Iranian Journal of Chemistry and Chemical Engineering (IJCCE)

A Study on the Possibility of Sodium Nitrite Substitution *Punica granatum var. pleniflora (Persian Golnar)* Extract in Sausage Formulation

Maleki kohaki, Alireza[1], Nateghi, Leila*[2], Kavian, Fatemeh[3].

[1]Ph.D. in Food Science and Technology, Department of Food Science and Technology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran

[*2] Associate Professor, Department of Food Science and Technology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran

[1]Ph.D. in Food Science and Technology, Department of Food Science and Technology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran

*Corresponding author:
Leila Nateghi Tel: +98-912587875 Fax: +98-2136733720 E-mail:leylanateghi@iauvaramin.ac.ir

ABSTRACT: *The study aimed to investigate the possibility of sodium nitrite substitution form (Persian Golnar) extract in sausage formulation. To this end, the amount of sodium nitrite allowed in sausages (120 ppm) was substituted for different concentrations (0, 20, 40, 60, 80, 100 %w/w) of (Persian Golnar) extract. The amount of flavonoid, IC_{50}, color characteristics (a^*, b^*, L^*), microbial characteristics (total microbial, mold, yeast, coliform, Clostridium perfringens and Staphylococcus aureus counts), and their sensory properties (taste, odor and general acceptance) were evaluated on days 1, 25, and 50 of storage at 4°C. The results showed that increasing the substitution percentage of (Persian Golnar) for sodium nitrite increased flavonoids and decreased IC_{50}. Furthermore, increasing the substitution percentage of (Persian Golnar) instead of sodium nitrite decreased a^* and L^* and increased b^*. It is worth noting that the total microbial counts of all samples entire storage periods were within the acceptable range of Iranian National Standard, and there didn't observe any coliforms, Clostridium perfringens, Staphylococcus aureus, mold and yeast in any sample at all intervals. Substitution of (Persian Golnar) for sodium nitrite in sample 3 (60% sodium nitrite, and 40% (Persian Golnar), and sample 4 (40% sodium nitrite, and 60% (Persian Golnar) had the highest score of sensory properties (smell, taste, and overall acceptance). Thus, the substitution of all or part of the sodium nitrite used in the sausage formulation of (Persian Golnar) extract increased antioxidant properties, without any adverse effect on the sensory and microbial properties of the sausage.*

Keywords: *Punica granatum var. pleniflora*, *Persian Golnar*, Sodium nitrite, Sausage, Microbial properties, Antioxidant.

INTRODUCTION

Sausages and cold cuts are popular meat products that are a favorite of millions of consumers worldwide. In response to consumer demand for natural products and their willingness to pay more for natural foods [1], the meat industry is looking for natural solutions to reduce microbial spoilage, reduce oxidative spoilage, and extend the shelf life of its products [2]. Plants are good sources of natural antioxidants and antimicrobials and can be a good alternative to synthetic preservatives [3]. Fat oxidation is important because it reduces the quality of meat products [4] and negatively affect sensory properties such as color, aroma, texture and taste, as well as the nutritional quality of products, and adverse effects on the human body [5]. Therefore, numerous studies have been conducted to increase the stability of fats, the products for maintaining consumer health due to economic reasons. Using antioxidants is an easy way to reduce fat oxidation [6]. Sodium nitrite added to these products has antimicrobial and antioxidant roles [7, 1]. Herbs containing active ingredients have long been used for therapeutic purposes as well as food flavorings worldwide, and the positive effects of their preservation on foods have been unintended because of such compounds [8].

Punica granatum var. pleniflora (Persian Golnar) belongs to the *Puniaceae* family. Ineffective pomegranate flowers are important herbs in traditional medicine. Plant phenolic compounds contain various groups, including flavonoids such as anthocyanins, flavonols, flavones, and non-flavonoids such as phenolic acids and lignins. According to studies, Alginic acidis the most important chemical compound in pomegranate peel, and its structure and phenolic nature cause its strong antioxidant activity [9]. There are substances such as gallic acid, ursolic acid, triterpenoids such as maslinic acid,asiatic acid, and phenolic compounds such as punicalagin in pomegranate flowers and all of them can cause antimicrobial activity of the plant components [10]. The antioxidant activity of phenolic compounds is due to the reduction-oxidation (redox) properties, and chemical structures of these molecules that can play important roles in neutralizing free radicals, chelating heavy metals, and suppressing singlet and triplet oxygen ($^1O2$ and $^3O2$) [11]. Other researchers have also conducted studies on the evaluation of antioxidant properties of pomegranate plant, including the evaluation of antibacterial activity and determination of total phenolic content of hydroalcoholic extract of *P. granatum var. pleniflora* [11]. Karimizadeh and Dastgheib [12] studied the Sodium Nitrite substitution for pomegranate peel extract and stated that pomegranate peel with high levels of antioxidants such as anthocyanins, flavonoids, ascorbic acid, and folic acid can be used as a natural preservative in processed meat products.

The production of meat products is very advanced in the world, and these products are also more reasonably priced compared to ordinary fresh meat in addition to having desired sensory characteristics. Meat products contain nitrite. Reducing nitrite in meat emulsions is desirable for the negative effects, but it promotes the lipid oxidation reaction. This reaction is destructive, causes unpleasant odor and taste, then removes desirable color pigmentation of meat products, and also causes the growing demand of consumers to use natural additives as a substitute and preservative in foods due to their safety compared to synthetic additives. Plant extracts have been used by the ancient Egyptians and Asian countries such as India and China for centuries, but the medicinal uses of these compounds have been of paramount importance for their taste and odor in recent years. The extract of *P. granatum var. pleniflora* can fulfill the properties of sodium nitrite without any adverse effect on the consumers' health due to its antimicrobial and antioxidant properties and by creating red pigment. The present study aimed to remove all or part of sodium nitrite from sausage formulation and replace it with *P. granatum var. pleniflora* which was studied for the first time in Iran.

EXPERIMENTAL SECTION

Materials

Persian Golnar was purchased from Saveh local market and approved by the herbarium of medicinal plants of the Faculty of Pharmacy, University of Shahid Beheshti, Iran, with herbarium number 8016 and scientific name of *P. granatum var. pleniflora*. Beef 55% (Sterla brand, Brazil), sunflower oil (Oila, Iran), wheat flour (Kordan, Iran), milk powder (Pegah, Iran), potassium phosphate (Merck, Germany), salt and spices (Black and red pepper, and mustard) (Golha Company, Iran), and garlic from the local market were the production of sausage. The chemicals such as tetracycline, aluminum chloride, DPPH solution, ethanol, sodium carbonate solution, hydrochloric acid, quercetin, sodium acetate, cyanidin-3-glucoside, potassium acetate solution, and a variety of buffer solutions with culture media, including Mueller-Hinton agar, Cooked Meat, DRBC Agar, Plate Count Agar, Giolitti-Cantoni Broth (GB), Violet Red Bile Lactose (VRBL) agar, and Sabouraud agar (SC) were purchased from Merck, Germany. Furthermore, *Staphylococcus aureus* (PTCC: 143), *Clostridium perfringens* (PTCC: 1766) and *Escherichia coli* (PTCC: 1338), were prepared in lyophilized forms from the microbial collection of the Iranian Scientific and Industrial Research Organization.

Preparation of *P. granatum var. pleniflora* (Persian Golnar)

Persian Golnar sample was placed in an oven at 45 °C for 24 hours to reduce the moisture content up to 8%. Then, the dried parts were passed through Pars Khazar mill 320P (Iran) and sieved with 500-micron mesh. Extraction of effective compounds from Persian Golnar was performed according to the factorial method with three independent variables, namely solvent type (water and 80% methanol), time (at three levels of 12, 24, and 48 hours), and temperature (at three levels of 20, 35, and 50 °C). The ratio of Persian Golnar powder to solvent was 1 to 5. Therefore, the solvent and Persian Golnar were transferred to a 250-ml conical Erlenmeyer flask, and then the extraction conditions of Persian Golnar were provided. To prevent solvent evaporation, the Erlenmeyer lid was closed tightly with a polyethylene cover during the extraction time and then kept in anaerobic shaker, model 22 (Germany) at appropriate temperature and time for complete pigment extraction. It is worth mentioning that different ratios of Persian Golnar powder to solvent and the levels of variables were determined based on pretreatment. Then, the extract was filtered by *Whatman*1 filter paper, and the filtered extract was concentrated by IKA RV10 DS99 Rotary Evaporator (Germany) at 50 °C to 60 °Bx, and then dried in an oven at 40 °C until complete drying [13].

Obtaining the extract from *P. granatum var. pleniflora* (Persian Golnar)

Obtaining the Persian Golnar extract was performed based on the results of studies by Maleki *et al*. [14] reported that the optimal conditions for extracting the extract from Persian Golnar included the highest amount of antioxidant and antimicrobial compounds by soaking at 50 °C for 48 hours, and using methanol solvent.

According to the results reported in a study conducted by Maleki *et al.* [14] Persian Golnar extracted in the above-mentioned conditions had a flavonoid content of 8.6216 mg/g, an anthocyanin level of 5.7655 mmol/g, and an IC_{50} level (7.2793 mg/ml). The Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Persian Golnar extract obtained by soaking against *Staphylococcus aureus*, *Escherichia coli*, and *Clostridium perfringens* were equal to 468.5, 1875, 937.5, 3750, 937.5, and 5000 µg/ml respectively. The results of the study of inhibition zone halo of the extract proved more antimicrobial effects against *Staphylococcus aureus*

with the largest diameter of inhibition zone halo (13 mm) compared to *Clostridium perfringens*, and *Escherichia coli*.

Sausage sample production method

The materials with the following formulation were prepared to produce sausages: 55% beef with 8-10% fat, including 1.65 kg of meat, 0.54 kg of ice, 0.48 kg of sunflower oil, 0.15 kg of wheat flour, 0.06 kg of milk powder, 0.01 kg of potassium phosphate, 0.04 kg of salt, 0.05 kg of spices (black and red pepper, and mustard), and 0.06 kg of garlic to obtain 3.04 kg of dough. All materials were mixed in a cutter (Alfina, Germany) and packaged in a coating (synthetic polyamide coating with low water and gas permeability). The samples were then cooked at 74 °C. They were then cooled under running water and kept at refrigeration (4 °C) for a month [15].

To prepare other sausage samples with extracts of Persian Golnar by extraction method, they had the highest antioxidant compounds with concentrations of 0, 20, 40, 60, 80, and 100 %w/w instead of all or part of the nitrite used in the sausage formulation instead of nitrite, and it was compared with a control sample containing 120 ppm sodium nitrite (Based on the concentration used in sausage factories in Iran and in accordance with the permissible limit of the national standard in Iran).

The samples were transferred to the cooking room after filling in the coating. In the cooking room, the temperature of the product center reached 70-72 ºC for 1 hour and was kept at this temperature for about 15 minutes. The product temperature was then rapidly reduced with a cold shower and immediately transferred to a cold store above zero (temperature of 0-4 °C). Sausage samples were produced with 3 replications and the samples were compared with the control sample in terms of chemical properties (DPPH free radical scavenging activity, total flavonoid content), color (b*, a*, L*), and microbial and sensory properties (texture, odor, taste, and overall acceptance) on 1, 25, and 50 days at 4 °C after production.

Tests on sausage samples

Color of sausage samples

Hunter lab colorimeter (CR400 model made in Japan) was used to evaluate the color of the samples, and it was standardized by black and white tiles. All samples were cut transversely from the middle by two connected blades with a fixed distance to have equal diameters. The cut pieces were then placed in a special glass cup in a way that when they were placed at the sample location of the device, the area would be completely covered and there would be no holes. A matte black container called a light trap was then placed on the cup to prevent external light from interfering, and the color of the samples was evaluated as b*, a*, and L* [16]. The rates of color indices a* (redness), b*(brownness), L*(brightness) of sausage samples containing different percentages of Persian Golnar and sodium nitrite.

Total flavonoid content

The total flavonoid content in sausage flavonoids was measured using colorimetry. 0.5 ml of the extract prepared in the previous method was dissolved in 1.5 ml of methanol in a test tube, and then 0.1 ml of 10% aluminum chloride and 0.1 ml of 1M potassium acetate solution were added. Finally, 2.8 ml of distilled water was added to them and kept at room temperature for 30 minutes, and then the resulting mixture was read at a wavelength of

415 nm by spectrophotometry. Quercetin was used as the standard to draw the calibration curve. The flavonoid content was expressed in milligrams of quercetin equivalents per gram of dry sample [17].

DPPH Free radical scavenging activity

The ability to give a hydrogen or electron atom by *Ganoderma lucidum mushroom* was measured using decolonization of the DPPH ethanol solution. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a stable radical compound in purple that is converted to yellow diphenylpicryl hydrazine through reduction by electron or hydrogen donating elements (antioxidant compounds). In this method, DPPH was used as a reagent and a stable radical compound [18].

20 g of sausage sample was added to 20 ml of distilled water and mixed well with a mixer for 2 minutes, and the homogenized sample was placed in a centrifuge at 6000 g for 20 minutes, and 250 µl of the filtered solution with 3 ml of 60 µM DPPH solution was added to ethanol and the samples were read at 517 nm against the control after half an hour of incubation in the dark, and the percentage of free radical scavenging was calculated using Equation 1.

Equation 1:

$$\text{DPPH}= \frac{Control\ absorbance(\%) - Sample\ ansorbance(\%)}{Control\ absorbance(\%)} \times 100$$

IC_{50} factor as an extract percentage, which can neutralize 50% of DPPH free radicals, was used for better evaluation of the antioxidant activity of the extract.

Microbial test

Microbial tests were performed according to the National standard No. 2303 (2021) [19] (Sausages and cold cuts, tests characteristics, and methods). The total count of microorganisms, the presence or absence of *mold* and *yeast, coliform, Staphylococcus aureus*, and *Clostridium perfringens* were examined according to this standard.

Overall count of microorganisms

1 g of the sample was taken with a sterile metal spoon and poured into a beaker with 9 ml of normal salineand was completely homogenized. Therefore, a 0.1 dilution was obtained. The Plate Count Agar (PCA) was prepared according to the manufacturing instructions (23.5 grams PCA in 1000 ml distilled water). 0.1cc of the prepared dilutions was taken and cultured in them by surface culture method. The tube was shaken well before sampling from each dilution for homogenization. The lids of the plates were fixed (next to the flame), and the plates were incubated at 27-25ºC for 2-3 days. The plates were selected for counting and they had 30-300 colonies [20].

Total count per gram (Cfu/g)= Inoculation rate reversal × Dilution reversal× number of colonies

Mold and yeast count

1 g of the sample was taken with a clean metal spoon and poured into a beaker with 9 ml of normal saline and was homogenized well. Therefore, a dilution of 0.1 was obtained. 0.01 mL dilution was prepared from 0.1 mL dilution of normal saline and was homogenized by the vortex. To prepare a dilution of 0.001, 1 ml of the dilution was added to a tube containing 9 ml of normal saline, and the necessary dilutions were prepared for a dilution of

10^{-4}. Plates containing Dichloran Rose-Bengal Chloramphenicol Agar (DRBC) was prepared according to the manufacturing instructions (15.75g culture medium added to 500 ml of distilled water and heat to dissolve completely and then one vial SR0078E
reconstitute chloramphenicol supplement added). After that 0.1 cc was taken from the prepared dilutions and cultured in them by the surface culture method. The tube was shaken well before taking a sample from each dilution for homogenization. The lids of the plates were fixed (next to the flame) and the plates were incubated at 25-27 °C for 2-3 days. Plates were selected for counting to have 30-300 colonies [21].
Number of yeast and mold per gram (Cfu/g)= Inoculation rate reversal× Dilution rate reversal× Number of colonies

Coagulase-positive *Staphylococcus aureus* count

10 ml of the Giolitti-Cantoni Broth (GB) prepared in a test tube in the first step was diluted toa 10^{-1} dilution, and 1 loop of the GB was taken to Baird parker agar after 24-48 hours of incubation. If black spots were observed, the product contained *Staphylococcus* [22].

***Coliform* counting**

In order to identify and count coliform bacteria according to national standard number 11166 (National Standard Organization 2008), each of the desired dilutions were cultured in Violet Red Bile Agar culture medium and the plates were cultured for 24 hours at 37 °C. Grad was placed in a greenhouse, to confirm and count definite coliform, the colonies suspected of coliform that had grown in violet red bile agar medium were transferred to test tubes containing Durham and bright green broth culture medium containing lactose and after being kept in a greenhouse at a temperature of 37 °C for 24 hours, in the case of each of the Durham pipes, the gas had accumulated, the presence of coliforms was confirmed, and finally the number of coliforms per gram of the sausage sample was calculated [23].

***Clostridium perfringens* counting**

1 ml of test tube diluted at 10^{-1} in the first step was poured into the test tube containing 10 ml of cooked meat, and 1 loop of the cooked meat solution was transferred to selenite cystine (SC) agar after 24-48 hours of incubation. If white spots were observed, the product contained *Clostridium* [24].

Sensory evaluation

Samples of produced sausages were given to 8 semi-trained experts to assess the quality of the products and achieve the best formulation. The sensory properties of the sausages, including taste and odor, and general acceptance were evaluated by a nine-point hedonic scale method at room temperature (25°C). Sausage samples were given to the experts and they were asked to evaluate the traits and mention their opinions on the evaluation sheets. Therefore, a very good sample (very satisfactory) was scored 9, good (satisfactory): 8, medium (acceptable): 4, poor (unacceptable):1, and very poor (non-consumable): 0. [25] It should be noted that sausage samples were boiled in water for 5 minutes and then given to the experts for their safety.

Data analysis

Six treatments were using a completely randomized design. The tests were performed in three replications. The one-way Duncan tests (One-Way ANOVA) and two-way (Two-Way ANOVA) were used in Minitab 16 software.

RESULTS AND DISCUSSION

Physicochemical properties of Persian oleander extract

Evaluation of color indices

Color is a food appearance characteristic that affects the consumer's quality perception of a product and plays an important role in consumers' acceptance of products. Figure 1 is shown changes in color indices of a^*, b^*, and L^* in Mortadella samples with different concentrations of Persian Golnar and sodium nitrite during 50 days of storage. The rates of color indices a^*, b^*, L^* of sausage samples containing different percentages of Persian Golnar and sodium nitrite decreased significantly in 50 days of storage ($p\leq0.05$).

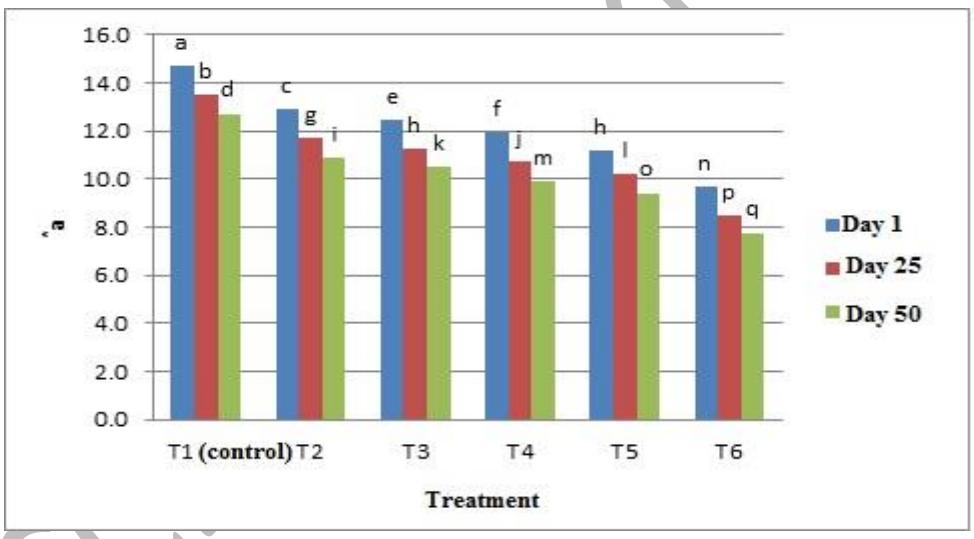
The results indicated that decreasing sodium nitrite decreased the amount of color index a^*. The highest color index a^* (12.71) belonged to the sample of sausage containing 100% sodium nitrite without Persian Golnar, and the lowest color index a^* (7.77) was achieved for the sample containing 100% Persian Golnar without sodium nitrite on the 50th day of storage, and they were significantly different from other treatments ($p\leq0.05$). The reasons for reduction of a^* (redness) included the gradual progression of lipid oxidation and accumulation of methemoglobins in all treatments during storage. Aliyari *et al.* [26] reported a strong negative correlation coefficient between lipid oxidation parameters and redness index. As a result of myoglobin oxidation, metmyoglobin is formed and the amount of redness decreases. Due to a series of special reaction sin the process of producing sausages by nitrite, pink color is obtained in the meat. Nitrite in the samples is converted to nitro peroxide and eventually to the myoglobin nitrosomet complex. It is then converted to nitroso-myoglobin with a bright red color under reduction. Therefore, the red component was less in treatments with lower nitrite percentage or without nitrite. Consistent with the results of this study, El-Nashi *et al.* [27] pointed out a reduction in the red component in treatments containing pomegranate peel powder in their study of pomegranate peel on hamburger characteristics during storage.

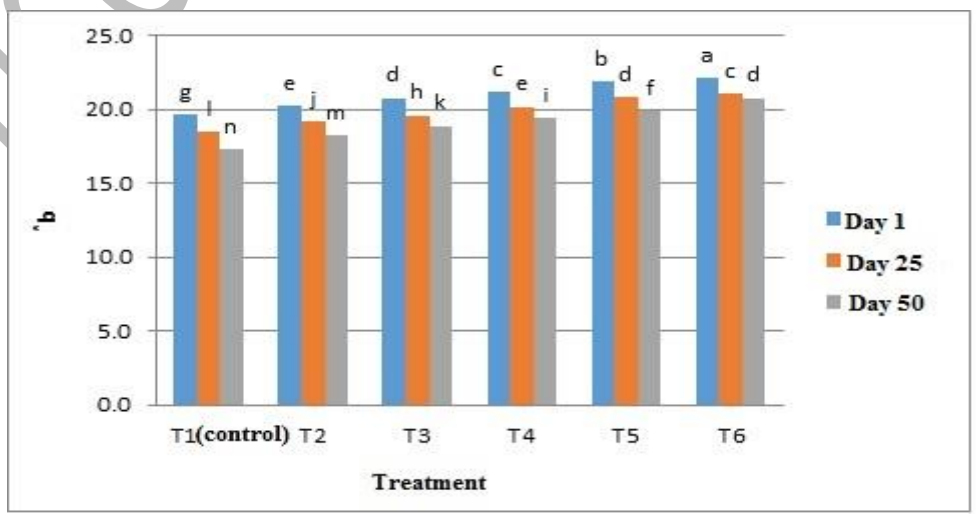
There was an inverse relationship between the percentage of sodium nitrite and color index b^*; hence, color index b^* increased due to the reduction of sodium nitrite. The lowest amount of color index b^* (17.28) belonged to the sample of sausage containing 100% sodium nitrite without Persian Golnar, and the highest amount (20.79) belonged to the sample containing 100% of Persian Golnar without sodium nitrite on day 50 of storage, and they were significantly different from other treatments ($p\leq0.05$). In the case of factor b^*, even though this index is systematically defined on a yellow-blue scale, the examination of this factor and sensory evaluation indicates the degree of browning of this product [28]. Reactions of Maillard browning and caramelization occur during the cooking and heating process, affecting the yellowness factor [29]. The results of the evaluation of index b^* indicated that the addition of Persian Golnar led to its increase, and its reduction reduced the amount of this factor compared to other variables. Khodaei and Khani, [30] studied the effect of substitution of a part of nitrite in sausage formulation using rosemary essential oil and red beet powder and reported that the highest amount of

yellowness index and the lowest amount of *brightness* index belonged to a treatment containing 60-ppm nitrite, 0.04% rosemary essential oil, and 3% red beet powder.

The highest amount of brightness index L^* (57.61) belonged to the sample of sausage containing 100% sodium nitrite and Persian Golnar, and the lowest amount of color index L^* (48.62) belonged to the sample of sausage containing 100% Persian Golnar without sodium nitrite on the 50th day of storage, and they were significantly different with other treatments ($p \leq 0.05$). Therefore, the reduction of nitrite decreased the amount of color index L^*. Based on the comparison of the transparency and brightness factors of the product (L^*) with Persian Golnar, it decreased the brightness of the samples. The luminosity factor in sausages depends on several important factors, including the concentration and type of available pigments, the water content of the pigments, and the moisture-absorbing properties of the pigments dissolved in the matrix [30].

Consistent with the results, Qin *et al*. [31] investigated the effect of pomegranate extract on the physicochemical properties of pork and found that increasing pomegranate extract decreased the factor L^*. Aliyari *et al*. [26] Examined the effect of using pistachio green peel extract and pomegranate peel as natural preservatives in sausages. The results indicated that the amounts of L^* and a^* decreased by increasing the amounts of extract in the treatments; hence, the lowest amounts of a^* and L^*were observed in samples without nitrite.





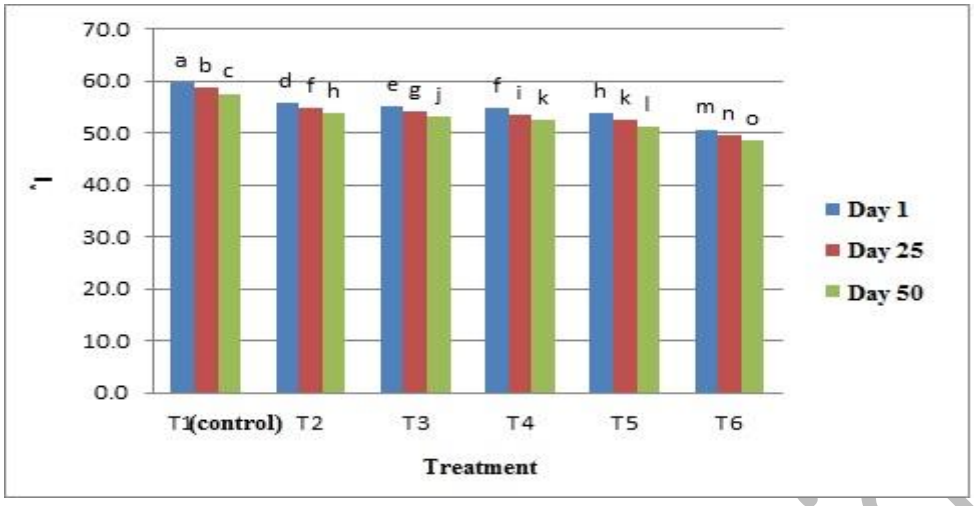


Figure1. Changes in color indices a*, b* and L*of sausage samples containing different concentrations of Persian Golnar extract and sodium nitrite on 50 days of storage

Different lower-case letters indicate a significant difference in each column

T1 (100%sodium nitrite), T2 (20% Persian Golnar extract, 80% sodium nitrite), T3 (40% Persian Golnar extract, 60% sodium nitrite), T4 (60% Persian Golnar extract, 40% sodium nitrite), T5 (80% Persian Golnar extract, 20% sodium nitrite), T6 (100% Persian Golnar extract)

Flavonoid compounds

Table (1) is shown changes in amounts of flavonoid compounds in sausage samples containing different concentrations of Persian Golnar extract and sodium nitrite on 50 days of storage. According to the results, enhancing the concentration of Persian Golnar extract significantly increased the amounts of flavonoids in all treatments ($p \leq 0.05$). Plants are a rich source of phenolic and flavonoid compounds which are among the most important natural antioxidants [32]. Zhang *et al.* [33] reported that the pomegranate plant contained at least 10 mg/g of flavonoid compounds and had 5 types of flavonoids, including Apigenin, apigenin-40-Ob-glucopyranoside, luteolin-40-Ob-glucopyranoside, luteolin-30-Ob-glucopyranoside, and luteolin-30-Ob-xylopyranoside.

According to the results, the lowest amount of flavonoid compounds (5.62 mg/g) belonged to the sausage sample containing 100% sodium nitrite, and the highest amount of flavonoid compounds (12.59 mg/g) belonged to the sample containing 100% Persian Golnar on the 50th day of storage. There was a significant difference ($p \leq 0.05$) with other treatments. The content of flavonoids decreased during storage in all samples because flavonoids along with poly phenolic compounds had antioxidant properties and were involved in maintaining the quality of meat against free radicals and oxidation during storage [34]. The content of flavonoids was not the same in all types of pomegranate flowers and it was a function of factors such as harvest time. Increasing plant growth enhanced the content of bioactive compounds, including flavonoids [33]. In this regard, Mohammed *et al.* [35] examined the effect of pomegranate peel powder extract treated with gamma rays at 0, 3, 6, and 9 kGy levels on the quality of beef sausages, and reported that the maximum amount of flavonoids equal to 3067.35 mg quercetin per 100 g belonged to samples treated with 6 kGy.

Table 1. Evaluation of changes in amounts of flavonoid compounds in sausage samples containing different concentrations of Sodium nitrite and Persian Golnar extract on50 days of storage

Sample	Sodium nitrite (%)	Persian Golnar extract (%w/w)	Day 1	Day 25	Day 50
T1 (control)	100	0	6.45 ± 0.09^{fA}	6.21 ± 0.06^{fB}	5.62 ± 0.07^{fC}
T2	80	20	9.78 ± 0.04^{eA}	8.66 ± 0.05^{eB}	7.46 ± 0.09^{eC}
T3	60	40	10.65 ± 0.06^{dA}	9.69 ± 0.07^{dB}	8.78 ± 0.09^{dC}
T4	40	60	12.87 ± 0.08^{cA}	11.52 ± 0.05^{cB}	10.90 ± 0.07^{cC}
T5	20	80	13.56 ± 0.07^{bA}	12.28 ± 0.04^{bB}	11.76 ± 0.09^{bC}
T6	0	100	14.12 ± 0.03^{aA}	13.22 ± 0.04^{aB}	12.59 ± 0.09^{aC}

Results are shown in mean±standard deviation.

Different lower case letters indicate a significant difference in each column.

Different uppercase letters indicate a significant difference in each row.

The IC_{50} of sausages containing Persian Golnar extract and sodium nitrite

Table (2) is shown changes in IC_{50} in sausage samples containing different concentrations of Persian Golnar extract and sodium nitrite during 50 days of storage. The results indicated that the IC_{50} level increased significantly ($p\leq0.05$) during storage. Given that IC_{50} represents the concentration of the extract, which can remove 50% of free radicals, increasing time due to enhancing fat and protein oxidation processes during storage and reduced antioxidant compounds increased the production of more free radicals and IC_{50} level. The increase of IC_{50} during of storage is due to the oxidation process and the reduction of concentrations of antioxidant compounds in sausages. However, the sample containing 100% Golnar had lower IC_{50} on day 50 than other treatments, and the control sample showed the activity of compounds such as flavonoids in Persian Golnar [34]. The antioxidant power of phenolic compounds and flavonoids is due to their important roles in adsorption and neutralization of free radicals, and the removal of singlet oxygen ($^1O_2$) from the reaction [36].

According to the results, increasing the concentration of Persian Golnar extract significantly decreased the IC50 level ($p\leq0.05$). The highest IC_{50} (12.20 mg/ml) is belonged to the sausage sample containing 100% sodium nitrite, and the lowest IC_{50} (8.15 mg/ml) was obtained for to the sausage sample containing 100% Persian Golnar extract on day 50, and they were significantly different from other treatments ($p\leq0.05$).

The results indicated that increasing the concentration of Persian Golnar extract in the treatments significantly enhanced the antioxidant properties of the treatments ($p\leq0.05$) probably due to the presence of flavonoid compounds in Persian Golnar extract. Therefore, increasing the antioxidant activity of the treatment containing 100% Persian Golnar compared to the treatments containing lower amounts and with a higher percentage of nitrite indicated the presence of high levels of antioxidant compounds such as phenolic compounds in Persian Golnar.

According to the results of a study by Huang *et al.* [37], pomegranate peel extract obtained by the microwave method had strong antioxidant properties with IC_{50} of 0.187 mg/ml. Furthermore, Jouyandeh and Yade mellat [38] examined the antioxidant and antimicrobial effects of pomegranate peel extract on beef burgers during refrigerated storage and reported the total phenolic content of aqueous extract of pomegranate peel equal to 267.82±6.23 mg of tannic acid per gram of dry powder of the extract as well as IC_{50} of 97.42±3.41 mg of tannic acid per gram of dry powder of the extract.

Table 2. Evaluation of changes in IC_{50} in sausage samples containing different concentrations of Persian Golnar extract and sodium nitrite on 50 days of storage

Sample	Sodium nitrite (%)	Persian Golnar extract (%w/w)	Day 1	Day 25	Day 50
T1 (control)	100	0	10.68 ± 0.02^{aC}	11.38 ± 0.03^{aB}	12.20 ± 0.05^{aA}
T2	80	20	9.51 ± 0.02^{bC}	10.37 ± 0.03^{bB}	11.68 ± 0.05^{bA}
T3	60	40	8.73 ± 0.05^{cC}	9.51 ± 0.03^{cB}	10.92 ± 0.05^{cA}
T4	40	60	7.19 ± 0.05^{dC}	8.38 ± 0.04^{dB}	9.91 ± 0.03^{dA}
T5	20	80	6.72 ± 0.02^{eC}	7.59 ± 0.06^{eB}	8.91 ± 0.04^{eA}
T6	0	100	6.37 ± 0.06^{fC}	7.21 ± 0.02^{fB}	8.15 ± 0.04^{fA}

Results are shown in mean±standard deviation.

Different lower case letters indicate a significant difference in each column.

Different uppercase letters indicate a significant difference in each row.

Microbial count of sausages containing Persian Golnar extract and sodium nitrite

It is important to investigate the microbial population of food products and find out the possibility of the presence of bacteria that produce food poisoning in terms of ensuring the quality and health of foods [38]. Table (3) shows changes in the total count of microorganisms in sausage samples containing different concentrations of Persian Golnar extract and sodium nitrite during 50 days of storage. According to the results, the total count of microorganisms increased significantly in sausage samples containing different concentrations of Persian Golnar and sodium nitrite during 50 days of storage ($p\leq0.05$). The highest total count of microorganisms (3.178 log cfu/g) belonged to the sausage sample containing 100% Persian Golnar, and the lowest total count of microorganisms (3.002 log cfu/g) belonged to the sample containing 100% nitrite on day 50 of storage. According to Standard 2303 [19], the acceptable level was equal to 10^5 CFU/g for the total microbial count in sausages. The total microbial count of all treatments was within the acceptable standard range after 50 days of storage. According to the results of the present study and other researchers, this property was due to the presence of phenolic compounds and flavonoids in Persian Golnar that had antimicrobial properties and led to the reduction of the number of microorganisms and their growth rate during storage, indicating their antimicrobial effects like nitrite. Phenolic compounds can denature the enzyme. These compounds also bind to carbohydrates, minerals, and vitamins, and make them inaccessible to microorganisms. Furthermore, phenolic compounds are absorbed by the cell walls of microorganisms and cause the destruction of cell membranes [40]. In this regard, Aliyari *et al*. [26] observed an increase in the total microbial count in all formulations produced to substitute nitrite for natural preservatives of pomegranate peel and pistachio green peel in sausages. On the 15th day of storage, treatments containing pomegranate peel had a significantly lower microbial population (2.97 ± 0.06 log cfu g^{-1}) than control samples. In confirmation of the results, Hussien *et al*. [41] investigated the effect of tomato peel powder and pomegranate peel powder on the microbial load of chicken sausage and reported that the microbial loads in samples containing tomato peel powder and pomegranate peel powder were significantly lower than the control sample (without sodium nitrite, tomato peel powder, and pomegranate peel powder).

Evaluation of the results of c*oliform microbes, Staphylococcus aureus, and Clostridium perfringens,yeast,* and *mold* in sausages containing different concentrations of Persian Golnar extract and sodium nitrite

The examination of microbiological results of sausages containing different concentrations of Persian Golnar extract and sodium nitrite indicated that *coliforms, Staphylococcus aureus*, and *Clostridium perfringens*, *mold,* and *yeast* were not observed in any of the treatments.

Table 3. Examination of changes in the total counts of microorganisms in sausage samples containing different concentrations of Persian Golnar extract and sodium nitrite during 50 days of storage

Sample	Sodium nitrite (%)	Persian Golnar extract (%w/w)	Day 1	Day 25	Day 50
T1 (control)	100	0	2.845 ± 0.00^{aC}	3.003 ± 0.00^{aB}	3.178 ± 0.00^{aA}
T2	80	20	2.845 ± 0.00^{aC}	2.997 ± 0.00^{bB}	3.161 ± 0.00^{bA}
T3	60	40	2.845 ± 0.00^{aC}	2.975 ± 0.00^{cB}	3.144 ± 0.00^{cA}
T4	40	60	2.845 ± 0.00^{aC}	2.965 ± 0.00^{dB}	3.110 ± 0.00^{dA}
T5	20	80	2.845 ± 0.00^{aC}	2.958 ± 0.00^{eB}	3.046 ± 0.00^{eA}
T6	0	100	2.845 ± 0.00^{aC}	2.947 ± 0.00^{fB}	3.0024 ± 0.00^{fA}

Results are shown in mean±standard deviation.

Different lower case letters indicate a significant difference in each column.

Different uppercase letters indicate a significant difference in each row.

Sensory evaluation of heated sausage containing of Persian Golnar extract and sodium nitrite

Table (4) presents the changes of sensory evaluation in sausage samples containing different concentrations of Persian Golnar extract and sodium nitrite on 50 days of storage. According to the results, increasing the concentration of Persian Golnar extract significantly decreased the odor sensory score in all treatments ($p\leq0.05$). According to the results, the lowest sensory odor score (5.77) belonged to the sausage sample containing 100% Persian Golnar extract, and the highest score (7.77) belonged to the sample containing 60% sodium nitrite with 40% Persian Golnar extract on the 50th day of storage, and it was significantly different from other treatments ($p\leq0.05$).

Increased microbial load in the control sample compared to the samples treated with Persian Golnar was the cause of faster spoilage in the control sample. Furthermore, the products of lipid oxidation and ammonia production by hydrolysis of proteins by microorganisms can cause more severe odor reduction in the control sample (without Persian Golnar) [41].

Vaithiyanathan *et al.* [42] studied the effect of phenolic compounds of pomegranate juice (Punica granatum) on the characteristics of chicken meat during 28 days of refrigeration and observed and expressed a decrease in the sensory evaluation of chicken meat from day 4 in all samples. Odor properties in the treated sample were higher than in the control sample.

Table 4. Evaluation of changes in odor sensory evaluation in sausage samples containing different concentrations of Persian Golnar extract and sodium nitrite on 50 days of storage

Sample	Sodium nitrite (%)	Persian Golnar extract (%w/w)	Day 1	Day 25	Day 50
T1 (control)	100	0	7.11 ± 1.16^{aC}	7.66 ± 0.50^{aA}	7.22 ± 0.66^{aB}
T2	80	20	8.00 ± 0.70^{aA}	7.22 ± 0.44^{aB}	7.11 ± 0.60^{aC}
T3	60	40	8.44 ± 0.52^{aA}	7.88 ± 0.92^{aB}	7.77 ± 0.97^{aC}
T4	40	60	8.44 ± 0.52^{aA}	7.22 ± 0.66^{aB}	7.11 ± 0.78^{aC}
T5	20	80	6.66 ± 0.50^{aA}	5.88 ± 0.92^{aB}	5.77 ± 0.97^{aC}
T6	0	100	$6.55\pm.52^{aA}$	5.88 ± 0.78^{aB}	5.77 ± 0.66^{aC}

Results are shown in mean±standard deviation.

Different lower case letters indicate a significant difference in each column.

Different uppercase letters indicate a significant difference in each row.

Table (5) presents changes in taste sensory evaluation in sausage samples containing different concentrations of Persian Golnar extract and sodium nitrite on 50 days of storage. According to the results, increasing the concentration of Persian Golnar extract significantly decreased the sensory evaluation of taste in all treatments ($p\leq0.05$).

According to the results, the lowest taste sensory evaluation score (5.77) belonged to the sausage sample containing 100% Persian Golnar extract, and the highest score (8.22) belonged to the sample containing 60% sodium nitrite with 40% Persian Golnar extract on the 50th day of storage, and the result was significantly different from other treatments ($p\leq0.05$).

El-Gharably and Ashoush, [43] studied the antioxidant effects of pomegranate peel powder and red beet powder on the quality of sausages and reported that the sensory properties (except for taste and juiciness) of the samples were not affected by the types and amounts of compounds and only a small statistically significant difference was seen in the edible quality of the production samples. Khodaei and Khani, [44] studied the sensory properties of substitution of a part of nitrite in sausage formulation using rosemary essential oil and red beet powder and reported that the highest score belonged to the treatment of 60-ppm sodium nitrite with 3% beet+ 0.04% rosemary.

Table 5. Evaluation of changes in sensory evaluation of taste in sausage samples containing different concentrations of Persian Golnar extract and sodium nitrite on 50 days of storage

Sample	Sodium nitrite (%)	Persian Golnar extract (%w/w)	Day 1	Day 25	Day 50
T1 (control)	100	0	7.33 ± 1.41^{bC}	7.77 ± 0.44^{aA}	7.66 ± 0.50^{abB}
T2	80	20	8.22 ± 0.83^{aA}	7.33 ± 0.50^{aB}	7.22 ± 0.44^{bC}
T3	60	40	8.44 ± 0.52^{aA}	7.88 ± 0.920^{aC}	8.22 ± 0.66^{aB}
T4	40	60	8.44 ± 0.52^{aA}	7.22 ± 0.66^{aB}	7.11 ± 0.78^{bC}
T5	20	80	6.66 ± 0.50^{bA}	5.88 ± 0.78^{bB}	5.77 ± 0.97^{cC}
T6	0	100	6.55 ± 0.52^{bA}	5.88 ± 0.60^{bB}	5.77 ± 0.66^{cC}

Results are shown in mean±standard deviation.

Different lower case letters indicate a significant difference in each column.

Different uppercase letters indicate a significant difference in each row.

The overall acceptance factor of products is an important aspect in the ability to produce food products with new formulations instead of conventional formulations, but a project will not be applicable if the results of other tests are successful and acceptable but the overall acceptance is not high. Table (6) presents changes in the general acceptance in sausage samples containing different concentrations of Persian Golnar extract and sodium nitrite on 50 days of storage. According to the results, increasing the concentration of Persian Golnar extract significantly decreased the overall acceptance in all treatments ($p \leq 0.05$). According to the results, the lowest score of overall acceptance evaluation (5.77) belonged to the sausage sample containing 100% Persian Golnar extract, and the highest score (8.11) belonged to the sample containing 60% sodium nitrite with 40% Persian Golnar extract on the 50th day of storage, and the result was significantly different from other treatments ($p \leq 0.05$).

According to the report by Naveena *et al*. [2] on the comparison of the effects of pomegranate nectar, pomegranate peel powder extract, and butyl hydroxyl toluene on the characteristics of chicken in treatment and control samples (without additives), the addition of pomegranate nectar, and pomegranate peel powder extract had no significant difference in sensory evaluation, including appearance, odor, taste, and aroma of chicken. Furthermore, the overall acceptance of the treated samples was equal to the control.

Table 6. Evaluation of changes in the overall acceptance evaluation of sausage samples containing different concentrations of Persian Golnar extract and sodium nitrite on 50 days of storage

Sample	Sodium nitrite (%)	Persian Golnar extract (%w/w)	Day 1	Day 25	Day 50
T1 (control)	100	0	7.33 ± 1.41^{bC}	7.77 ± 0.44^{aA}	7.55 ± 0.72^{abB}
T2	80	20	8.22 ± 0.83^{aA}	7.33 ± 0.50^{aB}	7.11 ± 0.60^{bC}
T3	60	40	8.44 ± 0.52^{aA}	7.77 ± 0.97^{aC}	8.11 ± 0.78^{aB}
T4	40	60	8.44 ± 0.52^{aA}	7.22 ± 0.66^{aB}	7.11 ± 0.78^{bC}
T5	20	80	6.44 ± 0.52^{cA}	5.88 ± 0.78^{bB}	5.77 ± 0.97^{cC}
T6	0	100	6.33 ± 0.50^{cA}	6.00 ± 0.50^{bB}	5.77 ± 0.66^{cC}

Results are shown in mean±standard deviation.

Different lower case letters indicate a significant difference in each column.

Different uppercase letters indicate a significant difference in each row.

CONCLUSION

In the present study, sodium nitrite in sausage formulations with different concentrations (0 (control), 25, 50, 75 and 100 %w/w), and the optimal extract of Persian Golnar extract were substituted by the soaking method. Therefore, 6 treatments were designed according to a completely randomized design and the flavonoid content, IC50, color characteristics (b^*, L^*, a^*), microbial characteristics (total microbial count, *mold* and *yeast*, *coliforms*, *Clostridium perfringens*, *and Staphylococcus aureus*), and their sensory properties were evaluated at 4 °C on days 1, 25, and 50 of storage. The results indicated that increasing the substitution percentage of Persian Golnar for sodium nitrite increased the flavonoid content and decreased IC50. Furthermore, increasing the substitution percentage of Persian Golnar for sodium nitrite decreased a^* and L^*, and increased b^*. It is worth noting that the total count in all samples in all periods was with in the acceptable range of the Iranian national standard (No. 2303), and coliforms, Clostridium perfringens, *Staphylococcus aureus*, *mold*, and *yeast* were not observed in any samples in the periods. Substitution of Persian Golnar for sodium nitrite in sample 3 (60% sodium nitrite and 40% Persian Golnar) and sample 4 (40% sodium nitrite and 60% Persian Golnar) had the highest score of sensory

properties (odor, taste, and overall acceptance). The results indicated the possibility of substitution of a part of sodium nitrite for the Persian Golnar extract. Despite the desire to completely remove nitrite from the sausage formulation, a synergistic state of sodium nitrite and Persian Golnar leads to the production of a product with higher antioxidant and lower microbial properties and can meet all consumer expectations in terms of safety, quality, and sensory properties.

REFRENCES

1- Sebranek J., Bacus J., Natural and organic cured meat products: regulatory, manufacturing, marketing, quality and safety issues. *American meat sci associa white paper seri*, **1:** 1-16. (2007).

2- Naveena B.M., Sen A.R., Vaithiyanathan S., Babji Y., Kondaiah N., Comparative efficacy of pomegranate juice, pomegranate rind powder extract, and BHT as antioxidants in cooked chicken patties. *Meat science*, **80**(4):1304-1308. (2008).

3- Nunez de Gonzalez M.T., Boleman R.M., Miller R.K., Keeton J.T., Rhee K.S., Antioxidant properties of driedplum ingredients in raw and precooked pork sausage. *J Food Sci*. **73:** 63-71. (2008[a]).

4- Raghavan S, Richards M.P. Comparison of solvent and microwave extracts of cranberry presscake on the inhibition of lipid oxidation in mechanically separated turkey. *Food Chem*, **102:** 818-826. (2007).

5- Nunez de Gonzalez M.T, Hafley B.S, Boleman R.M, Miller R.K, Rhee K.S, Keeton J.T. Antioxidant properties of plum concentrates and powder in precooked roast beef to reduce lipid oxidation, *Meat Sci*. **80:** 997-1004. (2008[b]).

6-Tomaino A., Cimino F., Zimbalatti V., VenutiV., Sulfaro V., Pasquale A.De., Saija A., Influence of heating on antioxidant activity and the chemical composition of some spice essential oils, *Food Chem*, **89**(4)**:**549-554. (2005).

7- Kanatt S.R., Chander R., Sharma A., Chitosan and mint mixture: A new preservative for meat and meat products, *Food Chem*. **107:** 845-852. (2008).

8- Shan B., Cai Y., Brooks J.D., Corke H., Antibacterial properties and major bioactive components of cinnamon stick (Cinnamomum burmannii): activity against foodborne pathogenic bacteria. *J Agricull and Food Chem*, **55** (14)**:**5484 – 5490. (2007).

9- Salahvarzi Y., Tehranifar A., Jahanbakhsh V., The relationship between antioxidant and antifungal activities of extracts of different parts of pomegranate (*Punica granatum L.*) with its phenolic content. *Iranian Medici and Aromatic Plants Res*. **27** (1): 47-56. (2011).

10- Jurenka J., Therapeutic applications of pomegranate (Punica granatum L.): *a review*. *Alternative Medicine Review*. **13**(2):128-144. (2008).

11- Ahmadi A., Abdollahi A., Fassihi Ramandi M., Namdar N., Mousavi M., Samizadeh B., Evaluation of antibacterial activity and determination of total phenol content of hydroalcoholic extract of Punica granatum var. pleniflora flower. *J Fasa Uni Medic Sci.* **6** (3):319-325. (2016).

12- Karimizadeh M., Dastgheib-Beheshti M.K., Production of sausages by examining the possibility of substitution sodium nitrite for pomegranate peel extract. *Agricul Sci, Enviro Sci, Enviro (General*). MDCONF01. (2016).

13- Ebrahimzadeh M.A., Pourmorad F., Hafezi S., Antioxidant activities of Iranian corn silk. *Turkish J Biolog*, **32:** 43-49. (2008).

14- Maleki A., Nateghi L., Rajaei P., Investigation of the effect of the soaking method on the extraction of flavonoid compounds, anthocyanins, antioxidant and antimicrobial activity of Persian Golnar. *J Food Res*, **33**(1)**:** 1-15. (2021).

15- Zarringhalami S., Sahari M.A., Hamidi-Esfehani Z., Partial replacement of nitrite by annatto as a color additive in sausage. *Meat sci.* **1:81**(1)**:**281-4. (2009).

16- Viuda-Martos M., Ruiz-Navajas Y., Fernández-López J., Pérez-Álvarez J.A., Effect of adding citrus wastewater, thyme, and oregano essential oil on the chemical, physical and sensory characteristics of bologna sausage. *Innov Food Sci and Emerg Technol.* **10**(4)**:** 655–660. (2009).

17- Chang C.C., Yang M.H., Wen H.M., Chern J.C., Estimation of total flavonoid content in propolis by two complementary colorimetric methods, *J Food and Drug Analys.* **10**(3)**:** 178-182. (2002).

18- Yao X., Zhang D., Zu Y., Fu Y., Luo M., Gu C., Li C. M. F., Efferth T., Free radical scavenging capability, antioxidant activity and chemical constituents of Pyrola incarnata Fisch. Leaves. *Indust Crops and Prod*. **49:** 247– 255. (2013).

19- National standard of Iran. Standard No. 2303. Sausages and cold cuts, Features, and Test Methods. First edition, second revision, Karaj, 17. (2021).

20- National standard of Iran. Standard No. 5272-1. Food chain microbiology. A comprehensive method for counting microorganisms. First Edition. (2014).

21- National standard of Iran. Standard No. 10899-1. Microbiology of foods and animal feed. A comprehensive method for counting molds and yeasts. First Edition. (2008).

22- National standard of Iran. Standard No. 6806-1. Microbiology of foods and animal feed. Coagulase-positive *Staphylococcus aureus* count. First Edition. (2005).

23- National Standard of Iran. Standard No. 11166. Microbiology of foods and feed. A comprehensive method for detecting and counting *coliforms*. First Edition. (2008).

24- National Standard of Iran. Standard No. 9432. Microbiology of foods and animal feed. A comprehensive method for counting sulfite-reducing bacteria under anaerobic conditions. First Edition. (2007).

25-Yam K.L., Papadakis S.E., A simple digital imaging method for measuring and analyzing the color of food surfaces. *J Food Eng*. **61** (1)**:** 137-142. (2004).

26- Aliyari P., Bakhshi Kazaj F., Barzegar M., Ahmadi Gavlighi H., Production of functional sausage using pomegranate peel and pistachio green hull extracts as natural preservatives. *J Agricul Sci and Technol*. **22**(1)**:** 159-172. (2020).

27- El-Nashi H.B., Fattah A.F., Rahman N.R., Abd El-Razik M.M., Quality characteristics of beef sausage containing pomegranate peels during refrigerated storage. *Annals Agricul Sci*. 16 (2)**:**403-12. (2015).

28-OSullivan M., Byrne D.V., Martens H., Gidskehaug L., Evaluation of pork color: Prediction of visual sensory quality of meat from instrumental and computer vision methods of color analysis. *Meat Sci*. **65**(2)**:**909-9018. (2003).

29-Goli A., Barzegar H.M., Sahari M.A., Antiodant activity and totalphenolic compounds of pistachio (Pistachia vera) hull extracts. *Food chem.* **92**: 521-525. (2005).

30- Khodaei S., Khani M.R., The effect of substitution of a part of nitrite in sausage formulation using rosemary essential oil and red beet powder. *J Food Indus Res*. **28**(1)**:**105-120. (2018).

31- Qin Y.Y., Zhang Z.H., Li L, Xiong W, Shi J.Y, Zhao T.R, Fan J. Antioxidant effect of pomegranate rind powder extract, pomegranate juice, and pomegranate seed powder extract as antioxidants in raw ground pork meat. *Food sci and biotechnol*, **1:22(4):**1063-9. (2013).

32- Masjedi M., Nateghi l., Berenjy sh., Eshaghi M.R., Optimization of Extraction of Flavonoid, Total Phenolic, Antioxidant, and Antimicrobial Compounds from Ganoderma Lucidum by Maceration Method. *Iran. J. Chem. Chem. Eng.* (*IJCCE),* **41**(119)**:**3127-3140. (2022).

33- Zhang L., Gao Y., Zhang Y., Liu J., Yu J., Changes in bioactive compounds and antioxidant activities in pomegranate leaves. *Scientia Horticul*. **123**(4)**:**543-546. (2010).

34- Fernandes R.P., Trindade M.A., Lorenzo J.M., Munekata .PE., De Melo M.P. Effects of oregano extract on oxidative, microbiological and sensory stability of sheep burgers packed in modified atmosphere. *Food Cont.* **1(63):**65-75. (2016).

35-Mohammed I.A., Hassanien F.R., Afify M.A., Mohammed A.M., Keeping quality of beef sausage using ethanolic extract of gamma-irradiated pomegranate peel powder. *Acta Periodica Technol*. **(47):**29-50. (2016).

36-Khaleghi A., Rezaei K., Kesai M.R., Khosravi Darani K., Soleimani M., Investigating the antioxidant

properties of black barberry extract on the amount of fat oxidation in sausages stored in the refrigerator. *J Nutr Sci and Food Indus Iran*, **7**(5)**:**345-353. (2013).

37-Huang J., He W., Yan C., Du X., Shi X., Microwave assisted extraction of flavonoids from pomegranate peel and its antioxidant activity. InBIO Web of Conferences 8:03008. EDP Sciences. (2017).

38- Jovandeh H., Yidmelt M., Antioxidant and antimicrobial effect of pomegranate peel extract on beef burger during refrigerated storage. *Food indus res.* **28**(1):135-144. (2018).

39- Taheri A., Jalalinejad S., Hosseini V., Ahmadi A., Naseri F., A study of bacterial population of Iranian fish sauce (Mahyawa), *J Shahid Beheshti Uni*. **5:** 273-280. (2014).

40-Basiri S., Shekarforoush S.S., Aminlari M., Akbari S., The Effect of Pomegranate Peel Extract (PPE) on the Polyphenol Oxidase (PPO) and Quality of Pacific White Shrimp (Litopenaeus vannamei) during Refrigerated Storage. *LWT-Food Sci. Technol.* **60:**1025-1033. (2015).

41- Hussien G.M, Abdel-Samiem M.A, AbdElSalam B.A, Goneim S.I., Production of healthy sausage with tomato peel powder and pomegranate peel powder. *Sinai J Appl Sci*, **8**(1)**:**51-64. (2019).

42- Vaithiyanathan S., Naveena B.M., Muthukumar M., Girish P.S., Kondaiah N., Effect of dipping in pomegranate (Punica granatum) fruit juice phenolic solution on the shelf life of chicken meat under refrigerated storage (4 ^{0C}). *Meat sci*, **88**(3)**:**409-14. (2011).

43- El-Gharably A.M.A., Ashoush I.S., Utilization Impact of adding pomegranate rind powder and red beet powder as a natural antioxidant on quality characteristics of beef sausage. *World J Dairy & Food Scis*, **6**(1)**:** 86-97. (2011).

44-Khodai S., Khani M.R., The effect of partially replacing nitrite in sausage formulation using rosemary essential oil and red beetroot powder. *Food indus res*. **28**(1)**:**105-120. (2018).