryan, Rezazadeh Bari, Almasi, & Amiri (2017) investigated the effect of sodium caseinate coating containing cinnamon essential oil on increased shelf life of chicken breast fillet for 12 days in refrigerator. The results showed that over time, in all treatments, TVN has a significant increasing trend ($P<0.05$) (Ranjbarayan *et al.*, 2017).

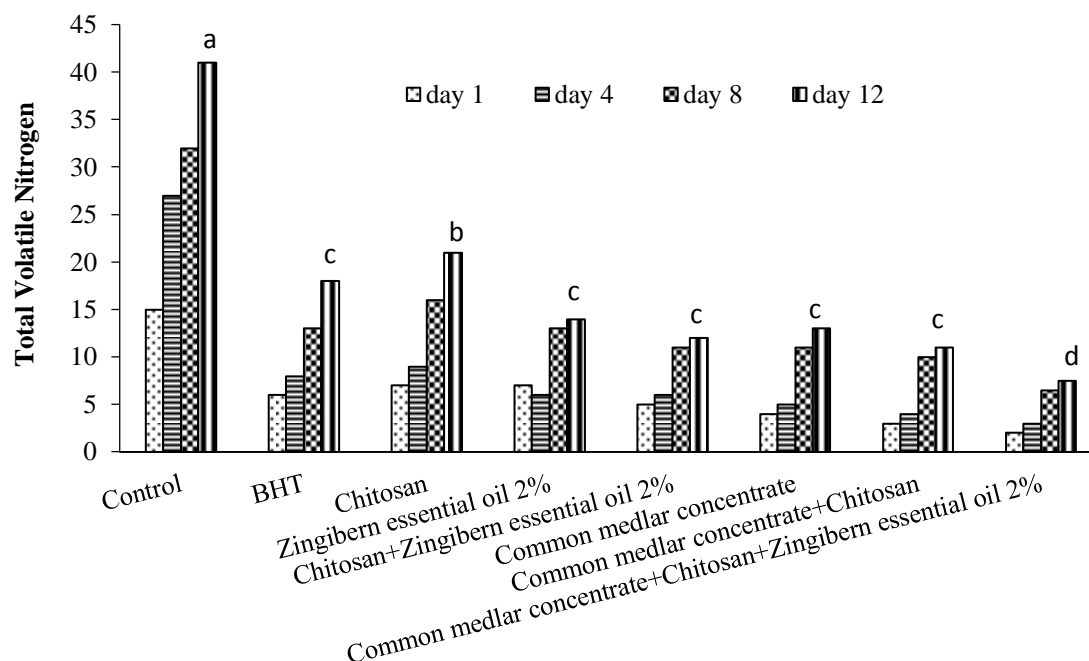


Fig. 4. Total volatile nitrogen bases changes in different chicken breast fillets stored in refrigerator (4 ± 1 °C)
 *Different lowercase letters (a, b, c) in each chart indicate a significant difference ($P<0.05$) in various treatments.

Among the treatments, TVN rate during storage periods in treatments with coating and essential oil was lower than other treatments that is due to the strong antibacterial property of cinnamon. Hakim, Fazlara, & Tadayoni (2017) reported that chitosan containing mountainous essential oil could reduce TVB-N rate in chicken meat significantly ($P<0.05$), so that the control sample became corrupted in 9 days, but chitosan coated sample containing mountainous essential oil in 15 days became corrupted (Hakim *et al.*, 2017) and this is consistent with our results. Also, due to low pH of common medlar concentrate in treatments, bacterial growth reached to the minimum level and this led to enhanced quality of treatments over the storage period.

Investigating microbiological properties

The means of aerobic mesophilic bacteria and cryophilic bacteria over the storage period are presented in Table (2). Generally, over the storage period in

refrigerator, microbial flora significantly increased in all samples ($P<0.05$) and this increase was higher in the control sample. Over the storage period, aerobic mesophilic bacteria number after 8 days reached to 7 log cfu/g while in other treatments, after 12 days, it reached to 6.75 log cfu/g. BHT and Zingiber essential oil 2% showed to significant antimicrobial effect ($P>0.05$). The minimum increase was observed in chitosan + essential oil 2% + common medlar concentrate that after 12 days reached to 4.1 log and showed the maximum antimicrobial effect. About cryophilic bacteria, after 12 days, the control bacteria were higher than 8 log while the highest decrease was related to chitosan+essential oil 2%+common medlar concentrate that after 12 days, this index reached to 4.32 log. The minimum rate was related to chitosan, BHT, essential oil 2% and chitosan + essential oil 2% and no significant difference was observed.

Table 2. Aerobic mesophilic bacteria and cryophilic bacteria logarithm (Log cfu /g) in various chicken fillet treatments over the storage period

Microbial tests	Treatments	Test days			
		0	4	8	12
Aerobic mesophilic bacteria	Control	3.45±0.23	4.23±0.50	7.00±0.21	-
	BHT	3.34±0.40	3.21±0.50	5.10±0.02	6.78±0.14 ^{A*}
	Chitosan	3.22±0.56	3.75±0.01	5.50±0.13	6.27±0.02 ^B
	Zingiber essential oil 2%	3.10±0.14	3.55±0.62	5.60±0.12	6.46±0.04 ^A
	Chitosan+Zingiber essential oil 2%	2.90±0.20	3.20±0.15	5.10±0.07	6.20±0.09 ^B
	Common medlar concentrate	2.70±0.06	3.10±0.14	4.80±0.12	5.60±0.10 ^C
	Chitosan+common medlar concentrate	3.20±0.13	2.80±0.20	3.90±0.11	4.80±0.30 ^C
	Chitosan+common medlar concentrate+Zingiber oil 2%	2.03±0.10	2.22±0.10	3.10±0.02	4.10±0.01 ^D
Cryophilic bacteria	Control	4.20±0.02	4.26±0.22	6.12±0.32	8.10±0.01 ^A
	BHT	3.90±0.21	4.21±0.12	4.90±0.56	6.92±0.02 ^B
	Chitosan	4.20±0.02	4.76±0.21	5.02±0.82	7.20±0.11 ^B
	Zingiber essential oil 2%	4.00±0.01	4.20±0.74	5.20±0.12	7.30±0.20 ^B
	Chitosan+Zingiber essential oil 2%	4.10±0.23	4.25±0.45	5.00±0.09	7.10±0.30 ^B
	Common medlar concentrate	3.20±0.12	3.90±0.52	4.20±0.02	5.02±0.10 ^C
	Chitosan+common medlar concentrate	2.65±0.16	3.20±0.25	3.85±0.22	4.90±0.02 ^C
	Chitosan+common medlar concentrate+Zingiber oil 2%	2.20±0.12	2.82±0.16	3.52±0.23	4.32±0.31 ^D

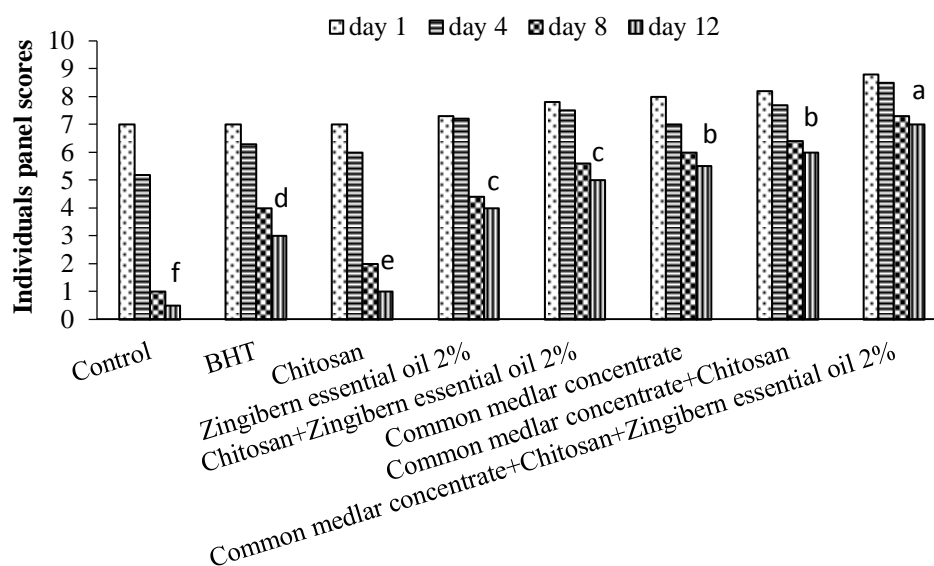
* Upper cases (A, B, C) in each column show a significant difference ($P<0.05$) in different treatments.

Bacterial counting results are consistent with Duan, Cherian, & Zhao (2010) who reported that using chitosan coating, aerobic mesophilic and cryophilic bacteria decrease significantly over the storage period (Duan *et al.*, 2010). Yingyuad *et al.*, (2006) obtained similar results in using chitosan coating 2% in pork (Yingyuad *et al.*, 2006). The results of this study are consistent with Yingyuad *et al.* (2006) and Fan *et al.* (2009) who showed that the use of chitosan coating in mean samples decreases pH relative to the control samples that is due to acidic chitosan coating on meat and its microbial growth inhibition properties (Fan *et al.*, 2009; Yingyuad *et al.*, 2006). In this study, employing common medlar concentrate that naturally has organic acids and phenolic compounds, the antimicrobial effects are enhanced. The antimicrobial activity mechanism of common medlar concentrate, in addition to reducing pH, leads to phenolic compounds reaction with microbial cells membrane protein and inhibition of glycosyltransferases that finally leads to microbial cell membrane decomposition (Ismail, Sestili, & Akhtar, 2012).

Assessment of sensory index

Sensory assessment scores showed a

considerable decrease in all samples until the end of the storage period (Fig. 5). In chicken samples, sensory scores above 10 were acceptable for consumption. In this assessment, the control sample received lower scores than other treatments. Also, composite treatments showed higher acceptability than other treatments, so that until the 12th day, they were acceptable for the panel members while the control sample, was unacceptable on the 8th day and the sample with chitosan coating containing Zingiber essential oil 2% and common medlar concentrate received the highest scores on the last day. Generally, samples containing chitosan coating and common medlar concentrate and essential oil, in addition to chemical tests, were effective in sensory tests compared with other treatments in maintaining chicken breast fillet quality. In a study by (Latou, Mexis, Badeka, Kontakos, & Kontominas, 2014), chicken fillets coated with chitosan with packaging in the modified atmosphere, could be acceptable until the 14th day in terms of sensory index while the control sample lost its acceptability after 5 days (Latou *et al.*, 2014).



Sensory evaluation

Fig. 5. Sensory score changes in terms of total acceptability in various chicken breast fillet treatments stored in the refrigerator

*Different lowercase letters (a, b, c) in each chart indicate a significant difference ($P < 0.05$) in various treatments.

Conclusions

According to the results of this study, it was specified that simultaneous use of common medlar concentrate, chitosan with Zingiber extract 2% increases shelf life of chicken fillet for 12 days compared with the treatments without coating. Therefore, with more studies, these coatings can be used in food industry and the related sciences for optimal use of plant compounds and other effective compounds and replace them with chemical preservatives.

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ارزیابی فساد شیمیایی و میکروبی فیلۀ مرغ پوشش داده شده با کیتوزان، اسانس زنجبیل و کنسانترۀ ازگیل طی نگهداری در دمای یخچال

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چکیده

اکسیداسیون لیپیدها و رشد میکروبی از جمله عوامل مؤثر بر خصوصیات کیفی و فسادپذیری گوشت در طی نگهداری است. در این مطالعه به کارگیری پوشش خوراکی کیتوزان، کنسانترۀ ازگیل، اسانس زنجبیل به تنهایی و در ترکیب با یکدیگر بر کاهش فساد شیمیایی و افزایش زمان ماندگاری گوشت مرغ نگهداری شده در دمای یخچال مورد بررسی قرار گرفت. ۸ گروه مورد بررسی طی یک دورۀ ۱۲ روزه در دمای یخچال نگهداری شده و از نظر شاخص‌های شیمیایی اکسیداسیون اولیه (PV)، ثانویه (TBA) و شاخص بازهای فرار (TVB-N) و میکروبیولوژیکی (شمارش باکتری‌های مزوفیل هوازی و سرما دوست)، ارزیابی حسی در روزهای صفر، ۴، ۸ و ۱۲ مورد بررسی قرار گرفتند. تست توتال فنل و قدرت احیا کنندگی نیز به منظور بررسی خواص ضد اکسایشی انجام گردید. براساس نتایج حاصل از دستگاه گاز کروماتوگراف-طیف‌سنج جرمی (GC-MS) ترکیبات اسانس زنجبیل آلفا-زینجیبرن (۳۶/۵۴ درصد)، بتا-سکوایفلاندرن (۱۶/۴۵ درصد) و ترانس-گاما-کادینن (۱۰/۲۷ درصد) بودند. نتایج نشان داد که در تیمارهای پوشش داده شده با کیتوزان حاوی اسانس زنجبیل ۲ درصد و کنسانترۀ ازگیل شمارش شاخص‌های میکروبی ذکر شده در مقایسه با گروه کنترل به صورت معنی داری کاهش یافت ($P < 0.05$) و شاخص‌های اکسیداسیون در مدت زمان نگهداری، تغییرات کمتری داشته و با اختلاف معنی دار ($P < 0.05$)، قوی‌ترین اثر ضد اکسایشی و حسی نسبت به سایر گروه‌ها را به خود اختصاص داد. نتایج میکروبیولوژیکی، شیمیایی و حسی این مطالعه نشان داد که اثر پوشش کیتوزان همراه با کنسانترۀ ازگیل و اسانس ۲ درصد باعث حفظ کیفیت و افزایش ماندگاری به مدت ۱۲ روز در شرایط یخچالی شد.

واژه‌های کلیدی: اسانس زنجبیل، پوشش کیتوزان، فساد، فیلۀ مرغ، کنسانترۀ ازگیل

Development of a Novel Edible Surface Coating Made by *Lepidium sativum* Seed Gum and Comparison of its Effect with Traditional Glazes of Sorghum Gluten-Free Bread

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Abstract

Glazing process can improve the baked texture and overall quality of bread. In this study, the effects of traditional glazes (water, oil, cheese powder, xanthan gum) on the physicochemical and sensory parameters of sorghum gluten-free bread were compared with *Lepidium sativum* seed gum. Specific volume, porosity, water activity, moisture, firmness and sensory parameters of breads were evaluated. Results showed cheese powder had the lowest moisture and vegetable oil treated samples had the lowest water activity. *Lepidium sativum* seed gum and xanthan gum provided the high effect on specific volume and porosity. Evaluation of crumb firmness implicated water, *Lepidium sativum* seed gum diminished the bread staling and these treated samples had the lowest firmness. Application of glazing ingredients did not have any significant ($P>0.05$) effect on odor and taste. *Lepidium sativum* seed gum as a novel glaze was more effective than xanthan gum and its application was better than the usual glazes to improve the crust and overall quality of gluten-free bread.

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Key words

Glaze

Gluten-free bread

Lepidium sativum

Porosity

Introduction

The proposition of a diet for celiac people is not easy, since the most commonly baked products are usually prepared with wheat flour and are consumed on an every-day basis by most people but the effective treatment for this patient's lifetime is a strict adherence to gluten-free diet (Gallagher, Kunkel, Gormley, & Arendt, 2003). Gluten-free flours reduced the freshness and other parameters of loaves of bread. Meanwhile, studies on the use of methods to improve quality and acceptability of gluten-free loaves of bread were very rare (Shittu, Aminu, & Abulude, 2009). Among these, application of glazes seems to be one of the effective processes

to improve gluten-free bread quality. However, only very limited researchers have reported the effect of glazing on quality of bakery products especially the items that have gluten-free and wheatless flours (Casper, Oppenheimer, Weber, Erickson, & Ray, 2006; Hahn, Huang, Goedecken, & Sierzant, 2001; Hayes-Jacobson, 2003). Glazing process can also be increased the baked specific volume (Lonergan, 1999). Lang, Eberhardt, Entenmann, & Shipman (1987) found that glazes can be used to provide a glossy appearance. Lonergan (1999) reported that glazing was comprised of about 20-90% water, 0.1-5% of reducing sugar, 0.1-15%

of an edible hydrocolloids, and 10-80% vegetable oil. The glazing concentration of 8-10% or 3-10% of dough weight was recommended. According to these studies, finding new sources that could improve the quality and quantity of bakery products is still a need. *Lepidium sativum* is annual herb, which belongs to the Cruciferae family, which grows widely in the Middle East, Europe and USA (Karazhiyan *et al.*, 2009). *Lepidium sativum* seed exhibits a quick adsorption when soaked in water and produces a large amount of mucilaginous substance (Sahraiyani, Naghipour, Karimi, & Davoodi, 2013). The macromolecular component of *Lepidium sativum* seed gum (as a new hydrocolloid) is nearly as rigid as xanthan with regard to chain conformation emulsifying and foaming properties which reminds unchanged after heating. Heating increased in viscosity of solution of this gum (Naji & Razavi, 2014; Sahraiyani *et al.*, 2013). In addition, *Lepidium sativum* seed is an important medicinal source use in traditional medicine for the treatment of various diseases, such as antibacterial, anti-asthmatic, aphrodisiac, and aborti-facient (Doke & Guha, 2015). Therefore in the present study the behavior and alterations in physicochemical and sensory parameters of sorghum gluten-free bread affected by using glazing method and compared *Lepidium sativum* seed gum as a novel gum with xanthan gum, cheese powder, vegetable oil, and water.

Materials and methods

Materials

Sorghum flour with 11% moisture, 10.11% protein, 3.2% lipid, 1.35% ash and 0% wet gluten were procured from Khorasan Razavi agricultural and natural Resources research education center Mashhad, Iran. *Lepidium sativum* seeds with 7.1% moisture, 11% ash, 2.09% protein, 2.2% fat and 77.5% carbohydrate were procured from the traditional medicinal plant market. The seeds were manually cleaned

to remove all foreign matter such as dust, dirt, stones, chaff, immature and broken seeds then Aqueous *Lepidium sativum* seed gums were extracted from whole seeds using distilled water (Karazhiyan, Razavi, & Phillips, 2011). Cheese Powder (Pegah Co., Tehran, Iran), vegetable oil (Narges Co., Shiraz, Iran), fresh yeast (Razavi Co., Mashhad, Iran), Guar and xanthan gum (Pars Behbod Asia Co, Mashhad, Iran) and other ingredients such as salt, sugar, powdered egg whites and soybean isolated protein were procured from local market.

Methods

Preparation of bread with gluten free flour (Sorghum bread)

Gluten-free breads were produced by sorghum flour (100 parts), guar gum (1.5 parts), xanthan gum (1 part), soybean isolated protein (9 parts), egg white powder (4 parts), fresh yeast (4 parts), salt and sugar (1 part), vegetable oil (1 part) and water (100 parts because of gluten-free bread). The gluten-free bread. These breads were produced by mixing all ingredients (6 min), fermenting for 30 min at 45 °C and 85% relative humidity, dough dividing (loaf weight was 500 g) and rounding, sheeting (size of dough was 70 cm length, 25 cm width and 2 cm thickness). Then, resting in fermentation cabinet for 20 min and baking at 220 °C for 13 min. The size of dough before baking was 71.0×25.5×3.1 cm and the weight of dough was 497 g. After cooling the samples were packed in polyethylene bags and stored at 25 °C (Maleki, Vetter, & Hoover, 1981).

Preparation of glazing

The glazing composition (Table 1) (vegetable oil, water, cheese powder, and xanthan and *Lepidium sativum*) were added to the cold water under agitation. The mixture was heated to 80 °C and stored at temperature 25 °C before using (4 h). The formulations (Control (without glaze) vegetable oil (100%), water (100%),

vegetable oil+cheese powder (90+10%), vegetable oil+water+xanthan (20+75+5%) and vegetable oil+water+*Lepidium sativum* seed gum (20+75+5%) may be applied to the top of surface of dough by wiping (1 mL of coating was equivalent 0.1 mm thick (Razavizadegan Jahromi *et al.*, 2012)) before the final proofing step (Lang *et al.*, 1987).

Table 1. Preparation of different glazing formulas

Materials	Formula (g)				
	1	2	3	4	5
Vegetable oil	100	-	90	20	20
Water	-	100	-	75	75
Cheese powder	-	-	10	-	-
<i>Lepidium sativum</i>	-	-	-	5	-
Xanthan	-	-	-	-	5

Bread quality

Moisture

Moisture was determined following AACC methods (AACC, 2000). The following equation was applied to determine the moisture. For this purpose, 10 g of whole bread was placed into an oven (Jeto Tech, model OF-O2G, South Korea) with the temperature of 100-105 °C for 2 h after 1 and 3 days of storage (5 °C).

$$MC = \frac{m_1 - m_2}{m_0} \times 100 \quad (1)$$

Where m_0 indicates the samples' weight, m_1 expresses the weight of sample before placing in oven and m_2 demonstrates the plate's plus sample's weight after passing oven stage.

Porosity

To evaluate the porosity value of gluten-free bread's internal part, the piece of bread in the size (area) of 2×2 cm of internal part of the produced bread were provided with the use of an electric saws (model 41600, 120 w, England), then using a scanner (model HP Scanjet G3010, American), the required images were taken with the clarity of 300 pixel. The taken images were analyzed by Image J (ordered by National Institute of Health-the USA), with activation of part Bit of the software, and

the grey images were created. To convert the grey images to Binary ones, a Binary section of the software got activated. These images are a collection of bright and dark points where the proportion of bright points to dark ones is an index of the porosity of the samples. It is obviously understood that, the more porosity of the achieved breads might exist. Actually with activation of the relevant part of the software, this proportion might be determined and as a result, the porosity value of the samples could be measured Specific volume was determined by rapeseed displacement method. It was determined at 2 h's after baking (Bárceñas & Rosell, 2006; Sabanis, Tzia, & Papadakis, 2008).

Water activity (aw)

The water activity has been detected with the use of an equipment (Novasina ms1-aw, model AXZIR Ltd, Switzerland) after 1 and 3 days of storage (5 °C) (Sahraiyen *et al.*, 2013). For this purpose, 5 g of whole bread was placed into cell for 80 s.

Crumb texture evaluation

A texture analyzer (Farnell Model QTS-CNS, UK) was used to measure the force required for penetration of a round-bottom (2.5 cm diameter×1.8 cm height) probe at a velocity of 30 mm/min and descended 30 mm (a sufficient distance to pass through the slice of 10×10 cm of bread) into the bread. Trigger value 0.05 N (Pourfarzad *et al.*, 2011).

Sensory analysis

For this purpose, 10 different panelist from the faculty of Khorasan Razavi Agricultural Research and Education Center (Mashhad-Iran) according to triangular test and Gasola and sink 1984 approach, were determined. The pieces in the size (area) of 5×5 cm of the produced bread were provided with the use of an electric saws, then used for the sensory analysis. A hedonic scale of nine points

(1: dislike extremely, 9: like extremely) was used for overall acceptability. Here, the overall quality of breads was evaluated by considering the upper surface properties, odor, taste and overall quality.

Statistical analysis

All samples were evaluated in three batches. In order to assess significant differences among treatments, a complete randomized design of triplicate analyses of six samples was performed using the MSTATC program (version 1.41). Duncan's new multiple range tests were used to study the statistical differences of the means with 95% confidence.

Results and discussion

Water activity

Water activity was indicated in Fig. (1). The values were significantly ($P < 0.05$) differed in the range of 0.74-0.89 and 0.69-0.82 at first and third days of storage. *Lepidium sativum* had the highest water

activity in the first day but only *Lepidium sativum* seed gum had the effect to keep the level of water activity three days after baking. This behavior may be pertaining to ability of *Lepidium sativum* seed gum to prevent cracking of dough surface and protect moisture during baking and storage. Razavizadegan Jahromi *et al.* (2012) found that using oil on the dough surfaces had an unpleasant effect on moisture barrier property, since they tend to crack upon the handling or during the temperature changes. Cracking of dough surface lead to exiting of moisture and consequently, reduction in water activity. Hahn *et al.* (2001) indicated that using oil and fat as a coating can acts as a physical barrier, which indirectly impairs the water activity. Sahraiyani *et al.* (2013) reported that, using *Lepidium sativum* seed gum in composite bread formula due to creating thick surface and Slight shrinkage might prevent the reduction of product's moisture and water activity.

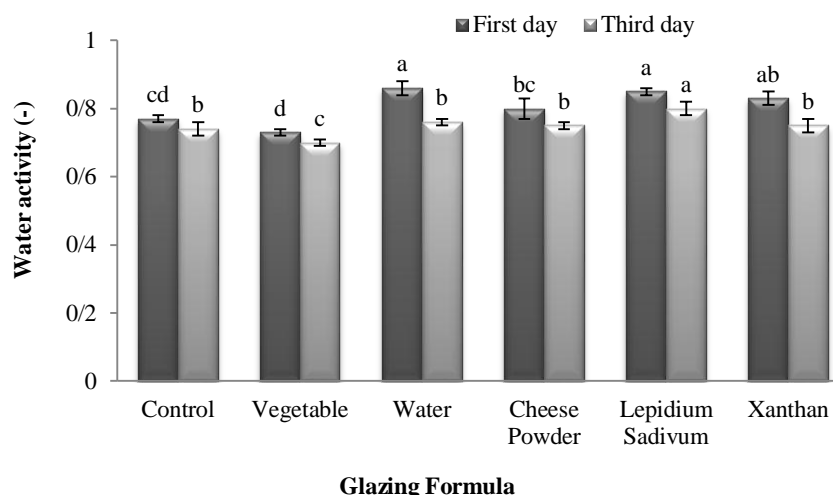


Fig. 1. Effect of different glazing formulas on water activity after storage for 1 and 3 days at 5 °C of gluten-free bread,

* Different letters show the statistically significant differences ($P < 0.05$).

Moisture content

Different glazes had the significant ($P < 0.05$) effect on the moisture content of gluten-free bread (Table 2). The Moisture content of the control sample was $22.96 \pm 1.13\%$. After glazing, the moisture content has differed in the range of 20.42-

28.71 and 18.04-26.67 at first and third days of storage. The Moisture content of all samples was significantly higher than control except vegetable oil and cheese powder. The vegetable oil treated samples were as same as control at first day. The moisture of both treatments was more than

cheese powder. At the third day, control and cheese powder treated samples had the lowest moisture. Whey protein (β -lactoglobulin) is most important in the formation of gel in 75 °C (Invensys, 2002) and to improve the quality of glaze. Cheese powder had the lowest β -lactoglobulin. So, it isn't capable to protect the moisture of sample because of low quality gel formation. Averbach (1992) reported that oil and fats as coating surface tend to crack upon the handling or during the temperature changing and have some disadvantages on the moisture barrier. Due to cracking the crust of sample, a lot of water exits from the vegetable oil-treated samples. *Lepidium sativum* seed gum had the highest moisture at first and third days. Also, there were no significant differences ($P<0.05$) between water and xanthan in moisture content on the first day but xanthan is better than water three days after baking because of xanthan treated

samples had higher moisture than water treated samples after 3 days storage. This improvement in moisture content by hydrocolloids is attributed to water retention ability of this polymer which is as the result of their hydrophilic nature (Bárcenas & Rosell, 2006). Mohammadi, Sadeghnia, Azizi, Neyestani, & Mortazavian (2014) investigated the effect of xanthan gum and carboxymethyl cellulose (CMC) on quality parameters of gluten-free bread, based on rice and corn starch. The bread containing xanthan showed the highest moisture content, so using xanthan gum was more effective in decreasing hardness, in both fresh and stored bread. Naji-Tabasi & Mohebbi (2015) reported xanthan and *Lepidium sativum* seed gum were hydrated in cold water and produced a viscose solution. This gel had proper texture characteristics to link with water and keep it during baking and storage.

Table 2. Effect of different glazes on moisture, specific volume and porosity of gluten-free bread

Treatments	Moisture (%)		Specific volume (cm ³ /g)	Porosity (%)
	First day	Third day		
Control	22.96±1.13 ^b	18.23±0.32 ^d	1.90±0.3 ^c	36.3±1.1 ^c
Vegetable oil	22.48±0.87 ^b	19.97±1.01 ^c	1.89±0.1 ^c	37.1±0.7 ^c
Water	26.93±0.93 ^a	24.91±0.79 ^b	2.45±0.4 ^b	41.0±0.4 ^b
Cheese powder	20.42±1.22 ^c	18.04±0.53 ^d	1.90±0.3 ^c	38.4±0.9 ^{bc}
<i>Lepidium sativum</i>	28.71±1.14 ^a	26.97±1.07 ^a	3.40±0.2 ^a	45.3±0.4 ^a
Xanthan	26.68±0.39 ^a	25.33±0.57 ^b	2.97±0.5 ^b	43.7±1.2 ^a

Different letters show the statistically significant differences ($P<0.05$).

Specific volume and porosity

The results of specific volume and porosity analysis of samples are presented in Table (2). The Binary images of treatments were shown in Fig. (2). Results indicated that specific volume was enhanced by water, xanthan and *Lepidium sativum* gum. The highest porosity of gluten-free bread (binary images showed the most proportion of bright to dark points) was related to *Lepidium sativum* treatment. These increasing behaviors may be pertaining to the ability of *Lepidium sativum* gum to permit dough expansion during the baking process. The hydrocolloids give stability to interface upper surface which conferees

additional strength to the gas cells through baking. Consequently, the gas losses will be reduced and in turn, bread volume will improve (Bárcenas & Rosell, 2006). Generally, Lonergan (1999) reported that the glaze helps to keep the outer surface of the dough malleable without the addition of water, thereby delaying the setting of the outer dough surface, while not affecting the amount of water absorbed by the outer dough surface. Naji-Tabasi & Mohebbi (2015) studied the effect of *Lepidium sativum* (cress seed) gum and xanthan on gluten-free bread. The results exhibited that hydrocolloids, by foaming thick layer, influenced the stability of gas

cells and caused more regular pores (increasing specific volume and porosity) in gluten-free bread which was more noticeable in bread containing cress seed gum. Demirkesen *et al.* (2014) studied the effect of hydrocolloids and hydrocolloids blend on gluten-free bread. The highest number of pores and lowest average area of pores were obtained from gluten-free bread prepared with the addition of xanthan, CMC, xanthan-guar gum, xanthan-locust bean gum and hydroxypropyl methylcellulose (HPMC), which is associated texture of these crumbs. So the use of hydrocolloids especially *Lepidium sativum* gum represents the most widespread approach used to glaze in manufacture of gluten free bread.

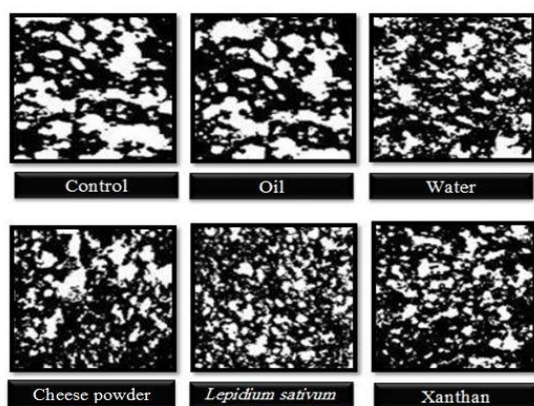


Fig. 2. Effect of different glazing formulas on binary images of gluten-free bread

Texture

The results showed significant differences in crumb firmness of samples ($P < 0.05$), which varied between 1.59 ± 0.17 and 3.13 ± 0.1 N (Fig. 3). The outcomes indicated that firmness reduced with using water, xanthan and *Lepidium sativum* seed gum. Among all treatments, *Lepidium sativum* seed gum formula had the lowest and cheese powder formula had the highest firmness. The improving effect of precipitated whey protein on softness, yellowness (b^* value) and sensory quality of lavash bread has been reported by Jooyandeh (2009). The adverse effect of

whey protein concentrate (WPC) (over 5% level) on the quality characteristics of parotta (UFB) especially softness has been found by (Indrani, Prabhasankar, Rajiv, & Rao (2007)). These researchers reported an unpleasant increment in surface thickness of WPC-treated samples which increased crumb firmness. On the other hand, vegetable oil treated sample did not show the significant difference compared to the control. The anti-staling effect of water, xanthan and *Lepidium sativum* seed gum may be related to their ability to retain the water on the top of dough surface. Mariotti, Pagani, & Lucisano (2013) studied the influence of HPMC on the bread making properties of some commercial gluten-free bread. The results showed the presence of HPMC proved useful in reducing diffusion and loss of water from crumb and crust, resulting in a softer gluten-free crumb and slower staling kinetics during storage. Hydrocolloids consist the number of water-soluble polysaccharides with varied chemical structures providing a wide range of fictional properties such as increasing specific volume and porosity and decreasing crumb hardness (Li & Nie, 2016).

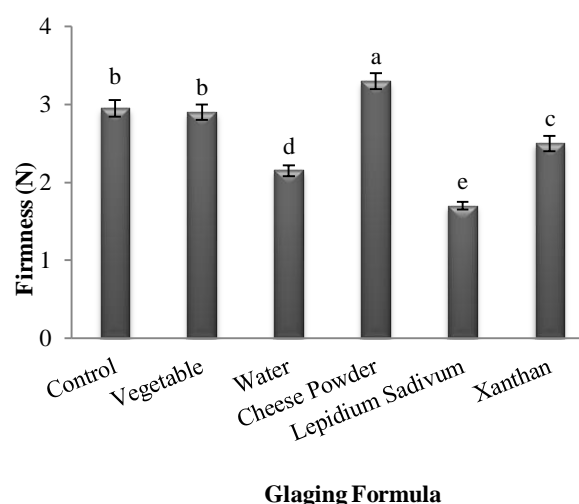


Fig. 3. Effect of different glazing formulas on firmness of gluten-free bread, different letters show the statistically significant differences ($P < 0.05$)

Sensory analysis

Outcomes indicated *Lepidium sativum* seed gum treated samples had the highest upper surface and overall quality score (Table 3). Based on the present result, it might be conveyed that the produced surface in the presence of *Lepidium sativum* seed gum, might express higher moisture content and as a result higher brightness of the product was achieved. In this regard, Purlis & Salvadori (2009) reported that the moisture

maintenance ability during the cooking process, might lead to the production of the smooth surface, as a result of it the light reflex of the products' surface increases and the brightness of the produced product increases. Furthermore, the achieved results presented that the general acceptance is in the middle of moisture content and water activity, indicating the positive effect of these two parameters on the general acceptance of final products.

Table 3. Effect of different glazes on sensory properties of gluten-free bread

Treatments	Properties		
	Upper surface	Odor and taste*	Overall quality
Control	5.2±0.20 ^c	6.4±1.01 ^a	5.4±0.26 ^c
Vegetable oil	5.5±0.37 ^c	5.9±1.16 ^a	5.4±0.32 ^c
Water	6.2±0.90 ^b	6.5±1.41 ^a	5.9±0.14 ^c
Cheese powder	5.1±0.30 ^c	7.1±0.74 ^a	5.7±0.30 ^c
<i>Lepidium sativum</i>	7.8±0.24 ^a	7.3±1.25 ^a	7.8±0.92 ^a
Xanthan	6.5±0.76 ^b	7.0±0.69 ^a	6.9±0.28 ^b

Values are the average of three replicates samples; all scores were from 0 to 9, with 9 being the highest value.

* $P < 0.05$, not statistically significant.

Conclusions

The quality properties of different glazing such as water, vegetable oil, cheese powder, xanthan and a native hydrocolloid (*Lepidium sativum* seed gum) on gluten-free bread were evaluated. Some glazing treatments probably provided the layer of the moisture barrier on the surface of the dough and restricted migration of water from crumb to crust, therefore increasing the specific volume, porosity and moisture

while decreasing firmness of gluten-free bread. Finally, outcomes indicated *Lepidium sativum* seed gum (a novel and local hydrocolloid) is the most effective than the traditional glazes to improve gluten-free bread quality and quantity. Due to the results of this project, the glazes have better effects on sensory and technological properties of part baked gluten-free bread. Then, it is recommended to use glazes in these products.

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توسعه کاربرد پوشش خوراکی جدید حاوی صمغ شاهی و مقایسه اثرات آن با رومال‌های سنتی نان بدون گلوتن بر پایه آرد سورگوم

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چکیده

رومال‌ها جهت بهبود بافت و ویژگی‌های ظاهری نان استفاده می‌شوند. در این پژوهش اثرات رومال‌های سنتی (آب، روغن، پودر پنیر و صمغ زانتان) و رومال حاوی صمغ شاهی بر ویژگی‌های فیزیکوشیمیایی و خصوصیات حسی نان بدون گلوتن مقایسه شدند. بدین‌منظور حجم مخصوص، تخلخل، فعالیت آبی، رطوبت، سفتی بافت و ویژگی‌های حسی نان بررسی گردید. نتایج نشان داد، نمونه‌های حاوی پودر پنیر و روغن کمترین میزان رطوبت و نمونه‌های حاوی صمغ شاهی و زانتان بیشترین حجم مخصوص و تخلخل را داشتند. همچنین نتایج حاکی از آن بود نمونه‌های تیمار شده با رومال آب و صمغ شاهی دارای کمترین میزان بیاتی و سفتی بافت بودند. این در حالی بود که تمام رومال‌های استفاده شده در این پژوهش اثر معنی‌داری ($P > 0.05$) بر بو و مزه نمونه‌های تولیدی نداشتند. در نهایت باید گفت، صمغ شاهی به‌عنوان یک رومال جدید عملکرد بهتری در مقایسه با صمغ زانتان داشت و از رومال حاوی این صمغ می‌توان جهت بهبود سطح نو ویژگی‌های کیفی و ظاهری نان بدون گلوتن استفاده نمود.

واژه‌های کلیدی: تخلخل، رومال، صمغ شاهی، نان بدون گلوتن

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State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

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The methodology must be clearly stated and provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Results and discussions (bold)

The findings and arguments of the work should be explicitly described and illustrated. Supporting figures, tables and images of the results (no more than eight figures and eight tables) may be included in the extended abstract.

Tables and Figure

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article.

Please avoid using vertical rules and shading in table cells. for the tables, they should also be numbered (Table 1) and the table header should be placed at the top.

Examples:

Table 1. Effect of modified atmosphere packaging on quantity and quality properties of strawberry

Statistical sources	DF	Mean of squares				
		Weight loss (%)	Texture firmness (n)	Soluble solids (brix)	Titrateable acidity (%)	pH
Gas combination	2	0.003144**	0.00274**	2.02374**	1.85096**	0.129980**
Temperature	1	0.014569**	1.81134**	13.48001**	2.29402**	0.248067**
Thickness	2	0.009124**	0.0458 ^{ns}	1.64067**	0.95045**	0.002230 ^{ns}
Temperature×Gas combination	2	0.000009 ^{ns}	0.12647**	4.98624**	0.36987*	0.006939 ^{ns}
Gas combination×Thickness	4	0.000330 ^{ns}	0.87377**	1.01431**	1.02597**	0.112063**
Thickness×Temperature	2	0.000810*	0.16010**	0.09060*	0.06511 ^{ns}	0.097067**
Gas combination×Thickness×Temperature	4	0.000079 ^{ns}	0.31488**	0.42611**	0.20161*	0.76389**
Error	36	0.000128	0.01429	0.02488	0.04833	0.003937
coefficient of variations	2	17.2	4.5	1.4	2.9	1.4

ns: non-significant, *($P \leq 0.05$) and ** ($P \leq 0.01$)

The tables and figures should be centered. Figures should be numbered (Fig. 1) and figure headers should be placed under the figure. Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

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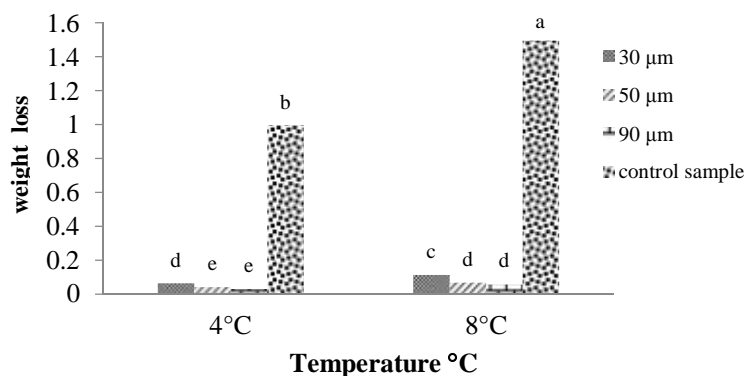


Fig 1. The interaction effect of temperature and coating thickness on strawberry weight loss percentage (Non-similar letters indicate a significant difference between treatments)

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4. Give the meaning of all symbols immediately after the equation in which they are first used.
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11. The repeated writing of chemical formulae in the text is to be avoided where reasonably possible; instead, the name of the compound should be given in full.

Conclusion (bold)

Conclusions should include (1) the principles and generalisations inferred from the results, (2) any exceptions to, or problems with these principles and generalisations, (3) theoretical and/or practical implications of the work, and (4) conclusions drawn and recommendations.

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Alinasabhematabadi, L. (2015). *Protein oxidation in Atlantic mackerel (*Scomber scombrus*) during chilled and frozen storage*. (master's thesis), NTNU, Retrieved from https://ntnuopen.ntnu.no/ntnu-xmlui/bitstream/handle/11250/2351628/8731_FULLTEXT.pdf?sequence=1&isAllowed=y

Holopainen-Mantila, U. (2015). *Composition and structure of barley (*Hordeum vulgare* L.) grain in relation to end uses*. (Doctoral dissertation), Department of Biosciences, Faculty of Biological and Environmental Sciences, Plant Biology, VTT Technical Research Centre of Finland, University of Helsinki, Retrieved from <https://helda.helsinki.fi/bitstream/handle/10138/153489/S78.pdf?sequence=1&isAllowed=y>

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Ghorbani Hassan Sariei, A., Shahidi, F., Bahadorghousi, H., & Motamedzadegan, A. (2012, October). *Potentials of different omega-3 fatty acids in food enrichments*. Paper presented at the 2nd Food Security Conference, Islamic Azad University, Savadkuh Branch. (in Persian) https://www.civilica.com/Paper-FSS02-FSS02_320.html

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چکیده فارسی با قلم B Nazanin11 در قالب یک پاراگراف، پس از اسامی نویسندگان (گان) قرار گیرد. چکیده باید در عین مختصر بودن، به روشنی گویای محتوای مقاله بوده و صنعتی‌بودن طرح در مقاله اشاره شود و با تأکید بر روش‌ها، بیانگر نتایج و اهمیت کاربرد آنها باشد و در آن از کلمات اختصاری مبهم استفاده نشود. از ذکر منابع نیز خودداری گردد. تعداد کلمات چکیده بین 150 تا 250 کلمه است. واژه‌های کلیدی با قلم B Nazanin11 به صورت برجسته و به ترتیب حروف الفبا نگارش شوند. تعداد واژگان کلیدی از 5 مورد بیشتر نباشد. از آوردن پاورقی در عنوان، چکیده فارسی و انگلیسی اجتناب گردد (سبک چکیده)

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نشریه پژوهش و نوآوری در علوم و صنایع غذایی

شماره پروانه: ۹۲/۲۰۵۱۹

جلد ۸، شماره ۴، سال ۱۳۹۸

صاحب امتیاز: مؤسسه پژوهشی علوم و صنایع غذایی

مدیر مسئول: دکتر رسول کدخدایی

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بازدهی استخراج بتا - دی - گلوکان از ضایعات قارچ دکمه‌ای (آگاریکوس بیسپوروس) و توانایی آن در جذب آفلاتوکسین B₁ ۳۱۵

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