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An Experimental Study on the Effect of Thermal Shield on Energy Saving in Cooking Pot

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Abstract
The present study aims at reducing the energy consumption in the cooking process by using a simple technical method, a thermal shield around the cooking pot. The research has conducted experimentally and the effect of the thermal shield on the thermal efficiency was investigated regarding the geometric parameters of the pot (diameter and height) and the amount of fluid in the pre and post-boiling stages. The results showed that the thermal shield has a positive effect on pre-boiling stage leading to an average amount of 20% energy savings independent of geometry and the amount of fluid. It was also shown that the effect of thermal shield in the boiling stage is a function of liquid height inside the container. Also, for a constant value of thermal energy, the effect of thermal shield increases with the container height without limitation. It was also shown that the effect of the thermal shield in boiling stage is a function of the height of liquid inside the container. Finally, an economic investigation for Iranian households showed that utilization of a thermal shield in cooking process will save energy consumption equivalent to 12.5 million barrels oil per year.

Keywords
Cooking Pot
Energy
Energy Consumption
Energy Saving
Fossil Fuel

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Introduction
Energy savings has become one of the critical international policies in the field of research and management. The limitation of fossil fuels, the pollution resulting from using these fuels, and the impact of political changes on energy resources are some of the important parameters in this area. A lot of researches have been devoted to improve the energy consumption pattern in various fields, annually. Part of research in this area is performed with the purpose of facilitating the transition of energy resources from fossil fuels to renewable energies; some other research has been focused on the optimization of fossil fuel and renewable energy consumptions. Also, several researchers have focused on the energy recovery in order to design and implement approaches to recover a part of energy loss in thermodynamic systems. Generally, energy consumption optimization is a strategic policy in both global and national scales. Besides considering the energy consumption quality, a significant portion of efforts
has been devoted to hazard elimination, increasing the system efficiency, and decreasing the production of carbon oxides. According to the literature, more than 30% of energy consumption is related to the household energy use 80% of which is associated to cooking (Moshiri et al., 2011). In some research it has been reported that up to 90% of household energy consumption is attributed to cooking in developing countries (Nahar & Gupta, 1991). Thus, the considerable growth in solar cookers can be attributed to this fact, because these systems provide the possibility of using the solar energy for cooking instead of fossil fuels (Panwara et al., 2011). In this regard, other renewable energies such as biomass have been taken into consideration (McKendry, 2002). Based on the considerable contribution of fossil fuels in this area, a great deal of researches have been carried out to reduce the energy consumption in cooking process; for instance, increasing the heat transfer surface in cooking pots or cook stoves. In less developed regions, plans for utilization of green energy in energy-efficient stoves have been proposed (Thacker et al., 2017). Also, in developing countries, as a result of sustainable development, pots equipped with thermal shield are used to save thermal energy as much as possible (World Bank, 2014). Despite the existence of several studies in the area of optimal energy consumption in cooking pots, investigating the utilization of thermal shields both experimentally and numerically is increasingly attracting attention. Using an appropriate cooking method not only should successfully resolve the high energy consumption and production of environmental pollutants challenges, but also has to be consistent with the cooking local traditions. Various studies have proven that the three sides of an ideal cooking, that is optimal energy consumption, minimal production of pollutants, and consistency with cooking traditions, can be assumed as functions of the pot shape, the heat transfer mechanism to pot, and the energy source. Thus, the proper variables of cooking system can be defined. Many researchers have focused on the simultaneous effect of geometry and heat transfer mechanism to the pot in order to achieve an optimal cooking process. In this regard, Cadavid et al. (2014) have simulated the thermal efficiency of a pot as a function of geometrical properties (height, diameter and material) via an experimental-numerical approach using ANSYS-fluent software. Their results showed that the best thermal efficiency was related to a pot with shortest wall and largest diameter. Also, Hannani et al. (2006) have mathematically investigated the effect of the pot diameter, corner angle, and wall slope on the thermal efficiency using neural network algorithm. They showed that increasing the pot diameter, bottom wall curvature and pot wall slope leads to an increase in thermal efficiency. Also, their results showed that increasing the ratio of pot diameter to pot height improves the thermal efficiency. According to the obtained results, the height of cooking material in the pot has an insignificant impact on the quality of heat absorption. In addition to the pot geometry, the cooking traditions directly affect the thermal efficiency; for instance, closing the lid during cooking, using all the volume of container and utilization of thermal isolation can decrease the energy consumption. Also, in addition to the thermal efficiency which is a function of pot geometry, the amount of pollutant production may be effectively reduced by properly designing the pot
An Experimental Study on the Effect of Thermal Shield …

(Karunanithy & Shafer, 2016). For this purpose, one can refer to Arora et al. (2014) wherein three modern technologies of high efficiency cooking are compared with the traditional brick-mud cook stove; the obtained results showed that the carbon monoxide emission was reduced in modern cook stoves. Arora et al. (2014) have evaluated the effect of chemical components and humidity of fuel on the thermal efficiency. Furthermore, the quality of heat transfer to cooking pot has been considered as a key factor in optimization of cooking process. In this regard, Kanjanapongkul (2017) has used ohmic heating to uniformly cook rice. They compared the performance of ohmic heating with other modern devices such as electric rice cooker and microwave. Daigoglou et al. (2012) have investigated both induction and natural gas cook stoves’ pot to optimize the geometry and material of pots and propose novel designs. In addition to above mentioned research, which studied the effect of pot geometry, heating mechanism, and traditions related to how a cooking pot is used, some other studies have been devoted to the proper cooking standards in underdeveloped areas, rural and suburb areas. Regarding the fact that the fuel used in these regions is highly polluting, the main focus of research has been on the optimization of burning process in cook stoves. It should be mentioned that approximately around one-third of world population does not have access to proper cooking devices with low level of pollution production. It seems that this ratio does not change until 2030, (MacCarty et al., 2010). Burning the biological materials leads to emission of hazardous pollutants including carbon monoxide, nitrogen oxides, phosphor oxide and other airborne particles jeopardizing the human health (WHO, 2016). In the last decade, considerable amount of effort has been taken in developing countries to develop biomass cook stoves. According to the fact that almost 3 billion people around the world are still using fire or wood fuel, charcoal or other solid fuel cook stoves, a genuine motivation has been created in this research area (WHO, 2016). Utilization biomass cook stoves have been considered as one of global warming factors. Around 22% of airborne soot particles are produced because of burning biomass. However, fossil fuels only produce 7% of total soot. Previous studies showed that the adverse effect of biomass burning is the cause of death of around 3 million people in the world, annually (Kshirsagar & Kalamkar, 2015). The biomass cook stove performance improvement plan focuses on the reduction of pollutant emission in indoor environment, reducing the production of greenhouse gases, and efficient use of fuel. Based on these efforts, a considerable improvement has been achieved in the areas of thermal efficiency and cleanliness standards (Kshirsagar & Kalamkar, 2014; Funk, 2000). In the present paper, the effect of thermal shields on thermal energy absorption efficiency for cooking pot in an axial natural gas fueled cook stoves is investigated, experimentally. The idea of using a thermal shield to improve the heat absorption in the pot can be a proper approach to achieve a fast and cheap cooking process. Therefore, the main innovation of present study is to propose a simple approach to reduce the household energy consumption and analyzing its influence in national scale.

Problem description
In present study, the thermal energy absorption efficiency in a pot equipped with thermal shield and containing
water is investigated. According to Figure (1), the mixture of fuel and warm air move in the radial direction and transfer the heat to the central and other areas in the bottom of container. Next, the low energy flow of combustion gases and hot air moves toward the pot edge. Utilization of thermal shield results in reduction in thermal energy loss to the ambient and thus increases the quality of heat absorption by the container. In the present paper, the effects of geometric parameters including the pot diameter, pot capacity, and also the height of water inside the container on the thermal efficiency in two stages; before and after boiling are investigated.

![Figure 1](image1.png)

**Figure 1.** (a) The schematic representation of water heating including the container, thermal shield, stand, and Bunsen burner, and (b and c) Two dimensional views of thermal shield and the container

![Figure 2](image2.png)

**Figure 2.** The experimental equipment including gasometer, Bunsen burner, and double wall container

**Material and methods**

In order to investigate the effect of geometrical parameters of container, three steel pots were used with diameters of 19.4, 24 and 29.3 cm and total volumes of 1750, 3500 and 5500 mL, respectively. Also, as shown in Table (1), the various volumes of water are used. A household cook stove equipped with gasometer is used for heating. For the first step in each experiment, a simple container with known volume of water is employed and the results are recorded for two stages. In the first stage, the variations in temperature are recorded until the water reaches the boiling point. Then, the amounts of evaporated water and consumed fuel were recorded in a period of 10 min. In second step, similar to previous stage, the experiments were performed with the aim of investigating the effect of thermal shield on the value of energy absorption as a function of container capacity and the volume of water inside the pot. However, in the second step, the criterion for end of experiment was considered to be the time when the consumed fuel was equal to the one in first stage.
Table 1. The experiments conducted on the various pots

<table>
<thead>
<tr>
<th>Type</th>
<th>Performed experiments based on the volume of pot (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large</td>
<td>500 1000 1500 2000 2500 3000 3500 4000 4500 5500</td>
</tr>
<tr>
<td>Medium</td>
<td>500 1000 1500 2000 2500 3000 3500 4000 4500 5500</td>
</tr>
<tr>
<td>Small</td>
<td>250 500 1000 1500 2000 2500 3000 3500 4000 4500</td>
</tr>
</tbody>
</table>

Because of the oscillation in flow rate of urban gas pipelines, using this method can minimize the difference between energy consumed for warming water in simple containers and the ones equipped with thermal shield.

Accuracy of measuring devices
The ambient temperature $T_a$ was measured by testo 605 thermometer with an accuracy of $\pm 0.5 \, ^\circ C$. The temperature of water in the container was measured using a two-channel testo 922 thermometer equipped with k-type thermocouple (NiCr-Ni) with accuracy of $\pm 0.5 \, ^\circ C + 0.3\%$ mv. According to the international standards, the thermocouple sensor should be located inside the water and 5 cm upper than the bottom of container (Funk, 2000). The natural gas flow rate was measured by a laboratory flow meter, VINCI, WG series. The flow meter capacity in each round was 25 dm$^3$, the flow range was 30-90 l/h, and its accuracy was $\pm 0.5\%$. This flow meter worked in ambient pressure and temperature conditions. In order to investigate the effect of thermal shield on the quality of energy absorption, a thermal efficiency parameter for containers with thermal shield was defined. Then, an overall estimation of energy saving was made to investigate the amount of resultant energy savings if this approach was used publicly.

Result and discussion
The performance of thermal shield before boiling
The relationship of heat transfer to a single-phase substance can be written as follows:

$$Q_{pot} = mc \frac{\Delta t}{\Delta t}$$

(1)

Where $Q_{pot}$ is a part of combustion energy ($Q_{com}$) absorbed by the pot. Therefore, the above relation can be written in the following relation:

$$\dot{q}_{pot} = \eta \dot{q}_{com}$$

(2)

By assuming that the variation of temperature regarding time is linear, thus for a specific increase in water temperature ($\Delta T$), a constant rate of thermal energy production ($Q_{com}=\text{cte}$), and a specific mass of water ($m$), the efficiency of thermal energy absorption ($\eta$) can be written as follows:

$$\eta = \frac{mc \Delta t}{Q_{com} \Delta t}$$

(3)

To quantitatively define the thermal value of using a thermal shield, the efficiency parameter ($\varepsilon$) can be expressed as the ratio of difference between energy absorption efficiencies in a pot equipped with thermal shield and a simple pot to the energy absorption efficiency of the simple pot; thus:

$$\varepsilon \equiv \frac{\eta_s - \eta_{sh}}{\eta_{sh}}$$

(4)

or

$$\varepsilon = \frac{\Delta t_s}{\Delta t_{sh}} - 1$$

(5)

Where $\Delta t_s$ and $\Delta t_{sh}$ are the times when the temperature reaches to 93 $^\circ C$ in simple and shielded pots, respectively. It is obvious that if the thermal shield effectively manages the thermal energy absorption, the time period of achieving a specific temperature in shielded pot becomes less than in simple pot. Figure (3) shows the variations of $\varepsilon$ as a function...
of pot size and the volume of water content in the pot. According to Figure (3), one can deduce that in the pre-boiling stage, the utilization of thermal shield results in 20% improvement in quality of heat absorption. Also, Figure (3) shows that there is no meaningful correlation between pot geometry, water content, and the quality of energy absorption. In fact, employing a thermal shield in pre-boiling stage always guaranties a 20% increase in thermal energy absorption. The obtained results from the experiments are consistent with the previous reports in which utilization of thermal shield was accounted as an effective factor to reduce the fuel consumption by 25-30% (MacCarty et al., 2010).

\[ \varepsilon = \frac{m_{sh} - m_{si}}{m_{si}} \]  

(7)

For a known period of time from the time boiling has started, Eq. (7) can be written as follows:

\[ \varepsilon = \frac{\Delta m_{sh}}{\Delta m_{si}} - 1 \]  

(8)

Where \( \Delta m_{si} \) and \( \Delta m_{sh} \) determine the evaporated water in simple pot and the pot equipped with thermal shield, respectively. The more effectiveness of the thermal shield reduces the thermal loss from the pot, the more increase in the ratio of \( \Delta m_{sh}/\Delta m_{si} \) is achieved and thus the efficiency of thermal shield increases. In order to analyze the effect of thermal shield on the heat absorption in boiling stage, the amount of water evaporated in 10 min as a function of pot diameter (with pot capacities of 1750, 3500 and 5500 mL), the height of material inside the pot (according to table 1) is calculated for both simple and shielded pots. The results of all 44 tests are presented in Figure (3). In Figure (3), except for the specific interval of 2\text{cm} \leq H^* \leq 3\text{cm} in which the influence coefficient decreases with increasing the volume of liquid, increasing the liquid

\[ H^* = \frac{V_w}{A_{pot}} \]  

(9)

Where \( V_w \) and \( A_{pot} \) are the volume of water and the bottom area of cylindrical container, respectively. As it can be seen in Figure (3), in contrast with the pre-boiling stage, there is a meaningful correlation between the efficiency parameter, the height of liquid and the size of container. According to Figure (3), except for the specific interval of 2\text{cm} \leq H^* \leq 3\text{cm} in which the influence coefficient decreases with increasing the volume of liquid, increasing the liquid
volume, and especially around to the ultimate capacity of the pot, leading to a linear and homological (in all dimensions of the container) increase in pot’s influence coefficient. The decrease in influence coefficient in a specific interval of liquid height can be attributed to the concentration of temperature distribution along the thermal boundary layer developed between two concentric cylinders. In the space between the thermal shield and the pot. In fact, at constant value of roughness in the interface of solid-fluid and at constant ambient pressure, the energy absorption in nucleate boiling depends on the number of active bubble formation points. The temperature concentration points with the potential of bubble formation can lead to activation of these points and alternative formation of bubbles. Possibly, for the liquid height between 2 and 3 cm, the concentration points of heat transfer to the pot are located in the points where the hot gas enters into the annulus space between two cylinders and also at the top of this space where there is no contact between the liquid and the wall. Therefore, in a specific interval near the free surface, the bubbles could not be formed in proportion with increase in height of the liquid. In fact, because the wall roughness is constant, in order to maintain growth trend of energy absorption coefficient as a function of liquid height similar to the interval of zero to 2 cm, the number of potential points for the bubble formation should increase in proportion with increase in volume of water. It is possible that in the mentioned interval, the growth in energy absorption coefficient did not occur because of the location of heat transfer concentration. When the liquid height passes the critical interval (2 cm ≤ H* ≤ 3 cm), according to Figure (4), it can be inferred that for a specific thermal energy, because of the presence of thermal shield, increasing the size of pot results in a decrease in the energy loss.

According to Figure (4), in order to achieve an optimal performance of thermal shield, the height of materials inside the pot should be larger than a specific value (here, 3.5 cm). For this purpose, the amount of water inside the pot should be increased or for a small volume of material a smaller pot should be used.

Investigation of the economic efficiency of thermal shields
Moshiri et al. (2011) performed a research in the field of allocation of national energy resources and prediction of energy demand in Iran. They calculated the energy consumption per family for the time period of 2005 to 2030. The results were shown in Table (2), which included the details of household demand for energy based on various types of energy resources.
Based on the data presented in Table (2), the economic potential of using a thermal shield for high efficiency cooking pot can be investigated. According to Table (2), the consumption of natural gas, LPG, and kerosene for cooking in 2017 was equal to 33, 3.7 and 25.5 mboe, respectively. Therefore, utilization of thermal shield in cooking pots in 2017 could reduce the energy consumption to around 12.5 million oil barrels (approximately equal to 4 days of oil production in current time). This estimation has been made by assuming a 20% increase in thermal energy absorption in cooking process which only includes the process of warming the food. However, the cooking process involves the boiling stage. Considering the boiling process, using a properly designed thermal shield can increase the heat absorption more than 20% (according to figure 4, when the characteristic height is more than 5 cm).

Uncertainty analysis of experimental results

Uncertainty of each parameter in experiments might be due to the inaccuracy of measuring tools and repetition procedure which can be calculated as follows:

\[ \sigma_v = \sqrt{\left(\sigma_{v_{equ}}\right)^2 + \left(\sigma_{v_{rep}}\right)^2} \]  

(10)

The uncertainty for a multi-variable function such as \( k \) with variables of \( v_1 \), \( v_2 \), etc., \( v_n \) and \( v_m \); and the calculated uncertainties of \( \sigma_{v_1} \), \( \sigma_{v_2} \), etc., \( \sigma_{v_n} \) and \( \sigma_{v_m} \) can be defined as:

\[ k = \frac{\prod_{i=1}^{n} v_{i}^{\frac{1}{n}}}{\prod_{i=1}^{m} v_{i}^{\frac{1}{m}}} \]  

(11)

Where the uncertainties of parameters \( v_1 \), \( v_2 \), etc., \( v_n \) and \( v_m \) are
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independent. The overall uncertainty can be calculated as follows (Taylor, 1997).

\[
\frac{\sigma}{\varepsilon} = \sqrt{\left(\frac{\sigma_{\Delta T_{\text{in}}}}{\Delta T_{\text{in}}}\right)^2 + \left(\frac{\sigma_{\Delta T_{\text{at}}}}{\Delta T_{\text{at}}}\right)^2 + \ldots + \left(\frac{\sigma_{\Delta V_{\text{at}}}}{\Delta V_{\text{at}}}\right)^2}
\]

(12)

Because the purpose of experiments was investigation of the effect of container capacity and height of liquid on performance of thermal shield (Eqs. 5 and 8), the uncertainty of efficiency coefficient for Eq. (5) can be written as follows:

\[
\frac{\sigma_{e_1}}{e_1} = \sqrt{\left(\frac{\sigma_{\Delta T_{\text{in}}}}{\Delta T_{\text{in}}}\right)^2 + \left(\frac{\sigma_{\Delta T_{\text{at}}}}{\Delta T_{\text{at}}}\right)^2 + \ldots + \left(\frac{\sigma_{\Delta V_{\text{at}}}}{\Delta V_{\text{at}}}\right)^2}
\]

(13)

\[
\sigma_{e_1} = \sqrt{\left(\frac{\sigma_{\Delta T_{\text{in}}}}{\Delta T_{\text{in}}}\right)^2 + \left(\frac{\sigma_{\Delta T_{\text{at}}}}{\Delta T_{\text{at}}}\right)^2 + \ldots + \left(\frac{\sigma_{\Delta V_{\text{at}}}}{\Delta V_{\text{at}}}\right)^2} = 0.057
\]

Accordingly, the uncertainty of efficiency coefficient in boiling stage can be written as:

\[
\frac{\sigma_{e_2}}{e_2} = \sqrt{\left(\frac{\sigma_{\Delta T_{\text{in}}}}{\Delta T_{\text{in}}}\right)^2 + \left(\frac{\sigma_{\Delta T_{\text{at}}}}{\Delta T_{\text{at}}}\right)^2} = 0.041
\]

(14)

Table 3 presents the accuracy, type, and uncertainty of measuring tools employed in this study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tool</th>
<th>Accuracy</th>
<th>Uncertainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>Graduated cylinder</td>
<td>±0.01 L</td>
<td>±0.01</td>
</tr>
<tr>
<td>Temperature</td>
<td>Thermometer Testo 922</td>
<td>±0.3 ℃</td>
<td>±0.05</td>
</tr>
<tr>
<td>Time</td>
<td>Digital clock</td>
<td>±1 s</td>
<td>±1</td>
</tr>
</tbody>
</table>

**Conclusion**

In the present paper, the effect of thermal shield on the quality of thermal energy absorption in cooking pots at two stages; pre and post-boiling was investigated. The experiments were performed for three different pots with capacities of 1750, 3500 and 5500 mL. The water content in the pots was also considered as an essential parameter affecting the quality of thermal energy absorption. The results showed that in the pre-boiling stage, the effect of utilization of thermal shield on quality of energy absorption was not significantly dependent on the pot geometry and the volume of liquid inside the pot. Therefore, it can be argued that regardless of operational parameters, the thermal shield always has a positive effect on energy absorption. The calculations based on the experimental data showed that utilization of thermal shield increases the heat absorption by 20% approximately. However, in boiling stage, the effect of thermal shield depended on the characteristic height (the ratio of liquid volume to pot diameter) and was independent of container shape. Increasing the ultimate capacity of pot at a specific thermal influx can result in higher efficiency. Considering the fact that the main energy resource in Iran meeting the energy demand in the household cooking sector is fossil fuels such as kerosene, LPG, and natural gas with annual consumption of 25.5, 3.7 and 33 million barrel oil, respectively, using thermal shield can result in energy saving equivalent to 12.5 million barrel oil per year; that is equivalent to 4 days of Iran’s oil sale in the global market. Based on the obtained results, using thermal shield for either warming the food or cooking in the stage of boiling is a simple and inexpensive solution for
energy saving. This proposal can be commercialized in two following ways:
- To design thermal shields as cooking device accessories based on the dimensions of standard pots in the market;
- Gradually, reengineering the current pots and producing double-wall pots based on the idea of present paper.

References


تحلیل تجربی اثر سری حرارتی بر کاهش مصرف انرژی در ظرف پخت غذا

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هدف این تحقیق کاهش مصرف انرژی در فرآیند پخت و یز از طریق استفاده از سری‌های حرارتی پیرامون ظرف قدامیت. در این تحقیق که برای اولین بار و به‌صورت آزمایشگاهی انجام شده است اثر سری حرارتی باتوجه به پارامترهای هندسی ظرف ( قطر و ارتفاع) و مقدار سیال، بر پایه حرارتی قبل و بعد از جوشش مورد بررسی قرار گرفته است. نتایج این تحقیق نشان داد که در مرحله قبل از جوشش استفاده از سری حرارتی مستقل از هندسه و میزان سیال می‌باشد و می‌تواند به‌طور متوسط 20 درصد انرژی مصرفی را کاهش دهد. همچنین نشان داد که اثر سری حرارتی در مرحله جوشش نسبت به ارتفاع سابع درون ظرف می‌باشد و برای یک مقدار مشخص انرژی حرارتی ثابت، با افزایش ارتفاع ظرف بدون محدودیت افزایش می‌یابد. در نهایت یک محاسبه اقتصادی برای خانوارهای ایرانی نشان می‌دهد که استفاده از سری حرارتی در فرآیند پخت و یز خانگی، موجب صرف‌جویی در مصرف منابع انرژی گرمایی به ارزش 12/5 میلیون بشکه نفت در سال خواهد شد.

واژه‌های کلیدی: انرژی، سوخت فسیلی، ظرف پخت غذا، کاهش مصرف انرژی، مصرف انرژی
The Effect of Sodium Caseinate and Microbial Transglutaminase Enzyme on Rheological, Physical and Sensorial Properties of Corn-based Gluten Free Bread

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Abstract
Celiac disease is a common autoimmune disorder which is triggered by receiving gluten. In this study, corn flour was used as gluten-free flour. Therefore, in order to simulate the properties of gluten, the use of enzymes with cross-linking ability such as microbial transglutaminase at levels of (0, 0.75 and 1.5%), with sodium caseinate at levels of (0, 3 and 6%) were used as a substitute for gluten and their effect on rheological properties of dough, physical and sensory properties of corn gluten-free bread was evaluated. The results indicated that with increasing angular frequency, both the storage and drop modules increased. The specific volume of breads produced from corn was obtained when the specific volume concentration of the transglutaminase enzyme and sodium caseinate were in the highest amount (1.460±0.02). The addition of enzyme and sodium caseinate to the bread formulation reduced the baking loss in comparison to the sample without these materials. The addition of the microbial transglutaminase enzyme to 0.75% resulted in a significant decrease in porosity and then the porosity increased by increasing the enzyme concentration. The addition of 3% of sodium caseinate along with 0.75% of the microbial transglutaminase enzyme to gluten-free bread resulted in the highest bread stiffness. The final acceptance of the produced samples decreased by increasing the enzyme and protein, so that the samples lacking these two compounds obtained the highest final acceptance score. Finally, according to the results, it can be concluded that using 0.75% of the microbial transglutaminase enzyme and 3% of sodium caseinate can be helpful for the production of gluten-free corn bread.

Keywords
Corn Flour
Image Processing
Sodium Caseinate
Transglutaminase

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Introduction
Bread is basic components of the diet in many countries, due to the regional, cultural, economic and social characteristics of the bread and provides the main part of daily energy and protein. In addition, the amount of vitamin, iron and calcium intake from bread is also significant (Rajabzade, 1993; Aminpour, 2006). Celiac patients cannot tolerate the gluten fraction of wheat in bread. Celiac disease is a
gastrointestinal disease, which gluten consumption causes inflammation and swelling of the small intestine and result in indigestion and lack of absorption of micronutrients and vitamins (Tayebi et al., 2014). The only way to overcome this problem is to follow a gluten-free diet (Deora et al., 2014). Several studies in the literature demonstrate the high quality flour is rich in protein, diet fiber, vitamins, magnesium, micro element and antioxidant produce bread (Badiu et al., 2014).

Since gluten is an essential component of bread, the main technological problem in this context is the removal of gluten and its replacement by other components. Gluten is the main constitutive protein of dough structure that is composed of gliadin and glutenin. The absence of gluten often leads to the production of relatively liquid dough and can result in the production of bread with crisp texture, poor color, low volume, and other quality defects. In recent years, research and developments are significantly increased to produce gluten-free breads that include the use of flour or a combination of gluten-free flours with hydrocolloids, enzymes, and dairy elements as gluten substitutes to improve the structure and durability (Rostamian et al., 2014).

The use of proteins with different sources in gluten-free breads formula leads to improved nutritional and functional properties. Moreover, proteins due to the formation of network structure react to gluten-free cereal proteins like wheat gluten network and can maintain the dough structure during fermentation. Also, proteins due to increased elastic modulus (through creating cross links), improved quality and taste (through increased millard browning reaction), improved structure and facilitation of foam formation in dough are added to gluten-free bread. Since the dough used in gluten-free bread should not be prepared from wheat, corn flour is a good alternative for this purpose (Sciarrini et al., 2010; Rostamian et al., 2014). The nutritional value of corn is significantly lower than wheat and its protein lacks gluten; therefore, it cannot be used to produce high-quality bread alone (Payan, 2004). For this reason, components that have the ability to mimic gluten properties will be used in corn flour dough to improve bread properties (Matos et al., 2013; Moore, 2006).

Among enzymes that are used in food industry, transglutaminase, due to modification and promotion of protein network in various food systems, has a special place (Renzetti et al., 2008; Deora et al., 2014). Microbial transglutaminase (MTG) enzyme produced from Streptoverticulum spp. The microbial transglutaminase enzyme catalyzes the acyl group transfer reaction of lysine glutamine bond in food proteins such as egg, milk, soya and wheat (Tseng & Lai, 2002). This molecular bond is such as other dipeptide bond and has non-effect on nutrition value (Pourmohammadi et al., 2012). Studies showed that whey protein and transglutaminase had good effect on quality of gluten-free bread (Dłużewska et al., 2015).

Sodium caseinate is a surfactant agent due to the fact that amphiphilic nature of the protein is applied as an emulsifier, thickener and foaming agent and increases water absorption in flour (Kenny et al., 2000).

Gallagher et al. (2003) studied the effect of adding rice starch and isolating milk protein on gluten-free-bread. They found this ingredient increased volume and improved appearance and this product was like wheat (Gallagher et al., 2003). Brites et al. (2010)
investigated the effect of different types of corn (i.e. regional and hybrid), milling process, formulation, and processing variables on the quality, sensitive and functional properties (i.e. special volume, texture, and color) of gluten-free bread. They stated that a significant difference exists between two types of corns in terms of protein, amylose, and minimum, maximum and final viscosity. Also, low speed of water milling compared with electric mill created a flour with less ash and higher minimum, maximum, and final viscosity. According to corn starch gelatinization, flour bleaching increases consistency, viscosity, and elasticity of dough (Brites et al., 2010).

Nunes et al. (2009), have studied the effect of low lactose dairy powder, sodium caseinate, milk protein isolate, isolate and whey protein concentrate (WPC) on the properties of rice-based gluten-free bread. This investigation showed that the reduction of hardness and increasing of specific volume over time by adding low lactose dairy powder, milk protein isolate and whey protein concentrate (WPC). Sodium caseinate had the opposite effect and hardness increased specific volume reduced (Nunes et al., 2009).

According to recent investigation and importance of celiac food, the objective of this study was to assess the impact of caseinate sodium and microbial transglutaminase enzyme on quality properties of corn-based gluten-free bread.

**Material and methods**

**Materials**

In this study, for the preparation of gluten-free bulk bread microbial transglutaminase enzyme (Artin Chemical, Iran), sodium caseinate (Iran Caseinate, Iran), guar gum (Sigma, Iran) and bakery yeast (Dr. Oetker, Turkey) have been used. Salt, sugar and corn flour were bought from local markets.

**Dough preparation and baking bread**

In this research sodium caseinate was added to the flour at 3 levels (0, 3 and 6%) and microbial transglutaminase enzyme was also added at 3 levels on (0, 0.75 and 1.5%) percent flour basis. This bread was produced using a method described by Ataye Salehi et al. (2012). Each sample was sieved and screened of 80 mesh (except sugar). Active yeast suspension was prepared by 20 ml water contain sugar at fermentation time 15 min, and then was added to the mixture. All components have been mixed in mixer for 3 min (in order to achieve the desired consistency of dough). The dough was placed in galvanized molds 4×6.5×10 cm that where the process of fermentation was continued in oven at 30 °C, relative humidity 70% for 20 min. Baking was proceeded in the oven in temperature 200 °C (high flame) and 190 °C min (low flame) for 35 min. After backing, the breads were cooled and packed in polypropylene bags in 18×20 cm and were stored in room temperature (Ataye Salehi et al., 2012).

**Corn-dough chemical properties**

The sample of corn flour was tested using methods of AACC (2000), moisture content with the number 16-44, the percentage of ash in the method of 7-8, the protein percentage by the method of 1-46, the percentage of fat by the method of 10-30 and the pH was evaluated according to the 52-02 method (AACC, 2000).

**Rheological properties of corn based gluten free dough**

The effects of sodium caseinate powder and microbial transglutaminase enzyme on some rheological properties of the
dough were evaluated with the rheometer following the method of Demirkesen et al. (2010). The loss modulus and the storage modulus measurements were conducted using a Physica Anton Paar rheometer (MCR 301, Austria) equipped with a parallel plate geometry. Frequency sweep test was determined in the range from 1 to 100 S⁻¹. All measurements were performed at 25 °C. In the case of the dynamic oscillatory experiments, first linear viscoelastic region was determined in range of 1 Hz (Demirkesen et al., 2010).

**Hardness**
The hardness of bread samples was measured with a texture Analyser at 25 °C. The slices with a thickness of 2×2×2 cm³ were cut from the center of the loaves, and then the slices were relaxed for 1.5 s. the bread slice was compressed 50% of first height. Start and final points respectively were 5 g and 10 mm (Lazaridou et al., 2007).

Measurements were done at the speed of movement of the head 1 mm/s with a cylinder of a diameter of 38.1 mm.

**Bread specific volume**
The specific volume of the breads was determined by rapeseed grape displacement method (Gujral et al., 2003).

**Weight loss**
The weight of the dough and the weight of the bread samples were measured after baking and cooling for 2-3 h weight loss ratio was calculated according Eq. (1), (Solemanifard et al., 2013).

Weight loss percentage = A-B/A×100

A: dough weight
B: bread weight after baking

**Porosity**
To evaluate the porosity of the samples, the image processing method was measured with a digital camera (12 megapixel, Canon, Japan) and Image J software (Shahidi et al., 2011). Vertical slice of bread was prepared placed in a wooden box of imaging chamber. The angle between lens axis of the camera and the light source was fixed in 45 degrees angle. The images were analyzed by Image J software. This test was performed after baking in three replications (Fani Sadrabadi et al., 2013).

**Sensory evaluation**
The sensory analysis was conducted with a group of 10 panelists. Breads were submitted for an acceptance test to determine the overall acceptance by using Eq. (2) panelists were asked to assess the breads acceptability by evaluation coefficients 1, 2, 2, 4, 3, 3, and 3, respectively for of color, springiness, porosity, color, stiffness, flavor and chewing properties, and to rate samples from 1 to 5 (1 unacceptable and 5 very acceptable) (Katina et al., 2006).

\[ Q = \frac{\sum (P \times G)}{\sum P} \]  

Q: Overall acceptance (Bread Quality Number), P: coefficient of characteristics rating and G: coefficient of characteristics Evaluation.

**Statistical Analysis**
The effect of sodium caseinate powder and microbial transglutaminase enzyme on the physical and sensory properties of corn-free gluten bread was carried out in a completely randomized design with factorial experiment (2×3). If significant difference was found out, Duncan's multiple comparison test was
used \((P \leq 0.05)\). Statistical analyses were performed using SAS software version 9.1 and drawing charts with Microsoft Excel (2013), 3 replications were used for the measurement of bread samples.

**Table 1.** Chemical properties of corn flour used in this research

<table>
<thead>
<tr>
<th>Properties</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content</td>
<td>10.79±0.11</td>
<td>0.42±0.1</td>
<td>7.365±0.05</td>
<td>1.25±0.08</td>
<td>5.83±0.01</td>
</tr>
</tbody>
</table>

**Viscoelastic properties**

Elastic modulus and loss modulus demonstrate flow behavior of material and both of these parameters are time-dependent. Figure (1) and (2), show effect of sodium caseinate and microbial transglutaminase enzyme on rheological properties of free-gluten dough.

The result showed that elastic moduli \((G')\) and loss moduli \((G'')\) increased in frequency sweep \((0-100 \text{ s}^{-1})\) and T1.5C6 \((1.5\% \text{ enzyme} + 6\% \text{ sodium caseinate})\) had low \(G'\) and \(G''\) compared with T1.5C3 \((1.5\% \text{ enzyme} + 3\% \text{ sodium caseinate})\) and controlled sample. Also, higher value of \(G'\) than \(G''\) shows its elastic behaviour. Matos *et al.*, (2014) reported \(G'\) and \(G''\) increased with the addition of caseinate sodium in rice-based gluten-free bread (Matos *et al.*, 2014). Several researches showed WPI and WPC reduce both \(G'\) and \(G''\) moduli while the sodium caseinate reduce \(G'\) and \(G''\) moduli. Addition of caseinate increased \(G''\) moduli more considerably than whey protein isolate. Also, WPC decreased \(G'\) moduli of frozen dough (Kenny *et al.*, 2000). Gujral *et al.* (2003) found microbial transglutaminase enzyme increased both \(G'\) and \(G''\) modulus and this increment was more prominent at higher concentration of enzyme. Protein had a significant effect on viscoelastic properties of the dough (Gujral *et al.*, 2003).

**Result and discussion**

**Chemical properties of corn flour**

Chemical properties such as moisture, ash, protein and fat (dried based) and pH showed in Table (1).

**Figure 1.** The effect of sodium caseinate powder and microbial trans-glutaminase enzyme on gluten-free paste based on corn flour (T0.75C3: 0.75% transglutaminase +3% sodium caseinate; T0.75C6: 0.75% transglutaminase +6% of sodium caseinate; T1.5C3: 1.5% transglutaminase +3% of caseinate sodium and T1.5C6: 1.5% transglutaminase +6% sodium caseinate)

**Figure 2.** The effect of sodium caseinate powder and microbial transglutaminase enzyme on moduli of corn based gluten free flour (T0.75C3: 0.75% transglutaminase + 3% of caseinate; T0.75C6: 0.75% transglutaminase +6% sodium caseinate; T1.5C3: 1.5% transglutaminase +3% of sodium caseinate and T1.5C6: 1.5% transglutaminase +6% sodium caseinate)
**Special volume**

Bread volume depended on the ability of the protein network to maintain the carbon dioxide gas during fermentation (Smerdel *et al.*, 2013; Katina *et al.*, 2006). The results showed all independent variables had a significant effect on the specific volume \( P \leq 0.01 \). Addition of 0.75% transglutaminase on the surface resulted in a significant increase in the specific volume of bread, while further increase in the enzyme had negative impact (table 2).

<table>
<thead>
<tr>
<th>Content of microbial transglutaminase enzyme (%)</th>
<th>Specific volume (ml/g)</th>
<th>Bread weight loss (%)</th>
<th>Tissue porosity (%)</th>
<th>Stiffness (g)</th>
<th>Overall score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.239&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1809&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.75</td>
<td>1.352&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2159&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.5</td>
<td>1.329&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2155&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.66&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by different letters within a column are significantly different \( P \leq 0.05 \).

According to findings Renzetti *et al.* (2008), addition of the transglutaminase enzyme in sorghum and corn bread resulted in an increase in specific volume. Also, according to other studies, addition of 1% transglutaminase resulted in the highest volume and increased in softness of rice-based gluten free bread to compare with other samples (Renzetti *et al.*, 2008).

According to studies by Mohammadi *et al.* (2014), with increasing in level of microbial transglutaminase to guar-based gluten free bread had a significant effect and reduced the specific volume of breads compared with control sample. The transglutaminase enzyme created a cross-link bound between the glutamine and lysine amino acids that can prevent the growth of gas cells during fermentation and reduce the specific volume of the breads.

The results of Pourmohammadi *et al.* (2012) showed that adding transglutaminates to wheat and barley flour-based breads, decreased the volume of breads, which can be attributed to the formation of cross-links between proteins.

In another study, the effect of adding the microbial transglutaminase on rice bread was tested and the results showed that increased enzyme concentration had a negative effect on the specific volume of bread, which is due to the high ability of transglutaminase in the conversion of weak gluten to strong gluten structure.

At high concentrations, transglutaminase has excessive cross-linking, and then dough becomes stronger. This explanation can also be justified in the case of corn meal dough when using 0.75% level compared to 1.5% (Gujral & Rosell, 2004). Table (3) shows that increasing in the concentration of sodium caseinate, the volume of bread reduce at first, which is in agreement with the findings of Nunes *et al.* (2009). The researchers reported the addition of sodium caseinate led to a reduction in specific volume. Several studies reported that sodium caseinate reduced specific volume. Caseinate Sodium is a highly soluble composition that can easily be dispersed in an aqueous and homogenized mixture containing of oil or fat. On the other hand, breads containing sodium caseinate are affected by the starch phase and protein network did not form in the bread structure. Casein is resistant to heat so it prevents the accumulation of casein to create a protein network during cooking.
The dominance of the starch phase on casein led to the formation of a weak gel that reduced bread volume. Therefore, the difference in the thermal stability of whey protein and casein can play a major role in the final structure of bread. The use of 6% protein levels in comparison with other levels resulted in a significant increase in specific volume in corn gluten-free breads.

Sahraeian et al. (2013), in studying the effect of water-based powder on gluten-free sorghum bread, showed that the addition of whey protein powder at a level of 6% compared to the control sample resulted in a significant increase in the volume. Table (4) showed the maximum specific volume of breads derived from corn were obtained when the levels of the enzyme transglutaminase and sodium caseinate were in their highest concentration.

**Table 3. Effect of sodium caseinate content on measured specimens**

<table>
<thead>
<tr>
<th>Sodium Caseinate Content (%)</th>
<th>Specific Volume (ml/g)</th>
<th>Bread Weight Loss (%)</th>
<th>Tissue Porosity (%)</th>
<th>Stiffness (g)</th>
<th>Overall Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.34</td>
<td>21.93</td>
<td>22.83</td>
<td>1864</td>
<td>4.29</td>
</tr>
<tr>
<td>3</td>
<td>1.23</td>
<td>14.75</td>
<td>18.60</td>
<td>2537</td>
<td>3.87</td>
</tr>
<tr>
<td>6</td>
<td>1.35</td>
<td>13.57</td>
<td>18.93</td>
<td>1722</td>
<td>3.59</td>
</tr>
</tbody>
</table>

Means followed by different letters within a column are significantly different (P≤0.05).

**Table 4. Interaction of the concentration of microbial transglutaminase and sodium caseinate in the measured parameters**

<table>
<thead>
<tr>
<th>Sodium Caseinate Content (%)</th>
<th>Content of microbial transglutaminase enzyme (%)</th>
<th>Specific Volume (ml/g)</th>
<th>Bread Weight Loss (%)</th>
<th>Tissue Porosity (%)</th>
<th>Stiffness (g)</th>
<th>Overall Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1.28±0.02</td>
<td>32.00±0.45</td>
<td>22.70±0.50</td>
<td>1567±2.33</td>
<td>4.59±0.01</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1.22±0.04</td>
<td>15.74±0.36</td>
<td>20.70±0.31</td>
<td>2071±0.99</td>
<td>4.01±0.01</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>1.21±0.01</td>
<td>14.04±0.21</td>
<td>22.10±0.18</td>
<td>1789±2.10</td>
<td>3.49±0.01</td>
</tr>
<tr>
<td>0</td>
<td>0.75</td>
<td>1.45±0.07</td>
<td>15.26±0.13</td>
<td>19.00±0.03</td>
<td>1448±1.61</td>
<td>4.40±0.01</td>
</tr>
<tr>
<td>3</td>
<td>0.75</td>
<td>1.22±0.00</td>
<td>14.54±0.22</td>
<td>17.30±0.61</td>
<td>3451±7.80</td>
<td>4.00±0.04</td>
</tr>
<tr>
<td>6</td>
<td>0.75</td>
<td>1.38±0.07</td>
<td>13.47±0.50</td>
<td>17.40±0.11</td>
<td>1580±2.22</td>
<td>3.79±0.03</td>
</tr>
<tr>
<td>0</td>
<td>1.5</td>
<td>1.30±0.01</td>
<td>18.53±0.70</td>
<td>26.80±0.81</td>
<td>2576±3.13</td>
<td>3.89±0.01</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>1.23±0.00</td>
<td>13.98±0.01</td>
<td>17.80±0.20</td>
<td>2091±1.55</td>
<td>3.60±0.01</td>
</tr>
<tr>
<td>6</td>
<td>1.5</td>
<td>1.46±0.02</td>
<td>13.19±0.09</td>
<td>17.30±0.61</td>
<td>1799±4.25</td>
<td>3.50±0.02</td>
</tr>
</tbody>
</table>

Means followed by different letters within a column are significantly different (P≤0.05).

**Bread weight loss**

The results showed that by increasing the enzyme levels in the formulation, a significant decrease in the percentage of bread weight loss was initially observed (table 2). As the protein levels increased, the percentage of bread weight loss decreased due to their ability to maintain moisture (table 3).

Fani Sadrabadi et al. (2013) stated that the addition of 4% of caseinate sodium, in comparison with 2% of this protein, produced the lowest amount of bread weight loss. By increasing sodium caseinate, moisture content in bread increased and because of its high ability to maintain moisture, the percentage of bread weight loss decreased.

Bread weight loss indicates weight loss due to baking or evaporation in bread, which is economically important because of the final weight loss of the bread (Solemanifard et al., 2013). Table (4) also showed that the lowest amount of bread weight loss was obtained, with the highest concentrations of enzyme and protein used in the formulation.

**Texture porosity**

Porosity is one of the most important parameters for determining bread quality, especially bread crumb. (Armero & Collar, 1996). The results showed (table 2) that the addition of the
enzyme transglutaminase microbial to 0.75% significantly decreased in porosity, and then the porosity increased with increasing enzyme concentration. Table (3) also showed that the addition of caseinate sodium initially decreased and then increased porosity. Fani Sadrabadi et al. (2013) observed that sodium caseinate in comparison with other dairy powders, had the lowest porosity which was measured in gluten-free bread samples. Increasing the porosity with the increase in protein content can be attributed to the strengthening of the dough cells by the protein network, which leads to a decrease in the loss of gas produced and increase in the level of the cavities (Fani sadrabadi et al., 2013).

Dłużewska et al. (2015) who detected addition of microbial transglutaminase enzyme to gluten-free bread at 1 unit with whey proteins resulted in significant increase of porosity in bread crumb and the addition of the enzyme at 10 units compared to unit 1 resulted in a greater positive effect on the porosity of gluten-free bread crumb.

According to previous studies, during the first mixing when the flour becomes hydrated and converted to a viscous paste, the soluble and insoluble compounds placed on top of the bubble so the formation of the bubble in the whole process and the stability of the bubbles were impressed by them.

In addition, during the course of the dough, the bubbles that are produced during the mixing in the dough are expanded by yeast production. Therefore, the stability of bubbles is the most important issue, the main reason for their instability being the interconnection of single-gas cells the presence of thick layers on bubbles causes repulsion between them and the chance of interconnection decreases (Ozkoc et al., 2009). Also, the results of interaction of enzyme and protein concentration showed that the highest porosity of bread was obtained when the concentration of enzyme and caseinate sodium was 1.5 and zero% respectively (table 4).

**Hardness**
Hardness is the resistance of the bread crumb to deformation, which is considered as an important indicator in staling. Due to the role of gluten in preventing the staling, this phenomenon is more common in gluten-free bread (Furlan et al., 2013). The results of Table (2) showed that addition of 0.75% of the enzyme in comparison to 1.5% resulted in a higher increase in the hardness of bread tissue, and the addition of 3% of caseinate sodium also produced the highest degree of hardness (table 3).

About the interaction effect on the hardness of bread tissue, adding 3% of sodium caseinate and 0.75% of microbial transglutaminase enzyme to gluten-free bread increased in hardness of the bread (table 4).

In studies done whey protein powder on gluten-free products resulted in 6% protein had a lower hardness compared with control sample at a time interval of 2 h of baking (Sahraeian et al., 2013). Also, according to other researchers, some proteins such as whey protein had reduced the amount of hardness in gluten-free bread (Deora et al., 2014).

Gallagher et al. (2003) who studied the addition of caseinate sodium (high protein content) briefly increased the hardness of gluten-free bread, this fact was attributed to the high protein content of sodium caseinate which has strong cross-linking with water and reduction in the migration of moisture from the brain to the crust (Gallagher et al., 2003).

Dłużewska et al. (2015) reported that the effect of adding the glycojenic transglutaminase enzyme on gluten-free breads showed that the structure of the tissue became more effective, depending on the type of protein used as
a substrate for the enzyme of action and specifically the availability of protein and lysine and glutamine chains (Dluzewska et al., 2015).

Renzetti et al. (2008) studied the 10 units transglutaminase to compare with 1 unit resulted in an increase in the hardness of gluten-free sorghum bread. The results of Moore et al. (2006) on gluten-free bread showed that the increase in the level of the enzyme in the sample containing the skim-milk powder increased in bread hardness. Other reports also indicated more content of the enzyme resulted in increasing the hardness of rice-based bread (Gujral & Rosell, 2004; Sahraieyan et al., 2013)

Specific volume has an effect on firmness of the structure, decreasing the specific volume bread increased bread firmness (Gujral & Rosell, 2004). In this study, the concentration of 1.5% enzyme had the lowest specific volume and highest texture stiffness.

**Evaluation of Bread Properties**
The results showed different levels of enzyme and protein concentration and their interactions had a significant effect ($P \leq 0.05$) on the final acceptance of breads. The final acceptance rate of the samples reduced by increasing the enzyme and protein concentration (tables 2 and 3), so that samples without these two compounds obtained the highest final acceptance scores. According to Mohammadi et al. (2015), the addition of high levels of microbial transglutaminase had significant reduction in the volume and density of the bread compared to the control sample and enzyme which had the lowest overall score (Mohammadi et al., 2015). In another research Mahalleh (2016), the effect of isolated soy protein, egg white powder and transglutaminase enzyme on the sensory properties of corn-free gluten bread was studied and obtained similar results.

**Conclusion**
Rheological characterization (frequency sweep) showed a significantly increased in G' and G" values and corn flour had an elastic behaviour. Microbial transglutaminase had a negative impact on hardness. The results showed that the addition of this enzyme at first had an increase in specific volume, and then this parameter reduced, porosity and weight loss showed opposite trend. Sensory evaluation suggested low protein and non-enzyme samples had more acceptability. Milk protein isolate has a high nutrition value, water absorption and water holding capacity of dough. Therefore, it can be recommended to be used with free-gluten dough.

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تأثیر افزودن کازئینات سدیم و آنزیم ترانس گلوتامیناز میکروبی بر خواص رنلوزیکی، فیزیکی و حسی نان بدون گلوتین بر پایه آرد ذرت

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چکیده

به‌واسطه سلیکا بک اختجاج شایع خودایمنی است که با دریافت گلوتین پرانی‌بافته می‌شود. در این مطالعه آرد ذرت، آرد ذرت ذرت، ترانس گلوتامیناز میکروبی تراکم آنزیمی و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در مخصوص نان‌های تولیدی از ذرت (200 0/460±4) بود که میزان گلوتین ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میزان گلوتین آنزیم ترانس گلوتامیناز و کازئینات سدیم در بیشترین مقدار قرار داشت. افزودن آنزیم و کازئینات سدیم به فرمولاسیون نان موجب کاهش افت یک با میان...
Effects of Polyethylene Thickness, Gas Combination and Temperature on Shelf Life and Quality of Strawberry

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Abstract
In this research, the effect of modified atmosphere packaging on the quality and shelf life of strawberries was investigated. For packing strawberries 3 gas compositions (air, N₂75%+CO₂15%+O₂10% and N₂75%+CO₂10%+O₂15%), polyethylene lining in 3 thickness of 30, 50 and 90 µm and 2 storage temperatures 4±1 and 8±1 °C were used. The effect of treatments on weight loss, firmness, soluble solids, titratable acidity and pH in strawberries at 20 days after storage was studied in a completely randomized design with factorial experiment in 3 replications. Results showed that the effects of gas composition and temperature on all of the mentioned factors are significant ($P \leq 0.01$), but the effect of the thickness of the polyethylene (PE) lining is only significant on the percentage of weight loss, soluble solids and titratable acidity ($P \leq 0.01$). The quality and quantity characteristics of strawberries were maintained better at temperature of 4 °C due to reduced breathing, evaporation and transpiration. The polyethylene lining at thickness of 90 µm, maintained the qualitative and quantitative qualities of the product due to low permeability and maintaining the moisture around the product within the package. The first gas composition (N₂75%+CO₂15%+O₂10%) was more suitable for keeping the qualitative and quantitative characteristics of strawberries due to more CO₂ and reduced respiration. As a result, strawberries packed in a 90 µm thick polyethylene lining and a gas mixture (N₂75%+CO₂10%+O₂15%) at 4 °C had the best quality and highest durability.

Keywords
Gas Composition
Modified Atmosphere
Strawberry
Temperature

Introduction
Diet rich in fruits and vegetables provides protection against some diseases such as inflammatory, cancer and cardiovascular and due in particular to their antioxidant content include anthocyanins, flavonoids, flavonols and catechins, which can protect the human body against oxidation reactions and have antifungal, antioxidants and anticancer properties (Scalzo et al., 2005).

Fresh-cut fruits and vegetables are perishable due to respiratory and microbiological activity after harvest,
which reduce the shelf life of these products (Gross et al., 2004).

Postharvest vegetable and fruit start to ripen due to enzymes activities, which results in the production of ethylene and increasing the speed of ripening. At this time the produce’s quality first increases and then, quite immediately, diminishes (Thompson et al., 1972).

Equilibrium modified atmospheres within plastic packages can be beneficial in maintaining quality of the fresh-cut product and retail product. Equilibrium modified atmosphere packaging within control of gas combination (by a combination of increasing carbon dioxide and decreasing oxygen concentrations) reduce texture firmness, respiration rate and ethylene protection (Kader, 2002).

Ayhan et al. (2008) examined the effect of modified atmosphere packaging on the quality and shelf life of fresh-cut carrots. The dried carrots were packed in polypropylene bags using three different gas compositions: air (passive), high oxygen (O₂80%+CO₂10%+N₂10%) and low oxygen (O₂5%+CO₂10%+N₂85%). The packs were stored for 21 days in 4 °C. The results showed that packs with air and oxygen with high density had better qualities.

Nielsen & Leufven (2008) examined the effect of modified atmosphere packaging on the quality of strawberries. Strawberries were stored in polyethylene bags at 5 °C for 10 days. The packed strawberries showed no sign of weight loss; however, the unpacked ones lost 1.5% of their weight per day. The results showed that strawberry storage in a modified atmosphere 11-14% O₂ and 9-12% CO₂ can be used to maintain the quality for a longer time, than if kept in air in open containers.

Jing-jun et al. (2012) examined effects of active modified atmosphere packaging on quality of mushrooms stored at cold temperature (4 °C). The gas components were EMAP1: O₂2%+CO₂7%, EMAP2: O₂2%+CO₂10% and EMAP3: O₂2%+CO₂13%. The results showed that active modified atmosphere packaging could increase the shelf life of mushrooms to 17 days and the high concentration of CO₂ could improve the postharvest quality of mushrooms. EMAP2 treatment with decreased respiration rate, delayed decrease in firmness, soluble sugar and vitamin C, and with reduction in the activities of the poly phenoloxidase (PPO) and browning of the mushrooms guarantees better quality.

Caner et al. (2008) investigated the impact of equilibrium-modified atmosphere packaging (EMAP) technology on extension quality (pH, acidity, brix, color) and texture profile analyses of fresh strawberries during storage. The overall results expressed that strawberry quality can be maintained effectively at least for 10 days using various polymeric lid films. PET/EVOH-LAF and CPP were much more effective than LLDPE due to barrier properties during storage periods. Quality of strawberry packaged with suitable high-barrier lid films have been prolonged significantly.

Juki & Khazaei (2014) examined the effect of low-dose gamma radiation and active modified atmosphere packaging EMAP1: CO₂10%, O₂5% and N₂85% and EMAP2: CO₂5%, O₂10%, N₂85% on quality of strawberry fruits stored at 4 °C. Quality parameters were analyzed on days 1, 7, 14 and 21. Result showed strawberries kept in active EMAP1 maintained their texture and appearance better than those packaged under air and EMAP2. The strawberries packed in packages with active compounds EMPA1 and EMPA2 were firmer than those stored in air during the storage time (21 days). The irradiated strawberry was not attacked by fungus
(Botrytis cinerea) during 7 days of storage time. Also, the result showed low-dose gamma irradiation in combination with EMAP retained quality.

Finally, it can be claimed that gamma radiation as well as active gas compound increases the post-harvest longevity of strawberries to 14 days without fungus attack. Maghoumi et al. (2009) studied effect of modified atmosphere packing with high CO₂ on storing features of the strawberry cv. selva. They used two types of packaging, polyethylene and polyamide, as well as a two-gas combination, (air and O₂2% with CO₂15% and N₂83%), for packaging the strawberries. The samples were stored in a temperature of 4 °C and the relative humidity of 85-90% for 20 days, while their features were being checked every 5 days. The results showed that packing with modified atmosphere considerably reduced the weight loss and perishing of the produce. CO₂ with high density and polyethylene retained the quality of the fruit better than other treatments (Maghoumi et al., 2009). Another way to preserve the freshness of the fruits and vegetables in storage is storing the produce in equilibrium modified atmospheres and storing in low pressure atmosphere (vacuuming) and packing the produce in modified atmosphere. Storage in low pressure affects the breathing and ethylene production of the fruits and increases the longevity of the produce by reducing the waste during the storage. However, aforementioned storing methods are not economically reasonable and can be adopt to store fruits in large scale, not for retailing. As after releasing from the storing condition the breathing and microbial activities reignite, which in terms, reduces the longevity of the fruits in retailing. Hence, packaging in small scales as well as controlling the conditions in the packages is deemed the best way to increase the longevity of the product (Kader, 2002). The objectives of the present study were to evaluate the effects of polyethylene thickness, active and inactive gas combination and temperature on weight loss, firmness, soluble solid, titratable acidity, pH, shelf life and quality of strawberry.

Material and methods
Strawberries from cultivar Paros grown in greenhouse of Gardening Department of Sari University of Agricultural Sciences and Natural Resources were harvested. They were transported in to the laboratory. The strawberries were sorted to eliminate premature and rotten berries as well as any samples with obvious defects. 6 strawberries were placed in every bag. The controlled treatment was unpackaged and placed under normal conditions. 6 bags of strawberries stored at 4 °C and other stored at 8 °C.

In the present study polyethylene bags with three different thicknesses were used. The bags were 30, 50 and 90 μm. The packing was done in modified atmosphere. Two gas compounds, active and inactive, were used to pack the product. For the active compound three different gases oxygen, carbon dioxide and nitrogen with two different combinations were applied in packing the treatments.

The first combination included O₂ 10%, CO₂ in 15% and the rest uneffective gas, nitrogen (N₂75%+CO₂15%+O₂10%).

The second combination included O₂ 15%, CO₂ in 10% and the rest uneffective gas, nitrogen (N₂75%+CO₂1.0%+O₂15%).

To conduct the research, the samples were after placing the product in the desired coatings, weighed strawberries then placed in a vacuum packing machine (DZQ-400/2E, China).

First, considering the program of the device the gases existing in the vacuum and the packages were extracted and
then the gases, having been already combined in the cylinders connected to the device, were injected to the device after that the packages were automatically sealed. In inactive approach micron-size pores are made on the packing. In the present study applying a manual approach with a needle which had a tip of 80 μm diameter, 6 pores (3 on each side) were made on the pack. Having placed the packages in the environment the gases were emptied and they were packed without inserting gas. After packing all the samples, they were all weighed and prepared, along with control samples, to be placed in refrigerator with 4±1 ºC and the other half in a refrigerator with 8±1 ºC. The weight of all the bags was recorded the first day of storage and during the test. The weighing process was conducted by a digital scale (JADEVER, Taiwan), with precision of 0.01, and then the samples were put under the two temperature treatments, the weights of the packages were weighed daily by a scale of 0.01 with a precision. Method of Mostofi & Najafi (2004) were used to calculate the weight loss.

A texture analyzer, model FG-5020, connected with a testing tensile was used to measure the firmness of fruit texture applying a cylindrical penetration probe connected to the testing tensile from the tip. The maximum mean of the data were digitally recorded and reported according to Newtonian metric system. Total soluble solids (TSS) were measured in an atago digital refractometer (DBR0090, China), and the results are expressed in Brix (%).

The percentage of titratable acidity with 0.01 of normal caustic soda was measured. In this test 5 mL of filtered juice was mixed with distilled water to reach 100 cc, and then it was titrated with reagent phenolphthalein, titrated with a normal 0.01 soda. Every mL with 0.01 of normal caustic soda was deemed equal to 0.0067 g of citric acid (Parvane, 1992).

The pH of the strawberry homogenate was analyzed by a pH meter (Sartorius PB-11, USA) in duplicate measurements. The pH meter was calibrated with buffer solutions of 4.1, 7 and 9.2 and then the fruit extract was poured into beaker and after calibrating electrodes in the mixture, the pH was read. Having read each electrode, they were washed by distilled water and then passed through filter paper (Mostofi & Najafi, 2004).

The present study was conducted applying completely randomized design with factorial experiment in three replications tests. The treatments used in the study were two active gas combinations (CO₂15%+O₂10% and CO₂10%+O₂15%) as well as an inactive gas combination (air). Polyethylene bags with 3 different thicknesses were used. The bags were 30, 50 and 90 μm in 4 and 8 ºC.

Completely randomized design with factorial experiment in three replications test was used for multiple comparison and separation of means. Statistical analysis was carried out using the general linear models procedure of SAS (version 9) and Genstat (version 12).

Result and discussion
This study showed the important role played by polyethylene thickness, temperature and gas combination for determining the shelf life and quality of strawberry (table 1). All major effects on the measured traits at the probability level of (P≤0.01) were significant, except the effect of coating thickness on texture firmness and pH that was non-significant. Some of interaction effects on the measured traits at the probability levels of (P≤0.05) or (P≤0.01) were significant.
The analysis of variance (table 1) showed that there was a significant difference between different thicknesses of polyethylene, gas compositions and storage temperature at 1% level.

The strawberries packed with EMAP1 (CO₂15%+O₂10%) lost little weight compared to other gas combinations. Polyethylene with thickness of 90 μm lost very little weight compared to other thickness.

After storage at 4 °C, the weight of the strawberries was reduced by 0.49% whereas the weight of the strawberries at 8 °C decreased by 0.82%.

Statistical interactions between thickness and temperature indicated a significant effect on weight loss (P≤0.05) and interaction between gas combination and temperature, thickness and gas combination and three-way interactions among gas combination, temperature and thickness had no significant effect on weight loss. Figure (1) showed interactions between polyethylene with thickness of 50 and 90 μm and temperature at 4 and 8 °C had an insignificant effect. The lowest weight loss between two temperatures of 4 and 8 °C was observed in coating with the thicknesses of 90 and 50 μm at the temperature of 4 °C and the highest weight loss was observed in the control case at the temperature of 8 °C.

Low permeability of packaging coatings reduces the weight loss and creates an appropriate atmosphere for protecting fruits (Anon; 2003). Packaging coatings, with increased relative humidity around the product and decreased air velocity on the product surface, create a stable layer around the product and decrease pressure difference between environment and product texture followed by decreased evaporation and water and weight loss (Liu et al.; 2004).

Martínez & Artes (1999) examined the effects of polypropylene coatings with the thicknesses of 30 and 40 μm as perforated and unperforated with cooling by vacuum and active modified atmosphere with O₂5% and CO₂0% on lettuce and concluded that unperforated polypropylene coating (40 μm) without active modified atmosphere has the best performance in maintaining the appearance, preventing weight loss, and wilting of lettuce. At the temperature of 4 °C, since respiration, transpiration, and metabolic processes are slow, qualitative and quantitative properties of strawberries were preserved better compared with the temperature of 8 °C.
Texture firmness

Table (1) showed that temperature and gas combination had a significant effect on strawberry texture firmness ($P \leq 0.01$), but the effect of coating thickness on texture firmness was not significant. Mean comparison results showed that all treatments were softened during the storage period and reduced their stiffness. The first gas composition ($\text{CO}_215\%+\text{O}_210\%$) and control treatment showed the lowest texture firmness and the firmness level between the second gas composition ($\text{CO}_210\%+\text{O}_215\%$) and the inactive gas composition had no significant difference. The temperature of 4 °C was more suitable compared with the temperature of 8 °C to maintain strawberry texture firmness.

All mutual effects of temperature and gas composition and temperature and coating thickness and gas composition and coating thickness and also the interaction effect of temperature and gas composition and coating thickness on strawberry texture thickness were significant at the probability level of ($P \leq 0.01$) (table 1). The first gas composition ($\text{CO}_215\%+\text{O}_210\%$) in the coating with the thickness of 90 μm with the maintenance of texture firmness as 45% showed a better performance compared the control case (figure 2-A).

Storage of products in packages with higher CO$_2$ leads to decreased texture firmness that is consistent with the findings of Herner (1987) who concluded that tomato softening will be delayed with decreased respiration through increased CO$_2$ concentration.

Jouki & Khazaei (2014) found that strawberry packaged by gas composition with higher CO$_2$ concentration, maintained texture and appearance of strawberry better compared with inactive composition and gas composition with lower CO$_2$ concentration.

Martinez-Romero et al. (2003) stated that increased CO$_2$ concentration and decreased O$_2$ concentration minimize respiration and metabolic activities of the fruit and packaging in the modified atmosphere with the prevention of pectin degrading enzymes activities leads to fruit texture firmness. According to the interaction effect of temperature and gas composition on texture firmness, no significant difference existed between the first gas composition ($\text{CO}_215\%+\text{O}_210\%$) and the second gas composition ($\text{CO}_210\%+\text{O}_215\%$) and inactive gas composition at the temperature of 4 °C and the best performance in texture firmness maintenance was observed. Also, the control case showed strawberry texture softening at the temperature of 8 °C (figure 2-B).

Generally, low temperature facilitates
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decreased respiration, microorganism growth, and delay in metabolic activity of the plant texture (Villaescusa & Gill, 2003; Ooraikul & Stilles, 1991; Paine & Paine, 1992; Zagory & Kader, 1988). The interaction effect of temperature and polyethylene coating thickness on strawberry texture firmness showed that the thickness of 90 μm at the temperature of 4 °C prevented strawberry texture softening better than other thicknesses (figure 2-C).

Figure 2. The interaction effect of (A) polyethylene coating thickness and gas composition on strawberry, (B) temperature and gas composition, (C) temperature and polyethylene coating thickness; on strawberry texture firmness (Non-similar letters indicate a significant difference between treatments)

Ghorbani et al. (2017) found that by increasing the storage time and temperature, texture firmness decreases. In coatings with higher thickness, due to low permeability and humidity maintenance inside the package, qualitative and quantitative properties of the product are preserved better. Esmaeili (2012) found that polyethylene with the thickness of 50 μm compared with the thickness of 20 μm was more efficient in maintenance of texture firmness and decreased weight loss of packaged tomato.

The interactions among temperature, gas combination and coating thickness showed the passive inactive gas combination and first gas combination (CO215%+O210%) with polyethylene bags (50 and 90 μm thickness) at 4 °C retained firmness compared to other treatments Figure (3). Alonso & Alique (2003) used two types of coatings with different permeability for cherry packaging and the results showed that coating with smaller pores and low
permeability maintains acidity and firmness in packaged cherry and it turns black followed by decreased quality and increased decay.

![Image](image_url)

**Figure 3.** The interaction effect of temperature and gas composition and polyethylene coating thickness on strawberry (texture firmness PE30G1, PE50G1 and PE90G1: Polyethylene with thickness of 30, 50 and 90 μm respectively under first gas composition (CO215%/O210%); PE30G2, PE50G2 and PE90 G2: Polyethylene with thickness of 30, 50 and 90 μm respectively under second gas composition (CO210%/O215%) and PE30, PE50 and PE90: Polyethylene with thickness of 30, 50 and 90 μm respectively under inactive gas composition) (Non-similar letters indicate a significant difference between treatments)

**Soluble solids**

The results of analysis of variance showed that temperature, gas component and thickness had significant effects ($P \leq 0.01$) on total soluble solids of strawberries (table 1). The results of analysis of variance showed that strawberry soluble solids increased in all treatments at the end of the storage period. It seems that this is due to the product respiration during the storage period and conversion of sugar to starch metabolism. In the second gas composition (CO210%/O215%), soluble solid showed minimum increase and the control treatment showed the maximum increase. In the coating with the thickness of 30 μm, soluble solids showed minimum increase that is probably due to desirable atmospheric conditions for the product in the package. The temperature of 4 °C is better for the maintenance of soluble solids compared with the temperature of 8 °C. The interaction effect of temperature and gas composition and gas composition and coating thickness and the interaction effect of temperature, gas composition and coating thickness at the probability level of ($P \leq 0.01$) showed a significant difference but the interaction effect of temperature and coating thickness at the probability level of ($P \leq 0.05$) had a significant effect on soluble solids. In investigating the interaction effect of coating thickness and gas composition of the second gas composition (CO210%/O215%), in the thickness of 30 μm, the soluble solids have been maintained better than other soluble solids and the control treatment showed the highest increase in soluble solids (figure 4-A).

Aminzadeh et al. (2014) observed that thin packaging coatings maintain soluble solids better. Mohebi et al. (2015) found that gas composition with lower CO2 percentage maintains soluble solids better. The interaction effect of temperature and gas composition of the second gas composition (CO210%/O215%) at the temperature of 4 °C showed the minimum increase in soluble solids (figure 4-B).
According to the interaction effect of temperature and coating thickness, the thickness of 30 μm at the temperature of 4 °C showed the best performance in the maintenance of soluble solids (figure 4-C). At the temperature of 4 °C with decreased respiration and evaporation, better atmospheric condition is provided to maintain soluble solids.

![Figure 4. The interaction effect of (A) gas composition and polyethylene coating thickness, (B) temperature and gas composition, (C) temperature and polyethylene coating thickness; on strawberry soluble solids](image)

In investigating the interaction effect of temperature, gas composition and coating thickness, the thickness of 30 μm in the second gas composition (CO₂10%+O₂15%) and the temperature of 4 °C showed the best performance in the maintenance of soluble solids (figure 5). The modified atmosphere packaging led to the maintenance of soluble solids and prevention of its increase and also prevention of the metabolism related to the conversion of starch to sugar. Batu & Thompso (1994) reported that in a study, they found that the use of 50 μm polyethylene coating in tomato, after 60 days of storage in the cool warehouse led to the maintenance of soluble solids compared with uncoated samples. Aminzadeh et al. (2014) observed that thin packaging coatings maintain soluble solids better.
Titratable acidity

Strawberry titratable acidity decreased in all treatments at the end of the storage period. Titratable acidity decreases in ripe fruits. Investigating the results of analysis of variance in Table (1) showed that temperature, gas composition and polyethylene thickness had a significant effect on strawberry titratable acidity ($P \leq 0.01$).

The second gas composition (CO$_2$10%+O$_2$15%) performed better than the first gas composition and inactive composition in titratable acidity maintenance. Coating with the thickness of 90 $\mu$m showed the best performance in preventing decreased titratable acidity. The temperature of 4 $^{\circ}$C showed the best performance compared with the temperature of 8 $^{\circ}$C in the maintenance of titratable acidity. Among interaction effects, the effect of temperature and gas composition and also the interaction effect of temperature, gas composition and polyethylene coating at the probability level of ($P \leq 0.05$) and the interaction effect of composition and coating thickness at the probability level of ($P \leq 0.01$) had a significant effect on titratable acidity. The interaction effect of coating thickness and temperature did not show any significant effect on titratable acidity. According to the interaction effect of coating thickness and gas composition (figure 6-A), the second gas composition (CO$_2$10%+O$_2$15%) at 90 and 50 $\mu$m has the best performance in keeping the titres of acidity possible.

Nakhasi et al. (1991) reported the strawberries packed with modified atmosphere packaging lost little titratable acidity compared to controlled treatment. Alonso & Alique (2003) found that edible packaging with low permeability was useful to preserve quality of cherries through losses of titratable acidity.

Interaction of temperature and gas combination (figure 6-B) showed second gas composition (CO$_2$10%+O$_2$15%) at 4 $^{\circ}$C lost little titratable acidity compared to other treatments. Mohebi et al. (2015) concluded that titratable acidity is maintained better in the gas composition with low CO$_2$ percentage.

In interaction effect investigation among temperature, gas combination and polyethylene thickness showed strawberries sealed within polypropylene with thickness of 50 and 90 $\mu$m retained titratable acidity under second gas composition (CO$_2$10%+O$_2$15%) at 4 $^{\circ}$C compared to other treatment (figure 6-C). With increased coating thickness, permeability decreased and as a result,
respiration decreased and ripening delayed and acidity reduction was prevented. In a study by Batu & Thompson (1996) on tomato packaged under modified atmosphere and also studies by Majidi et al. (2011) and Tasdelen & Bayindirli (1998), titratable acidity in packaging coatings was maintained better. Tefera et al. (2007) stated that increased temperature decreased weight loss and enhanced qualitative properties of fruit such as pH and acidity.

Figure 6. The interaction effect of (A) gas composition and polyethylene coating thickness, (B) gas composition and temperature, (C) temperature and gas composition and polyethylene coating thickness on strawberry titratable acidity (PE30G1, PE50G1 and PE90G1: Polyethylene with thickness of 30, 50 and 90 μm respectively under first gas composition (CO₂15%+O₂10%); PE30G2, PE50G2 and PE90G2: Polyethylene with thickness of 30, 50 and 90 μm respectively under second gas composition (CO₂10%+O₂15%) and PE30, PE50 and PE90: Polyethylene with thickness of 30, 50 and 90 μm respectively under inactive gas composition)

(Non-similar letters indicate a significant difference between treatments)

pH
pH of all samples increased during storage time. Results indicated that temperature and gas combination had a significant effect (P≤0.01) on pH value of strawberries (table 1). Thickness of polyethylene had an insignificant effect on pH value. Strawberries under active first gas composition (CO₂15%+O₂10%) had lower pH than strawberries packaged under air and the control case had the highest increase in strawberry pH. Changes in pH at 4 °C were lower than changes at 8 °C. In investigating the interaction effects regarding pH, it was observed that except the interaction effect of temperature and gas composition that did not have any significant effect on pH, other interaction effects had a significant
effect on strawberry pH at the level of ($P \leq 0.01$). Investigating the effect of gas composition and coating thickness showed that the first gas composition ($CO_215\%+O_210\%$) at the thickness of 90 $\mu$m had the best performance in pH maintenance (figure 7-A).

High concentration of $CO_2$ due lower respiration rate retained pH value. These results are in agreement with the observation of Tabatabaekoloor et al. (2016) who reported that inactive gas combination with high concentration of $CO_2$ retained pH of tomatoes at 4 and 20 $^\circ$C. Interaction between thickness and temperature showed polypropylene with thickness of 30 and 90 $\mu$m at 4 $^\circ$C had lower pH value (figure 7-B) and the control treatment at the temperature of 8 $^\circ$C showed the highest increase in pH. The temperature of 4 $^\circ$C showed lower respiration, transpiration, and evaporation and is more suitable.

In the study of the interplay of the interactions among gas combination, temperature and coating thickness it was observed first gas combination ($CO_215\%+O_210\%$) in coating with a thickness of 90 $\mu$m at 4 $^\circ$C was better than other treatments it prevents the increase of pH (figure 7-C). In coatings with a thickness of 90 $\mu$m due to low permeability, products have better quality. Tefera et al. (2007) reported the decrease of temperature retained pH value.

**Figure 7.** The interaction effect of (A) gas composition and polyethylene coating thickness, (B) polyethylene coating thickness and temperature, (C) gas composition and temperature and polyethylene coating thickness; on strawberry pH (PE30G1, PE50G1 and PE90G1: Polyethylene with thickness of 30, 50 and 90 $\mu$m respectively under first gas composition ($CO_215\%+O_210\%$); PE30G2, PE50G2 and PE90G2: Polyethylene with thickness of 30, 50 and 90 $\mu$m respectively under second gas composition ($CO_210\%+O_215\%$) and PE30, PE50 and PE90: Polyethylene with thickness of 30, 50 and 90 $\mu$m respectively under inactive gas composition)
(Non-similar letters indicate a significant difference between treatments)
Conclusion
The results of this study showed that between two temperatures that tests were conducted at, the temperature of 4 °C due to decreased respiration, transpiration and evaporation in maintaining qualitative and quantitative properties of strawberry was better than the temperature of 8 °C. The polyethylene lining at thickness of 90 μm, maintained the qualitative and quantitative qualities of the product due to low permeability and maintaining the moisture around the product within the package. The first gas combination was more suitable for keeping the qualitative and quantitative characteristics of strawberries due to more CO₂ and reduced respiration. As a result, strawberries packed in a 90 μm thickness of polyethylene and first gas combination (N₂75%+CO₂15%+O₂10%) at 4 °C had the best quality and highest durability.

References


بررسی تأثیر ضخامت پلی اتیلن، ترکیب گازی و دما بر زمان ماندگاری و کیفیت توت فرنگی

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چکیده
در این پژوهش تأثیر ستون‌بندی تحت یک‌شیخیده بر کیفیت و طول عمر توت فرنگی مورد بررسی قرار گرفت. برای بسته‌بندی توت‌فرنگی‌ها از سه ترکیب گازی (هوای، ترکیب گازی اول: 15 درصد نیترژن و 85 درصد اکسیدکرین، ترکیب گازی دوم: 25 درصد نیترژن و 75 درصد اکسیدکرین و ترکیب گازی سوم: 35 درصد نیترژن و 65 درصد اکسیدکرین) در دمای 10 گردیده شد. تأثیر تبادلگاه یادگیر در قابل طرح کیفیتی شاخصی با آزمایش فاکتوریال در سه تکرار با پارامترهای ذکور کاهش وزن، سفته‌پات، مقدار جumlah محلول، اسیدیت pH قابل تغییر می‌باشد. نتایج آنالیز واریانس نشان داد که تأثیر ترکیب گازی و دما بر تغییرات فاکتورهای مورد اشاره در دمای 1 درصد معنی‌دار است، ولی تأثیر ضخامت پلی اتیلن فقط بر دمای کاهش وزن به‌کارگرفته مقدار جumlah محلول و اسیدیت قابل تغییر در دمای 1 درصد معنی‌دار است و در زمان دمای کاهش و دما در انتقال صورت گرفت. دمای 4 درجه سانتی‌گراد به‌دلیل کاهش نفوذیت، تبخیر و تعرق در حفظ خصوصیات کمی و کیفی توت‌فرنگی بهتری از دمای 8 درجه سانتی‌گراد عمل کرد. پوشش پلی اتیلن در ضخامت 90 میکرون به‌دلیل نفوذیت کم و حفظ CO2 رطوبت اطراف محصول درون بسته‌بندی صربیه و کمی محصول را بهتر حفظ نمود. ترکیب گازی اول به‌دلیل داشتن دمای بیشتر و کاهش نفوذ قابل حفظ خصوصیات کمی و کیفی توت‌فرنگی مناسب‌تر است. درنتیجه توت‌فرنگی‌هایی که در پوشش پلی اتیلن با ضخامت 90 میکرون و ترکیب گازی اول در دمای 4 درجه سانتی‌گراد بسته‌بندی شدند بهترین کیفیت و بالاترین ماندگاری را داشتند.

واژه‌های کلیدی: اتیل‌کردی، ترکیب گازی، توت فرنگی، دما
Evaluation of the Oxidative Stability of Frying Oil, Mixed with Purslane and Corn Seed Oil

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Abstract
One of the procedures for the stabilizing of frying oil, in order to preserve the synthetic antioxidants, is adding oils containing antioxidant and high oxidative stability compounds. The objective of this research was the quality evaluation of three frying oils mixture (Sunflower, Ladan and Iran) due to the addition of purslane and corn seed oils and frying over 12 h at 170±2 °C for this reason, the mixed frying oil, containing corn and purslane seed oil in the proportion of (70:15:15 w/w) called mixture 1, mixed frying oil containing corn and purslane seed oil in the proportion of (75:15:10 w/w) called mixture 2, corn seed oil, purslane seed oil and mixture of three frying oils in terms of qualitative indexes (peroxide, p-anisidine, totox, total polar compounds, oxidative stability and conjugate-DNs), were evaluated. At first the antioxidant activity rate of purslane seed oil was assessed and the value of 52.9±0.19 percent was measured. The comparison of two types of frying oil mixtures indicated that the values of peroxide, p-anisidine, totox and conjugate-DNs, indicators of mixture 1 and 2 were (25.83 meq/kg, 80.63, 132.29 and 15.01 μmol/g) and (28.13 meq/kg, 85.73, 141.99 and 17.17 μmol/g) respectively which had significant difference with frying and other oils (P<0.05). The higher ratio of purslane oil in the frying oil, mixture 1, increased its oxidative stability in contrast to oil mixture 2 and purslane preserved it against early degradation at high temperature.

Keywords
Corn Seed Oil
Oxidative Stability
Purslane Seed Oil

Introduction
Purslane (Portulaca oleracea) is the 8th abundant plant in the world (Coquillat, 1951). According to the report of the world health organization (WHO, 1990) it mainly contains unsaturated fatty acids and it is also considered as a botanical source, full of omega-3 fatty acid (Alam et al., 2014; Dweck, 2001). Due to the abundant quantity of unsaturated fatty acids, purslane is utilized to decrease cholesterol, prevent the growth of cancerous cells and treat psychological depression. Recently it has been recognized that purslane seed oil is a suitable source for omega-3 fatty acids, β-carotene, vitamin, essential amino acids, alkaloids, coumarin, flavonoids and polysaccharides (de Lorgeril et al., 2001; Keyvani &
Boland, 2015; Mazza et al., 2007). According to the report of Delfan-Hosseini et al. (2017), this oil has indexes of peroxide (meq oxygen/kg), iodine (Iodine g/100 g), acidic (Potassium g/oil g), saponification (Potassium g/g), refractive index and extraction efficiency (%) in values of 0.8, 135.9, 1.93, 181.05, 1.04805 and 59.37 respectively. Phenolic compounds (Gallic acid mg/oil k), antioxidant activity (%) and oxidative stability (h) are 66.51, 53.9 and 9.67 respectively. Furthermore, the values of saturated and unsaturated fatty acid are 21.95 and 78.39 respectively and its mono and poly unsaturated fatty acid are 21.08 and 57.31% respectively (Ren et al., 2015; Uquiche et al., 2008; Yoshida et al., 2005).

The observation of Fangfang et al. (2013) indicated that purslane seed oil contains α-linolenic acid 40.25%, linoleic acid 29.43% and oleic acid 15.61%. They also reported that saturated fatty acids included 13.94% of total oil content while mono unsaturated fatty acid and poly unsaturated fatty acid were 16.28 and 69.68%. Furthermore the content of linolenic acid (40.2570%) in purslane seed oil in comparison with camellia seed (0.27%) is too much (Houhoula et al., 2003; Yang et al., 2013). The content of linoleic acid (29.43%) in comparison with to other vegetable oils such as flax seed (15.45%), camellia seed (7.26%), grape seed (11.4%) and olive oil (0.56%), is too much (Fangfang et al., 2013). Linolenic and linoleic acids are omega-3 and omega-6 fatty acids respectively and both are essential fatty acids which have an important role in growth and development of human as well as preserving against various diseases (Dunbar et al., 2014). Furthermore the oils full of omega-3 are very useful for human health (Andrade et al., 2009). It is expected that purslane oil due to high quantity of omega-3 fatty acid, would influence on antioxidant and anticancer functions. Vegetable oils are rich resources for volatile Terpenoid and Phenolic compounds (Ebrahimzadeh et al., 2009). Corn is an herbal plant that among agricultural crops in terms of cultivated areas has third rank in the world. Among all vegetable oils, corn oil has tenth rank in terms of annual production and shares 2% of produced oil throughout the world.

According to Codex standard (1981), the iodine index of corn oil is 103-128, and its saponification number is 187-195. Five major fatty acids of corn oil in terms of quantity are respectively, linoleic, oleic, palmitic, stearic and linolenic. Despite of higher amount of unsaturated fatty acids, corn oil is very stable, and therefore it is suitable for different consumptions such as frying. The results obtained from evaluation of corn oil fatty acids distribution, indicated that 98% position 2 (β-position) in corn oil triglycerides, are unsaturated and only 2% are saturated (Malek, 2010). During frying, oils hydrolyzed and converted into free fatty acids, mono and diglycerides and by continuous application of oil concentrated in it. Furthermore, oxidized oil, hydroperoxide, conjugated dicianoic acids, epoxides, hydroxide and ketones are formed. Increase in the quantity of volatile compounds results in an increase in the index of peroxide, and free fatty acids in oil (Kress-Rogers et al., 1990). Hydroperoxides are first product of oil oxidization and they are also the most important flavor precursors, but their importance depends upon the relative stability and construction of compounds that are produced from them under various situation. In the primary stages of oxidation process, the quantity of these compounds are low, but later in the dispersion stage, the quantity of hydroperoxides intensively increased. In this stage the determination of peroxide number is a suitable index for recognition of oil oxidation condition. In
the final oxidation stage, hydroperoxides were disintegrated to secondary oxidation products and their quantity reduced and even could become zero (Chatzilazarou et al., 2006). Totally, the peroxide number is not capable of indicating the real oil destruction during frying and it is not recommended as an index for this objective. Anisidine index is used in order to recognize secondary compounds including low volatile aldehydes in particular 2,4-dienal and 2-alkanols (Tooranigholsalar, 2011). Hydroperoxides are instable chemical compounds and do not always have direct relation to oxidation during frying. Furthermore, the quantity of p-anisidine, which depends on the product resulted from secondary oxidation, is strictly an experimental evaluation method. Therefore, relying only upon the results of these tests is not adequate and consequently several methods together must be applied in order to find out the critical signs of frying oil destruction to replace or throw it out. Totox index is a proper indicator to the description of oils oxidation since it originated from combination of aldehydes and peroxides (Casal et al., 2010). Application of natural materials for prevention of frying oils destruction was recommended, which includes the addition of materials that are converted into non-saponification products, and resulted from olive, corn and wheat sprout. These materials can protect the frying oil against oxidation destruction during heating. The objective of this research is enhancing the stability and nutritional values of frying oil by mixing them with purslane and corn seed oils.

**Material and methods**

**Devices and materials used in this research**

Laboratory grinder house fryer (Euro max, Switzerland), chromatography gas, gas-liquid (Hewlett-pockard, model HP-5890, France), frying oil (Sunflower and Corn, Ladan and Iran) and purslane seed from Yazd. All chemical materials and solutions purchased from Germany.

**Preparation of the sample and oil extraction**

Purslane seeds collected from different regions of Yazd province and then dried out in room temperature. The seeds were packed in plastic bags and kept in the refrigerator until extraction process. Purslane oil extraction carried out by mechanical pressing method. The hydraulic cold pressing machine pressed the samples, dried by oven (105 °C) and ground (Moulinex, France) to obtain particles of 0.8±0.15 mm, at 10 MPa pressure for 10 min according to the method of Uquiche et al. (2008). Then the extracted oil was filtered and kept at -18 °C to perform experiments and operations (The samples were prepared according to table 1). The moisture content of utilized purslane seeds were 6% on wet basis. The efficiency value based on the seed dry weight and calculated by the Eq. (1) was 18.2%.

\[
\text{Efficiency Extraction} \, (\%) = \frac{\text{Extracted oil weight}}{\text{Dry weight of oil seed}} \times 100
\]

**Table 1. Treatments of experiment**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Compounds</th>
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<tbody>
<tr>
<td>1</td>
<td>Purslane seed oil</td>
</tr>
<tr>
<td>2</td>
<td>Corn oil</td>
</tr>
<tr>
<td>3</td>
<td>Frying oil (Sunflower, Ladan, Iran)</td>
</tr>
<tr>
<td>4</td>
<td>70% Frying oil + 15% Corn + 15% Purslane</td>
</tr>
<tr>
<td>5</td>
<td>75% Frying oil + 15% Corn + 10% Purslane</td>
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</table>
The preparation of oils mixture
In order to conduct efficiency evaluation of the mixed purslane and corn oils, mixed with frying oils (Sunflower, Ladan, Iran), two types of the mixture were prepared from 3 kinds of frying oils, with the ratio of (w/w), which include, corn (Ladan, Iran) and purslane. Mixture 1 and 2 were in the proportion of (70:15:15) and (75:15:10) respectively. All the proportions for oils combinations obtained from test, error and normalization of proportions. The weight of all oils, in order to perform process and pour in to the fryer container, were considered 1 kg. 12 h prior to the preparation of the mixtures, all above-mentioned oils were taken into the laboratory and after mixing them, the mixtures were prepared.

Determination of the frequency distribution of fatty acids
Based on the AOCS (1989) method, first the methyl ester of fatty acids was prepared.

The determination of the fatty acids compounds done by gas chromatography with capillary glass column (column length 30 m, inner diameter of column 0.22 mm), and with flame photometer. To carry out this operation, about 0.3 g oil was dissolved at 7 mm n-hexane and 2 mm potassium hydroxide solution in methanol, normality 2, was added and properly mixed at 50 °C. 0.4 mm at 150 °C was injected with split/splitless. After 10 min the initial temperature of the oven was 190 °C and at the rate of 4 °C per min its temperature was increased to 210 °C and then kept steady for 5 min at this temperature. Then at the rate of 1 °C per min increased up to 215 °C and was steadily kept 18 min at this temperature. The gas current containing nitrogen, was regulated at 1 mL per min. Eventually the obtained curve level from the device compared to the standard curve and the type and amount of each fatty acid in the structure of the oil was determined in terms of percentage over 95 min.

Frying operation
The frying operation was carried out in the house fryer (Euro max, Switzerland). The precise regulation of the temperature done via situating a thermal probe inside of the fryer container as well as controlling of import electrical current. The heating operation performed continuously at 170 °C for 12 h. Thermal stability evaluation of the discussed oils and their reactions assessment, carried out at time intervals of 0, 1, 2, 4, 6, 8, 10 and 12 h. From the oil in the container of the fryer some samples were taken in order to perform the chemical tests (peroxide, anisidine, conjugate-DN and totox). For every sample, 15 mL oil taken from the fryer container and put into the glass tubes with screw cap and after cooling down and coding the samples, they were transferred into a freezer for the future tests.

Measuring antioxidant activities
The antioxidant activity of samples investigated via 2,2 dipheny-1-picrylhydrazyl (DPPH) in order to perform this test, 0.1 mL purslane oil added into 3.9 mL DPPH solution of 0.1 mmol in methanol and kept at room temperature for about 60 min in the dark. The samples absorbance recorded at the wavelength of 517 nm. For control the DPPH solution, containing distilled water was used (Firestone, 1993). The percentage of free radicals were calculated according to the Eq. (2):

\[
\text{DPPH} \, (\%) = \frac{[\text{Control absorbance-Sample absorbance}]}{\text{Control absorbance}} \times 100
\]

Determination of oxidative stability time
For determination of oil stability against oxidation the rancimat machine (Metrohm 743, Switzerland) was used. In brief 2.5 g of oil sample were put
into the reaction chamber and heated up to 110 °C. The volatile compounds which had been released into the distilled water during oxidation reaction, increased the electrical conductivity of water. The inlet air current velocity regulated at 20 L/h. After sometimes the oil samples oxidized entirely and the diagram slope drawn by the machine was suddenly increasing and the mentioned time was recorded by machine. In this case the machine automatically closed the air current and presented the final result. The diagrams, in terms of time (h) and electrical conductivity (µS), were separately drawn for each air channel (Houhoula et al., 2003).

**Measuring total polar compounds (TPC)**
The measurement of the total polar compounds was carried out via the column chromatography. That was silicagel dried out at 160 °C, over 24 h and in the proportion of (0.5 silicagel: 9.5 water) mixed with water and then transferred into the determined column. The applied solvent system was isohexane and disopropyl in the proportion of 85 isohexane and 15 disopropyl. The oil sample mixed with toluene in the ratio of 1 to 9 and then 1 mL of it was transferred into the column. After termination of chromatography operation, the bottom of column was washed up with toluene and by the method of gravimetric the quantity of polar compounds were measured (AOCS, 1989).

**Measuring peroxide index**
Considering the quantity of oil peroxide, 0.5 up to 5 g of each sample weighted and mixed with 30 mL acetic acid chloroform in the ratio of 2 to 3. Then 0.5 mL of the saturated potassium iodide solution was added into the solution. After passing 1 min it was titrated with sodium thiosulphate, normality 0.1, in the presence of Starch glue and, the value of peroxide number was calculated with Eq. (3) (AOAC, 2005).

\[
PV = V \times N \times \frac{1000}{W}
\]

PV: the amount of peroxide (meq/kg)
V: sodium thiosulphate volume (mL)
N: normality of, sodium thiosulfate
W: sample weight

**Measuring p-anisidine**
The oil sample, depending on the oxidation intensity, was weighed 0.01 to 0.5 g. The selected sample (2.5 g), was weighed inside of the 25 mL volumetric flask and then it was diluted with glacial acetic acid. Then this solution was poured into each of two tubes and mixed properly and then to form the complex (p-anisidine, aldehyde and appearance of yellow color), 10 min was given. Eventually the absorbance at 350 nm of sample in comparison with the control (the absorbance difference with the oil sample without solvent) was measured and the index of p-anisidine was calculated with the following equation (Shahidi & Wanasundara, 2002).

\[
p-\text{AV} = 25(1/2\text{As} - \text{Ab})/W
\]

As: sample absorbance
Ab: control absorbance
W: sample weight

**Measuring totox index**
Totox index is exclusively used to measure the oxidative stability of refined oils. So that by applying two indexes of peroxide and p-anisidine in the Eq. (5), this index can be calculated and evaluated (Foglia et al., 1993).

\[
TV = 2PV + p-\text{AV}
\]

TV: totox index value
PY: peroxide index value
AV: p-anisidine index
of 1:60 with isoctane (w/v), then the absorbance difference of diluted sample was measured in comparison with the control (isoctane without oil) at the wavelength of 233 nm and the quantity of conjugate-DN compounds were calculated with Eq. (6) (Ronald, 2001).

\[
\text{C}_{\text{CD}} = \frac{A_{233}}{(\Sigma \times L)}
\]

(6)

C: conjugate-DN concentration, mmol/L; A: absorbance difference of the sample and control; \( \Sigma \): constant coefficient (linoleic acid hydroperoxide), the value is \( 2.525 \times 10^4 \) and L: Measuring of cuvette length in cm (1 cm). Compounds were calculated with Eq. (7) (Ronald, 2001).

\[
\text{CD value} = \frac{(C_{\alpha} \times (2.5 \times 10^4)) + w}{w}
\]

(7)

\( C_{\alpha} \): concentration mmol/mL, W: Sample weight in g and \( 2.5 \times 10^4 \): since the length of applied cuvettes are 1 cm, this factor equals to isoctane from \( \mu \text{mol} \) to mmol, exist at 25 mL of the sample volume.

Statistical analysis
All the tests in this experiment were carried out at three replications. The analysis of obtained data done in the completely randomized design and the comparison of means based on the compare means Duncan test performed by SPSS software (Version 16.0) at the 5% level of probability and the diagrams were drawn by Microsoft Excel software (Version 2013).

Result and discussion
Identifying and determining the frequency distribution of fatty acids, producing oils
The combination of fatty acids in every oil, determined the attributes and application of that oil (Tooranigholsalar, 2011). According to Table (2), considering the higher content of unsaturated fatty acids in purslane seed oil (79.69±0.22%), lower thermal stability was expected for this oil, but based on the measured tests and slower disintegration trend of oil mixture 1, which contains higher amount of purslane oil, was proved the reverse of this reality. The reason is probably the presence of antioxidant compounds in purslane seed oil (Ahn et al., 2012).

Table 2. Recognition and determination of fatty acids frequency, produced by different oils (%)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Purslane</th>
<th>Corn</th>
<th>Frying oil</th>
<th>Mixture 1</th>
<th>Mixture 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0</td>
<td>-</td>
<td>-</td>
<td>0.27±0.05</td>
<td>0.19±0.01</td>
<td>0.2±0.01</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.04±0.0</td>
<td>0.63±0.02</td>
<td>0.5±0.05</td>
<td>0.45±0.01</td>
<td>0.48±0.03</td>
</tr>
<tr>
<td>C16:0</td>
<td>16.86±0.04</td>
<td>13.23±0.01</td>
<td>33.93±0.14</td>
<td>28.27±0.07</td>
<td>29.72±0.07</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.14±0.04</td>
<td>0.42±0.01</td>
<td>0.3±0.01</td>
<td>0.29±0.01</td>
<td>0.3±0.01</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.12±0.0</td>
<td>-</td>
<td>-</td>
<td>0.02±0.03</td>
<td>0.01±0.0</td>
</tr>
<tr>
<td>C17:1</td>
<td>0.04±0.0</td>
<td>-</td>
<td>-</td>
<td>0.01±0.0</td>
<td>0.004±0.0</td>
</tr>
<tr>
<td>C18:0</td>
<td>4.12±0.02</td>
<td>0.79±0.03</td>
<td>4.05±0.04</td>
<td>3.57±0.02</td>
<td>3.57±0.01</td>
</tr>
<tr>
<td>C18:1</td>
<td>21.03±0.02</td>
<td>30.06±0.09</td>
<td>32.13±0.12</td>
<td>30.16±0.13</td>
<td>30.71±0.13</td>
</tr>
<tr>
<td>C18:2</td>
<td>35.29±0.02</td>
<td>54.23±0.13</td>
<td>26.38±0.14</td>
<td>31.89±0.09</td>
<td>31.45±0.09</td>
</tr>
<tr>
<td>C18:3</td>
<td>23.19±0.02</td>
<td>0.65±0.01</td>
<td>2.44±0.11</td>
<td>5.28±0.05</td>
<td>4.25±0.02</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.33±0.0</td>
<td>-</td>
<td>-</td>
<td>0.05±0.01</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.36±0.0</td>
<td>-</td>
<td>-</td>
<td>0.06±0.01</td>
<td>0.04±0.0</td>
</tr>
<tr>
<td>Saturated fatty acid</td>
<td>21.83±0.05</td>
<td>14.65±0.02</td>
<td>38.75±0.21</td>
<td>32.58±0.13</td>
<td>34.05±0.14</td>
</tr>
<tr>
<td>Unsaturated fatty acid</td>
<td>79.69±0.22</td>
<td>30.47±0.1</td>
<td>32.43±0.11</td>
<td>67.63±0.05</td>
<td>66.7±0.1</td>
</tr>
<tr>
<td>Polyunsaturated fatty acid</td>
<td>58.48±0.03</td>
<td>54.88±0.12</td>
<td>28.82±0.25</td>
<td>37.17±0.09</td>
<td>35.7±0.12</td>
</tr>
<tr>
<td>Mono unsaturated fatty acid</td>
<td>21.21±0.25</td>
<td>85.35±0.02</td>
<td>61.25±0.21</td>
<td>30.46±0.07</td>
<td>31.014±0.06</td>
</tr>
</tbody>
</table>

* The minor values represented as dash.
Purslane seed oil in comparison to corn oil contains higher amount of saturated fatty acids (21.83±0.05 and 14.65±0.02% respectively), but also contains higher percentage of unsaturated fatty acid, therefore it can be said with adding frying oil, containing maximum amount of saturated fatty acids (38.75±0.21%) and minimum amount of poly unsaturated fatty acids (32.43±0.11%), to corn and purslane oil, containing minimum amount of saturated fatty acids which has minimum thermal stability, it is possible to increase the thermal stability of the final oil mixture.

**Determining total antioxidant activity, oxidative stability and polar compounds of purslane seed oil**

Antioxidant characteristics of purslane seed oil has a great effect on its oxidative stability. There are various antioxidant compounds such as phenolic compounds, α-Tocopherol, β-carotene, ascorbic acid, glutathione in purslane seed oil (Keyvani & Bolandi, 2015; Mazza et al., 2007).

In this research the according to Table (3), the antioxidant activity of purslane seed oil was 52.9±0.19% which indicates the abundant presence of percentages of free radical absorbing compounds in this oil. Antioxidants can prevent the chain reactions by absorbing free radicals and therefore inhibit lipid oxidation (Samaram et al., 2015). There is direct relationship between the content of phenolic compounds and antioxidant activity of oils (Delfan-Hosseini et al., 2017). The most important factor to identify the quality of oil is the measurement of its oxidative stability which in purslane seed oil is 4.51±0.14 h. The oxidative stability also has a direct relationship with total polar compounds and antioxidant activity. Samaram et al. (2015), extracted more polar compounds in comparison with this research with treatment of purslane seed oil in microwave, which this phenomenon can be attributed to the partial destruction of cell membrane caused by microwave and the optimization of phenolic compounds release (Simopoulos et al., 1992). Also Yang et al. (2013) found out a direct relationship between induction period and total polar compounds of canola oil with different moisture content. The lower oxidative stability of purslane seed oil can be attributed to higher percentage of mono and poly unsaturated fatty acids in the structure of this oil.

<table>
<thead>
<tr>
<th>Table 3. Antioxidant activity, oxidative stability and polar compounds in total purslane seed oil</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oil type</strong></td>
</tr>
<tr>
<td>Purslane seed oil</td>
</tr>
</tbody>
</table>

Table data reported in mean±standard deviation

**Evaluating total polar compounds of different oils during frying**

In the Figure (1), the quantity of polar compound production during the thermal process has been illustrated. The total amount of polar compounds of studied oils has linearly increased over 12 h of frying operation. The oils oxidation behavior during the thermal process has been illustrated by using the oils linear equation and the correlation coefficient between heating time and the polar compounds growth rate. 4 h after thermal process, the production rate of these compounds in corn oil has reached 25% that this range (25%) is as oil unusable limit for frying (Brand-Williams et al., 1995). The polar compounds production of oil mixture number 1 did not catch critical
point 8 h after thermal process and still it was useable. The total polar compounds have a direct relationship with oxidative stability and antioxidant activity. The total amount of polar compounds increased during frying process and heating, but this quantity has differently been reported due to the presence of various factors such as the concentration of antioxidants, which in this experiment we encountered with higher antioxidant activity (51%) which certainly resulted from the presence of its tocopherols and polyphenolic compounds. This effect is more obvious in the oil mixture number 1. As it was said, the amount of various polar compounds increased accordingly with increase in the frying duration (figure 1), and the quantity of compounds resulted from hydrolysis which increases during frying, but decreases during heating process without water (Houhoula et al., 2003).

![Figure 1. Variation of total polar compounds of different oils during frying at 170± 2 °C](image)

**Figure 1.** Variation of total polar compounds of different oils during frying at 170± 2 °C
Corn oil: $10.079x + 3.4475y = 0.9893 = Z_R$; Purslane seed oil: $5.4032x + 3.1845y = 0.9874 = Z_R$; Frying oil: $7.5682x + 2.6334y = 0.9851 = Z_R$; Blend 1 oil: $5.465x + 2.2832y = 0.9925 = Z_R$; Oil mixture-1: $6.3204x + 2.3959y = 0.9859 = Z_R$

**Evaluation of oxidative stability of different oils prior to thermal process**
The oxidative stability results obtained from rancimat machine has been illustrated in Table (4). Purslane seed oil appeared lower thermal stability in contrast to corn oil, however this difference was not significant, but its reason could be attributed to the presence of fatty acid 18:3. Although purslane oil saturated fatty acid was more than corn oil but the effect of 18:3 fatty acid presence was much more and increased the oxidative stability of corn oil in comparison with purslane oil. The previous researches have demonstrated that unsaturated fatty acids in comparison with saturated fatty acids are more sensitive to thermal oxidation and oxidase quicker and can produce higher level of polar compounds during the process (Warner & Mounts, 1993). The oil mixture 1 had higher oxidative stability in spite of having more poly unsaturated fatty acids and lower saturated fatty acids than frying oil, that, the reason of which can be attributed to higher amount of purslane oil and the position of fatty acids in the triglyceride structure of both oils.
Table 4. The oxidative stability of different oils prior to the thermal process

<table>
<thead>
<tr>
<th>Oil type</th>
<th>Studied index Oxidative stability (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purslane seed oil</td>
<td>4.51±0.14a</td>
</tr>
<tr>
<td>Corn oil</td>
<td>4.57±0.08a</td>
</tr>
<tr>
<td>Oil mixture 1</td>
<td>6.62±0.12b</td>
</tr>
<tr>
<td>Oil mixture 2</td>
<td>6.51±0.59b</td>
</tr>
<tr>
<td>Frying oil</td>
<td>5.59±0.12c</td>
</tr>
</tbody>
</table>

Table data reported in mean±standard deviation.

Oil mixtures 1 and 2 contain frying oils of corn and purslane in the ratio of (70:15:15) and (75:15:10) respectively.

Changes of peroxide and p-anisidine indexes of various oils during frying

2 h after frying, considering the slope of peroxide index diagram (figure 2), the increase rate of peroxide index decreased, and 6 h after frying this index decreased, since in the eventual stages of oxidation, hydroperoxides disintegrated to the secondary oxidation products (Chatzilazarou et al., 2006). Peroxides indexes in fats and oils during their spontaneous oxidation, generally indicates the prime oxidation rate of fats and oils.

P-anisidine evaluation method in comparison with peroxide is more acceptable, since it measures the oxidation secondary products which are stable during thermal process (Ghazali et al., 2009). Among the methods that confirm disintegration of oil, many of them are not suitable for frying. For instance in order to measure peroxide index which is the characteristics of oil hydroperoxide and oxidation content, is not an appropriate index in the frying process, since at frying temperatures hydroperoxide is unstable. Furthermore hydroperoxide can increase after taking oil out of fryer, but it must be analyzed prior to complete cooling (Blumenthal, 1991). Tooranigholsalar, (2011) expressed that disintegration tendency rate of this compound in comparison with its production rate is much lower. In the first frying hours the values of this index in the oil mixtures 1 and 2 were more than purslane seed oil which is probably due to oxidative stability and antioxidant compounds of purslane oil, but 6 h after frying this process converted because of more stability of frying oil at higher temperatures. The peroxide index of purslane seed oil reached 40.5±0.65 meq/kg 6 h after frying at the temperature of 170±2 °C, and it changed the orientation in the time space of 6 to 8 h and reached 38.24±0.45 meq/kg. The reaction pattern of purslane oil and oil mixture 1 were different from others, since the range of orientation change in other oils were 4 to 6 h but in these oils the pattern varied and became 6 to 8 h. The results indicate that in the initial frying hours, p-anisidine index increases but 5 to 6 h after frying with peroxide disintegration, p-anisidine index suddenly and remarkably increases. The maximum registered value of p-anisidine was at the end of frying period which was 118.5 mol/gμ and 94.02±0.97 mol/gμ for corn and purslane oils respectively, so that purslane seed oil in comparison with corn oil revealed better thermal stability. Choe & Min (2006) found out that higher amount of linoleic acid causes plenty of changes in the amount of p-anisidine during frying period. They also observed with increased frying time, the value of p-anisidine index increased, so that the amount of fatty acid (18:2) was very effective in the promotion of this index. The results indicated that prior to the disintegration of hydroperoxides, the variation of these two factors were closely similar to each other and their correlation coefficient was 0.998 in purslane seed oil, and in the
corn oil, oil mixture 1 and 2, and frying oil was calculated 0.976, 0.989, 0.98 and 0.979 respectively, this correlation was significant at the level of 0.05 meq/kg. The increase pattern of $p$-anisidine index for oil mixture 1 was partially linear and ascending 2 h after frying, but other oils did not follow such a pattern. The $p$-anisidine test is a reliable test in order to assess of oxidation secondary products, since it reveals proper instability during thermal process (Al-Kahtani, 1991). Diminution of $p$-anisidine vale in the early frying hours, can be attributed to the escape of aldehydes with higher volatility in the initiation of thermal process. $p$-Anisidine indicator prior to

The disintegration of hydroperoxide anti aldehyde, reacts with non-volatile section of fatty acid. This test has higher sensibility to unsaturated aldehydes in particular 2,4-dienals, but it cannot measure the chiton products of secondary oxidation stage. The oil that has $p$-anisidine index lower than 10, has appropriate quality (Naghshineh et al., 2009). Russell (1983) reported that $p$-anisidine index is an applicable index for the evaluation of oils which have higher quantity of poly unsaturated fatty acids and by descending peroxide index production, the index of $p$-anisidine ascends suddenly and remarkably.

![Figure 2](image.png)

**Figure 2.** The variation of peroxide values in the different oils during frying at the temperature of 170±2 °C

The changes of different oils totox index during frying

Totox index expresses the oxidative deterioration of oils, since it is originated from the combination of aldehydes and peroxides. Hydroxides are unstable at higher temperatures which are encountered during frying. The disintegration of one peroxide results in the formation of 2 aldehydes, since 2 oxygen molecules participate in the construction of peroxides while 1 oxygen molecule participates in the construction of aldehydes (Patterson, 1989). Abdulkarim et al. (2007) reported that at 185 °C after 12 h, the totox index of soybean oil reached 142.37 which was higher in compare to oil mixture 1 and 2. According Figure (3), totox values increased considerably ($P<0.05$) by increasing frying temperature in all oils. The results demonstrate that the totox index of various oils have different processes, hence peroxide and $p$-anisidine indexes of these oils are different. 12 h after thermal process, the totox index of purslane seed oil reached 162.62±1.02 and corn oil 166.94±0.98 which indicated the thermal stability of purslane oil was better than corn oil.
According Figure (4), the totox index of oil mixture 1 with a mean value of 132.29±0.86 was in better condition in comparison with other oils at the end of frying process. The totox index of frying oil with a mean value of 146.83±1.18 was further than the totox index of mixtures 1 and 2.

**Variation of different oils conjugate-DN during frying**

Conjugate-DN acids were produced due to the movement of dual bonds during the oxidation of poly unsaturated fatty acids. Along with the increase of absorption by the spectrophotometer, the value of conjugate-DN increased which was proportionate to the rate of oxygen absorption by oil during frying and formation of hydroperoxides in the early stages of oxidation (Farmer, 1946). Tooranigholsalar, (2011) reported that conjugate-DN value of mixed oil (soybean, palm olein and corn) which had higher thermal and oxidative stability, was 18.52, 8 h after frying at 180 °C, but the values of this index in this research 8 h after frying were lower for all experimental oils except the corn oil. The values of oil conjugate-DN (mmol/mL) had considerable increase in the early hours of frying, but this trend was stable and slow in the final frying hours. The value of this index in the oil mixture 2 reached 15.01, 12 h after frying process that revealed very suitable stability and the reason is that the total amount of saturated fatty acids and mono unsaturated fatty acids was
65.064% which participated very little in the reaction. For the formation of conjugate-DN there must be at least two dual bonds in the oil structure, that due to the higher percentage of purslane oil poly unsaturated fatty acids, it is possible to prevent from the increase of conjugate-DN by increasing the proportion of frying oils (Schulte, 2004). Another effective factor is the presence of phenolic and free radical absorber compounds in purslane oil which prevented the increase of this factor. In another research carried out by Arkanit et al. (2006) it is demonstrated that the conjugate-DN values increase by expanding frying time. This issue was even demonstrated by absorbed oil in French fries. They also reported that by increasing the frying temperature, the production rate of conjugate-DN ascends, so that the value of conjugate-DN reached to the maximum in the early stage of frying process at 190 °C. The produced mixture 1 conjugate-DN during the thermal process, at 180 °C and zero time as well as 12 h after heating, were 3.83±0.33 and 15.01±0.2 respectively that these values obtained 4.01±0.12 and 19.34±0.55 for the frying oil. As it has been illustrated in Figure (5), mixture 2 diagram in comparison with oil mixture 1 situated a little upper, but the behavior of these 2 oils in the production value of this index can be considered the same, since in many points there are no significant differences between the results of these 2 oils. These results revealed the presence of purslane seed oil in the combination of oils mixture which had considerable effect in the oil oxidative stability in particular in the initial frying times. The controversial point about oil mixture 1 is its increase pattern on the production value of conjugate-DN in the range of 15 μmol/g, which indicates the reduction in the intensity of production process in this range. Purslane seed oil after corn oil showed he highest amount of conjugate-DN production among other oils, so that at the end of the frying process it reached 18.59±0.39 μmol/g and this increase occurred mostly in the terminal hours of frying. The reason of this issue can be attributed to the lower thermal stability of this oil than the frying oil, that due to the presence of antioxidant phenolic compounds in this oil, this stability was more than other oils at the lower temperatures and in the early hours, and also because of higher proportion of unsaturated fatty acids to saturated ones, revealed further thermal stability at higher temperatures.

Figure 5. The variation of conjugated-DNs values in different oils during frying at the temperature of 170 ± 2 °C
Conclusion

The objective of this research was the increase of oxidative stability of the frying oils (aimed at the elimination of synthetic antioxidants and adding the oils with natural antioxidative compounds and higher oxidative stability) via mixing with purslane and corn oil. The results revealed that the production value of primary oxidative compounds such as peroxides and conjugate-DN of oil mixture 1 (frying oils, corn and purslane in the proportion of 70:15:15) descended in comparison with the frying oil. The conjugate-DN number of oil mixture 1, reached 15.01 μmol/g whose value was better in comparison to other oils with significant difference at the level of 5%. The results demonstrated that the production of secondary oxidation compounds such as aldehydes in the frying oils decreased by mixing them with corn and purslane oils. While corn and purslane oil alone, revealed lower oxidative stability at higher frying temperature, however, the purslane seed oil revealed higher stability in comparison with other oils due to the presence of antioxidant phenolic compounds in the early hours of frying, but because of lower oxidative stability than other oils in the final frying hours, this process was converted. Considering the above-mentioned results, it is possible to prepare the special frying oil by mixing with purslane and corn oil which reveals proper stability in comparison with the frying oil and add nutritional properties such as antioxidant phenolic compounds.

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بررسی پایداری اکسیداتیو روغن سرخ‌کردنی مخلوط‌شده با روغن دانه خرده و روغن ذرت

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چکیده
یکی از راه‌های پایداری از روغن سرخ‌کردنی به منظور حذف آنتی-اکسیدان‌های سنتری، افزودن روغن‌های با ترکیبات
ضدایمندی و پایداری اکسیداتیو بالا می‌باشد. هدف از این پژوهش، ارزیابی کیفی مخلوط سه نوع روغن سرخ‌کردنی
(افتاب‌گردان، لاکنت و ایران) در اثر افزودن روغن دانه خرده و ذرت طی ۱۲ ساعت سرخ‌کردن در دمای ۱۷۰±۲ سانتی‌گراد بود.
برای این منظور روغن سرخ‌کردنی مخلوط حاوی روغن دانه ذرت و خرده با نسبت‌های (۱۵:۷۰ وزنی/وزنی) تحت عنوان
مخلوط ۱ و روغن سرخ‌کردنی مخلوط حاوی روغن دانه ذرت و خرده با نسبت‌های (۱۵۰/۷۵ وزنی/وزنی) تحت عنوان
مخلوط ۲ نمونه‌برداری شد. روغن دانه ذرت، روغن دانه خرده و مخلوط سه روغن سرخ‌کردنی از لحاظ شاخص‌های کیفی (براکسید، پلاژتیریدین، توتورکس،
ترکیبات فلزی کل، پایداری اکسیداتیو و دی‌های مزدوج) مورد ارزیابی قرار گرفتند. در این مطالعه فعالیت آنتی-اکسیدانی روغن
دانه خرده مورد بررسی قرار گرفته و درصد اندام‌گیری شد. مقایسه بین دو مخلوط روغن سرخ‌کردنی نشان داد
مقاول شاخص‌های پراکسید، پلاژتیریدین، توتورکس و دی‌های مزدوج مخلوط ۱ و مخلوط ۲ به ترتیب (۳۸/۱۹ میلی‌کیلوگرم
بر کیلوگرم، ۱۸/۸۲ درصد، ۱۷/۱۷ درصد و ۲۸/۱۳ میلی‌کیلوگرم بر کیلوگرم، ۴/۸۲ درصد) بود که تفاوت قابل مutyای داری
(۴/۰۵) با روغن سرخ‌کردنی و سایر روغن‌ها داشت. نسبت بالاتر روغن خرده در روغن سرخ‌کردنی مخلوط ۱، پایداری اکسیداتیو این روغن را نسبت به روغن مخلوط ۲ بالا برده و روغن دانه خرده از تخریب
زود هنگام ای ندر پر امداد ممنوعت می‌کند.

واژه‌های کلیدی: پایداری اکسیداتیو، روغن دانه خرده، روغن ذرت
Determination of Floral Origin Common Honey in Khorasan Razavi Province Based on Color Characteristics, Salinity, Electrical Resistance and TDS using Chemometrics Methods

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Abstract
Nowadays, food adulteration is important to marketing and production. The purpose of this study was to classify different types of honey (Thyme, Chaste tree, Jujube, Coriander, Barberry, Acacia and Alfalfa) with different herbal origin based on color characteristics, salinity, electrical resistance and total dissolved solids (TDS). For classification of honey, principal component analysis (PCA), linear discriminant analysis (LDA) and cluster analysis (CA) of chemometrics method, were used. The results showed the two principal components (PC1 and PC2) comprise 77.69% of the total variance, which indicates the distinction between different honeys based on physicochemical characteristics. It was also shown in the LDA method was able to classify different honeys with an accuracy of 75%. On the other hand, the method of CA in the distance of 85.58, honey was placed in 7 groups that contained thyme, coriander, acacia, barberry and jujube honey in separate groups and alfalfa honey and chaste tree honey were scattered among the groups.

Introduction
Honey cannot be a major meal for humans, but it can be considered as a nutritional supplement. Honey has also been used in traditional medicine from ancient times and has been reported to treat burn, asthma, infection, gastrointestinal disorders, skin lesions, various inflammatory processes, as well as cataracts and other eye diseases (Khalafi et al., 2016). Honey is a natural product that contains a large amount of sugars and minor amounts of minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids, enzymes and volatile compounds. The amount of these different compounds varies depending on the source of the flower and the geographical origin of the honey. In addition, honey combinations are also affected by processed and storage time (Gheldof et al., 2002).

Color is a variable feature of honey and is mainly influenced by the flower's source, but also depends on the ash content, temperature and storage time. Color is the first sensory assessment that consumers take, which can
determine whether or not to buy a product (Bogdanov et al., 2004). Honey lighting plays a very important role in consumer acceptance. Honey specialists are aware that honey color varies from bright to dark violet, but yellow amber, red and green are the most colorful colors of honey. In many countries, the price of honey is associated with its color. Honey with a light color has a higher price than dark honey, and in some areas dark honey is more popular (Tuberoso et al., 2014).

The electrical conductivity of honey has a high correlation with the amount of minerals, ash and acidity, indicating ion, protein and organic acids. Therefore, the presence of these compounds increases the electrical conductivity, which can be used as a quality indicator in the detection of counterfeit honey from natural honey (Karabagias et al., 2014).

Misleading labeling and cheating on honey has unfortunately become a problem all over the world. Cheating is usually the addition of water solution with sugar and syrup (such as corn syrup and high fructose corn syrup), as well as cheating another feeding bee with sugar and syrup, which produces artificial honey. Because of the difference in the price of honey on the basis of herbaceous origin, there is another type of fraud that is deliberately referred to as a flower or geographical origin. For this reason, regulatory agencies, manufacturers, retailers and consumers are interested in knowing the origin and quality of honey (Tewari & Irudayaraj, 2004).

Material and methods

Honey samples

44 samples from 7 different types of honey (Thyme, Chaste tree, Jujube, Coriander, Barberry, Acacia and Alfalfa honey) were obtained from the Agricultural Jahad of Mashhad, with emphasis on single flowering. The samples were stored in plastic containers within a refrigerator.
Determination salinity, electrical resistance and TDS

Electrical resistance, salinity, and total dissolved solids (TDS) were measured at 20 °C using a conductivity meter (Seven Easy, Mettler Toledo, Switzerland) and a solution prepared by dissolving 20 g of honey (dry matter basis) in 100 mL of distilled water twice (Saxena et al., 2010).

Colorimetric

To measure the color characteristics L (brightness), a (red-green), b (blue-yellow) and H (hugh angle) was used colorimetric (Colorimeter-WF-30, Iwave, China) with D65 light source and specular component included (SCI) optical mode for 9 °C.

Data preprocessing

Due to the lack of uniformity of the units and the variation in the range of variations of variables, the pre-processing problem was solved to solve this problem, and the data scale was changed and made identical. Eq. (1) was used for this.

\[ Y_i = \frac{x_i^2}{\sum x_i^2} \]  

(1)

Chemometric methods

Principal component analysis is based on the concentration of the data variance into a small number of principal components (PCs) by means of mathematical transformation. As a result, the first PC describes the maximum information from the data; the second PC describes the maximum amount of the residual variance. Each successive principal component is an orthogonal combination of the original variables which cover the maximum of the variance not accounted for by the previous component (Yucel & Sultanoglu, 2013). The aim of the method is to reduce the dimensionality of multivariate data whilst preserving as much of the relevant information as possible (Tsankova & Lekova, 2015). The plot of scores of PC1 versus PC2 is a common method for classifying samples from their properties measured (Yucel & Sultanoglu, 2013).

Cluster analysis was performed to classify samples on the basis of the similarities of their chemical properties. The method allowed displaying different classes from botanical origins according to the mineral profiles. In cluster analysis, the groups showed the relation of botanical origin of the honey samples. The similarity between samples was calculated from the Eq. (2) distance as follows:

\[ D_{ik} = \sqrt{\sum_{j=1}^{n} (x_{ij} - x_{kj})^2} \]  

(2)

Where \( x_{ij} \) and \( x_{kj} \) are the values of variables \( j \) for the samples \( i, k \) and \( n \) is the number of variables (Lachman et al., 2007).

In cluster analysis methods, samples are grouped in high dimensional space and form dendrogram. Gradually samples are joined into clusters up to the final cluster with all the samples. In the first step each sample forms a cluster, and then two objects closest together are joined. In the next step, either a third sample joins the first two or two other samples join together in a different cluster. Each step results in one lesser cluster than the step before until at the end all samples are in one cluster (Yucel & Sultanoglu, 2013).

LDA is used to determine which variables discriminate between two or more naturally occurring groups. This mathematical procedure maximizes the variance between groups and minimizes the variance within each group (Corbella & Cozzolino, 2006). For validation of the model, cross-validation was used.

Result and discussion

Honey color based on the color standards USDA (United States Department of Agriculture) is one of the early features of honey classification. Honey color, which has not been processed, depends primarily on the source of the origin
flower (Moniruzzaman et al., 2014). Table (1) shows color indicators (L, a*, b* and H) of different honey. The L indicator represents the brightness. Among the evaluated honey, Acacia honey had the highest L-value, which had a significant difference (P<0.05) with other honey (except Alfalfa honey). Alfalfa honey is also considered as light honey, and the darkest honey is Chaste tree honey. In addition, in terms of a redness index a*, yellowing index b* and Hyo H index respectively, Jujube, Chaste tree and Acacia honey had the highest amount.

And the range of changes in L*, a*, b* and H indexes was 22.20-28.52, 2.08-4.34, 5.81-11.08 and 61.76-78.29 respectively. Gonzalez-Miret et al. (2005) generally divided honey into two categories they were classified honey with a L index of more than 50, as bright honey, and honey with a L index of less than 50 as dark honey. Based on this categorization, honey is considered to be dark in this study. On the other hand, the color of honey is also associated with its taste, bright honey has a mild taste, while dark honey has a bitter taste. Honey contains potassium, calcium, sodium and phosphorus salts that are responsible for salinity production (Gheldof et al., 2002). The amount of salinity is correlated with electrical resistance and electrical conductivity, and the amount of these compounds is dependent on the source of honey (Corbella & Cozzolino, 2006). In the evaluation of honey in this study, honey with the highest salinity has the least amount of electrical resistance. Acacia honey had the lowest salinity (0.09±0.009) and the highest electrical resistance (omega-cm, 5.03±0.87) and had a significant difference (P<0.05) with other honey. On the other hand Barberry and Jujube honey, unlike Acacia, had the highest amount of salinity and the least amount of electrical resistance.

Total dissolved solids (TDS) content in honey include all organic and inorganic compounds that are molecular, ionized, and micro granular (colloidal solution) (Khalil et al., 2012). The range of total solids changes was measured in honey samples between 305.12 and 96.85 ppm, witch Barberry and Alfalfa honey having the highest and lowest total dissolved solids respectively. Islam et al. (2017) reported the amount of TDS in Bangladesh's honey ranging from 150-100 ppm (Islam et al., 2017). In a study by Nweze et al. (2017), the TDS was between 209-399 ppm was variable (Nweze et al., 2017).

Table 1. Characteristics of color, salinity, electrical resistance and TDS of different honey

<table>
<thead>
<tr>
<th>Honey</th>
<th>L</th>
<th>a*</th>
<th>b*</th>
<th>H</th>
<th>TDS (mg/L)</th>
<th>Salinity (ppm)</th>
<th>Electrical resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyme</td>
<td>25.94±1.33bc</td>
<td>3.1±0.51bc</td>
<td>7.15±1.31ed</td>
<td>68.54±4.36bc</td>
<td>162.52±4.78d</td>
<td>0.156±0.011d</td>
<td>3.02±0.09d</td>
</tr>
<tr>
<td>Chaste tree</td>
<td>25.45±2.4c</td>
<td>3.68±0.8ab</td>
<td>11.08±2.63a</td>
<td>70.99±5.77b</td>
<td>163.72±7.42d</td>
<td>0.15±0.076d</td>
<td>3.61±1.55b</td>
</tr>
<tr>
<td>Jujube</td>
<td>25.9±0.67bc</td>
<td>4.34±1.39a</td>
<td>8.27±1.58ed</td>
<td>61.76±3.51d</td>
<td>258.89±8.75b</td>
<td>0.25±0.008a</td>
<td>1.19±0.07b</td>
</tr>
<tr>
<td>Coriander</td>
<td>25.52±1.42b</td>
<td>3.37±0.21bc</td>
<td>9.69±2.91bc</td>
<td>71.73±7.12b</td>
<td>204.83±8.42c</td>
<td>0.19±0.008c</td>
<td>2.44±0.08d</td>
</tr>
<tr>
<td>Barberry</td>
<td>22.2±1.38d</td>
<td>2.89±0.44ad</td>
<td>5.81±0.6d</td>
<td>63.61±2.82ed</td>
<td>305.12±10.17d</td>
<td>0.29±0.011a</td>
<td>1.64±0.05d</td>
</tr>
<tr>
<td>Acacia</td>
<td>28.52±0.71a</td>
<td>2.08±0.35d</td>
<td>10.05±0.65ab</td>
<td>78.29±2.13a</td>
<td>96.85±14.44c</td>
<td>0.09±0.009c</td>
<td>5.03±0.87a</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>27.63±1.2ab</td>
<td>2.47±0.22ad</td>
<td>9.56±3.21bc</td>
<td>71.15±6.25b</td>
<td>131.4±27.99d</td>
<td>0.12±0.025c</td>
<td>3.77±0.96b</td>
</tr>
</tbody>
</table>

Data are means ± standard deviation. In each column, values with different letters indicate significant differences (P<0.05)
Determination of Floral Origin Common Honey in Khorasan Razavi …

Chemometrics
Principal component analysis (PCA)
Principal component analysis (PCA) is one of the methods of chemometrics and is commonly used to reduce high-dimensional data and display it in two or three-dimensional environments (Jandric et al., 2015). According to Table (2) and Figure (1), we consider components with a specific value greater than 1 as the main components.

Table 2. Variance and initial eigenvalues of the main components evaluated

<table>
<thead>
<tr>
<th>Component</th>
<th>Initial eigenvalues</th>
<th>Extraction sums of squared loadings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>Variance %</td>
<td>Cumulative %</td>
</tr>
<tr>
<td>1</td>
<td>4.334</td>
<td>61.911</td>
</tr>
<tr>
<td>2</td>
<td>1.105</td>
<td>15.787</td>
</tr>
<tr>
<td>3</td>
<td>0.954</td>
<td>13.634</td>
</tr>
<tr>
<td>4</td>
<td>0.394</td>
<td>5.628</td>
</tr>
<tr>
<td>5</td>
<td>0.119</td>
<td>1.695</td>
</tr>
<tr>
<td>6</td>
<td>0.091</td>
<td>1.307</td>
</tr>
<tr>
<td>7</td>
<td>0.003</td>
<td>0.038</td>
</tr>
</tbody>
</table>

The first main component comprise the 61.91% of the variance and the second main component comprise the 15.78% of the variance of the data. In total, the two main components comprise 77.69% of the total data variance. Jandric et al. (2015) evaluated the chemical properties of different honey and determined the main components that showed the two main components comprised 87.5% of the variance.

Figure (2) shows the dispersal of 7 different honey types in the two-dimensional space PC1 and PC2. Based on the main components, Barberry honey, Acacia, Alfalfa, Jujube, Thyme and Coriander were placed in separate groups and only Chaste tree honey was shown sporadically. The reason for the overlap of some honey can be due to the beekeeping method. In the case of migrating bees, the possibility of mixing honey is present and, given that the honey is prepared from different beekeepers, there is a potential for mixing. However, the main components were able to distinguish between different honeys.
Table (3) shows the effect of each of the characteristics evaluated on the two main components. According to this Table (3), the characteristics of L, H, salinity and electrical resistance are affected by the first main component and the characteristics a* and b* affect the second main component.

**Table 3.** Effect of the evaluated variables on the two main components

<table>
<thead>
<tr>
<th>Component</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>0.711</td>
<td>-0.003</td>
</tr>
<tr>
<td>a*</td>
<td>-0.418</td>
<td>0.812</td>
</tr>
<tr>
<td>b*</td>
<td>0.588</td>
<td>0.658</td>
</tr>
<tr>
<td>H</td>
<td>0.782</td>
<td>-0.055</td>
</tr>
<tr>
<td>TDS</td>
<td>-0.962</td>
<td>0.051</td>
</tr>
<tr>
<td>Salinity (ppm)</td>
<td>-0.962</td>
<td>0.055</td>
</tr>
<tr>
<td>Electrical</td>
<td>0.910</td>
<td>-0.151</td>
</tr>
<tr>
<td>resistance</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Linear discriminant analysis (LDA)**

Before the analysis with the LDA method, a step-by-step method was used to determine the effective variables, and in each step, the variable with the highest F value was selected (Ciappini *et al.*, 2016). According to Table (4), the variables TDS, electrical resistance and a* were selected as effective variables in the honey grouping. Based on LDA analysis, 75% of honey was correctly grouped. As shown in Table (5), Thyme, Jujube, Coriander and Barberry honey were correctly (100%) in there. And only Chaste tree honey was scattered among different groups, as can be seen in Figure (2).

**Table 4.** F value of each variable in each step

<table>
<thead>
<tr>
<th>Step</th>
<th>Variable</th>
<th>Tolerance</th>
<th>F to Remove</th>
<th>Wilks' Lambda</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TDS</td>
<td>1.000</td>
<td>34.796</td>
<td>0.285</td>
</tr>
<tr>
<td></td>
<td>TDS</td>
<td>0.242</td>
<td>18.353</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Electrical</td>
<td>0.242</td>
<td>6.874</td>
<td>0.151</td>
</tr>
<tr>
<td></td>
<td>resistance</td>
<td>0.242</td>
<td>17.743</td>
<td>0.165</td>
</tr>
<tr>
<td>3</td>
<td>Electrical</td>
<td>0.241</td>
<td>6.051</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td>resistance</td>
<td>0.948</td>
<td>4.200</td>
<td>0.070</td>
</tr>
</tbody>
</table>

Manzanares *et al.* (2017), by studying the physicochemical properties of various Spanish honey, were able to group honey with 95% accuracy by using the LDA method (Manzanares *et al.*, 2017). Rios *et al.* (2014) grouped Argentina honey with 84% accuracy (Rios *et al.*, 2014).

Cross-validation method was used to validate the model. In this method, the model was first created with all samples. Then all samples were placed in order to validate in the model. In this study, out of 31 samples from 44 honey samples in the validation phase were correctly predicted, indicating that the provider is 70.45% of the model’s validity.
Table 5. Grouping of different honey based on LDA

<table>
<thead>
<tr>
<th>Honey</th>
<th>Thyme</th>
<th>Chaste tree</th>
<th>Jujube</th>
<th>Coriander</th>
<th>Barberry</th>
<th>Acacia</th>
<th>Alfalfa</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyme</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Chaste tree</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Jujube</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Coriander</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Barberry,</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Acacia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

75.0% of original grouped cases correctly classified.

Cluster analysis (CA)
The cluster analysis is a multivariate analysis method used to find real groups. In this way, members who are more similar are grouped together (Santos et al., 2008). Figure (3) shows the dendrogram diagram of the grouping of honey. The ward method was used for cluster analysis and Euclidean distance was used to determine the distance between groups. In the distance of Euclidean 322.6, the honey was divided into 5 groups: Alfalfa honey and Thyme in one group, Chaste tree and Coriander honey in the other group, and Jujube, Acacia and Barberry honey in separate groups.

While at 85.58 Euclidean, the honey was placed in 7 different groups: Thyme, Barberry, Acacia, Jujube and Coriander honey were almost in separate groups, and Chaste tree honey and Alfalfa honey were scattered among the groups. Kivrak et al. (2017) measured the physicochemical properties of Turkish honey by means of the average linkage and rescaled spacing, they were able to divide the honey into 3 different groups. Group A included: Cedar, Lentil and Pine; group B: Multicolored, Lavender, Cranck and Chestnut; group C: Peony, Five Fingers, Sunflower and Heather (Kivrak et al., 2017).

Figure 3. Cluster analysis dendrogram diagram
(1: Thyme, 2: Chaste tree, 3: Jujube, 4: Coriander, 5: Barberry, 6: Acacia, 7: Alfalfa)
**Conclusion**

The results of color characteristics, electrical resistance, salinity and TDS of single-flowered honey with the aid of multivariate analysis showed that, honey can be grouped according to its origin flower but using a feature, cannot distinguish between different honeys.

Given the varying price of honey based on its origin flower, this method can be used to determine adulteration and ensure consumer food security. It is also possible to find out that PCA, LDA and CA chemometric methods have the ability to separate various honey.

**References**


تعیین منشأ گیاهی عسل‌های رایج استان خراسان رضوی بر اساس خصوصیات رنگ، شوری، مقاومت الکتریکی و مواد جامد تأم با استفاده از روش‌های کمومتریکس

اهسان اکبری، عادل بیگبایبی، مصطفی شهیدی نوقابی

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چکیده

در حال حاضر اکالت‌سنگی در مواد غذایی یک جالش مهم در بازاریابی و تولید آن می‌باشد. هدف از این مطالعه تقسیم‌بندی انواع عسل (آویشن، اقاقیا، گشنیز، زرشک، عنب، پونه و هندبید) با منشأ گیاهی مختلف بر اساس خصوصیات رنگ، شوری، مقاومت الکتریکی و مواد جامد محلول تأم (TDS) (میان‌بندی برای فاصله‌بندی عسل‌های تک‌گل از روش‌های کمومتریکس تحلیل مولفه اصلی (PCA)، آنالیز‌های خطی (LDA) و آنالیز خوشه‌ای (CA) استفاده گردید. نتایج نشان داد دو مولفه اصلی PC1 و PC2 69/77 درصد از کل واریانس را شامل می‌شوند. همچنین در پرسسو صورت گرفته‌ها با مند LDA توانایی تقسیم‌بندی عسل‌های مختلف با صحت 75 درصد را دارد. ازطرف دیگر مقد 75 درصد در فاصله اقلیدسی 85/158 عسل‌ها در گروه قرار گرفتند که عسل‌های آویشن، زرشک، اقاقیا، عنب و گشنیز تقریباً در گروه هما و یونجه و عسل‌های هندبید و پونه در بین گروه‌ها پراکنده بودند.

واژه‌های کلیدی: آنالیز چندمتغیره، اکالت‌سنگی، تقلیبات، عسل، کمومتریکس
Utilizing Pattern Recognition Methods for Detecting the Adulteration of Glucose and Fructose in Honey

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Abstract

The aroma of honey is one of the important parameters in honey grading and that is depended on several factors, such as geographical origin, climate, botanical and environmental conditions. The aim of this study was the development and evaluation of an electronic nose as a new, fast and nondestructive method for detecting adulteration in honey. In this research, the ability of electronic nose as a non-destructive system for detecting honey adulteration with different percentages (pure, 20% syrup, 40% syrup, 60% syrup and 80% syrup) was investigated. The developed electronic nose consists of 8 metal oxide semiconductor sensors (MOS) to detect adulterity in honey. After preprocessing the data obtained from the electronic nose the chemometric methods were utilized to classify different type of honey. Principle component analysis (PCA), hierarchical cluster analysis (HCA), linear discriminate analysis (LDA), were used to analyze the data obtained from electronic nose. Based on the results, the detection of adulteration was 98.4% of variance for PCA method, 99% accuracy for HCA method and 100% classification power by LDA method.

Introduction

Food quality is a complex concept referring to multiple characteristics that make a food product acceptable or more desirable to eat. Important food quality aspects are safety, nutritional value, functionality, and aesthetics (color, texture, aroma, appearance). While the first three are subjectively quantifiable, the last has an even more important subjective component, which makes it more difficult to describe and/or quantify. Aroma, is a very important component of this subjective quality (Lammertyn et al., 2004).

Traditional analytical and quantitative techniques for aroma analysis include HPLC, GC with headspace sampling and GC-MS analysis with solid phase microextraction. Numerous reports exist on the aroma analysis of a wide range of food products with these techniques and they have proven to give very precise and reliable results. It has been proven that they give these techniques, however, involve a lot of sample preparation, are time consuming and can only be carried out in a specially equipped laboratory environment by well-trained operators. Next to a
chemical characterization, aroma analysis often also includes a sensory evaluation by both trained taste panels and consumer panels. This type of evaluation is important in classifying aroma characteristics according to human perception and consumer behavior. Evidently, this is a very subjective and variable evaluation, which involves a very costly and time consuming procedure (Lammertyn et al., 2004).

Honey contains a viscous solution, as well as supersaturated sugar from flower nectars, which is collected and produced by the honeybee. According to the Iranian National Standard Rules, honey is defined as a pure substance, additives cannot be added to it. Due to the high demand of Iranian honey in the country as well as neighboring countries, this has led to an increase in the sale of honey from pure honey to adulterated honey. Adulteration with sweeteners is the most important issue to assess the authenticity of honey. Sweeteners that have been detected in honey are sugar syrup, corn molasses, sugar cane and sugar beet which is affected by the acid or enzyme. Various analytical techniques, including: isotopic (Padovan et al., 2003, Cabanero et al., 2006), chromatographic (Cordella et al., 2003; Morales et al., 2008) and thermal analysis (Cordella et al., 2005) have been implemented for the detection of honey adulteration. The strength of these methods in honey adulteration detection has been proven by numerous researches, however, they are time-consuming, destructive, and some of them expensive. Therefore, fast, non-destructive, and precise analytical methods are welcome to complement the existing techniques (Shafiee et al., 2016). One of the new techniques for food quality control is the e-nose technic, e-nose is an instrument designed to emulate the sense of smell and discriminate among complex odors by means of an array of gas sensors (which respond to gases and vapors generated by the sample) and multivariate data analysis methods (Kiani et al., 2016). There are several studies reporting the use of e-nose for quality and adulteration assessment of foods, aromatic plants and fruits such as olive oil (Oliveros et al., 2002; Melucci et al., 2016), milk (Yu et al., 2007), soy sauces (Gao et al., 2017), pumpkin (Zhou et al., 2017), honey (Lammertyn et al., 2004; Zakaria et al., 2011), saffron (Heidarbeigi et al., 2015; Kiani et al., 2017), tomato (Wang & Zhou, 2007), tea (Roy et al., 2012; Huo et al., 2014), apple (Ezhilan et al., 2018), coffee (Freitas et al., 2001), sesame oil (Hai & Wang, 2006) and tomato juice (Man et al., 2005). Therefore, the objective of this study was to assess the potential application of e-nose system for detection of honey adulterated by addition of sugar syrup.

**Material and methods**

All honey samples were collected from beekeepers of different provinces of southern region of Iran (Bushehr, Hormozgan, Khuzestan, Sistan and Baluchestan, Fars and Kerman), which are the main producers of *Ziziphus* honey. Adulterant solutions were produced by blending of the fructose-glucose mixtures 1:1 (w/w) were prepared by mixing fructose (HFCS 55, High fructose corn syrup) and glucose (DE 42, Dextrose equivalent) (Zar Fructose Company, Iran) completely. A set of honeys were subsampled and then adulterated with fructose-glucose adulterant solutions at four levels i.e. 20, 40, 60 and 80%. Finally, each group of samples was tested 20 times by the electronic nose.

The developed e-nose system consisted of a sample and sensor chamber, air circulation system, a set of
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gas sensors a data collection system and suitable data preprocessing programs written in LabVIEW software (figure 1).

The basis of e-nose systems is based on gas sensors. Semiconductor sensors metal oxide semiconductor (MOS) due to its high chemical stability, high sensitivity and suitable for a wide range of food and agricultural products were used in the e-nose system.

For designing the sensor array, about 20 different MOS gas sensors were purchased and each of them was evaluated using different honey samples, and 8 sensors that showed a large difference among different honey samples were selected and placed inside the sensor chamber.

The sensor array consists of 8 different MOS gas sensors that consists of 6 MQ sensors fabricated in (HANWEI Electronics Co., Ltd., Henan, China) and 2 TGS sensors supplied by (Figaro Engineering, Inc., Osaka, Japan) (table 1). The circuit of both type of sensors are presented in Figure (2). According to the manufacturer's recommendations In order to ensure the correct functioning of the sensors, an hour before the experiments the sensors were turned on to achieve a steady state.

![Figure 1. The developed e-nose](image)

Table 1. Specifications of the sensors utilized in the sensor chamber

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Detect</th>
<th>Detection range (PPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MQ-2</td>
<td>Methane, Butane, LPG, Smoke</td>
<td>200-5000</td>
</tr>
<tr>
<td>MQ-3</td>
<td>Alcohol, Ethanol, Smoke</td>
<td>0.05-10</td>
</tr>
<tr>
<td>MQ-4</td>
<td>Methane, CNG Gas</td>
<td>200-10000</td>
</tr>
<tr>
<td>MQ-5</td>
<td>Natural gas, LPG</td>
<td>200-10000</td>
</tr>
<tr>
<td>MQ-6</td>
<td>LPG, Butane gas</td>
<td>200-10000</td>
</tr>
<tr>
<td>MQ-136</td>
<td>Hydrogen Sulfide gas</td>
<td>1-200</td>
</tr>
<tr>
<td>TGS-2610</td>
<td>High sensitivity to LP and its component gases</td>
<td>500-10000</td>
</tr>
<tr>
<td>TGS-2620</td>
<td>For alcohol, toluene, xylene, other volatile organic vapors</td>
<td>500-5000</td>
</tr>
</tbody>
</table>

![Figure 2. The data sheet of gas sensor (a) MQ type (b) TGS type](image)

The schematic drawing of the olfactory machine system is shown in Figure (3), which shows how the system works. The measurement process in the experiments is divided into two phases: 1) saturation and measurement; and 2) purification.
About 250 g of sample was poured inside a sample chamber and its lid was sealed with a cork to prevent the outflow of gases from the honey space. Regarding the sampling step, the air flowed through the various paths controlled by a computer program. The sensor chamber is cleaned with dry air before the honey head space gasses were guided into the sensor chamber. At this stage, the pumps 1, 2 and 3 and the valves 1, 2 and 4 are switched on and the solenoid valve 3 is off. At this stage, the dry air passes through the sensor and sample chamber and cleans out the two compartments from any scent of aromas and eventually exhausts from the outlet. This takes about 600 s. In the next step, the pumps 2 and 3 are still on, but the pump 1 was turned off, as well as the electric switches 2 and 3 were switched on and the electric valves 1 and 4 were turned off. At this stage, the aroma of honey was saturated and circulates from the sensor chamber and the sensors are exposed to the honey head space gasses and the changes in the output of the sensors were stored. The time required to reach the steady state was 550 s, and this time was considered as the response time. After this phase, the sensor and sample chambers that were saturated with honey aroma were cleaned for 600 s using the dry air, to return the sensor response back to the baseline. The output of the sensors was collected using the Ardino Mega 2560 microcontroller, and the LabVIEW 2017 software was used to connect the microcontroller to the computer for data storage and preprocessing.

In this research, the Ardino mega 2560 was used to communicate between sensors and convert analogue data to digital output. The Mega 2560 Ardino board has a microcontroller board based on ATmega 2560. This board has a total of 54 digital PINs which fifteen of them can be used as pulse width modulation (PWM) outputs, as well as 16 analog inputs. The Mega 2560 board includes features that allow communication with the computer, other Arduino boards or other microcontrollers. The data collected from the sensors was sent to LabVIEW by the Arduino board. LabVIEW is a graphical programming language that is widely used for applications in military, educational, and laboratory industries as a standard model for data collection and analysis, as well as a tool for controlling and simulating systems. LabVIEW is a graphical programming language that is widely used for applications in military,
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educational, and laboratory industries as a standard model for data collection and analysis, as well as a tool for controlling and simulating systems. This program has two basic parts, first part is the software interface and operator, and the other part is the block diagram, which is the location of the codes and symbols that is the environment of programming. Figure (4) shows the codes written in the LabVIEW software for communicating the information between the e-nose and computer for analysis.

Figure 4. Codes written in the LabVIEW software

Signal preprocessing is used for extraction of relevant data from the obtained responses and also for preparation of the data for multivariate pattern analysis. The major aspects of this preprocessing are: (a) baseline identification and manipulation/determination, (b) compression, (c) normalization (Pearce et al., 2003).

\[
y_s(t) = \frac{x_s(t) - x_s(0)}{x_s(0)}
\]  

(1)

Differential, relative and fractional techniques are 3 different techniques for baseline manipulation. Fractional method is also widely used for MOS chemo-resistors. In this method, the baseline \( x_s(0) \) is first subtracted from the sensor response \( x_s(t) \) and then divided into the baseline. Fractional measurements are not only dimensionless but also normalized since the resulting response \( y_s(t) \) is a per-unit change with respect to the baseline, which compensates for sensors that have intrinsically large (or small) response levels. Fractional method was used in the current study (Sanaeifar et al., 2016).

Compression is a preprocessing stage in which the response of each sensor array is utilized as a feature vector or a fingerprint by decreasing the number of descriptors. In this study, the maximum response value for each sensor was individually extracted and analyzed. Normalization is the final stage of preprocessing which is applied to operate on the sensors signals to compensate for sample-to-sample variations due to the change in analytic concentration and drift in the sensors. On the other hand, normalization operates across the entire database for a single sensor (e.g., the complete history of each sensor), and is generally employed to compensate for differences in sensor scaling. In what follows, we
will denote it by \( x_s^{(k)} \) which is the response of sensor “s” to the k-th example in the database (Eq. 2). In Sensor normalization, the range of values for each individual sensor is set to [1, -1] (Sanacifar et al., 2016). Figure (5) shows the sensor responses for honey sample.

\[
y_s^{(k)} = \frac{x_s^{(k)} - \min_{k}[x_s^{(k)}]}{\max_{k}[x_s^{(k)}] - \min_{k}[x_s^{(k)}]}
\]

(2)

Figure 5. Sensors responses after preprocessing

After preprocessing the data principle component analysis (PCA), hierarchical clustering analysis (HCA) and linear discriminant analysis (LDA) studied in order to create the models and to discriminate between different honey samples through the sensor array responses of e-nose.

PCA is a statistical technique that is used for extracting information from a multivariate dataset. It transforms the original, measured variables into new uncorrelated variables called principal components (PCs), which retain the information present in the original data as much as possible. In this work, the PCs were selected to contain the maximum variance in the sensor array signals and subsequently as a sensor selection method. The first PC is oriented to explain as much variance in the data as possible and the second PC explains the next largest variance in the data (Kiani et al., 2016).

The HCA is one of the well-known unsupervised clustering methods and providing a succinct graphical representation of how well each object lies within its cluster, known as a dendrogram (Kiani et al., 2016).

LDA is one of the most widely used classification procedure. LDA, as a supervised method, has been used for feature extraction and variable selection in a dataset (Tudu et al., 2008). In this research, the past V3 software was used to analyze PCA and LDA also, R software was used for HCA analysis.
LDA is a technique that directly maximizes class separately; generating projections where the examples of each class, form compact clusters and the different clusters are far from each other (Sanacifar et al., 2016).

**Result and discussion**

**PCA test results**

PCA score plot was utilized to investigate clustering of data points within the multi-dimensional space of different samples. The level of adulteration was evaluated in 5 levels of 0, 20, 40, 60 and 80%. In the statistical analysis, 20 replicates were used for each sample using the e-nose system and a total of 100 samples were evaluated for evaluation. The first and second PCs (PC1-PC2) account for 98.4% of data variance for the original dataset (PC1=82.36% and PC2=16.04%) which means that the differences existing among honey samples along the first two axes are more significant. The PCA results are illustrated in Figure (6). That shows the clear discrimination between the samples except between pure honey and 20% syrup because of their similar aroma strengths.

![Plot](image)

**Figure 6.** The PCA score plot for different honey samples

**HCA results**

To check the PCA results, the HCA was applied to analyze the preprocessed data. The results of analysis can be displayed graphically using a tree diagram, known as a dendrogram, which shows all the steps in the hierarchical procedure in this method, all classes are initially connected and then categorized according to the distance between the data. Figure (7) shows the dendrogram of honey samples that were successfully discriminated based on their aroma strength. As depicted in Figure (7), HCA can successfully discriminate honey samples into 5 groups as it can be seen the pure honey, 20 and 40% syrup are classified in first branch and 60 and 80% syrup classified in second branches based on their aroma strength. By comparing the PCA and HCA results it can be concluded that pure honey and 20% syrup are more similar and from the Figure (7) it can be seen that one of the pure honey samples miss classified in to 20% syrup branches that indicates these two classes have the close aroma strength and the close aromatic quality. All the honey samples were discriminated based on their aroma with 99% accuracy.
LDA analysis results
The response of the sensors after applying the fractional preprocessing method was considered as input in the linear discrimination analysis method. LDA analysis results are shown in Figure (8). This Figure (8) represents analysis results on a two-dimensional plane, linear discriminant 1 (LD1) and linear discriminant 2 (LD2). The results obtained by LDA plot, provided a perfect classification. The first two LDs (LD1–LD2) account for 95.75% of data variance for the original dataset (LD1=74.54% and LD2=21.21%) which means that the differences existing among honey samples along the first two axes are more significant. As the Figure (8) shows, all the honey samples are completely separated and as expected from the PCA and HCA results the pure honey and 20% syrup groups are very close to each other. The confusion matrix of LDA is given in Table (2). As shown in Table (2), in the LDA method, the 100% discrimination between pure honey and adulterated honey is evident. Yu et al. (2007) examined the ability of the e-nose to detect milk adulteration. They found that milk adulteration using linear discriminant analysis (LDA) were better than principle component analysis (PCA).
Table 2. Confusion matrix of LDA analysis

<table>
<thead>
<tr>
<th>Samples</th>
<th>Pure honey</th>
<th>20% syrup</th>
<th>40% syrup</th>
<th>60% syrup</th>
<th>80% syrup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure honey</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20% syrup</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40% syrup</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60% syrup</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>80% syrup</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Discrimination accuracy %</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Conclusion
In this research, the portable e-nose system was built on the basis of metal oxide semiconductor (MOS) sensors. The e-nose, in contrast to conventional technologies, such as high-performance liquid chromatography (HPLC) gas chromatography (GC), which determines the aroma characteristics and components of each substance, do not have the problem of high cost, the need for an expert operator for running the systems and the laborious preparation of samples. Based on the results, the presented e-nose is a reliable tool for recording changes between different levels of adulterated honey and it’s easy to use. PCA, HCA and LDA methods were evaluated to evaluate the ability of the e-nose to detect levels of adulteration in honey. The results included 98.4% of the variance for the PCA method and 99% accuracy classification for the HCA method and 100% classification accuracy for LDA analysis. Also, from all the analytical methods employed in this study, it can be concluded that by increasing the amount of adulteration, the similarity of the pure honey with the adulterated honeys is reduced that is evidence of the ability of the e-nose to distinguish between different honey and the ability to detect adulteration in honey with high precision.

References


استفاده از روش‌های تشخیص الگو در آنالیز داده‌های حسگر بویایی جهت
شناسایی تقلبات گلعزک و فروکتوز در عسل

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چکیده

راهنما عمل یکی از پارامترهای مهم در طیف‌نگاری عمل به‌خاس می‌آید و بی‌کیفیت جغرافیایی و
ترکیبات تشکیل‌دهنده عمل می‌تواند منفی باشد. هدف از این تحقیق تشخیص و ارزیابی یک سامانه
بویایی برای سنجش نیازهای در عمل بود. برای این منظور از روش‌های حسگر جهت شناسایی تقلب در
عمل‌های با درصد‌های مختلف تقلب (خالص، ۲۰ درصد شربت، ۴۰ درصد شربت، ۶۰ درصد شربت) مورد
ارزیابی قرار گرفت. مانند بویایی ساخته‌شده شامل ۸ عدد حسگر نیمه‌هادی اکسید فلزی برای گیری
جمع‌آوری اطلاعات موجود در گازهای فضای فعالی عمل بود. بعد از پیش‌بردارش داده‌های بدست‌آمده
از مانند بویایی مدل‌های تشخیص الگو در
شناسایی تقلب الگودادگری قرار گرفتند. اجزای اصلی سامانه طراحی شده شامل سامانه تقصیر داده، الگوریتم‌های تشخیص الگو و
(LDA) تحلیل داده می‌باشد. تحلیل مؤلفه‌های اصلی (PCA) تجزیه و تکیه‌کننده خطي (HCA) و تکیه‌کننده به روش
روش‌های بویایی که برای تحلیل داده‌های بدست‌آمده از مانند بویایی مورد استفاده قرار گرفتند، با توجه به نتایج بدست‌آمده
دقت تشخیص تقلب شامل ۹۸/۴ درصد و ۹۹ درصد از روش PCA و HCA و۱۰۰ درصد قدرت
طبق‌نگاری به روش LDA بود. نتایج نشان داد مانند بویایی ساخته‌شده یک وسیله‌کارامد و قابل اطمینان در تشخیص تقلب
عمل است.

واژه‌های کلیدی: تشخیص الگو، تقلب، حسگر، عمل، مانند بویایی
The Effect of Wild Leek (*Allium Ampeloprasum*) on Growth and Survival of *Lactobacillus Acidophilus* and Sensory Properties in Iranian White Cheese

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Abstract
The survival ability of probiotic bacteria in food products is one of the most important challenges ahead. The wild leek (*Allium ampeloprasum*) herb of the Allium family contains various prebiotic compounds that can stimulate the growth of probiotic bacteria. In this research, sensory properties of cheese based on *Allium ampeloprasum* as a medicinal plant and flavoring was evaluated. For this purpose, after chemical tests on raw milk, different cheese treatments were prepared to determine the effect of fresh and dry concentrations of 1 and 2% of plants, as well as non-plant control containing probiotic bacteria *Lactobacillus acidophilus* (PTCC 1643) on the growth of *Lactobacillus acidophilus* bacteria. The results showed that probiotic bacteria *Lactobacillus acidophilus* had decreasing trend in all treatments and control during storage, but in the treatments containing *Allium ampeloprasum*, this trend was less. At the end of the 45th day, the lowest bacterial count (Log CFU/g) was observed in the treatment without plant (6.69) and the highest in the sample containing one percent dry plant (8.12). The results of pH assessment also showed that in all samples, the process of pH reduction observed naturally with time of the cheese ripening. However, in treatments containing plant, there was significant difference between 30 and 45 days with non-plant control ($P<0.05$). At the end of the 45th day, the lowest bacterial count (Log CFU/g) was observed in the treatment without plant (6.69) and the highest in the sample containing one percent dry plant (8.12). In sensory evaluation, samples containing 1% dry plant and probiotic bacteria had the highest score among different treatments and specified the addition of *Allium ampeloprasum* as a plant additive could increase the sensory properties of the product and could be successfully used to produce synbiotic cheese.

Keywords
*Lactobacillus acidophilus*, Prebiotic, White Cheese, Wild Leek

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Introduction
Probiotics are living microorganisms (bacteria or yeasts) that induce their health effects in the host's body if fed through food and at the right number (at least $10^6$-$10^7$ living microorganisms per
Among the microorganisms, some *Lactobacillus acidophilus* species, with probiotic properties, are the most common bacterial species used in the production of probiotic products (Isolauri, 2004). Lactobacilli are fermentative bacteria, most of whose products are lactic acid. These bacteria have an inhibitory effect on the growth of undesirable bacteria. Many studies have been conducted on dairy products with this bacterium to evaluate its shelf life. For instance, the studies by Yilmaztekin *et al.* (2004) on white cheese in brine, Buriti *et al.* (2007) in fresh Minas cheese, Mazinani *et al.* (2015) in ultrafiltrated cheese and Dehnavi *et al.* (2013) (Yilmaztekin *et al.*, 2004; Buriti *et al.*, 2007; Mazinani *et al.*, 2015; Dehnavi *et al.*, 2013).

There are indigestible compounds such as inulin, fructans, oligosaccharides, and so on that stimulate selectively the growth or activity of one or more of the microorganisms in the intestines, which are known as such probiotic (Ozcan *et al.*, 2016). Many studies have been conducted for using plant probiotics in food products. In a study by Ghaemi *et al.* (2010), synbiotic ultrafiltrated white cheese with *Lactobacillus acidophilus* and inulin has been examined (Ghaemi *et al.*, 2010). Masihinezhad *et al.* (2014) also studied the effects of prangos on *Lactobacillus casei* in yogurt (Masihinezhad *et al.*, 2014). *Allium ampeloprasum* L.spp Iranicum is a plant known as Yaglica in Azerbaijan and Kurayeh and Konival in Kurdistan, and used to taste traditional cheeses in some areas. Medicinal herbs and their derivatives have long been used in the treatment of diseases and their side effects. There are many proofs for the beneficial effects of *Allium ampeloprasum* in treating some diseases. It has been shown that its active ingredients have a protective effect against injuries induced by harmful agents, serum cholesterol lowering agents, establishing a basic condition for some body functions and vasodilator (Nguansangiam *et al.*, 2003; Roghani & Aghaie, 2007). This plant has a large amount of cysteine sulfoxides, which has anti-diabetic and antioxidant properties (Kumari Kumari & Augusti, 2002). On the other hand, considering some active ingredients, it is similar to garlic, which can be beneficial for serum glucose and lipids (Fritsch & Keugen, 2006). However, the significant point about this plant, from allium family, is that like onions, it is considered probiotic compounds by having fructan. In one study, whole plant parts contain approximately 9% fructan compounds, and the degree of polymerization of fructan is about 8 (Muir *et al.*, 2007). However, the amount of fructan of this plant changes drastically, depending on the plant body and the harvest season, so that in another study, it was reported to vary from 2.5 to 83.5% per gram (Bernaert, 2013). Therefore, using it for producing probiotic foods can be interesting.

Recently, designing and producing herbal based probiotic preparations have received great attention given their nutritional value (protein, fiber, vitamin and minerals), and diversification in production and consumption. It seems that the industrial production of these products with proper qualitative properties would be a great section of the studies to be conducted in the future (Ozcan *et al.*, 2016). However, one of the most important challenges in the production and processing of probiotic products is the low survival probiotic...
bacteria due to the sensitivity to specific conditions in the food product and the intestinal conditions for these organisms. One of the solutions to eliminate these factors is using probiotic compounds along with probiotic bacteria and creating a condition called synbiotic that increases the growth and survival of these bacteria (Zimer & Gibson, 1998). As Allium ampeloprasum is traditionally used in some regions of Azerbaijan for flavoring cheese, no studies have been done on the use of it in Iranian white cheese, and it is not studied on probiotic bacteria, the purpose of the study was to examine the effect of Allium ampeloprasum on the survival of Lactobasillus acidophilus and its effect on the sensory properties of Iranian white cheese. This is done to gain suitable efficacy as well as the desired effect on sensory properties; it can be used to produce cheese and new synbiotic products.

Material and methods

Chemical tests of raw milk used to produce cheese
After preparing the milk needed for cheese production, the chemical tests were done. In doing so, the fat value was considered according to the national standard of Iran 760 (Institute of Standards and Industrial Research of Iran, 2002), protein according to standard 639 (Institute of Standards and Industrial Research of Iran, 2002), pH and acidity measurements according to standard 2852 (Institute of Standards and Industrial Research of Iran, 2001).

Preparation of probiotic bacteria and cheese starter
Probiotic bacteria (Lactobasillus acidophilus PTCC 1643) was bought from the Iranian Research Organization for Science and Technology as lyophilized ampoules. Lyophilized primer culture and DVS-R-704, including Lactococcus lactis, under the species of Cremonis and Lactococcus lactis were purchased from Chr. Hansen Company. To prepare the probiotic bacteria, the contents of the lyophilized ampoules containing Lactobasillus acidophilus were transferred to a test tube containing 10 mL of MRS medium (Merck, Germany) and incubated at 37 °C for 24 h under anaerobic incubation conditions. Then, the bacterial cultures prepared were transferred into Erlenmeyer Flasks containing 95 mL of MRS liquid medium. This was repeated 2 to 3 times to reach a bacterial count of 10⁸-10⁹ CFU/mL. Microbial cells were then obtained by centrifugation at 1500 rpm for 15 min. The obtained bacteria were washed again with 0.1% sterilized peptone water and used for inoculation in milk at cheese-making stage (Krasaekoopt et al., 2004).

Preparing Allium ampeloprasum L.spp Iranicum
The fresh plant was collected from mountains around Oshnaviyeh. After washing, some part of it was crushed and stored in the freezer, and the other part was dried in shade at 25 °C. Dried leaves were then stored in a freezer until the test was done. The fresh and dried plants were pasteurized in the oven and Bain-Marie at 65 °C for 30 min, respectively, and added to cheese by 1 and 2% (Ocak et al., 2015).

Preparation of white cheese stored in brine
To prepare the cheese, after milk pasteurization, the temperature was adjusted to 35 °C and calcium chloride was added at a rate of 0.01% (v/w). For probiotics containing Lactobasillus acidophilus, 1 mL of the prepared suspension containing 10⁶-10⁹ CFU/mL bacteria described above was added to the milk and mixed up. After that, lyophilized primer culture was inoculated according to the recommended dose of the manufacturer for 1 L of milk at 0.005% (v/w). After that, the milk was kept at 35 °C to keep it at a pH of 6.4.
Then, various concentrations of *Allium ampeloprasum* were added to the milk and mixed. Then rennet was added to the milk as much as 0.001% (v/w). The clot was kept after 15 min for the removal of whey, and the clot was then compressed into a sterile bag of carbs and a press machine, and pressed gradually for two hours at a load of weights. After the cheese had reached the intended consistency, 100 g cheese pieces were first placed in 20% sterilized brine for 8 h at ambient temperature (20 to 24 °C). After that, they were transferred to sterilized 8% brine and stored for 15 days at a temperature of 12-14 °C. Ultimately, after the initial consistency period, the cheese pieces were stored in a refrigerator for 8% at 4 °C for 45 days (Institute of Standards and Industrial Research of Iran, No. 5772, 2011).

**Preparation of cheese samples for counting probiotic bacteria**

A specific culture medium was used for counting lactobacilli in cheeses produced from MRS-BC agar (Merck, Germany) containing 2% green bromocerosol (Sigma Company) and clindamycin (Sigma Company). In doing so, 5 mg of clindamycin was mixed in 100 mL of water and 2 mL per liter of MRS was added using sterile syringe filter. After dilution and surface cultivation, incubation was done at 37 °C for 48 to 72 h in an anaerobic way and counted from day zero to 45 every 5 days (Phillips *et al*., 2006).

**Sensory evaluation**

Although the main value of probiotic products is their biotic features, their sensory properties are important as well, since the consumer has the main role in selection. Taste and odor are of the effective features in cheese market. Therefore, sensory testing was performed simultaneous to bacterial counting. Odor, taste, color and texture (consistency) of the samples were determined by a group of 10 trained sensory evaluators using the consumer inclination test and the 5-point Hedonic method. From each treatment, 5 identical samples were prepared and along with the form submitted to the referees. In doing so, 5 was given to excellent and 1 to very poor quality. The overall consumer evaluation was presented as a numerical value (IDF, 1997).

**Statistical Analysis**

The experiments were performed in 3 iterations. Data analysis was done in SPSS (version, 19) and mean comparison was done using Duncan's test with p-value less than 0.05 considered significant. The corresponding charts were drawn by Microsoft Excel software (version, 2016).

**Result and discussion**

**Physicochemical analysis of raw milk**

The results of the physicochemical properties of milk used to produce cheese are shown in Table (1). As milk compounds and its properties affect the properties of the produced cheese and may even affect the durability of the probiotic bacterium, analyses were done on the raw milk used for producing cheese.

<table>
<thead>
<tr>
<th>Test</th>
<th>Fat (%)</th>
<th>Dry matter (%)</th>
<th>pH (1:2)</th>
<th>Acidity (°D)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td>3.7±0.6</td>
<td>11.9±0.7</td>
<td>6.2±0.02</td>
<td>17.1±0.8</td>
<td>3.01±0.6</td>
</tr>
</tbody>
</table>
Counting the probiotic bacteria

The results of counting probiotic bacteria in Iranian white cheese are shown in Figure (1). As indicated in the chart, the probiotic bacteria *Lactobacillus acidophilus* had decreasing trend in all treatments and during storage.

![Figure 1. Changes in counting Lactobacillus acidophilus in different days at 4 °C](image)

However, in the treatments containing *Allium ampeloprasum*, this trend was less so that on the 30th control day, the bacterial count was lower 7.15 CFU/g with a significant difference with other treatments (*P*≤0.05). On day 45, counts of the control and 2% fresh plants treatment reached below 8 CFU/g, whereas in other treatments, probiotic bacteria were higher with a significant difference with these 2 treatments (*P*≤0.05). Additionally, the results of day 45 showed no significant differences between the treatments containing dry matter 1 and 2% in terms of probiotic bacteria (*P*≥0.05). The small differences between the effects of dry and fresh plants should be found in the mechanism for the release of fructan compounds and the effective ingredients in the growth of probiotic bacteria were distinguished. Many studies have been conducted on the use of different compounds as probiotic in dairy products. Araujo *et al.* (2009) used inulin to enhance the livestock recovery and tissue alteration of cottage cheese containing *Lactobacillus delbrueckii* and increase the viability of the probiotic bacteria mentioned (Araujo *et al.*, 2009). In another study, Ghaemi *et al.* (2010) studied the synbiotic white ultrafiltrated cheese containing *Lactobacillus acidophilus* and inulin, and the results showed that the addition of inulin increased the bacterial survival (Ghaemi *et al.*, 2010). The probiotic effects of garlic fructan in a laboratory environment were examined by Zhang *et al.* (2013). Their results showed that garlic fructans increased the count of bacterial bacteria in the intestine (Zhang *et al.*, 2013). China *et al.* (2012) examined the antimicrobial activity of *Sesbania grandiflora* extract on some pathogen bacteria and evaluated the stimulatory effect of growth on the probiotic organism *Lactobacillus acidophilus*. Their results showed that *Sesbania grandiflora* polyphenol extract has inhibitory effect on pathogens and stimulates the growth of probiotic bacteria (China *et al.*, 2012). Masihinezhad *et al.* (2014) studied the effects of prangos on *Lactobacillus casei* in yogurt, and the results showed that the maximum bacterial survival was at 20% concentration of the plant (Masihinezhad *et al.*, 2014). Zomorodi *et al.* (2015) studied the survival of bacteria *Lactobacillus acidophilus* in synbiotic
yogurt using apple and wheat fiber, and the results showed that the use of fiber of wheat and apple can cause an increase in the number of probiotic bacteria (Zomorodi et al., 2010). Hap & Gutierrez (2012) showed that the extracts of Kiwi, Strawberries, Blackberry and Acca sellowiana significantly increased the probiotic bacteria except Bifidobacterium (Hap & Gutierrez, 2012). Marhamatizadeh et al. (2009) examined the survival of Lactobasilus acidophi

lus and Bifidobacterium and their count in probiotic milk and yogurt. The results showed that during the 20 days of probiotic preservation, the bacteria remained alive and the growth rate and survival rate of bacteria in the sample containing Lactobasilus acidophilus were higher than that of Bifidobacterium bifidom (Marhamatizadeh et al., 2009). Kasimoglu et al. (2004) showed that the lactoobacillus survival rate declined during the first 15 days of arrival, the researchers reported the decrease in moisture content, increased salt content and reduced storage temperature, which are in line with the results of this study (Kasimoglu et al., 2004). Another study by Sutherland et al. (2009) showed that from among about 100 extracts studied, garlic and pepper extracts had an increasing effect on the growth of the probiotic bacteria Lactobacillus Reuteri (Sutherland et al., 2009). As the results showed, the bacterial survival was greater than 10^7 CFU/g in samples of different concentrations of Allium ampeloprasum. If this cheese is consumed at a dose of 30 g per day, the probiotic amount will be between 10^9 to 10^10 CFU/g, higher than the recommended amount for therapeutic effects (Boylston et al., 2004; FAO/WHO, 2002). Many researchers have reported similar results in various types of cheese (Bergamini et al., 2005; Buriti et al., 2007). As the results of most studies indicate, using a specific concentration of the plant in most cases increased the survival and survival of probiotic bacteria that is in agreement with the results of the present study. The mechanism of action of Allium ampeloprasum extract is similar to that of garlic and onion from the allium family, and the effect of increasing the extract of Allium ampeloprasum on the growth of Lactobacillus bacteria is due to the presence of some active compounds such as phenolic compounds and fructans in it. Similar results have been reported by other researchers on other plant extracts (Molan et al., 2009; Zomorodi et al. 2015; Rosendale et al., 2008). Concerning the mechanism of action of active compounds in the growth of the growth of probiotic bacteria, there is no complete understanding, but active compounds appear to act as an energy source and also as antioxidants.

Measuring the pH of probiotic-containing cheeses

The pH of the probiotic bacteria is one of the most important factors in its survival. The effects of various concentrations of Allium ampeloprasum and control samples on pH of the cheese produced with acidophilus bacteria are shown in Figure (2). As the results show, in all samples, pH decreases naturally with cheese ripening time. However, there was a significant difference in planting treatments between days 30 and 45 (P≤0.05). There was a significant difference in different pH treatments between different treatments during days of storage, so that on day 15 and 30, the lowest pH of the cheese sample containing dry matter was 2%, and on day 45, the sample had 1%. Furthermore, on day 45, the highest pH was related to the treatment without plant with a significant difference with other treatments. The pH reduction while storage may be due to the acidic
activity of probiotic strains and commercial starter (Ong et al., 2007). The pH decreased due to the production of organic acids by carbohydrate fermentation bacteria and decreased more in the treatments with the plant samples, which can be due to the effect of increasing the growth of probiotic compounds of *Allium ampeloprasum* on the bacteria producing acid. Reductions in pH have also been reported by other researchers (Buriti et al., 2007; Akin et al., 2003). Thus, following increased acidity production and reduction in pH, probiotic bacteria are affected as well.

In a study to examine the effects of commercial probiotics Raftiline and Raftilose on pH changes in the skim milk and the peptone-containing *Lactobacillus acidophilus* model, Olson & Aryana (2012) found that adding probiotics reduced pH compared to the control sample (Olson & Aryana, 2012), in line with the results of this study. Similar results were also obtained by Zhang et al. (2013) in examining fructan extracted from garlic on the pH changes of the *Bifidobacterium* in the culture medium (Zhang et al., 2013).

The results of the present study showed the effect of increasing the growth of the probiotic composition of *Allium ampeloprasum*, but given the important effect of fructan in the plant as the key determining factor, it is necessary that more studies be done to determine the amount of fructan in the whole cheeses produced.

![Figure 2](image.png)

**Figure 2.** Changes in pH in cheese containing *Lactobacillus acidophilus* in different treatments during storage days at 4 °C

**Sensory evaluations**

Sensory properties of food products are of the most significant effects on the part of consumers. The results of the flavor evaluation in cheese samples containing *Lactobacillus acidophilus* during the storage days at 4 °C are presented in Table (2). Overall, adding *Allium ampeloprasum* to cheese has changed the taste of cheese samples, so that on the first day of the experiment, the flavored scores decreased and a significant difference was observed with the control ($P \leq 0.05$). On the 15th day, the planting treatments increased and on the 30th and 45th days, this trend continued. Therefore, the highest score in these days was for treatments containing 2% dry matter, 1% dry and fresh plants and 1% dry plant. Thus, adding *Allium ampeloprasum* significantly improved the taste of cheeses from the viewpoint of evaluators. The results of tissue evaluation in cheese samples containing *Lactobacillus acidophilus* during the storage days are shown in Table (3) as well. The results show that there are no significant differences between treatments at zero and 15 days ($P \geq 0.05$). Nevertheless, from the 30th and 45th days of cheese ripening, the score for
control sample tissue decreased, whereas the proportion of specimens containing *Allium ampeloprasum* increased. Overall, the addition of *Allium ampeloprasum* as an additive to cheese causes tissue alteration, and this is evident in the results of the sensory test as well.

The most significant factors affecting the tissue of the cheese are the total solids content and the gradual decomposition of proteins (Çelik et al., 2008). In the present study, it seems that the addition of the plant has changed the total amount of solids in the cheese and thus affects the tissue results. However, it is recommended that tissue analysis methods be used in later studies by devices.

**Table 2.** Results (mean ± standard deviation) of flavor evaluation in cheese samples containing *Lactobacillus acidophilus* during storage days at 4 °C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>No plant</td>
<td>3.6±0.54a</td>
<td>3.4±0.54a</td>
<td>3.2±0.44a</td>
<td>3.0±0.00a</td>
</tr>
<tr>
<td>Fresh plant 1%</td>
<td>3.4±0.54ab</td>
<td>3.4±0.54a</td>
<td>5.0±0.00c</td>
<td>4.6±0.54ad</td>
</tr>
<tr>
<td>Fresh plant 2%</td>
<td>3.2±0.44ab</td>
<td>4.0±0.00b</td>
<td>4.4±0.54bc</td>
<td>3.6±0.54b</td>
</tr>
<tr>
<td>Dry plant 1%</td>
<td>3.0±0.00ab</td>
<td>3.4±0.54a</td>
<td>5.0±0.00a</td>
<td>5.0±0.00b</td>
</tr>
<tr>
<td>Dry plant 2%</td>
<td>2.8±0.44ab</td>
<td>3.8±0.44a</td>
<td>3.8±0.44ab</td>
<td>4.2±0.44c</td>
</tr>
</tbody>
</table>

* Non-similar letters in each column show significant differences (P≤0.05).

**Table 3.** Results (mean ± standard deviation) of tissue evaluation in cheese samples containing *Lactobacillus acidophilus* during storage days at 4 °C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>No plant</td>
<td>5.0±0.54a</td>
<td>4.4±0.54a</td>
<td>2.4±0.54a</td>
<td>3.0±0.00a</td>
</tr>
<tr>
<td>Fresh plant 1%</td>
<td>5.0±0.54a</td>
<td>5.0±0.00b</td>
<td>5.0±0.00b</td>
<td>4.8±0.44b</td>
</tr>
<tr>
<td>Fresh plant 2%</td>
<td>5.0±0.44a</td>
<td>4.6±0.54b</td>
<td>4.6±0.54b</td>
<td>4.6±0.54b</td>
</tr>
<tr>
<td>Dry plant 1%</td>
<td>5.0±0.00a</td>
<td>5.0±0.00b</td>
<td>4.8±0.44b</td>
<td>5.0±0.44b</td>
</tr>
<tr>
<td>Dry plant 2%</td>
<td>5.0±0.44a</td>
<td>4.4±0.54a</td>
<td>4.6±0.44b</td>
<td>4.6±0.54b</td>
</tr>
</tbody>
</table>

* Non-similar letters in each column show significant differences (P≤0.05).

The results of odor evaluation in cheese samples containing *Lactobacillus acidophilus* during the storage days at 4 °C are shown in Table (4). In the days 0 and 15 of cheese ripening the lowest scores related to odor was for the samples of fresh plant of 2% sample, whereas 1% sample had the highest score and the other samples did not differ significantly (P≤0.05). On days 30 and 45, the highest score was for the sample containing 1% dry matter. The lowest score was for the control sample without plant, with the other samples not differing significantly (P≤0.05). Finally, for a better comparison, the mean of total results of sensory tests in cheese containing *Lactobacillus acidophilus* in different treatments during storage days at 4 °C is shown in Figure (3). The results showed no significant differences between treatments on days zero and 15, but on days 30 and 45, the treatments containing *Allium ampeloprasum* had significantly higher sensory scores than the control group. The highest score was obtained by fresh 1% treatment on day 45 with a significant difference with non-plant treatment, whereas the lowest was for control treatment (P≤0.05). Overall, due to different compounds in the case of addition to food products as additive, aromatic herbs such as *Allium ampeloprasum* cause organoleptic changes in the product, which in some cases is a satisfactory change. Few studies have been done regarding the effect of plant probiotics on the sensory properties of...
synbiotic cheeses. In one case, Mazinani et al. (2015) studied the sensory features of synbiotic ultrafiltrated white cheese containing *Origanum vulgare* powder and *Spirulina plasticum* algae. The results showed that the highest acceptance among algae samples was for 0.3% algae samples and 1% *Origanum vulgare* (Mazinani et al., 2015). On the contrary, a review by Araujo et al. (2009) showed that adding inulin to cottage synbiotic cheese does not cause any organoleptic alteration (Araujo et al., 2009). Azambuja et al. (2013) examined the sensory properties of fresh cheese containing *Bifidobacterium* as probiotic and polydextrose probiotic. The results showed that sensory evaluators found no significant differences between treatments (Azambuja et al., 2013). In another study on cheese with *Allium ursinum*, the results showed that the addition of garlic to other than the change in cheese texture did not have a significant effect on other sensory properties (Tarakci et al., 2011). Furthermore, El-Khalek et al. (2016) on probiotics with probiotics containing zinger as a prebiotic showed that ginger containing treatments had a higher sensory score than the control group (El-Khalek et al., 2016). Overall, as the results of similar studies and the present study show, the additions of plant compounds to dairy products not only produce a variety of products, but also in many cases, in addition to the probiotic properties added to the product, increase the sensory scoring. In the present study, although the positive effects of *Allium ampeloprasum* on the sensory properties of Iranian white cheese were determined according to the participants in the sensory test, proving this requires more tests and conducting the exact device tests.

Table 4. Results (mean ± standard deviation) of odor evaluation in cheese samples containing *Lactobacillus acidophilus* during storage days at 4 °C

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>No plant</td>
<td>3.4±0.54</td>
<td>3.0±0.00</td>
<td>2.8±0.44</td>
<td>2.2±0.44</td>
</tr>
<tr>
<td>Fresh plant 1%</td>
<td>4.0±0.00</td>
<td>4.0±0.00</td>
<td>4.6±0.54b</td>
<td>4.6±0.54b</td>
</tr>
<tr>
<td>Fresh plant 2%</td>
<td>2.2±0.44a</td>
<td>2.4±0.54a</td>
<td>5.0±0.00c</td>
<td>4.8±0.44b</td>
</tr>
<tr>
<td>Dry plant 1%</td>
<td>3.0±0.00b</td>
<td>3.6±0.54b</td>
<td>5.0±0.00c</td>
<td>5.0±0.00c</td>
</tr>
<tr>
<td>Dry plant 2%</td>
<td>3.4±0.54b</td>
<td>3.2±0.44b</td>
<td>4.4±0.54b</td>
<td>4.6±0.54b</td>
</tr>
</tbody>
</table>

* Non-similar letters in each column show significant differences \( P \leq 0.05 \).

Figure 3. Mean of the overall results of sensory tests in cheese containing *Lactobacillus acidophilus* in different treatments during storage days at 4 °C. Non-identical Latin letters per day show a significant difference \( P \leq 0.05 \).
Conclusion
The results indicated that tertiary as a prebiotic in cheese, Allium ampeloprasum L.spp Iranicum can contribute to the survival of probiotic and beneficial microorganisms in unsuitable storage conditions and improve the tissue and sensory properties of the product. Thus, it is suggested that in the production of probiotic white Iranian cheese Allium ampeloprasum, with prebiotic, antimicrobial and nutritional features, be used to increase the probiotic shelf life, to reduce the risks of food-related diseases, and to create the organoleptic desirable characteristics.

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تأثیر گیاه تره‌کوهی بر رشد و ماندگاری باکتری پرپیوتوکین لاکتوباسیلوس
اسیدوفیلوس و ویژگی‌های حسی پنیر سفید ایرانی

تورج مهدیزاده ۱، مینا رضوی ۲، محمود اسماعیلی کوت‌مهر ۳

چکیده
قابلیت زندگی باکتری‌های پرپیوتوکین در محصولات غذایی یکی از مهم‌ترین جالش‌های پیشرو می‌باشد. گیاه تره‌کوهی از خواص آلوده‌زایی در ترکیبات مختلف پرپیوتوکین بوده که برای می‌بایسته تحکیک رشد باکتری‌های پرپیوتوکین شود. در این پژوهش، ویژگی‌های حسی پنیر سفید ایرانی بر پایه تره‌کوهی به‌عنوان یک گیاه دارویی و کاربردی مورد بررسی قرار گرفت. نتایج نشان داد که نتایج تردید باکتری پرپیوتوکین لاکتوباسیلوس اسیدوفیلوس (PTCC 1643) در بررسی‌های حاوی گیاه و کنترل بدون گیاه در طول زمان (تیمارهای حاوی گیاه و کنترل بدون گیاه در طول زمان گردیداری (۲۵ روز) رشد کاهشی را داشته ولی در تیمارهای حاوی گیاه تره‌کوهی این رشد کاهشی کمتر بود. نتایج که در پایان روز ۹۴ بسته باکتری (گل‌کرم/g) و بیشترین در نمونه حاوی ۱ درصد گیاه (CFU/g) نیز نشان داد که در تمام نمونه‌ها با طی زمان رشد رشته‌پدید رشد کاهش به طور ترکیبی مشاهده گردید. ولی در تیمارهای حاوی گیاه در روزهای ۴۰ و ۶۵ اختلاف معنی‌داری با نمونه کنترل بدون گیاه دیده شد. نتایج نشان داد که ویژگی‌های حسی پنیر سفید ایرانی از نظر رشد نشان دهنده اثرات افزایش میزان رشد باکتری پرپیوتوکین لاکتوباسیلوس اسیدوفیلوس (PTCC 1643) در بررسی‌های حاوی گیاه و کنترل بدون گیاه. نتایج نشان داد که با افزایش میزان رشد باکتری پرپیوتوکین لاکتوباسیلوس اسیدوفیلوس (PTCC 1643) در بررسی‌های حاوی گیاه و کنترل بدون گیاه می‌تواند بهبود خصوصیات حسی محصولات به‌طور مشخص ایزی برای درمان پنیر سفید و باکتری پرپیوتوکین لاکتوباسیلوس اسیدوفیلوس نشان دهد.
Optimization of Hydrolysis Condition of Pumpkin Seeds with Alcalase Enzyme to Achieve Maximum Antioxidant and Nitric Oxide Inhibition Activity

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4- Professor, Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Abstract
In this research, optimization of pumpkin (Cucurbita pepo) hydrolysis condition was investigated in order to achieve maximum 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and nitric oxide inhibition activity using Design Expert software and response surface method. For this purpose, hydrolysis conditions including concentration of alcalase enzyme were selected within 0.7-3.30%, temperature 32-58 °C and hydrolysis time 30-290 min as independent variable. The results showed that optimum hydrolysis conditions to achieve the maximum DPPH radical scavenging and nitric oxide inhibition activity were temperature of 44 °C, hydrolysis time of 260 min, and enzyme to substrate concentration of 3% which under this condition the DPPH radical scavenging and nitric oxide inhibition activity of hydrolyzate was 72.03 and 89.34%, respectively that was largely similar to the results proposed by the software (75.33 and 84.71%). According to the results, pumpkin seed protein hydrolyzate showed high antioxidant and nitric oxide inhibitory properties and can be used as a suitable ingredient in food formulations.

Keywords
Alcalase
Antioxidant
Enzyme Hydrolysis
Nitric Oxide Inhibition
Pumpkin Seeds

Introduction
Proteins are an important source of nutrition for humans. These compounds provide nitrogen, amino acids and energy for the normal function of the body systems. Hydrolysis acts to break down proteins into free amino acids and peptides of a different size. The most common way of producing peptides is to hydrolyze proteins with the help of enzymes (Korhnen & Pihlanto, 2006). Nowadays, bioactive peptides derived from the hydrolysis of various dietary proteins are known to have various biological roles such as antihypertensive (Li et al., 2007), relaxing (Ohinata et al., 2007), anticholesterol (Cho et al., 2008), antioxidant (Kou et al., 2013) and anti-
cancer (Meisel & FitzGerald, 2003), which has led to the consideration of proteins, especially plant types, in the food and drug sector for the production and purification of bioactive peptides. One of the properties of these peptides is inhibition of nitric oxide, which acts as an intermediate compound in the production of nitrosamines. Nitrate and nitrite salts, which are used as an additive to stabilize color and inhibit the growth of some microorganisms in meat products, are very strong oxidants. As a result of the reaction of nitrite with the secondary and tertiary amines, the nitrosamine carcinogen compound is formed; if there is an antioxidant compound in processed meat, it reacts much more rapidly than amines with nitrite and prevents from the formation of nitrosamine (Hauser et al., 1980; Coss et al., 2004).

Pumpkin seeds (Cucurbita pepo) are known as a rich source of protein and contain significant amounts of minerals such as zinc, potassium, calcium, magnesium, iron, copper, phosphorus and β-carotene. The protein isolated from pumpkin is rich in antioxidants and is effective in reducing the malignant effects of malnutrition (Mohamed et al., 2009). Nourmohammadi et al. (2016) hydrolyzed the protein of pumpkin seed meal using pepsin enzyme using three variables of temperature, time and enzyme concentration. The results showed that produced peptides had more antioxidant power in hydrolysis conditions including 1% concentration of enzyme, temperature of 30 °C and hydrolysis time of 2 h, compared to other treatments obtained from the response surface method and central composite designs. Piri Ghashlaghi et al. (2015), Mehregan Niko et al. (2013) and Etemadi et al. (2014) respectively produced hydrolyzed proteins from whey protein concentrate, crucian carp and soybean meal by alcalase enzyme and the effect of temperature, time, and the ratio of enzyme to substrate on antioxidant activity was investigated.

The results of various experiments have shown that the hydrolyzed proteins derived from the protein hydrolysis of fish (Khattaphant & Benjakul, 2008), the hydrolyzed protein of tracheal meat (Klompong et al., 2009), soybean protein (Chen et al., 1995), milk proteins such as α-lactalbumin and β-lactoglobulin (Hernandez-Ledesma et al., 2005), egg white protein (Davalos et al., 2004) and gluten protein (del Castillo et al., 2007) showed high antioxidant activity. Lee et al. (2012) hydrolyzed the proteins derived from oysters (Ruditapes philippinarum) by 8 protease enzymes and ultimately obtained peptides with the highest nitric oxide inhibition property by the alcalase enzyme.

The pumpkin seeds are commonly used in the production of oil. Meal obtained from pumpkin seed oil contains high percentage of protein, which is currently not processed and is a cheap source of protein for the consumption in animal feed. Considering the high potential of this material in the production of bioactive peptides, this study aimed to optimize the enzymatic hydrolysis of pumpkin meal by alcalase enzyme in order to produce protein hydrolyzate with the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and nitric oxide inhibition activity.

**Material and methods**

**Materials**
Pumpkin seed meal was purchased from Abkar Gorgan Company. The alcalase enzymes and DPPH radical obtained from Sigma and NaOH, hydrochloridric acid, sulfonilamide, phosphoric acid, sodium nitroprusside and ethanol from Merck, and naphthyl ethylenediamine dihydrochloride were obtained from Ridley Company, Germany. All materials
used were of laboratory grade.

**Preparation of pumpkin seed meal**
After removing the foreign material, the meal was turned into flour by milling machine (Perten, 3100, Germany) and passed through a sieve with 30 meshes. The resulting flour was de-defatted using hexane with the ratio of 1:3 for 16 h, and after that it was kept at room temperature for 48 h in order to remove the solvent residues completely, and finally kept in the refrigerator (Kaur & Singh, 2007).

**Pumpkin meal flour**
The defatted meals were ground at this stage by a grinding machine (Perten, 3100, Germany) so that the flour particles could pass through the sieve with 70 meshes. Samples were stored in sealed polyethylene bags at temperature of 4 °C in the refrigerator (Glew et al., 2006).

**Preparation of pumpkin protein concentrate**
The defatted pumpkin seed meal was dispersed in water with the ratio of 1 to 10 and then the pH of the solution was adjusted to 10 by 1 N NaOH and mixed at room temperature for 1 h. The resulting mixture was centrifuged at 5000g using a refrigerated centrifuge (Combi 514R, South Korea) for 20 min at the temperature of 4 °C. In order to precipitate pumpkin seeds proteins, the pH resulting supernatant was adjusted to pH=5 by 1 N HCL, and centrifuged under similar condition. Then the precipitate obtained was washed with distilled water and dried using a freezing dryer (FD4 model, Operon Company, South Korea) and then kept at freezer at temperature -18 °C for further experiments (Zivanovic et al., 2011).

**Hydrolysis of pumpkin seed protein concentrate**
Protein concentrate with the proportion of 5% (w/v) was dispersed in a tris-hydrochloridric acid buffer with pH=8 (Optimal pH of alcalase enzyme) and the enzyme was added at concentration of 1-3%. Then, the hydrolysis was carried out in the temperature range of 35-55 °C and time of 60-260 min in a shaking incubator (VS-8480 model, South Korea) with speed of 200 rpm. Finally the enzymatic reaction was stopped at 85 °C for 10 min, and the resulting mixture was centrifuged at the 5000g for 30 min to remove the insoluble compounds (Villanueva et al., 1999). The resulting supernatant was kept at the temperature of -18 °C and freeze dried.

**Determination of chemical composition of pumpkin meal**
Moisture, ash and protein were determined using the AACC methods of 15-44, 01-08 and 12-46, respectively (AACC, 1999) and the fat content was measured by soxhlet method (Parvane, 2006).

**Measurement of DPPH radical scavenging activity**
1000 μL of hydrolyzed protein was mixed with 1000 μL of DPPH (0.1 mM) prepared in 96% ethanol and kept at room temperature in the dark for 60 min and finally the absorbance of solution was measured at a 517 nm using spectrophotometer (Bougatef et al., 2009). In the control sample, 1000 μL of distilled water was used instead of the hydrolyzed protein sample. The DPPH radical inhibition activity was calculated as Eq. (1):

\[
\text{DPPH radical scavenging activity} = \frac{[\text{absorption of control-sample absorption}]}{\text{absorption of control}} \times 100
\]

**Measurement of nitric oxide inhibition activity**
60 μL of hydrolyzed protein was mixed with 60 μL of sodium nitroprusside in phosphate buffer (0.025 M) and placed
in the plates and kept in the incubator for 150 min at room temperature (ascorbic acid was used as a control sample). Then, the equal amount (120 μL) of Grease reagent (including sulfanilamide, naphthyl ethylene dihydrochloride and phosphoric acid) was added to plates and the absorbance was measured at 546 nm (Tsai et al., 2007) and the inhibition activity was calculated as Eq. (2):

\[
\text{Nitric oxide inhibitory percent} = \frac{(\text{absorption of control} - \text{sample absorption})}{\text{absorption of control}} \times 100
\]

Optimization of enzymatic hydrolysis conditions
In order to optimize the process from the viewpoint of maximizing the antioxidant and nitric oxide inhibition properties, Design Expert software version 11 and the response surface method with central composite designs were used for 3 independent variables of time ($X_1$), temperature ($X_2$) and the ratio of enzyme to substrate ($X_3$) at 5 levels (-α, -1, 0, +1 and +α). Approximate amount of each independent variable was obtained according to the experimental tests results. The examined responses were DPPH radical scavenging and nitric oxide inhibition activity. Total 20 treatments were randomly selected by the software with six replications at the central point. The different levels of the independent variables and the relevant treatments are presented in Tables (1) and (2), respectively.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>The limits of change</th>
</tr>
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<tr>
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<td>-α</td>
</tr>
<tr>
<td>Hydrolysis temperature (°C)</td>
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</tr>
<tr>
<td>The ratio of enzyme to substrate (%)</td>
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</tr>
</tbody>
</table>

The value of α is equal to 1.3.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Time (min)</th>
<th>Hydrolysis temperature (°C)</th>
<th>The ratio of Enzyme to Substrate (%)</th>
<th>DPPH radical scavenging activity (%)</th>
<th>Nitric oxide inhibitory activity (%)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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<td>3</td>
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<td>88.19</td>
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<td>81.45</td>
<td>77.13</td>
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</tbody>
</table>
The regression models 1 and 2 were proposed to predict the first and second responses (DPPH radical scavenging and nitric oxide inhibition activity) by the following Eq. (3 and 4):

\[ R_1=b_0+b_1x_1+b_2x_2+b_3x_3 - b_1x_1^2 - b_2x_2^2 - b_3x_3^2 \]  

\[ R_2=b_0+b_1x_1+b_2x_2+b_3x_3+b_1x_1x_2+b_1x_1x_3+b_2x_2x_3+b_3x_3x_1+b_1x_1^2+b_2x_2^2+b_3x_3^2 \]

In above Eq. (3 and 4), R1 and R2 are the responses or dependent variables (DPPH radical scavenging and nitric oxide inhibition activity, respectively), b0 is a constant, and b1, b2 and b3 are linear effects, b11, b22 and b33 are quadratic effects and b12, b13 and b23 are interaction effects.

Analysis of variance, regression coefficients, plotting and optimization were performed by Design Expert software and the significance of the tests were analyzed by Duncan multiple range test and SPSS V16 software at a significant probability level of (P<0.05).

**Result and discussion**

According to the results of Table (3),

<table>
<thead>
<tr>
<th>A matter</th>
<th>Protein</th>
<th>Fat</th>
<th>Moisture</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defatted Meal</td>
<td>51.8±1.19</td>
<td>9.65±0.15</td>
<td>7.03±0.09</td>
<td>7.63±0.02</td>
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<tr>
<td>Protein Concentrate</td>
<td>76±1</td>
<td>1.29±0.46</td>
<td>12.56±0.01</td>
<td>0.815±0.015</td>
</tr>
</tbody>
</table>

* Results are the average of three replicates.

Linear Eq. (5) and (6), taking into account the regression coefficients for the DPPH radical scavenging activity and nitric oxide inhibition property, respectively, which were suggested by the following factors according to the significance of coefficients (table 4).

\[ \text{DPPH radical inhibition activity} = +79.33 - (0.72\times t) + (6.21\times \text{temperature}) + (3.78\times \text{the ratio of enzyme to substrate}) - (2.46\times \text{time}\times \text{temperature}) - (0.28\times \text{time}\times \text{the ratio of enzyme to substrate}) - (2.84\times \text{the ratio of enzyme to substrate}) \]

\[ \text{Nitric oxide inhibition activity} = +76.92 + (0.61\times t) - (0.52\times \text{temperature}) - (0.60\times \text{the ratio of enzyme to substrate}) - (0.89\times \text{time}\times \text{temperature}) + (0.10\times \text{the ratio of enzyme to substrate}) + (0.51\times \text{temperature}\times \text{the ratio of enzyme to substrate}) + (4.79\times \text{time}\times \text{temperature}) + (2.09\times \text{temperature}\times \text{temperature}) + (2.86\times \text{the ratio of enzyme to substrate}\times \text{the ratio of enzyme to substrate}) \]
The value of R² was calculated 0.7811 for the first equation (Eq. 5) and 0.7365 for the second equation (Eq. 6), which indicates the relatively suitable distribution of the data. The lack of fitness, which is a criterion for the suitability of the presented model, was 0.1625 and 0.0521 in the first and second equations (Eq. 5 and 6), respectively. The high level of lack of fitness compared to the significant level of probability (95%) or, in other words, the non-significant of this factor indicates the appropriateness of the proposed model and fitness of the model based on the considered responses.

Table 4. Analyze of variance related to DPPH radical scavenging and nitric oxide inhibition test

<table>
<thead>
<tr>
<th>Factor</th>
<th>Degree of freedom</th>
<th>Sum of squares</th>
<th>Average of squares</th>
<th>F-Value</th>
<th>P-Value</th>
</tr>
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<tr>
<td>DPPH radical scavenging activity</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Model</td>
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<td>122.39</td>
<td>3.97</td>
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<td>5.88</td>
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<td>438.30</td>
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<tr>
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<td>162.98</td>
<td>5.28</td>
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<td>48.27</td>
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<tr>
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<td>0.62</td>
<td>0.020</td>
<td>0.8900</td>
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<tr>
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<td>148.23</td>
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<td>94.25</td>
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<tr>
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<td>19.82</td>
<td>0.64</td>
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<td>30.87</td>
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<td>44.40</td>
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<tr>
<td>Total</td>
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<tr>
<td>Nitric oxide inhibition</td>
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</tr>
<tr>
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<td>4.08</td>
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<td>2.06</td>
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The effect of independent variables on DPPH radical scavenging activity
In this section, the effect of temperature, hydrolysis time and enzyme to substrate ratio on the DPPH free radicals scavenging activity has investigated. Therefore, using the 3D-graphs obtained by Design Expert software, the effect of mentioned parameters on the antioxidant property was investigated and interpreted and the results were compared and evaluated with the results of previous studies. It should be noted that in each figure (1, 2 and 3) the effects of the two parameters are investigated when the third parameter was at the optimal point.

The effect of enzyme concentration and hydrolysis temperature on DPPH radical scavenging activity is shown in Figure (1). As it can be seen, the increase in enzyme concentration and
also increase in hydrolysis temperature led to increase in DPPH radical scavenging activity, but the increase in enzyme concentration was less effective than temperature increase in DPPH radical scavenging activity. Therefore, it can be said that the increase of these two parameters has a direct effect on the increase of the amount of free radical inhibition; thus the highest free radical inhibitory power was achieved at temperature of 55 °C and enzyme concentration of 3%.

Figure 1. The effect of enzyme concentration and hydrolysis temperature on DPPH radical scavenging activity at optimum hydrolysis time

The DPPH radical scavenging activity is used to check the hydrogenation capability of hydrolyzed proteins. Free radical removal is a mechanism by which antioxidant compounds are capable to prevent oxidative reactions. DPPH radical is one of the few stable radicals at room temperature. When this compound is exposed to a hydrogen donator compound like an antioxidant, it accepts a hydrogen, converts to a stable compound and after radical inhibition, a notable color change from purple to yellow and absorption reduction at 517 nm can be observed (Taheri et al., 2011). The type of initial material, the specificity of the enzyme, the hydrolysis conditions, and the size, amount and structure of amino acids and peptides produced are the factors affecting the antioxidant activity (Mehregan Niko et al., 2013). On the other hand, the antioxidant power of hydrolyzed materials depends highly on the structural arrangement and specific amino acid sequences of the peptide chain. Some researchers believe that histidine, hydrophobic amino acids, and peptides with sequences of proline-histidine-histidine have antioxidant potency (Phelan et al., 2009).

The effect of enzyme concentration and hydrolysis time on the DPPH radical scavenging activity is shown in Figure (2). According to Figure (2), the amount of DPPH free radical scavenging activity increased with increasing enzyme concentration, while with increasing hydrolysis time up to 160 min, the scavenging activity reached its highest level and then, by increasing the time, the amount of free radical scavenging activity decreased. Reduction of DPPH radical scavenging activity by hydrolyzed protein with increasing the hydrolysis time can be due to the progression of the hydrolysis and the greater effect of the enzyme on the protein substance, which causes breaking the chain of some antioxidant peptides formed in the early stages of hydrolysis (Taheri et al., 2011). After optimization of production of hydrolyzed protein from tuna fish waste using the response surface method, Ovissipour et al. (2009) reported that with increasing hydrolysis time, hydrolysis intensity reduced and after that remained constant. Guerard et al. (2002) stated that by increasing the concentration of enzyme, the production of antioxidant peptides increases. They also stated that by increasing hydrolysis time, the degree of hydrolysis increases, and after that the intensity and speed of hydrolysis reduced due to the reduction of the available peptide bands to the enzyme, reduction in the enzyme activity and the formation of inhibitors compounds.
Figure 2. The effect of enzyme concentration and hydrolysis time on DPPH radical scavenging activity at optimum hydrolysis temperature

Figure 3 shows the effect of temperature and hydrolysis time on DPPH radical scavenging activity. As it can be seen in the Figure (3), by increasing the temperature the antioxidant power increases, but the increase in the time up to 160 min increases and then reduces DPPH radical scavenging activity. In a study by Sun et al. (2011) on the antioxidant properties of peptides derived from hydrolysis of pig hemoglobin by flavourzyme, papain, alcalase, pepsin and trypsin, the highest DPPH radical scavenging activity (67%) was associated with hydrolyzed produced by pepsin after 60 min of hydrolysis. Nourmohammadi et al. (2016) obtained the highest antioxidant activity in peptides derived from hydrolysis of pumpkin seed meal by pepsin after 2 h, hydrolysis.

According to the results, it can be said that the hydrolysis temperature and the concentration of the alcalase enzyme have a direct effect on the DPPH radical scavenging activity of hydrolysed product, thereby by increasing these two parameters the antioxidant activity can be increased. However, the increase of hydrolysis time up to a certain limit (160 min) can cause an increase in antioxidant activity of pumpkin seed protein; but with the excessive increase in hydrolysis time, the intensity of hydrolysis increases and antioxidant peptides can be degraded, therefore the antioxidant activity decreases (Taheri et al., 2011).

The effect of independent variables on nitric oxide inhibitory activity

In this section, as in the previous section, the effect of temperature, hydrolysis time and alcalase enzyme concentration on the nitric oxide inhibition capability was investigated. Therefore, in the same way as previously mentioned, using the three-dimensional graphs obtained by Design Expert software, the effect of hydrolysis parameters on the nitric oxide inhibitory power were investigated and interpreted. It should be noted that in each Figure (4, 5 and 6) the effects of the two parameters are investigated when the third parameter is at its optimal point. The effect of enzyme concentration and hydrolysis temperature on the nitric oxide inhibition property is presented in Figure (4). According to the Figure (4), a decrease in nitric oxide inhibition activity has been observed at lower temperatures (35 to 45 °C) and increase in the enzyme concentration up to 2%, and then, with an increase in temperature and enzyme concentration, the amount of inhibition activity increased with a moderate slope. Thus, the lowest nitric oxide inhibition activity was observed at temperature of 45 °C and the enzyme concentration of 2%. Thus, according to the graph, the
highest nitric oxide inhibition activity (87%) is observed at temperature close to 35 °C and the enzyme concentration of 1%. The nitric oxide inhibitory property depends on the antioxidant property of that compound. The antioxidant property of peptides is related to the selectivity of the protease used, the degree of hydrolysis, the nature of released peptides (such as molecular weight, amino acid sequence and peptide structure), and other characteristics, such as the ability to bond to free radicals, the activity of metal ions, chelation and electron donating power, the structural arrangement and sequence of amino acids and the presence of specific amino acids in their peptide chains. The nitric oxide inhibition property also depends on these factors. The highest nitric oxide inhibition activity observed at the lowest temperature and concentration of pepsin enzyme (35 °C and 1%), but the increase in enzyme concentration (at a certain limit 2%) caused higher enzymatic activity and consequently further breaking of antioxidant and nitric oxide inhibitor peptides occur; so the nitric oxide inhibition activity decreases.

Lee et al. (2012) hydrolyzed the protein derived from oyster by 8 protease enzymes and ultimately peptides with the highest nitric oxide inhibition property obtained by the alcalase enzyme. The hydrolyzed product was then purified using high performance liquid chromatography (HPLC) and finally it was recognized that nitric oxide inhibitory peptide show a sequence of glutamine-cysteine-glutamine-glutamine -alanine-valine-glutamine-serine -alanine -valine at their N terminal section. Therefore, considering the mentioned findings in this section and the research carried out by Lee et al. (2012), it can be concluded that by increasing the hydrolysis time, enzyme activity and degree of hydrolysis increases and eventually the peptides with specific sequences obtain that results an increase in the nitric oxide inhibition activity of hydrolyzed products.
nitric oxide inhibition activity. In the initial times (before 160 min) and by increasing the temperature up to 45 °C, the nitric oxide inhibition activity shows a descending trend, and by increasing the hydrolysis time to 160 min and temperature to 45 °C, the nitric oxide inhibition shows an ascending trend. Therefore, the maximum nitric oxide inhibition activity can be achieved at hydrolysis time of 260 min and temperature of 35 °C. Low nitric oxide inhibition activity of hydrolyzed samples in the early stages of hydrolysis (before time of 160 min) may be due to low hydrolysis time that is insufficient to affect the substrate and to produce peptides with nitric oxide inhibitory activity. With the increase in the hydrolysis time and decrease in the size of produced peptides, the ability to inhibit free radicals by peptides enhances (Nourmohammadi et al. 2016).

![Figure 6](image_url)

**Figure 6.** The effect of temperature and hydrolysis time on the nitric oxide inhibition activity at optimum enzyme concentration

Phelan et al. (2014) through enzymatic hydrolysis of milk proteins achieved a peptide with amino acid sequence of alanine-valine-proline-tyrosine-proline-glutamine-arginine and tyrosine-tyrosine-alanine-lysine-proline -alanine-alanine-valine-arginine with proper nitric oxide inhibition property. Also, Kim et al. (2013) hydrolyzed the protein of shellfish with 8 different protease enzymes and investigated the nitric oxide inhibition activity of final products. Then, by purification using chromatography system they reported that the peptide which showed high nitric oxide inhibitory property contained 10 amino acids with sequence of glycine-valine-serine-leucine-leucine-glutamine-glutamine-phenylalanine-phenylalanine-leucine. According to the findings for achieving proper nitric oxide radical inhibitory activity of final product, the optimum hydrolysis condition include hydrolysis time of 260 min, temperature 35 °C and enzyme concentration of 1%.

**Optimization and validation of the model**

Optimal conditions were obtained by Design Expert software. Hydrolysis conditions for preparation of hydrolyzed protein with the highest DPPH radical scavenging activity and nitric oxide inhibition activity were obtained in accordance with temperature of 44 °C, hydrolysis time of 260 min and enzyme to substrate concentration of 3%, which corresponded to the nitric oxide inhibition activity and DPPH free radical scavenging activity of 4.71 and 75.33%, respectively. In order to confirm the suitability of conditions proposed by the software, additional experiments were carried out in the predicted situations obtained by the model. The DPPH free radical scavenging activity and nitric oxide inhibition activity of hydrolysed product were obtained 72.03 and 89.34%, respectively. The obtained results showed almost similar values with the predicted values proposed by the model, which indicated using predictive models it is possible to predict optimal conditions for the production of hydrolyzed protein with high antioxidant and nitric oxide inhibition activity from pumpkin seeds protein. Nourmohammadi et al. (2016) stated that the measured antioxidant activity of peptides produced using enzymatic hydrolysis of pumpkin seeds by pepsin at optimal conditions (82.07%) was largely similar to that
proposed by software (80.31%).

**Conclusion**

Millions of tons of valuable by-products are produced every year during processing of food products. One of these valuable by-products is pumpkin seed meal, which has so far been used as supplement in animal feed. In order to increase the value of this by-product which is economically important and due to its high protein content with the appropriate combination of amino acid, this study attempted to use the meal as a suitable protein source for preparation of protein hydrolyzate with high bioactive potential. In this study, optimization of production of hydrolyzed protein with high antioxidant and nitric oxide inhibition activity using alcalase enzyme was performed by response surface method. According to the results, preparation of pumpkin seeds protein hydrolyzate with high antioxidant and nitric oxide inhibition activity is influenced by reaction conditions including temperature, time and the enzyme concentration. Results showed that optimum conditions to achieve maximum DPPH radical scavenging activity and nitric oxide inhibition activity is temperature of 44 °C, hydrolysis time 260 min, and enzyme to substrate concentration of 3%. Under these conditions the antioxidant and nitric oxide inhibition property of protein hydrolyzate were 72.03 and 89.34%, respectively which were largely similar to the results proposed by the software (75.33 and 84.71%). Hence, the pumpkin seed protein hydrolyzate with high antioxidant and nitric oxide inhibitory activity can be used as an alternative to synthetic preservatives in different food formulations including meat products. Considering the consumer's tendency toward functional foods and present concerns about application of synthetic additives, the results of this study lead to the production of a functional ingredient that can be used in various types of food formulation. This product is particularly interesting regarding its nitric oxide inhibitory property which eliminates concerns about the nitrate and nitrite residues and prevention of nitrosamines formation in meat products such as sausage. Also due to its protein structural nature its application increases the nutritional value and creates a product with health promoting advantages.

**References**


به‌هم‌یابی شرایط هیدرولیز پروتئین دانه‌‌کدو با آنزیم آلکالاز به‌منظور دستیابی به حداکثر فعالیت ضاک‌سایشی و مهار‌کننده‌ی اکسید آنتی‌ویدرولیز

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چکیده

در این پژوهش، به‌هم‌یابی شرایط وظیفه‌ی پروتئین دانه‌‌کدو (Cucurbita pepo) به‌منظور کاهش قابلیت ضاک‌سایشی و مهار‌کننده‌ی اکسید آنتی‌ویدره (DPPH) با استفاده از آزمایش دی‌پپکریل هیدراژین (DPPH) و مهار‌کننده‌ی کلسیم نیتریک دستیابی به‌منظور دستیابی به حداکثر خصوصیات مشابه تا حدود زیادی ممکن می‌باشد.

واژه‌های کلیدی: آلکالاز، آنزیم آنتی‌ویدره، دانه‌‌کدو، مهار‌کننده‌ی اکسید آنتی‌ویدرولیز آنزیمی
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**Table 1.** Effect of modified atmosphere packaging on quantity and quality properties of strawberry

<table>
<thead>
<tr>
<th>Statistical sources</th>
<th>DF</th>
<th>Weight loss (%)</th>
<th>Texture firmness (n)</th>
<th>Soluble solids (brix)</th>
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ns: non-significant, *\(P\leq0.05\) and **\(P\leq0.01\)

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![Figure 1](image)

**Figure 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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**Acknowledgements**

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تأثیر افزودن کازنین سدیم و آنژیم ترانس گلوبنیژیا بر خواص رندهای گلوتینی، فیزیکی و حسی نان بدون گلوتین

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بررسی پاداری اکسیداتو روغن سرخ کردنی محصولات با روغن دانه خرما و روغن ذرت

تعیین منشأ گیاهی عمل‌های رایج استان خراسان رضوی براساس خصوصیات رنگ، شوری، مقاومت الکتریکی و مواد جامد نام با استفاده از روش‌های گوناگون

استفاده از روش‌های تشخیص الگو در آنانیز داده‌های حسگر بویایی جهت شناسایی تقلب‌های گلوز و فرورتوز در عمل

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