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The Effect of Sodium Nitrite Replacement with Lycopene Pigment in German Sausage and Evaluation of Its Physicochemical, Antimicrobial and Sensory Properties

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ABSTRACT

Background: Meat products contain sodium nitrite. Consumer demand for natural preservatives instead of synthetic ones in foods has been growing in recent years because of their safety. Today, good quality meat products are produced worldwide, and in addition to desirable sensory properties, they are cheaper than fresh meat. Sausage with 40% red meat is one of these products which can partially meet the need for animal proteins. **Methods:** In this study, sodium nitrite was replaced by lycopene pigment at concentrations of 200, 400 and 600 ppm in 40% meat sausage. Physicochemical properties of sausage samples including moisture, protein, ash contents and pH value were measured according to National Standard. Microbial tests (*Clostridium*, mold, yeast and *Clostridium perfringens* count) were performed according to National Standards. The colorimetric lightness index (L*) of the sausage samples was measured. Sensory properties (smell and taste) were compared with control sample (40% meat sausage contain 120 ppm sodium nitrite) after 30 days of storage. Data were analyzed using Duncan's one-way analysis of variance at 95% confidence level through Minitab 16 software. **Results:** After 30 days of storage, no significant (P>0.05) difference was found in physicochemical properties, microbial tests, lightness index (L*), and sensory properties among the samples. **Conclusions:** Since no significant difference was observed in microbial load and sensory scores between sausage sample containing 600 ppm lycopene pigment and control sample, 600 ppm lycopene pigment can be used in sausage formulation.

Keywords: *Sodium Nitrite; Lycopene; Antibacterial agents; Antioxidant activity*

Introduction

Meat products are widely consumed today due to their desirable sensory properties and lower price compared to fresh meat. German sausage is among these products which may partially meet the need for animal proteins. Meat products contain nitrite. Sodium nitrite is a key component in processed products (Yaldagard *et al.*, 2008) which develops the specific flavor and smell of processed products and delays the development of taste and odor associated with rancidity via inhibiting lipid oxidation. It gives processed meat products a distinct pink color by reacting with

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myoglobin in meat producing nitroso-hemochrom (Krause *et al.*, 2011). Nitrite is also able to inhibit the growth of pathogenic bacteria, especially *Clostridium* species. However, despite the mentioned advantages, consumption of high amounts of sodium nitrite added to meat products is harmful to health. Moreover, nitric acid may be produced through hydration of nitric oxide by reduction of sodium nitrite (NANO2) (Hammes, 2012). When reacting with secondary amines and amino acids present in meat muscles, nitrite forms N-nitrous, especially nitrosamines. These compounds are important for their carcinogenic and mutagenic properties. These potential negative effects increase the tendency to replace and reduce nitrite (Hord *et al.*, 2009). The presence of nitrate and nitrite in foods causes methemo-globinemia in young children. The lethal amount of nitrate entering the human body by food is 330 mg/kg body weight , which is 10 times more toxic than nitrate (Esmaeilzadeh *et al.*, 2012). In Iran, permissible concentrations of nitrite and nitrate in meat products on the first day of production are 120 ppm and 500 ppm, respectively (Bahadoran *et al.*, 2016).

Unfortunately, reducing nitrite in meat emulsions, despite inhibiting the above-mentioned negative effects, promotes lipid oxidation reaction. It is one of the most destructive reactions which causes undesirable odor and taste and loss of pigments (hemoglobin and myoglobin) in meat products (Uddin *et al.*, 2021). The compounds resulting from oxidation reaction can react with oxygen at a high rate. The rate of reaction can be decreased by adding antioxidants (Da Young Lee *et al.*, 2021).

Lycopene is a red pigment found in many fruits and vegetables. It is a powerful antioxidant which reduces oxidative damage to DNA, thereby decreasing the incidence of cancers such as prostate and breast cancers. Lycopene can be a good substitute for sodium nitrite (Gassara *et al.*, 2016). Sausage is a stable mixture of animal meat (Halal), fat, water, and permitted additives covered with natural or synthetic coatings under appropriate conditions and is submitted to proper heat and other processes. In any case, the main component used in the production of sausages, quantitatively and qualitatively, is meat. Technologically, these products are classified into four categories: heated, raw, cooked, and processed meats (Karim *et al.*, 2021). Lycopene can play an antimicrobial role due to its antioxidant activity. It is one of the popular pigments highly accepted by food industry as an additive and regarding its health benefits (Kim *et al.*, 2015). Nateghi demonstrated that the replacement of *Monascus purpureus* pigment with nitrite in 40% meat sausage resulted in a superior treatment due to its antioxidant and microbial properties (Nateghi *et al.*, 2020). This study aims to evaluate the replacement of sodium nitrite with lycopene pigment in German sausage with 40% meat and measure its physicochemical, colorimetric, antimicrobial, and sensory properties.

Materials and Methods

Design and setting: Red meat was purchased from a store. Liquid oil (Golbahar-e-Isfahan, Iran), wheat flour (Derakhshan-e-Qom, Iran), Soybean (Khsok bar, Tasty, Iran), gluten (Ardineh-e-Isfahan, Iran), spices (Naderi, Iran), salt (Delchasb, Iran), garlic (khoshkbar, ordinary, Iran), sodium phosphate (stpp, Iran), sodium nitrate (pouya shimi-e-Hegmatan, Iran), lycopene pigment (Mycology Department of Isfahan University of Medical Sciences, Iran) and other chemicals (Merck, Germany) were also purchased. Sausage with 40% red meat was produced according to the standard formulation used for Pardis meat products presented in **Table 1**. The samples filled in casing were transferred to the cooking room where the temperature of the product center reached 70-72 °C for 1 h and was kept at the same temperature for about 15 minutes. The product temperature was then rapidly reduced using a cold shower and immediately transferred to a cold store above 0 °C (0-4 °C). Sausage samples were produced with 5 replications. The samples were refrigerated (4 °C) for 30 days and their physicochemical, microbial, colorimetric and sensory properties were assessed.

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Table 1. Sausage formulation and components.

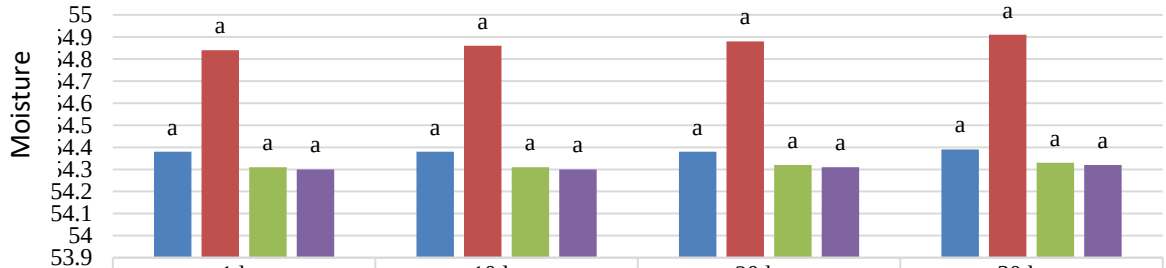
Treatments	Chicken Meat (g)	Oil (g)	Wheat Flour(g)	Soybean (g)	Gluten (g)	Ice (g)	Spices (g)	Garlic (g)	NaCl (g)	Sodium Phosphate(g)	Sodium Nitrite(ppm)	Lycopene Pigment(ppm)
T_1 (Control)	40	6.5	4	2	1	14.8	0.6	0.4	0.5	0.2	120	0
T_2	40	6.5	4	2	1	14.8	0.6	0.4	0.5	0.2	0	200
T_3	40	6.5	4	2	1	14.8	0.6	0.4	0.5	0.2	0	400
T_4	40	6.5	4	2	1	14.8	0.6	0.4	0.5	0.2	0	600

Measurements: Physicochemical properties of sausage samples including moisture, protein, ash contents, and pH value were measured according to Iran National Standard No. 2303 (Riazi *et al.*, 2016). Microbial tests were performed according to Iran National Standards No. 1-10899 and 2197 (Microbiology of Food and Feed). According to this standard, the presence or absence of Clostridium, mold, yeast and *Clostridium perfringens (C. perfringens)* count were measured (Riazi *et al.*, 2016). The lightness index (L*) of the sausage samples was measured by Hunter lab colorimeter (Model D25/DP9000, Hunter Lab Co., USA) (Hemmati *et al.*, 2021). Sensory properties of the samples were measured by 15 semi-trained panelists using the 8-point hedonic scale. Smell and taste of the sausage samples after 30 days of storage were evaluated as excellent (score 8), good (score 6), neither good or bad (score 4), bad (score 2) and very bad (score 0).

Data analysis: The data were analyzed using Duncan's one-way analysis of variance at 95% confidence level through Minitab 16 software.

Results

The results of changes in the moisture of sausage samples are shown in **Figure 1**. The moisture content of all treatments decreased slightly during storage, showing no significant (P>0.05) difference. The decrease could be the result of vacuum sealing because vacuum pressure applied to the treatment expelled some of the remaining water over time. After 30 days of storage, the highest (54.91%) and the lowest (54.32%) moisture contents were observed for the treatments containing 200 ppm and 600 ppm lycopene pigment, , showed no significant (P>0.05) difference. According to National Standard No. 2303, the maximum moisture content of sausages with 40% meat is 57%.



	1day	10days	20days	30days
0ppm	54.38	54.38	54.38	54.39
200ppm	54.84	54.86	54.88	54.91
400ppm	54.31	54.31	54.32	54.33
600ppm	54.3	54.3	54.31	54.32

Figure 1. Changes in moisture of sausage samples during 30 days of storage. Different lowercase letters indicate significant differences.

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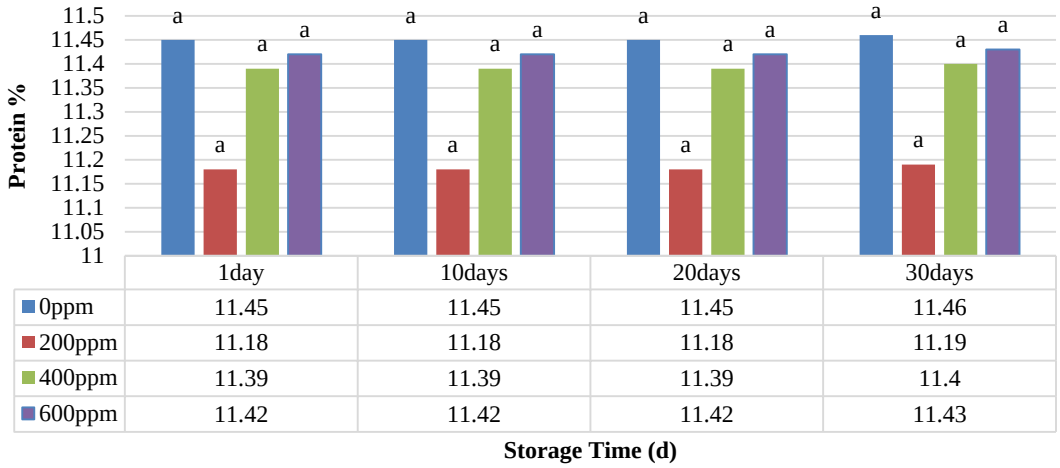
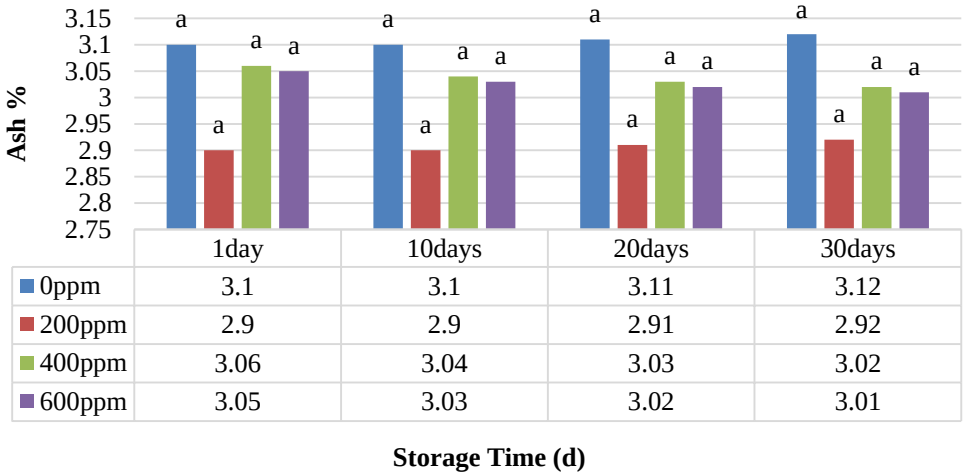
The results of changes in the protein content of sausage samples are shown in **Figure 2**. According to Standard No. 2303, the minimum amount of protein in products with 40% red meat is 10%; therefore, the protein content of all the treatments was within the acceptable standard range. The results revealed that after 30 days of storage, the highest (11.43%) and the lowest (11.19%) (following control sample) amount of protein contents were found in the treatments containing 200 ppm and 600 ppm lycopene pigment, respectively showing no significant (P>0.05) difference. The protein content of all treatments did not change significantly over storage.



	1day	10days	20days	30days
0ppm	11.45	11.45	11.45	11.46
200ppm	11.18	11.18	11.18	11.19
400ppm	11.39	11.39	11.39	11.4
600ppm	11.42	11.42	11.42	11.43

Figure 2. Changes in protein of sausage samples during 30 days of storage. Different lowercase letters indicate significant differences.

The results of changes in ash content of sausage samples are shown in **Figure 3**. According to Iran National Standard No. 2303, the maximum amount of ash in products with 40% red meat is 3.2%. During storage, the ash content showed increasing or decreasing trends for different treatments. The observed changes were not significant (P>0.05). After 30 days of storage, the lowest (2.92%) and highest ash contents (3.12%) were found for the treatment containing 200 ppm lycopene pigment and control sample, respectively.



	1day	10days	20days	30days
0ppm	3.1	3.1	3.11	3.12
200ppm	2.9	2.9	2.91	2.92
400ppm	3.06	3.04	3.03	3.02
600ppm	3.05	3.03	3.02	3.01

Figure 3. Changes in ash content of sausage samples during 30 days of storage. Different lowercase letters indicate significant differences.

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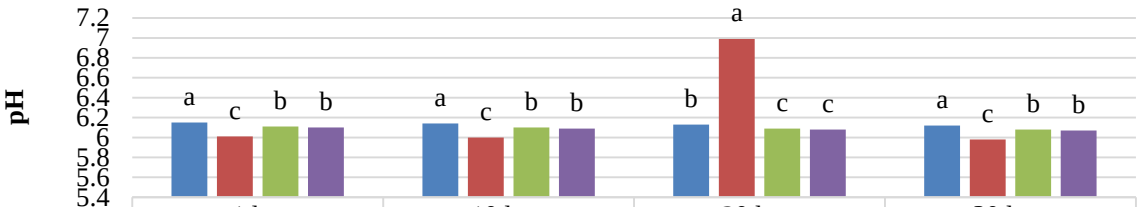
The results of changes in pH value of sausage samples are shown in **Figure 4**. According to National Standard No. 2303, the pH of sausage samples containing 40% red meat is 3.6-6.5. Therefore, pH value of all sausage samples after 30 days of storage was in an acceptable range. After 30 days of storage, the lowest (5.98) and highest (6.12) pH values were found for the treatment containing 200 ppm lycopene pigment and control sample, respectively. One of the factors affecting the pH of sausages is smoking before packaging.

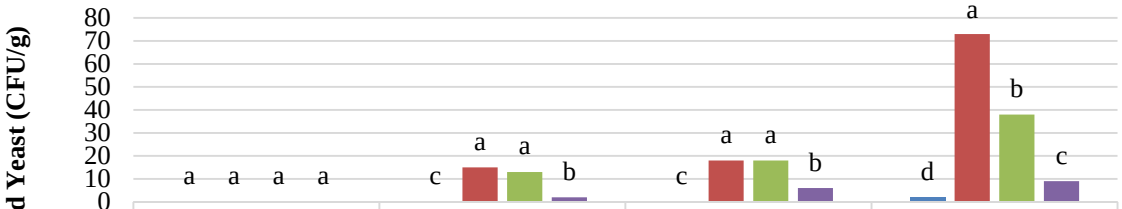


	1day	10days	20days	30days
0ppm	6.15	6.14	6.13	6.12
200ppm	6.01	6	6.99	5.98
400ppm	6.11	6.1	6.09	6.08
600ppm	6.1	6.09	6.08	6.07

Storage Time (d)

Figure 4. Changes in the pH of sausage samples during 30 days of storage. Different lowercase letters indicate significant differences.

The results of changes in different levels of lycopene pigment and time regarding mold and yeast count in the sausage samples are shown in **Figure 5**. The results showed that on the first day of production no mold and yeast grew in all treatments. On days 10 and 20, no significant (P>0.05) difference was observed in mold and yeast count between the treatments containing 200 and 400 ppm lycopene pigments. With increasing the concentration of lycopene pigment, mold and yeast count decreased significantly. After 30 days of storage, the lowest growth rate of mold and yeast (following control sample) was observed for the treatment containing 600 ppm lycopene pigment. According to National Standard No. 2303, maximum growth limit for mold and yeast in meat products is 100 CFU/g, so mold and yeast count in all treatments was within the standard range.



	1day	10days	20days	30days
oppm	0	0	0	2
200ppm	0	15	18	73
400ppm	0	13	18	38
600ppm	0	2	6	9

Storage Time (d)

Figure 5. Interactive effects of lycopene pigment and time on mold and yeast count in sausage samples during 30 days of storage. Different lowercase letters indicate significant differences.

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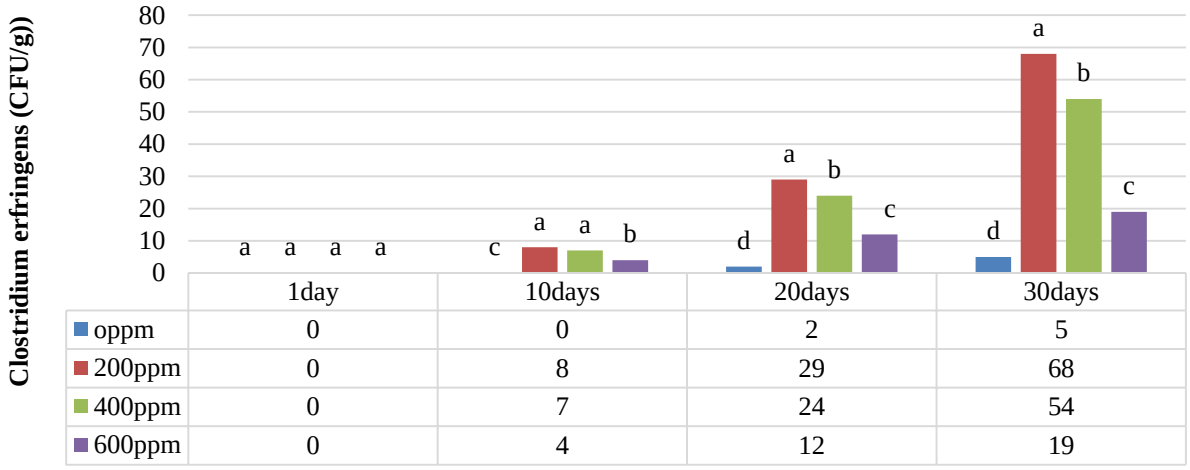
The results of changes in different levels of lycopene pigment and time with regard to *C. perfringens* count in sausage samples are shown in Figure 5. On the first day of production, no *C. perfringens* grew. On days 10 and 20, no significant (P>0.05) difference was observed in *C. perfringens* concerning the treatments with 200 ppm and 400 ppm lycopene pigment. According to the results, with increasing concentration of lycopene pigment, *C. perfringens* count decreased significantly as well; this might be due to antimicrobial properties of lycopene pigment. *C. perfringens* count showed an increasing trend in all treatments from day 1 to day 30, as after 30 days of storage, the lowest growth rate of *C. pefringens* (19 CFU/g) (following control sample) was found for the sausage sample containing 600 ppm lycopene pigment. According to National Standard No. 2303, the maximum growth limit of *C. perfringens* in meat products is 50 CFU/g. Therefore *C. perfringens* count on day 30 of all the treatments was within the national standard acceptable range except the sausage samples containing 200 and 400 ppm pigment lycopene. *C. perfringens* is a fastidious microorganism; i.e. it has complicated environmental requirements including humidity, low oxygen content, and sufficient nutrients. The treatment containing 200 ppm lycopene met all the requirements, and therefore, *C. perfringens* count exceeded the acceptable range.



	1day	10days	20days	30days
oppm	0	0	2	5
200ppm	0	8	29	68
400ppm	0	7	24	54
600ppm	0	4	12	19

Figure 6. Interactive effects of lycopene pigment and time on *C. perfringens* count in sausage samples during 30 days of storage. Different lowercase letters indicate significant differences.

The results of changes in lycopene pigment levels and time with regard to the L* value of sausage samples are shown in **Figure 7**. The highest L* index was observed for sausage samples on the first day of storage. L* index of all treatments decreased significantly (P<0.05) during storage time. The use of lycopene and its increased concentration in sausage samples decreased the L* of samples; therefore, at the end of 30 days of storage, the highest and lowest L* values were found for control sample and the one containing 600 ppm lycopene pigment, respectively. The reason was the presence of pigments in the sausage sample which reduced lightness.

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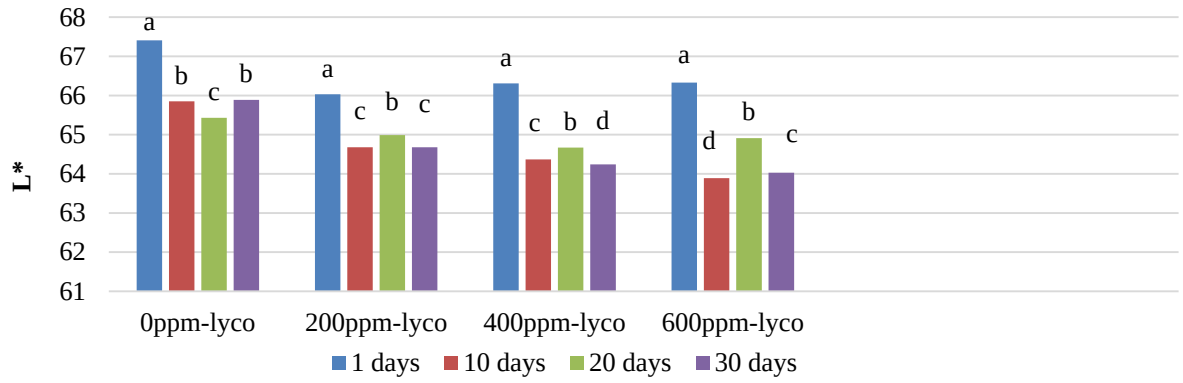

Figure 7. Interactive effects of lycopene levels and time on L* value of sausage samples during 30 days of storage. Different lowercase letters indicate significant differences.

The results of changes in taste and smell of sausage samples after 30 days of storage are shown in **Table 2**. The highest (7.57) and lowest scores (6.01) for taste and smell were given to control sample with 120 ppm sodium nitrite and the sample with 200 ppm lycopene pigment, respectively.

The results of changes in the total acceptance score of sausage samples are shown in **Table 3**. The highest (7.47) and lowest (6.10) overall acceptance scores were given to control sample containing 120 ppm sodium nitrite and the sausage sample containing 200 ppm lycopene pigment.

Table 2. Effect of storage time on taste and smell of sausage samples during 30 days of storage.

Sausage samples	Taste and smell (score)
ppm 120 sodium nitrite	7.54 ± 0.84^{a}
ppm 200 lycopene pigment	6.01 ± 0.57^{b}
ppm 400 lycopene pigment	6.28 ± 0.96^{ab}
ppm 600 lycopene pigment	7.18 ± 0.77^{ab}

The results are shown as mean±SD; Lowercase letters indicate significant differences; Different uppercase letters indicate significant differences in each line.

Table 3. Effect of storage time on total acceptance of sausage samples during 30 days of storage.

Sausage samples	Total acceptance score)
ppm 120 sodium nitrite	7.47 ± 0.51^{a}
ppm 200 lycopene pigment	6.10 ± 0.43^{b}
ppm 400 lycopene pigment	6.58 ± 0.26^{b}
ppm 600 lycopene pigment	7.40 ± 0.65^{a}

The results are shown as mean±SD; Lowercase letters indicate significant differences; Different uppercase letters indicate significant differences in each line

Discussion

The results of changes in the moisture of sausage samples are shown in **Figure 1**. The moisture content of all treatments decreased slightly during storage, showing no significant (P>0.05) difference. The decrease could be the result of vacuum sealing, because the vacuum pressure applied to the treatment expelled some of the remaining water over time. After 30 days, the highest (54.91%) and lowest (54.32%) moisture contents were observed for the treatments containing 200 ppm and 600 ppm lycopene pigment with no significant (P>0.05) difference. According to National Standard No. 2303, the maximum moisture content of sausages with 40% red meat is 57% (Krause *et al.*, 2011). Therefore, the moisture content of the studied sausage samples was slightly lower than the standard range. (Kamkar *et al.*, 2005) examined physicochemical properties of sausages and reported that the moisture content of sausage with 51-60% red meat was 38.2% higher than the acceptable range. (Yaghoubifar *et al.*, 2009) compared the quality and safety of sausages marketed in Sabzevar and showed that moisture content of sausage samples was 29.7% higher than the Iranian acceptable range. Changes in moisture content of sausage samples during the storage period can be caused by the type of sausage formulation and wrapping(Qi and Zhou, 2013).

The results of changes in pH value of sausage samples are shown in **Figure 4**. According to

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National Standard No. 2303, the pH of sausage samples with 40% of red meat is 3.6-6.5 (Nateghi *et al.*, 2020). Therefore, the pH value of all sausage samples after 30 days of storage was in the standard range. After storage, the lowest (5.98) and highest (6.12) pH values were related to the treatment containing 200 ppm lycopene pigment the and control sample. One of the factors affecting the pH of sausages is smoking before packaging. Smoke contains acidic compounds; so, it can change the pH of sausage samples. The reason for pH changes during storage may be growth and proliferation of microorganisms which break down and utilize the nutrients contained in the medium. On the other hand, reactions such as the breakdown of proteins, fats, etc. can also affect changes in pH levels (Nazemi *et al.*, 2011). The results of this study were in agreement with the findings obtained by other researchers (Ferysiuk and Wójciak, 2020). The results of changes in the levels of lycopene pigment and time regarding mold and yeast count in the sausage samples are shown in **Figure 5**. Accordingly, on the first day of production, no mold and yeast grew in all treatments. On days 10 and 20, no significant (P>0.05) difference was observed in mold and yeast count between the treatments with 200 and 400 ppm lycopene pigments. Following the increase in concentration of lycopene pigment, mold and yeast count decreased significantly. After 30 days of storage, the lowest growth rate of mold and yeast (following control sample) was observed for the treatment containing 600 ppm lycopene pigment. According to National Standard No. 2303, the maximum growth limit for mold and yeast in meat products is 100 CFU/g (Nateghi *et al.*, 2020). Therefore, mold and yeast count in all treatments was within the acceptable range; this was because of the presence of preservatives and antimicrobial compounds in lycopene pigment (Britannica, 2009, Qi and Zhou, 2013). On the other hand, smoking can be one of the most effective factors in reducing mold and yeast count (Maktabi *et al.*, 2016). Longer time of hot smoke can kill higher number of fungi and microorganisms. It also leads to more water loss, thereby decreasing moisture level for microorganisms to grow and survive (Hong *et al.*, 2008). In addition, smoke contains antimicrobial substances such as phenols, aldehydes, etc., which affects the growth of microorganisms (Vatandoost *et al.*, 2012). The results of changes in different levels of lycopene pigment and time regarding *C. perfringens* count in samples are shown in **Figure 5**. On the first day of production, no *C. perfringens* grew, and on days 10 and 20, no significant (P>0.05) difference was observed in *C. perfringens* regarding the treatments containing 200 ppm and 400 ppm lycopene pigment. After increase in concentration of lycopene pigment, *C. perfringens* count decreased significantly, maybe due to the antimicrobial properties of lycopene pigment (Deda *et al.*, 2007). *C. perfringens* count showed an increasing trend in all treatments from day 1 to day 30; after 30 days, the lowest growth rate of *C. perfringens* (19 CFU/g) (following control sample) was found for the sausage sample containing 600 ppm lycopene pigment. According to National Standard No. 2303, maximum growth limit of *C. perfringens* in meat products is 50 CFU/g (Nateghi *et al.*, 2020). Therefore *C. perfringens* count was within the acceptable range on day 30 in all treatments except sausage samples with 200 and 400 ppm pigment lycopene. The results of changes in taste and smell of sausage samples after 30 days of storage are shown in **Table 2**. The highest and lowest scores for taste and smell were given to the control sample with 120 ppm sodium nitrite and the sample with 200 ppm lycopene pigment. Due to the fact that sodium nitrite gives the sausage its distinct and desirable aroma, it had a higher score of taste and smell compared with other treatments (Gassara *et al.*, 2016). The reason for lower scores of taste and smell associated with lower lycopene content of sausage samples could be the increased growth and proliferation of microorganisms in these samples. The growth of anaerobic microorganisms as well as microorganisms such as *Lactobacilli* gives the treatments a bad taste. In addition, chemical reactions in the product are taken into account. Reactions such as the breakdown of proteins and

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fats change the taste of treatments (Wójciak *et al.*, 2019). The reason for lower scores of 200 and 400 ppm treatments compared to those containing 600 ppm might be the small amount of the extract that caused the reactions to occur earlier in the product. The treatment containing 600 ppm lycopene extract was given the highest scores for taste and odor following the control sample. The results of changes in the total acceptance score of sausage samples after 30 days of storage are shown in **Table 3**. The highest and the lowest overall acceptance scores belonged to the control sample with 120 ppm sodium nitrite pigment and the sausage sample with 200 ppm lycopene pigment. Because sodium nitrite prevents oxidative spoilage and unpleasant taste development and the subsequent loss of desirable pigments in meat products (Kim *et al.*, 2015), the control sample was given the highest overall acceptance score followed by the sample containing ppm 600 lycopene pigments; this could prevent the development of microorganisms as well as fat oxidation and improve the color of sausage samples (Esmaeilzadeh *et al.*, 2012).

Conclusion

The aim of this study was to replace sodium nitrite with 200, 400 and 600 ppm natural lycopene pigment in the formulation of sausage with 40% red meat and compare it with the control sample (120 ppm sodium nitrite). The use of lycopene pigment and increase in its concentration has no significant effect on physicochemical properties (moisture, protein and ash). It has a significant effect on reduction in mold, yeast, and *C. perfringens* count and increase in taste, smell, and overall acceptance scores. Since no significant difference was observed in sensory properties between sausage samples containing 600 ppm lycopene pigment and the control one, lycopene at the concentration of 600 ppm can be used in place of sodium nitrite without any adverse effect on sensory properties.

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Authors' contribution

Nateghi L designed the study, prepared the manuscript's first draft and guided the composition, Zarei F conducted the experimental research; Pahlevan Afshari K Performed data analysis. All Authors reviewed the paper and confirmed it.

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Nothing

Conflict of interest

There was no any conflict of interest in this study.

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