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The Effect of Replacing Sodium Nitrite with Monascus Purpureus Pigment in German Sausage

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ABSTRACT

Background: Many common cancers, such as prostate, breast, and colon, are caused by harmful substances and compounds contained in processed foods, including nitrite-treated meats. The aim of this study was to investigate the possibility of replacing sodium nitrite with *Monascus purpureus* pigment in sausage and to assess its antimicrobial, antioxidant, color, and sensory properties. **Methods:** The antioxidant properties (Thiobarbiturics), microbiology tests, color (*L**, *a**, and *b**), and their sensory evaluation were measured 30 days after production (storage in a refrigerator condition) and compared with the control sample (without *Monascus purpureus* pigment and with 100% nitrite). The data were analyzed using Duncan's one-way analysis of variance at 95% confidence level through Minitab 16 software. **Results:** Replacement of *Monascus purpureus* pigment with nitrite had no significant effect on the sensory properties of treatments and caused a slight increase in the thiobarbituric index during the storage period, which was not significantly different with the control sample before its replacement up to 60%. The microbial load was increased during the storage in all treatments. The microbial load of samples in which nitrite was replaced with 0%, 40%, and 60% *Monascus purpureus* pigment was within the acceptable range of standard after 30 days of storage. *L** index was decreased, but *a** and *b** indices were increased by replacing *Monascus purpureus* pigment with nitrite. **Conclusion:** The sample containing 60% *Monascus purpureus* pigment was selected as a superior treatment because the antioxidant properties, microbial load, and sensory properties had no significant difference with the control sample.

Keywords: *Sodium nitrite; Sausage; Monascus purpureus pigment*

Introduction

Currently, meat products are one of the most consumed foods in the world. In the United States and most European countries, meat is one of the costliest foods. Meat accounts for 35% of household expenses in Denmark, France, and Belgium; 30% in Italy, Spain, and Ireland; and 25% in the UK and the Netherlands (Jiménez-Colmenero *et al.*, 2001). Thus, production of meat products is developing rapidly in the world since they are more affordable than the ordinary fresh

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meat. In this regard, 40% red meat sausage is one of these products, which can partly eliminate the need for animal proteins (Hashemi and Shokraneh, 2013). Meat products contain nitrite that is a key ingredient in the processed meat products. Nitrite has different functions (NANO2) in meat products. The initial application of nitrite is to create and develop a special taste of meat products and prevents bad smells caused by oxidation of lipids (Esmaeilzadeh *et al.*, 2012). In the next step, nitrite reacts with meat myoglobin and produces a special pink color with the production of nitrosohemochrome. Nitrite can also prevent the growth of pathogenic bacteria, especially clostridium species. Despite the benefits mentioned above, high nitrite levels in meat products are harmful to health. Furthermore, Nitro acid may be produced as a result of nitro oxide hydration by sodium nitrite reduction and turn to N-nitroso compounds especially nitrosamines in reaction with type 2 amines and amino acids in the muscle. These compounds are remarkable for their carcinogenicity. Such negative potential effects increase the tendency to replace and reduce the contribution of nitrite to meat products (Toldrá *et al.*, 2009). Based on the standard limit for meat products, the limit for nitrites should not exceed 500 ppm for meat products (60%) (Al-Shuibi and Al-Abdullah, 2002). Although reduction of nitrite in meat emulsions is desirable for the aforementioned negative effects, it promotes the lipid oxidation reaction. The compounds produced by the oxidation reaction are capable of reacting with oxygen at high speed. The reaction speed can be delayed by adding antioxidants. Types of synthetic antioxidants, such as Tertiary butyl hydroquinone, Butyl hydroxyl anisole, and Butyl hydroxyl toluene are used in the food industry to prevent lipid oxidation, but their use in the industry is limited by the potential for health and toxicity (Domínguez *et al.*, 2019). There is an increasing demand in consumers to use natural additives as a substitute and preservative factor in foods during the recent years due to their safety in comparison to synthetic additives. Some of these compounds are extracted from plants or some of the pigments, particularly in some microbial, animal or plant pigments known as safe compounds (Stchigel *et al.*, 2004). From the production point of view, microbial pigments are economical and inexpensive and their extraction is simple. Moreover, if an appropriate strain is selected for extraction, the microbial pigments can have a high yield. In terms of raw materials, no shortage was observed in the production of microbial pigments. Moreover, seasonal changes do not have any impact on their production process. Regarding safety and health, microbial pigments do not pose a risk on the human. Some microbial pigments can be useful since they can have antioxidant, anti-cancer, antimicrobial, and anti-inflammatory effects. They also can be vitamin precursors along with coloring the food. Microbial pigments can be extracted from bacteria, molds, yeasts, as well as seaweed and sea molds (Nazemi *et al.*, 2011). Microbial pigments are used in the food industry for processing all kinds of foods. One of these important pigments is Anka's mold pigment called *Monascus*, which is a red intracellular pigment. Application of this fungus has long been used in eastern countries to produce color (Erdoğrul and Azirak, 2004). The meaning of the term in ancient Chinese is *Monascus purpureus*, i.e., red yeast rice. The red pigment is important in industry and especially in the meat industry, because it can replace the illegal artificial colors (Akihisa *et al.*, 2005). Many researches indicated that *Monascus* pigments exhibit biological activities, like anticancer, antihyperlipidemic, and anti-inflammatory activities (Hong *et al.*, 2008). According to (Wójciak *et al.*, 2019), intake of 200 ppm of Khuzestani sativa in the 60% meat Frankfurter sausage formulation has a favorable effect on the prevention of lipid oxidation, similar to that of 500 ppm sodium nitrite. Considering the disadvantages of nitrate and the advantages of *Monascus purpureus Pigment*, the overall aim of this study was to investigate the possibility of replacing sodium nitrite with *Monascus purpureus* pigment in 40% meat German sausage and to assess its antimicrobial, antioxidant, color, and sensory properties.

Materials and Methods

The *Monascus purpureus purpureus* CMU001 pigment was prepared from the mycology group of Isfahan University of Medical Sciences. Calf meat was purchased from Brazil. The starch was prepared from Fara-Daneh Inc. (Shiraz, Iran). The spices, sugar, salt, and wheat flour were purchased from Golestan company, Iran. Sodium nitrite was purchased from shanghai chemex group ltd, China. Phosphate and ascorbic acid were purchased from Sigma-Aldrich (U.S.A). All chemicals and microbials materials needed to perform tests were bought from Merck Company, Germany.

Sample preparation: The *Monascus purpureus* pigment was prepared by alkali method using the Haghparast method (Sharmila *et al.*, 2013). By this method, the *Monuscus pigment* was extracted from the powdered pericarp by shaking in a 0.13 molar solution of potassium hydroxide. Later, the filtering was done to remove impurities, pigments were precipitated with hydrochloric acid, and the solution was filtered and dried. To remove bad odors, dried sediments were washed with petroleum ether. At the end, pigments were mixed with alkali and dissolved in water.

The treatments were prepared according to the usual method of producing 40% meat sausage in Pardis factory by replacing 0, 20, 40, 60, 80, and 100% nitrite with *Monascus purpureus* pigment. The frozen meat was ground. The ground meat entered the Laska cutter. In the next step, ice was added to the cutter with *Monascus purpureus* pigment, nitrite, spices, salt, and phosphate. The cauterization process continued for 2 to 3 minutes. Later, the oil was added and after several rounds (3 minutes) of cauterization, filler materials (wheat flour and starch) and other additives (sugar and ascorbic acid) were added and the process was completed for 4 to 5 minutes until a fully emulsified paste was created with a temperature of 4 to 5 degrees celsius. The dough was entered to the Wolfkin filler and filled with 24 caliber polyamide coatings. The product was transferred to the baking room for thermal processing. After baking, carried out at 80 °C for a period of 45 minutes, the samples' temperature were reduced by cold water showers and the samples were kept in a refrigerator at 4°C for 1, 10, 20, and 30 days for analysis (Hashemi and Shokraneh, 2013).

Thiobarbituric acid (TBA): It was measured according to a previous study (Ulu, 2004).The total counting of microorganisms was carried out according (Sharifi-Yazdi *et al.*, 2016) using the Plate Count Agar (PCA) culture medium. The mold and yeast were measured according to (Maktabi *et al.*, 2016) using Dichloran Rose Bengal Chloramphenicol (DRBC) medium. *Staphylococcus aureus* was measured according to (Javadi and Safarmashaei, 2011). To this end, three factors of a^*, b^*, and L^* were measured by color measurement model CR-400 Minolta, Japan (Yam and Papadakis, 2004). The sensory properties of the samples were evaluated based on the 5-point hedonic method and using 10 trained evaluators to assess overall acceptability of the samples (Yam and Papadakis, 2004).

Data analysis: The results of the tests were evaluated in three replications. To compare the mean of data, Duncan's one-way analysis of variance was used at 95% confidence level by Minitab 16 software as well as Excel software to plot the graphs.

Results

***Analyzing antioxidant properties of the produced sausage samples*:** According to **Table 1**, no significant difference was found between the amount of thiobarbituric acid in all treatments on the first day of storage ($P \leq 0.05$). The amount of thiobarbituric acid during the storage period was increased in all treatments. In other words, the highest amount of thiobarbituric acid (2.68 mg malondialdehyde per kg) after 30 days of storage belonged to the treatment containing 100% *Monascus purpureus* pigment. However, the lowest amount of thiobarbituric acid (2 mg malonaldehyde per kg) belonged to the treatment containing 100% nitrite (control), which had a significant difference with each other. Thiobarbituric acid content was increased in all treatments after increasing the content of

Monascus purpureus pigment and decreasing nitrite content, but this trend was not significant by replacing 60% nitrite with *Monascus purpureus* pigment ($P \leq 0.05$).

The microbial properties of samples of sausages produced: According to **Figure 1**, the total count of bacteria, *staphylococcus aureus*, molds, yeasts, and coliforms had an increasing trend in all treatments during the storage period. So, the lowest total count of bacteria, *staphylococcus aureus*, molds and yeasts, as well as coliforms were observed among treatments in samples containing 100% sodium nitrite.

Evaluating the color properties of produced sausages L* index: The results of **Figure 2** indicate the effect of different concentrations of *Monascus purpureus* pigments on the mean of *L** index. According to the findings, application of *Monascus purpureus* pigment instead of nitrite used in sausage formulation and an increase in its concentration caused a slight decrease in *L** index compared to the control sample. However, this reduction was not statistically significant up to 60% replacement concentration of *Monascus purpureus* pigment instead of nitrite. A significant difference was observed between the levels of *L** between the control sample and treatments containing 80% and 100% *Monascus purpureus* pigments.

Figures 3 illustrates the effects of using different amounts of *Monascus purpureus* pigment instead of nitrite on changes in *a** index (red color) and *b** index (yellow color) in the formulation of German sausages. The results showed that by increasing the concentration of *Monascus purpureus* pigment instead of nitrite in the formulation of German sausages, the *a** and *b** index increased significantly. In other words, the highest rates of *a** and *b** indices were observed in the sample containing 100% *Monascus purpureus* pigment, which had a significant difference with the control sample (100% nitrite). According to the results, *a** index had a slight increase during the storage period in all treatments, which was not statistically significant. The *b** index had a slight decrease during the storage period in all treatments, which was not statistically significant.

Sensory evaluation (Overall acceptance): **Figure 4** represents results of the overall acceptance of sausage samples containing different concentrations of *Monascus purpureus* pigment instead of nitrite. The overall acceptance score of sausage samples containing different concentrations of *Monascus purpureus* pigment was slightly higher than the control sample, which was not statistically significant ($P \leq 0.05$). Therefore, no significant difference was observed between the overall acceptance score of samples containing different concentrations of *Monascus purpureus* pigment and the control sample. The results indicated that the overall acceptance score of the treatments showed a slight increase or decrease during the storage period, in which these changes were not statistically significant ($P > 0.05$) compared to the first day of production.

Table 1. Comparison of mean (±SD) of Thiobarbituric acid (mg MDA/Kg) of treatment groups

Treatments	Day 1	Day 10	Day 20	Day 30
100% Nitrite	0.58 ± 0.04^{aD}	0.90 ± 0.08^{aC}	1.38 ± 0.09^{aB}	2.00 ± 0.13^{aA}
20% *Monascus purpureus* + 80% Nitrite	0.57 ± 0.07^{aD}	0.90 ± 0.05^{aC}	1.39 ± 0.04^{aB}	2.05 ± 0.10^{aA}
40% *Monascus purpureus* + 60% Nitrite	0.59 ± 0.05^{aD}	0.94 ± 0.06^{aC}	1.42 ± 0.05^{aB}	2.08 ± 0.12^{aA}
60% *Monascus purpureus* + 40% Nitrite	0.58 ± 0.03^{aD}	0.97 ± 0.07^{aC}	1.46 ± 0.08^{aB}	2.15 ± 0.16^{aA}
80% *Monascus purpureus* + 20% Nitrite	0.61 ± 0.04^{aD}	1.16 ± 0.04^{bC}	1.59 ± 0.06^{bB}	2.32 ± 0.08^{bA}
100% *Monascus purpureus*	0.63 ± 0.05^{aD}	1.34 ± 0.09^{cC}	1.72 ± 0.03^{cB}	2.68 ± 0.17^{cA}

Small letters indicate a significant difference in each column and capital letters indicate a significant difference in each row.

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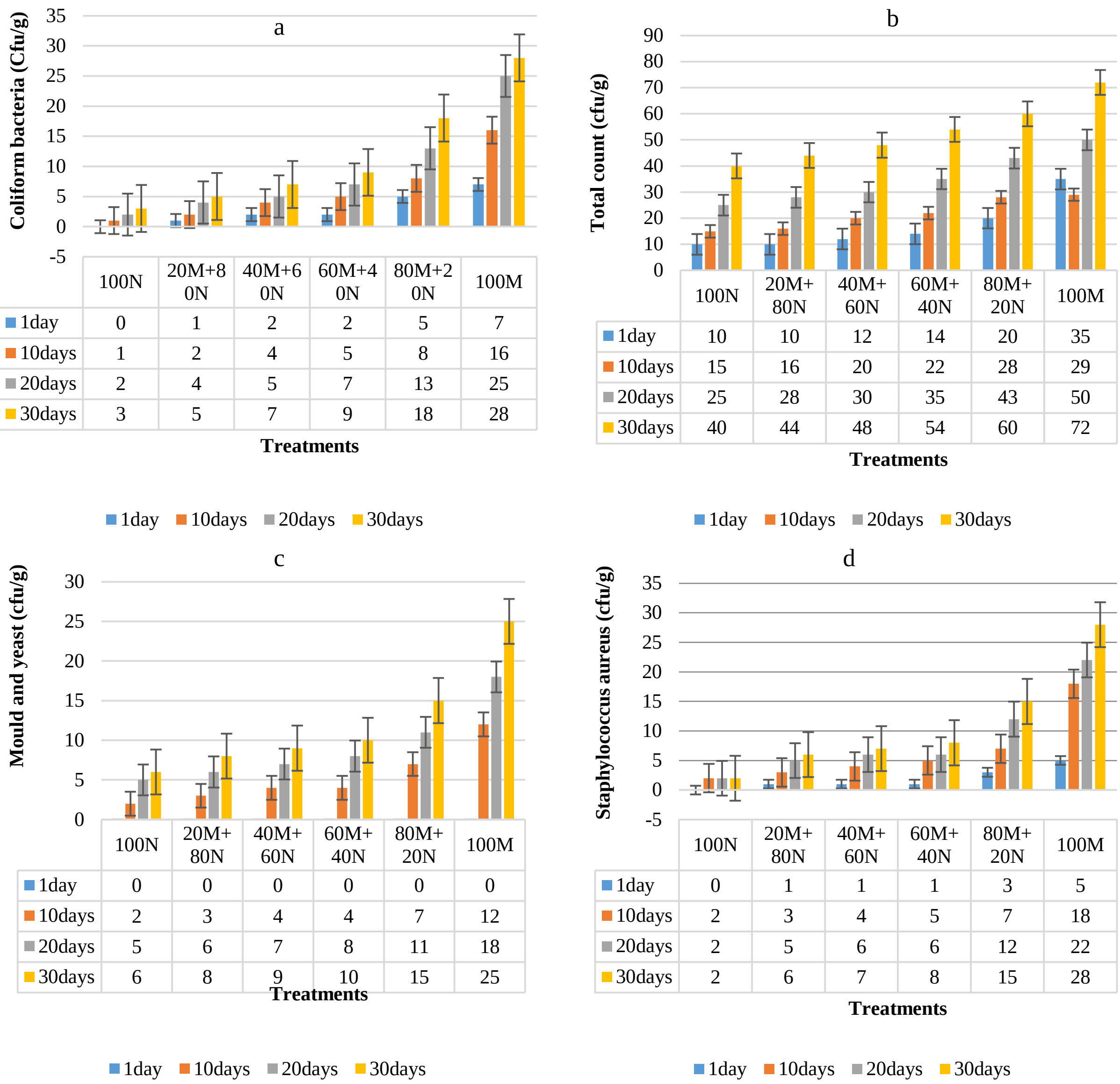

a

	100N	20M+80N	40M+60N	60M+40N	80M+20N	100M
1day	0	1	2	2	5	7
10days	1	2	4	5	8	16
20days	2	4	5	7	13	25
30days	3	5	7	9	18	28

b

	100N	20M+80N	40M+60N	60M+40N	80M+20N	100M
1day	10	10	12	14	20	35
10days	15	16	20	22	28	29
20days	25	28	30	35	43	50
30days	40	44	48	54	60	72

c

	100N	20M+80N	40M+60N	60M+40N	80M+20N	100M
1day	0	0	0	0	0	0
10days	2	3	4	4	7	12
20days	5	6	7	8	11	18
30days	6	8	9	10	15	25

d

	100N	20M+80N	40M+60N	60M+40N	80M+20N	100M
1day	0	1	1	1	3	5
10days	2	3	4	5	7	18
20days	2	5	6	6	12	22
30days	2	6	7	8	15	28

Figure 1. The results of microbial changes: a) coliform bacteria, b) total count c) mold and yeast d) *staphylococcus aureus* of German sausage with different levels of *Monascus purpureus pigment* and Nitrite during storage time

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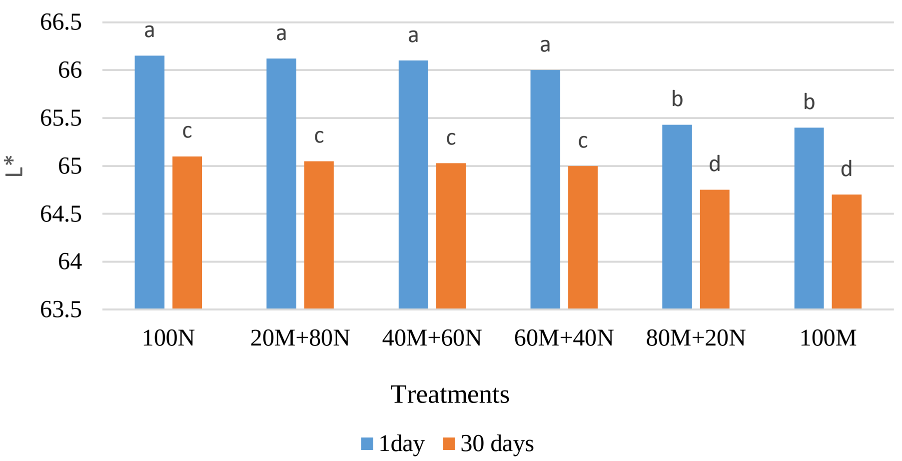

Figure 2. The results of the changes of L* in German sausage with different levels of *Monascus purpureus* pigment and Nitrite during storage time

*a** and *b** index

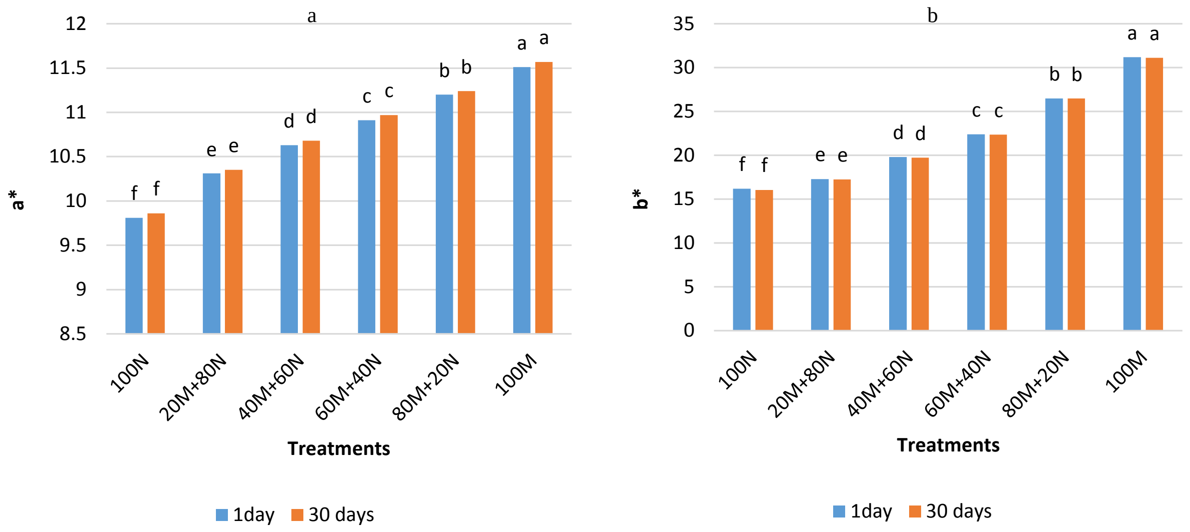

Figure 3. Average comparison under different levels of *Monascus purpureus* pigment on the average a* and b*

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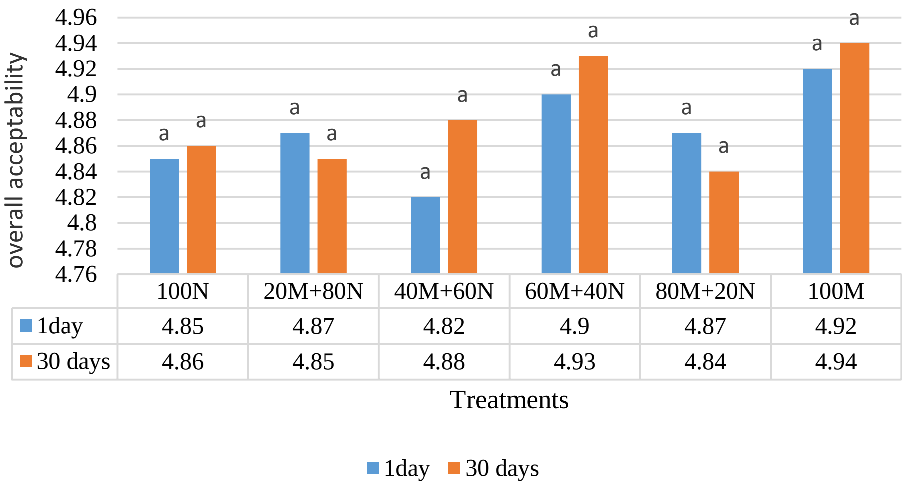

Figure 4. Average comparison different levels of *Monascus purpureus* pigment on the average of overall acceptability

Discussion

Replacement of nitrite up to 60% with *Monascus purpureus* pigment showed that the antioxidant properties were similar to the control sample. The presence of phenolic compounds in *Monascus purpureus* pigment could prevent the oxidative degradation of the treatments to some extent (Vatandoost *et al.*, 2012). (Maleki *et al.*, 2017) used celery extract as a substitute for nitrite in the formulation of chicken cocktail sausage and reported that the amount of thiobarbituric acid was increased by increasing the extract concentration and decreasing the nitrite level, which was attributed to the low content of phenolic compounds in the celery.

Jayawardana examined the effect of Adzuki bean extract on the cooked pork sausage and reported that the thiobarbituric acid index reduced significantly by increasing the amount of extract in the sausages from 0% to 0.3. (Jayawardana *et al.*, 2015) Qi reported that the thiobarbituric levels decreased with increasing the concentration of cedar seed shell extract in the sausage (Qi and Zhou, 2013).

Monascus purpureus is a plant with many nutritional and medicinal properties and has a very pleasant scent. The compounds in this plant and their properties were investigated in various studies with confirmed presence of antimicrobial compounds, such as phthalides (Vatandoost *et al.*, 2012). The highest amount of *coliforms* was observed after 30 days of storage in treatments containing 100% *Monascus purpureus* pigments. According to the National Iranian Standard (No. 2303) the maximum allowed coliforms in sausages should be less than 10 cfu. Therefore, the treatments' total amount of coliforms in which sodium nitrite was replaced with 0, 20, 40, and 60% *Monascus purpureus* pigments was within the acceptable standard range.

The highest total count was observed after 30 days of storage in treatments containing 100% *Monascus purpureus* pigments. According to National Iranian Standard (No. 2303), the maximum total number of microorganisms in sausages should be less than 10^5 cfu. Therefore, the total count of all treatments tested was within the acceptable standard range.

The highest amount of mold and yeast was observed after 30 days of storage in treatments containing 100% *Monascus purpureus* pigments. According to National Iranian Standard (No. 2303), the maximum mold and yeast in sausages should be less than 100 per gram. So, the amount of mold and yeast of all treatments was within the acceptable standard range.

The highest *staphylococcus aureus* was observed after 30 days of storage in treatments containing 100% *Monascus purpureus* pigments. According to the National Iranian Standard (No. 2303), the maximum permitted coagulase-positive staphylococcus aureus in sausages should be less than 10 cfu. As a result, the amount of coagulase-positive *staphylococcus aureus* was within the acceptable standard range in treatments that sodium nitrite was replaced with 0, 20, 40, and 60% *Monascus purpureus* pigments.

The results of this study indicated that the anti-microbial properties of *Monascus purpureus* up to 100% replacement with nitrite could prevent the total growth of bacteria, *clostridium perfringen*s, molds, and yeasts. Furthermore, their replacement up to 60% with nitrite could prevent the growth of *staphylococcus* aureus and coliforms. The antimicrobial properties of *Monascus purpureus* pigment could be attributed to the presence of anti-microbial compounds, such as the compounds in the phthalides group, especially ligustilide and flavonoids (Sagoo *et al.*, 2002).

No evidence showed the effect of natural preservative *Monascus purpureus* on the changes in microbial load of the studied cooked meat sausages. However, in many articles conducted on other meat products, especially variety of raw and fermented meat products, this effect was confirmed. In addition, the superior ability of chitosan in preventing the growth of bacterial, was proved in particular gram-positive bacteria (Darmadji and Izumimoto, 1994).

Maleki reported that the celery extract had an optimal antimicrobial effect and could replace up to 60% of the sodium nitrite used in meat cocktail sausages (Maleki *et al.*, 2017).

Based on the findings, replacement of nitrites with *Monascus purpureus* pigment resulted in a more than 60% reduction in *L** index. Some study reported that increasing the amount of beetroot powder in beef sausages decreased the *L** index (El-Gharably and Ashoush, 2011). On the other hand, Kim reported that increase of the wheat fiber dyed with pigments extracted from sunflower decreased L* index (Kim *et al.*, 2015). The findings showed that the storage time had a significant effect on the decrease of *L** index in all treatments. Maleki *et al*. observed that adding celery extract to chicken cocktail sausages decreased L* index significantly during the storage period (Maleki *et al.*, 2017).

It was reported that using olive leaf extract instead of nitrite in the formulation of meat mortadella, had more transparency than the control sample (containing nitrite). Furthermore, the degree of transparency in all samples decreased over time (Al Marazzeq *et al.*, 2015).

In this regard, increasing the concentration of *Monascus purpureus* pigment instead of nitrite in the formulation of German sausages increased the *a** and *b** indices significantly. In another study, cochineal and paprika natural dyes were used in Frankfurter sausages. Researchers reported that it is possible to produce Frankfurter sausages with a color similar to the control sample, without or with a low level of nitrite (Hoseinpoor *et al.*, 2013).

The results indicated that using *Monascus purpureus* pigment instead of nitrite in the formulation of German sausages did not have any significant effect on the overall acceptability. Qi and Zhou added different concentrations of Adzuki bean to pork sausages and reported that using different concentrations of Adzuki beans did not have any significant effect on the sensory properties of tested treatments, including smell (Qi and Zhou, 2013). De Almeida *et al.* reported no significant difference in the sensory properties of bologna sausage containing 0.5, 0.75, and 1% jabuticaba peel extract during 35 days of storage in terms of smell, taste, and texture compared to the control sample (de Almeida *et al.*, 2015). Seong reported that adding propolis extract to sausages had no negative effect on the sensory analysis of sausages. Moreover, the other studies showed that sausagesnwith 2 and 3% *Monascus* pigments had an off-flavor taste that ended in their crucially lower over-all acceptability ratings (Seong *et al.*, 2017).

Conclusion

In this research, *Monascus purpureus* pigment was added to the formulation of German sausage of 40% red meat instead of nitrite at levels of 20, 40, 60, 80, and 100%. Replacement of *Monascus purpureus* pigment with nitrite caused a slight increase in the thiobarbituric index during the storage period, which did not have a significant difference with the control sample up to 60% of replacement. The microbial load of all treatments was increased during the storage period. By replacement of *Monascus purpureus* pigment with nitrite, the transparency of all treatments (L*) reduced and the red (a*) and yellow (b*) colors increased. The findings showed that samples containing 60% *Monascus purpureus* pigment had no significant difference with the control samples in terms of antioxidant properties, microbial load, and sensory properties. Therefore, the amount of nitrite used in sausage with 40% meat can be reduced using *Monascus purpureus* pigment instead of nitrite up to 60% without having undesirable effects on its qualitative and sensory properties.

Conflict of interest

There was no conflict of interest in this study.

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Authors' Contribution

Nateghi L designed the study and guided the composition and analyses; Maleki Kahaki A conducted the experimental research and data analysis. Zarei F prepared the manuscript's first draft. Authors reviewed the paper and confirmed it.

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