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Investigating the Possibility of Producing Nitrite-Free German Sausages Containing Different Concentrations of Cochineal Pigment Alone or in Combination with Cumin Essential Oil

*Leila Nateghi; PhD**[1]

[1] *Department of Food Science and Technology, Facult of Agriculture, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran.*

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Corresponding author:
l.nathegi@iauvaramin.ac.ir
Department of Food Science and Technology, School of Agriculture, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran.

Postal code: 74895-33817
Tel: +98-9125878775

ABSTRACT

Background: Many of the most common cancers, such as prostate and breast cancer are due to the presence of harmful agents and compounds in processed foods, including nitrite. In this research the physicochemical, antioxidant, and color properties of sodium nitrite-free sausages containing different concentrations of cochineal pigment alone or in combination with cumin essential oil were studied and compared with the control sample containing sodium nitrite. **Methods**: The extract of cochineal pigment in concentrations 200 ppm, 400 ppm, and 600 ppm alone or with 0.004 % w/w of essential oil of cumin were added to 40% red meat German sausage and physico-chemical, antioxidant, and color changes of the samples during 1, 10, 20, and 30 days after production (in refrigerator conditions) were compared with the control (without cochineal pigment, essential oil of cumin, and 120 ppm sodium nitrite) sample. **Results**: The findings of the study showed that this substitution did not have a significant (P > 0.05) effect on physico-chemical properties, and by increasing the concentration of cochineal pigment in the samples, the antioxidant properties of the samples significantly increased. The samples containing 400 or 600 ppm of cochineal pigment with 0.004 %w/w of cumin in the formulation were not significantly different from control sample in terms of antioxidant properties and were selected as superior treatment. **Conclusion**: It is possible to produce a healthy German sausage without nitrite by using cochineal pigment and cumin essential oil.

Keywords: *Cochineal pigment; Cumin essential oil; Red Mmat; Sausage; Sodium nitrite*

Introduction

Sausages are a type of ready-to-eat food consisting of minced meat (beef, veal, lamb, and chicken), fat, water, preservatives, and spices. They are stuffed into natural or artificial casings and are prepared for human consumption after thermal process. German sausage is a type of sausage containing 40-50% of meat (Institute of Standard and Industrial Research of Iran, 2005).

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Today, preservatives play an important role in producing healthy and ready-to-eat foods. Increasing demand for ready-to-eat foods as well as long storage of processed foods have made the use of chemical preservatives in foods inevitable (Seong *et al.*, 2017). Nitrite and sodium nitrite are used as preservatives against clostridium and other pathogenic foodborne bacteria.

In accordance with the national standard of Iran No. 2303, the permissible limit in meat products is 120 ppm (Institute of Standard and Industrial Research of Iran, 2005). There are many reports on the effect of nitrite on human health. Nitrite may react with nitrosatable compounds in the stomach, such as secondary or tertiary amines or amides in foods. It has been demonstrated that 85% of the 209 nitrosamines and 92% of the 86 nitrosamines are carcinogenic. Most nitrosamines cause liver cancer, while some of them cause cancer in certain organs (bladder, lungs, esophagus, nasal cavities, etc.). Exposure to nitrosamines has been associated with increased risk of esophagus, stomach, and bladder cancers (Sharmila *et al.*, 2013). Generally, meat is processed with compounds based on salt, sugar, and other sweeteners as well as nitrite to develop the specific flavor and texture of the processed meat products. Nitrite is converted to nitric oxide and develops the desired color of the meat products (Kim *et al.*, 2015). However, the high amount of nitrite in meat products is harmful to health. Hydration of nitrous oxide may produce nitrous acid which reacts with secondary amines and amino acids and is converted to nitrous form especially to nitrosamine, which causes cancer (Vatandoost *et al.*, 2012). Chang et al. (2020) reported that in order to reduce nitrite consumption in sausages, in industrial conditions, beta-carotene, and cochineal pigments were used as alternatives in red meat (Chang *et al.*, 2020).

The cochineal is an insect in the suborder *Sternorrhyncha*, from which the natural dye carmine is derived. It is native to tropical and subtropical regions of South America feeding on dark opuntia cacti and the natural color carmine used in foods (Maaza, 2014). Cochineal dye has been recognized as safe by the FDA and is used in the food industry (Chattopadhyay *et al.*, 2008). Economically viable natural pigments of plant origin may be cultivated in farm land and saffron and cochineal (Jamora and Rhee, 2002).

Water-soluble form of carmine is calcium carmine used in alcoholic beverages and insoluble form is used in a wide range of products along with carmine ammonium found in processed meat, sausage, and chicken. Since the addition of preservatives is one of the methods of food preservation, it has long been considered by experts in the food industry. Given the increasing use of natural preservatives, many scientific studies have been conducted on natural products, including plant essential oil (Chen and Hoover, 2003). Cumin is an annual, small, herbaceous plant with a height of about 60 cm that grows in Iran, Turkey, India, and China. Cumin essential oil contains pinene, alpha-terpineol, apigenin flavonoids, and etc. It also has antimicrobial properties (Moradi *et al.*, 2012).

The present study aims to produce sodium nitrite-free German sausages containing different concentrations of cochineal pigment alone or in combination with cumin essential oil and compare with the control sample containing sodium nitrite during 30 days of storage in the refrigerator.

Materials and Methods

Ingredients for preparing sausage samples included red meat (Brazil), cochineal pigment (Kristin Hansen, Denmark), cumin essential oil (Adonis, Iran), liquid oil (Golbahar, Iran), wheat flour (Derakhshan, Iran), salt, garlic powder and soybeans (Tasty, Iran), gluten (Ardineh, Iran), spices (Naderi, Iran), sodium phosphate (Stupipi, China), and sodium nitrite (Pouya Shimi Hegmatan, Iran), which were purchased from listed companies.

Preparation of sausage samples: In order to produce 50 kg of control German sausages, 20 kg of red meat, 14.8 kg of ice, 6.5 kg of liquid oil, 4 kg of wheat flour, 2 kg of soybeans, 1 kg of gluten, 0.6 kg of spices, 0.5 kg of NaCl, 0.4 kg of garlic powder, and 0.2 kg of sodium phosphate were mixed in a cutter (Alfina, Germany). Then, 120 ppm sodium nitrite was added to the mixture. To prepare the tested treatments, cochineal pigment at concentrations of 200, 400, and 600 ppm alone or with 2 ml cumin essential oil (0.004% w/w) were added to the treatments based on the total weight of sausage mixture in the control formulation (**Table 1**). The samples were transferred to the cooking room after filling in the casings. In the cooking room for 1 hour, the temperature of the center of the product reached 70-72 ºC and was kept at this temperature about 15 min. The temperature of the product was then rapidly reduced with a cold shower and immediately transferred to a cold store (0-4 °C).

Physicochemical tests: Physicochemical tests, including moisture, protein, fat, and ash content were measured according to Iranian National Standard of Sausage, specifications, and test methods (Institute of Standard and Industrial Research of Iran, 2005).

Lipid oxidation test by thiobarbituric acid (TBA) method: Lipid oxidation of sausage samples was performed by thiobarbituric acid method. Ten g of the sample was weighed and mixed with 50 ml of distilled water. The resulting mixture was transferred to distillation erlenmeyer with 47.5 ml of distilled water. Then, 2.5 ml of hydrochloric acid 4 N with anti-foam and anti-boiling agents were added to the mixture and Erlenmeyer was added to the distillation apparatus. Fifty ml of distillate mixture was collected. After that, 5 ml of distilled material and 5 ml of TBA reagent were transferred to "closed" tubes and placed in boiling water for exactly 35 min after complete shaking. Simultaneously, all of these steps were repeated for the control sample. The samples were cooled for 10 min after 35 min boiling then light absorption (Model GT211-NV203 Nv20 (Ningbo Mflab, USA) was read at 538 wave length by spectrophotometer. The amount of thiobarbituric acid per kg of sample was calculated based on mg of malondialdehyde (MDA) (Maghsoudlou *et al.*, 2013).

TBA = Optical density×7.8 (mg of MDA/kg)

Evaluation of the color of the samples: Color measurement by digital photography was performed in a box with dimensions 50 × 50 × 60 (Length, width and height) under controlled conditions. The samples were cut transversely from the middle by two connected blades at a fixed distance, making all the tested samples equal in diameter. The cut pieces were then placed in a special glass cup, so that when they were placed at the location of the device sample, the area was completely covered and there were no holes. Then a black matte container called a light trap was placed on the cup to prevent outside light from interfering. Then, by pressing the reading key of the CR-400 model (Konica Minolta, Japan), the color of the samples was shown as numbers related to L*, a* and b* (Viuda-Martos *et al.*, 2009).

Data analysis: In this study, a completely randomized design was used to design the treatments, so 7 treatments were designed and the test results were evaluated on days 1, 10, 20, and 30 after production with 3 replications. Duncan test was also used to compare the mean data by Minitab 16 software and excel software was used to draw the graphs. A P-value less than 0.05 was considered significant.

Results

The effect of formulation on moisture changes in German sausages: The results of moisture changes in sausage samples are shown in **Table 2**. According to the results, the effect of storage time on moisture changes in German sausages was not significant ($P > 0.05$), but the replacement of nitrite with cochineal pigment and cumin essential oil had a significant ($P \leq 0.05$) effect on moisture content. The highest amount of moisture (55.99%) after 30 days of storage was observed in treatment T6 (40% red meat sausage + 400 ppm cochineal pigment + 2 ml of cumin essential oil), and the lowest was observed in the control sample.

Effect of formulation on protein changes in German sausages: The results of protein changes in sausage samples are shown in **Table 3**. According to the results, the effect of storage time on protein changes was not significant ($P > 0.05$). The replacement of nitrite with cochineal pigment and cumin essential oil did not have a significant (P > 0.05) effect on protein content.

The effect of formulation on the rate of fat changes in German sausages: The results of fat changes in sausage samples are shown in **Table 4**. According to the results, a slight decrease in fat content was observed during storage time in all the treatments, which was not statistically significant (P > 0.05), it should be noted this reduction was milder in the control sample and samples containing cumin essential oil with higher concentrations of cochineal pigment. This reduction may be due to lipid oxidation, which is less in control sample and samples containing cumin essential oil with higher concentrations of cochineal pigment.

After 30 days of storage, the lowest (23.11) and the highest (23.21) amount of fat were observed in the treatments T2 (40% red meat sausage + 200 ppm cochineal pigment), and T7 (40% red meat sausage + 600 ppm cochineal pigment + 2 ml of cumin essential oil), respectively.

The effect of formulation on the rate of change of German sausage ash: The results of ash changes of sausage samples are shown in **Table 5**. According to the results, the effect of nitrite replacement with cochineal pigment and cumin essential oil in German sausage and storage time did not have a significant effect (P > 0.05) on ash content.

The effect of formulation on changes in lipid oxidation of German sausage: The results of lipid oxidation experiments by thiobarbituric acid of sausage samples are shown in **Table 6**. According to the results, lipid oxidation significantly (P ≤ 0.05) increased by increasing storage time.

Replacement of nitrite with cochineal pigment and cumin essential oil also had significant (P ≤ 0.05) effect on lipid oxidation. The results showed that the highest and the lowest amount of thiobarbituric acid in all days were belonged to treatments T2 (40% red meat sausage + 200 ppm cochineal pigment) and treatments T7 (40% red meat sausage + 600 ppm cochineal pigment + 2 ml cumin essential oil), respectively.

The effect of formulation on the color changes (L, a* and b*) of German sausage:* According to the results (**Table 7**), replacement of nitrite with cochineal pigment significantly (P ≤ 0.05) decreased L* color index. The amount of L* in samples with the same amount of cochineal pigment, which contained cumin essential oil was significantly (P ≤ 0.05) higher. The reason for more L* index in the samples containing cumin essential oils can be due to the reduction of oxidation in the mentioned samples.

The L* index decreased significantly in all treatments during the storage time. This reduction may be due to lipid oxidation of the treatments during storage. After 30 days of storage, the highest and the lowest L*index was observed in T5 (sausage 40% red meat + 200 ppm cochineal pigment + 2 ml cumin essential oil) and T4 (sausage 40% red meat + 600 ppm cochineal pigment), respectively. By oxidation of fats, the free radicals are formed.

The results of changes in the color index properties of a* sausage samples are shown in **Table 7**. According to the results, the color index a* significantly (P ≤ 0.05) decreased during storage time. Replacement of nitrite with cochineal pigment and cumin essential oil also had a significant (P ≤ 0.05) effect on color index a*. The highest a* index was observed in the samples with the highest amount of cochineal. The results showed that the highest values (15.14) of a*, after 30 days of storage was observed in T7 (40% red meat sausage + 600 ppm cochineal pigment + 2 ml of cumin essential oil) and the lowest values (6.143) of a* was observed in T2 (40% red meat sausage + 200 ppm cochineal pigment).

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Table 1. The study Treatments.

Sample	Sodium nitrite (ppm)	Cochineal (ppm)	Cumin essential oil (% w/w)
T_1	120	-	-
T_2	-	200	-
T_3	-	400	-
T_4	-	600	-
T_5	-	200	0.004
T_6	-	400	0.004
T_7	-	600	0.004

Table 2. Moisture (%) changes of the sausage samples during storage.

Sample	Day 1	Day 10	Day 20	Day 30
T_1	54.60 ± 0.40^{bA}	54.35 ± 0.14^{dA}	54.50 ± 0.25^{bA}	54.60 ± 0.47^{cA}
T_2	54.50 ± 0.15^{bA}	54.47 ± 0.15^{cdA}	54.56 ± 0.18^{bA}	54.61 ± 0.07^{bcA}
T_3	55.88 ± 0.74^{abA}	55.22 ± 0.29^{abA}	55.76 ± 0.74^{aA}	55.91 ± 0.15^{abcA}
T_4	55.14 ± 0.10^{abA}	55.07 ± 0.22^{abcA}	55.25 ± 0.10^{abA}	55.89 ± 0.09^{abcA}
T_5	55.07 ± 0.30^{abA}	54.97 ± 0.30^{bcdA}	55.21 ± 0.15^{abA}	55.96 ± 0.12^{abA}
T_6	55.72 ± 0.36^{aA}	55.70 ± 0.18^{aA}	55.48 ± 0.23^{aA}	55.99 ± 0.43^{aA}
T_7	55.15 ± 0.09^{abA}	55.34 ± 0.36^{abA}	55.27 ± 0.13^{abA}	55.87 ± 0.16^{abcA}

Results are shown as mean ± standard deviation; Different lowercase letters indicate a significant difference in each column; Different uppercase letters indicate a significant difference in each row.

Table 3. Protein (%) changes of the sausage samples during storage.

Sample	Day 1	Day 10	Day 20	Day 30
T_1	9.30 ± 0.14^{aA}	9.26 ± 0.08^{aA}	9.26 ± 0.08^{aA}	9.33 ± 0.11^{aA}
T_2	9.31 ± 0.03^{aA}	9.25 ± 0.08^{aA}	9.27 ± 0.02^{aA}	9.30 ± 0.03^{aA}
T_3	9.25 ± 0.09^{aA}	9.28 ± 0.21^{aA}	9.29 ± 0.05^{aA}	9.31 ± 0.08^{aA}
T_4	9.28 ± 0.05^{aA}	9.29 ± 0.10^{aA}	9.30 ± 0.02^{aA}	9.32 ± 0.06^{aA}
T_5	9.27 ± 0.10^{aA}	9.29 ± 0.07^{aA}	9.28 ± 0.17^{aA}	9.31 ± 0.03^{aA}
T_6	9.32 ± 0.11^{aA}	9.26 ± 0.06^{aA}	9.29 ± 0.05^{aA}	9.30 ± 0.04^{aA}
T_7	9.26 ± 0.32^{aA}	9.28 ± 0.29^{aA}	9.27 ± 0.13^{aA}	9.30 ± 0.19^{aA}

Results are shown as mean ± standard deviation; Different lowercase letters indicate a significant difference in each column; Different uppercase letters indicate a significant difference in each row.

Table 4. Fat (%) changes of the sausage samples during storage.

Sample	Day 1	Day 10	Day 20	Day 30
T_1	23.28 ± 0.21^{aA}	23.25 ± 0.18^{aA}	23.23 ± 0.23^{aA}	23.20 ± 0.23^{aA}
T_2	23.30 ± 0.28^{aA}	23.20 ± 0.07^{aA}	23.15 ± 0.28^{aA}	23.11 ± 0.28^{aA}
T_3	23.28 ± 0.19^{aA}	23.25 ± 0.10^{aA}	23.20 ± 0.15^{aA}	23.18 ± 0.05^{aA}
T_4	23.28 ± 0.37^{aA}	23.26 ± 0.26^{aA}	23.23 ± 0.24^{aA}	23.21 ± 0.22^{aA}
T_5	23.29 ± 0.24^{aA}	23.21 ± 0.09^{aA}	23.17 ± 0.25^{aA}	23.13 ± 0.24^{aA}
T_6	23.29 ± 0.25^{aA}	23.26 ± 0.20^{aA}	23.21 ± 0.25^{aA}	23.19 ± 0.20^{aA}
T_7	23.29 ± 0.05^{aA}	23.27 ± 0.04^{aA}	23.24 ± 0.05^{aA}	23.21 ± 0.07^{aA}

Results are shown as mean ± standard deviation; Different lowercase letters indicate a significant difference in each column; Different uppercase letters indicate a significant difference in each row.

Table 5. Ash (%) changes of the sausage samples during storage.

Sample	Day 1	Day 10	Day 20	Day 30
T_1	3.14 ± 0.10^{aA}	3.11 ± 0.07^{aA}	3.23 ± 0.07^{aA}	3.22 ± 0.11^{aA}
T_2	3.25 ± 0.15^{aA}	3.11 ± 0.08^{aA}	3.20 ± 0.13^{aA}	3.23 ± 0.07^{aA}
T_3	3.10 ± 0.04^{aA}	3.08 ± 0.06^{aA}	3.21 ± 0.08^{aA}	3.19 ± 0.11^{aA}
T_4	3.20 ± 0.05^{aA}	3.16 ± 0.06^{aA}	3.25 ± 0.05^{aA}	3.27 ± 0.08^{aA}
T_5	3.10 ± 0.08^{aA}	3.08 ± 0.03^{aA}	3.15 ± 0.15^{aA}	3.21 ± 0.09^{aA}
T_6	3.12 ± 0.09^{aA}	3.18 ± 0.07^{aA}	3.22 ± 0.10^{aA}	3.23 ± 0.07^{aA}
T_7	3.21 ± 0.06^{aA}	3.16 ± 0.04^{aA}	3.25 ± 0.05^{aA}	3.25 ± 0.05^{aA}

Results are shown as mean ± standard deviation; Different lowercase letters indicate a significant difference in each column; Different uppercase letters indicate a significant difference in each row.

Table 6. Oxidation changes (mgMDA/kg) of sausage samples by thiobarbioturic method during storage.

Sample	Day 1	Day 10	Day 20	Day 30
T_1	1.74 ± 0.03^{cdD}	2.13 ± 0.09^{deC}	2.43 ± 0.03^{eB}	2.71 ± 0.07^{eA}
T_2	2.45 ± 0.11^{aC}	3.12 ± 0.16^{aB}	3.74 ± 0.23^{aA}	4.02 ± 0.11^{aA}
T_3	1.96 ± 0.13^{bcC}	2.84 ± 0.07^{abB}	3.28 ± 0.04^{bcA}	3.35 ± 0.20^{cA}
T_4	1.73 ± 0.11^{cdC}	2.43 ± 0.11^{cdB}	3.03 ± 0.09^{cdA}	3.22 ± 0.05^{cdA}
T_5	2.22 ± 0.09^{abC}	2.86 ± 0.07^{abB}	3.52 ± 0.10^{abA}	3.63 ± 0.03^{bA}
T_6	1.86 ± 0.04^{cdD}	2.63 ± 0.08^{bcC}	3.18 ± 0.08^{cB}	3.37 ± 0.05^{bcA}
T_7	1.63 ± 0.08^{dD}	1.93 ± 0.13^{eC}	2.72 ± 0.09^{deB}	3.04 ± 0.03^{dA}

Results are shown as mean ± standard deviation; Different lowercase letters indicate a significant difference in each column; Different uppercase letters indicate a significant difference in each row.

Table 7. Color changes of the sausage samples during storage.

Sample	Day 1	Day 10	Day 20	Day 30
*L**				
T_1	57.32 ± 0.00^{dA}	56.13 ± 0.01^{dB}	55.54 ± 0.03^{dC}	53.29 ± 0.03^{bD}
T_2	57.93 ± 0.00^{bA}	57.15 ± 0.02^{bB}	57.00 ± 0.02^{bC}	56.86 ± 0.01^{bD}
T_3	55.28 ± 0.00^{eA}	55.23 ± 0.00^{eB}	55.12 ± 0.01^{eC}	55.00 ± 0.01^{dD}
T_4	51.68 ± 0.01^{gA}	51.57 ± 0.02^{gB}	50.26 ± 0.02^{gC}	50.11 ± 0.01^{gD}
T_5	59.69 ± 0.01^{aA}	59.23 ± 0.02^{aB}	59.00 ± 0.03^{aC}	58.81 ± 0.02^{aD}
T_6	57.36 ± 0.01^{cA}	$57.03 \pm 0.\ 00^{cB}$	56.84 ± 0.02^{cC}	56.68 ± 0.04^{cD}
T_7	53.23 ± 0.01^{fA}	53.10 ± 0.02^{fB}	52.75 ± 0.03^{fC}	52.35 ± 0.02^{eD}
*a**				
T_1	13.14 ± 0.8^{dA}	12.09 ± 0.02^{dB}	11.94 ± 0.03^{dBC}	11.84 ± 0.01^{dC}
T_2	7.07 ± 0.03^{gA}	6.18 ± 0.05^{fB}	6.16 ± 0.02^{fB}	6.14 ± 0.01^{fB}
T_3	12.20 ± 0.06^{eA}	11.92 ± 0.05^{dB}	11.70 ± 0.09^{dB}	11.64 ± 0.02^{dB}
T_4	15.12 ± 0.05^{bA}	14.91 ± 0.07^{bB}	14.67 ± 0.06^{bC}	14.40 ± 0.10^{bD}
T_5	8.56 ± 0.08^{fA}	7.80 ± 0.03^{eB}	7.44 ± 0.03^{eC}	7.22 ± 0.02^{eD}
T_6	13.47 ± 0.01^{cA}	12.94 ± 0.02^{cB}	12.77 ± 0.01^{cC}	12.55 ± 0.01^{cD}
T_7	16.11 ± 0.01^{aA}	15.88 ± 0.01^{aB}	15.72 ± 0.08^{aB}	15.14 ± 0.01^{aC}
*b**				
T_1	19.35 ± 0.01^{dA}	18.62 ± 0.21^{dB}	18.47 ± 0.01^{dB}	18.45 ± 0.02^{cBC}
T_2	23.81 ± 0.06^{bA}	22.22 ± 0.08^{bB}	21.75 ± 0.03^{bC}	21.54 ± 0.02^{bD}
T_3	17.53 ± 0.01^{eA}	17.44 ± 0.02^{eA}	17.14 ± 0.03^{eB}	16.91 ± 0.02^{eC}
T_4	14.87 ± 0.08^{gA}	14.38 ± 0.01^{gB}	13.46 ± 0.02^{gC}	13.14 ± 0.02^{gD}
T_5	25.19 ± 0.06^{aA}	24.52 ± 0.03^{aB}	24.10 ± 0.06^{aC}	23.82 ± 0.01^{aD}
T_6	19.44 ± 0.02^{cA}	19.04 ± 0.01^{cB}	18.54 ± 0.02^{cC}	18.15 ± 0.03^{dD}
T_7	16.45 ± 0.07^{fA}	16.12 ± 0.01^{fB}	15.96 ± 0.04^{fC}	15.55 ± 0.04^{fD}

Results are shown as mean ± standard deviation; Different lowercase letters indicate a significant difference in each column; Different uppercase letters indicate a significant difference in each row.

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Discussion

According to Iranian National Standard No. 2303, the permissible range of moisture in sausages should be a maximum of 55 % (Institute of Standard and Industrial Research of Iran, 2005). The moisture content of T2 and control treatments was in the standard range. This means that treatments containing high amounts of cochineal and cumin essential oil had the highest moisture content. A study (Pidaei *et al.*, 2019) investigated the effect of parsley extract powder as a substitute for sodium nitrite in sausage production. They found that there was no significant difference between the water activity of sausage samples during storage, which is not in line with the present study. The water activity was constant until the end of the storage period due to minor changes in the formulation of sausages and as expected neither the addition of parsley extract nor the addition of sodium nitrite had any effect on the water activity of the produced sausage. Wojciak *et al* investigated the use of acidic whey and mustard seeds as a nitrite substitute during the production of cooked sausages (Wójciak *et al.*, 2019). The results showed that acidic whey and mustard seeds had no effect on the water activity of cooked meat products during 30 days of storage. Similar to the results of current study, a study showed a constant water activity (0.98) for pork sausages during refrigerated storage (Metaxopoulos *et al.*, 2002). According to the Iranian National Standard No. 2303 the permissible protein limit should be 9.5-11.5, so the protein content of all the treatments was within the permissible range of the Iranian National Standard (Institute of Standard and Industrial Research of Iran, 2005). Baldin investigated the effect of microencapsulated jabuticaba (*myrciaria cauliflora*) extract to fresh sausage as natural dye and reported that there was not any significant different of the fat content of the control sample and the sample containing natural dye (Baldin *et al.*, 2016). Consistent with the current results, the effect of using microencapsulated Jabuticaba dye in sausage formulation was investigated and no significant difference was observed between the ash content of the control sausage sample and the sausage sample containing natural pigment (Jabuticaba) (Baldin *et al.*, 2016).

Lipid oxidation in meat is one of the reasons for the deterioration of meat quality during storage. The presence of free radicals in meat leads to the formation of aldehydes, which are responsible for the spread of fat spoilage and changes in meat color (Al-Shuibi and Al-Abdullah, 2002). According to results, the treatments containing higher amounts of cochineal and also cumin essential oil had higher antioxidant power, which is due to the antioxidant properties of cochineal and cumin. The main constituent of the cochineal pigment is carminic acid, which has significant activity as a free radical receptor in aqueous media and methanolic solutions. This activity is comparable to known antioxidants, such as quercetin, ascorbic acid, and trolox (Gonzalez *et al.*, 2011). Another study investigated the antioxidant activity of cumin essential oil. Antioxidant activity was assessed using two DPPH tests and total phenol content. Their results showed that cumin essential oil had significant antioxidant activity and can act as a suitable alternative to synthetic antioxidants in foods (Souri *et al.*, 2008). The main active ingredients of cumin essential oil, which was identified by GC/MS method, were cumin aldehyde (25.2%), Para-Menta-1 and 4-Din-7-L (16.6%), Gamma terpenin (19%), para-menta 1 and 3 dain-7 -L (13%), and betapenine (10.3%). Haghiroalsadat *et al.* reported that the amount of phenolic compounds in cumin essential oil was 90.2 mg gallic acid per gram of essential oil that proved the antioxidant properties of cumin essential oil (Haghiroalsadat *et al.*, 2011). Some studies evaluated the effect of different amounts of nitrite on the prevention of lipid oxidation in pork sausages during 14-week storage at 25 °C and 40 °C. During the oxidation, oxidized iron atoms or myoglobin molecules are denatured that all these factors are effective in the formation of color compounds (Al-Shuibi and Al-Abdullah, 2002, Lynch and Faustman, 2000). To confirm the results, natural dyes of cochineal and paprika were investigated to create color in low-nitrite and nitrite-free frankfurter sausages. The results of their research showed that the sample containing 40 mg/kg nitrite and 0.002% cochineal, the sample containing 40 mg/kg nitrite and 1 mg/kg paprika, and the non-nitrite sample containing 0.015% cochineal were not significantly different in terms of color compared to the control sample. In general, it can be concluded that, especially from the point of view of the color sensory evaluator group, it is possible to produce frankfurter sausages with a color close to the control sample, without the use of nitrite or with low nitrite (Calvo *et al.*, 2008).

Nabavi investigated the effect of grape seed extract, nettle essential oil, and lycopene on the quality of Frankfurt sausages and reported that the use of these extracts reduced oxidation and peroxide index and consequently increased the color index L* (Nabavi *et al.*, 2020). The oxidation of pigments may be accelerated by the oxidation of fats in sausage samples during storage time. By oxidation of sausage samples, iron atoms are oxidized, leading to the denaturation of the myoglobin molecule; all of these factors can affect color changes (Lynch and Faustman, 2000). Consistent with the present study, Terns also observed a decrease in a* during storage (Terns *et al.*, 2011). The reduction in red color during storage can be attributed to the dependence between lipid oxidation and color oxidation in meat.

Hoseinpoor in their study, used the natural colors of cochineal and paprika in frankfurter sausages (Hoseinpoor *et al.*, 2013). They reported that the replacement of pigments in sausage samples with the replacement rate of 1 mg of paprika and 40 mg of nitrite or samples with 0.002% cochineal and 40 mg nitrite were similar to the control sample with 120 mg nitrite and the a* index or the amount of redness was similar in them. However, this rate decreased by increasing the amount of replacement. On the other hand, they reported that by increasing the storage time, the amount of red index significantly decreased in all the samples.

A study investigated the replacement of nitrite by anato as a color additive in sausages (Zarringhalami *et al.*, 2009). Their results showed that the sample containing 60% anato was the best sample in terms of color characteristics (highest a* and lowest b*).

Conclusion

The aim of this study was to replace sodium nitrite with cochineal pigment and cumin essential oil in German meat sausages. This substitution had no significant effect on physicochemical properties. Replacement of nitrite with cochineal pigment significantly affected color (L*, a* and b*) changes. The use of cumin essential oil and increasing the concentration of cochineal pigment significantly decreased the lipid oxidation of sausage samples during storage time. The results of this study proved the possibility of producing nitrite-free sausages with desirable qualitative properties and also healthier.

Conflict of interest

There is no conflict of interest in this study to declare.

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References

Al-Shuibi A & Al-Abdullah B 2002. Substitution of nitrite by sorbate and the effect on properties of mortadella. *Meat science.* **62 (4)**: 473-478.

Baldin JC, et al. 2016. Microencapsulated jabuticaba (Myrciaria cauliflora) extract added to fresh sausage as natural dye with antioxidant and antimicrobial activity. *Meat science.* **118**: 15-21.

Calvo M, García ML & Selgas MD 2008. Dry fermented sausages enriched with lycopene from tomato peel. *Meat science.* **80 (2)**: 167-172.

Chang S-H, Chen C-H & Tsai G-J 2020. Effects of chitosan on Clostridium perfringens and application in the preservation of pork sausage. *Marine drugs.* **18 (2)**: 70.

Chattopadhyay P, Chatterjee S & Sen SK 2008. Biotechnological potential of natural food grade biocolorants. *African journal of biotechnology.* **7 (17)**.

Chen H & Hoover D 2003. Bacteriocins and their food applications. Compr Rev Food Sci Food Safety 2: 82–100. *Comprehensive reviews in food science and food safety*. **2 (3)**: 82-100.

Gonzalez NJ, Adhikari K & Sancho-Madriz MF 2011. Sensory characteristics of peach-flavored yogurt drinks containing prebiotics and synbiotics. *LWT-food science and technology*. **44 (1)**: 158-163.

Haghiroalsadat F, et al. 2011. The indigenous cuminum cyminum L. of Yazd province: chemical assessment and evaluation of its antioxidant effects. *Journal of Shahid Sadoughi University of Medical Sciences*. **19 (4)**: 472-481.

Hoseinpoor S, Eskandari MH, Mesbahi G, Shekarforoush S & Farahnaky A 2013. Study on use of cochineal and paprika as natural colors for producing color of nitrite-free and low-nitrite frankfurter. *Iranian food science and technology research journal* **9(3)**: 243-252.

Institute of Standard and Industrial Research of Iran 2005. Sausage, specifications and test methods.

Jamora J & Rhee K 2002. Storage stability of extruded products from blends of meat and nonmeat ingredients: Evaluation methods and antioxidative effects of onion, carrot, and oat ingredients. *Journal of food science*. **67 (5)**: 1654-1659.

Kim H-W, et al. 2015. Wheat fiber colored with a safflower (Carthamus tinctorius L.) red pigment as a natural colorant and antioxidant in cooked sausages. *LWT-food science and technology*. **64 (1)**: 350-355.

Lynch M & Faustman C 2000. Effect of aldehyde lipid oxidation products on myoglobin. *Journal of agricultural and food chemistry*. **48 (3)**: 600-604.

Maaza M 2014. Natural dyes for photonics applications. *Novel plant bioresources: Applications in food, medicine and cosmetics*. 479-493.

Maghsoudlou Y, Asgharpoor A & Ariaiee P 2013. Effect of satureja khosestanica essential oil on bacterial, chemical and sensory properties of frankfurter sausages. *Research and innovation in food science and technology*. **2 (3)**: 279-294.

Metaxopoulos J, Mataragas M & Drosinos E 2002. Microbial interaction in cooked cured meat products under vacuum or modified atmosphere at 4 C. *Journal of applied microbiology*. **93 (3)**: 363-373.

Moradi B, Mashak Z, Akhondzadeh Basti A & Barin A 2012. The survey of the effect of Cuminum cyminum L. essential oil on the growth of Bacillus cereus in a food model system. *Journal of medicinal plants*. **11 (41)**: 93-102.

Nabavi SM, et al. 2020. Food Additives and Human Health. Bentham Science Publishers.

Pidaei F, Sharifan A & Masoud R 2019. The effect of Cold Plasma (Combined Argon/Helium Gases) on Microbial Contamination and Physicochemical Properties of Minced Sheep Meat. *Journal of medical microbiology and infectious diseases*. **7 (1)**: 12-18.

Seong P-N, et al. 2017. Effects of additions of Monascus and Laccaic acid on the color and quality properties of nitrite-free emulsion sausage during refrigerated storage. *Korean journal for food science of animal resources*. **37 (1)**: 10.

Sharmila G, Nidhi B & Muthukumaran C 2013. Sequential statistical optimization of red pigment production by Monascus purpureus (MTCC 369) using potato powder. *Industrial crops and products*. **44**: 158-164.

Souri E, Amin G, Farsam H & Barazandeh TM 2008. Screening of antioxidant activity and phenolic content of 24 medicinal plant extracts. *DARU journal of pharmaceutical sciences*. **16 (2)**: 83-87.

Terns MJ, Milkowski AL, Rankin SA & Sindelar JJ 2011. Determining the impact of varying levels of cherry powder and starter culture on quality and sensory attributes of indirectly cured, emulsified cooked sausages. *Meat science*. **88 (2)**: 311-318.

Vatandoost H, et al. 2012. Identification of chemical constituents and larvicidal activity of Kelussia odoratissima Mozaffarian essential oil against two mosquito vectors Anopheles stephensi and Culex pipiens (Diptera: Culicidae). *Experimental parasitology*. **132 (4)**: 470-474.

Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J & Pérez-Álvarez J 2009. Effect of adding citrus waste water, thyme and oregano essential oil on the chemical, physical and sensory characteristics of a bologna sausage. *Innovative food science & emerging technologies*. **10 (4)**: 655-660.

Wójciak KM, Stasiak DM & Kęska P 2019. The influence of different levels of sodium nitrite on the safety, oxidative stability, and color of minced roasted beef. *Sustainability*. **11 (14)**: 3795.

Zarringhalami S, Sahari M & Hamidi-Esfehani Z 2009. Partial replacement of nitrite by annatto as a colour additive in sausage. *Meat science*. **81 (1)**: 281-284.