GIDA
THE JOURNAL OF FOOD
E-ISSN 1309-6273, ISSN 1300-3070

Research/ Araştırma
GIDA (2020) 45(4) 825-835
doi: 10.15237/gida.GD20064

RESIDUAL NITRITE CONTENT OF HEAT-TREATED SUCUK AS AFFECTED BY CHARD POWDER INCORPORATION AND PROCESSING

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Received/ Geliş: 04.05.2020; *Accepted*/ Kabul: 20.07.2020; *Published online*/ Online baskı: 11.08.2020

Öztürk-Kerimoğlu, B., Serdaroğlu, M. (2020). Residual nitrite content of heat-treated sucuk as affected by chard powder incorporation and processing. GIDA (2020) 45(4) 825-835 doi: 10.15237/gida.GD20064

Öztürk-Kerimoğlu, B., Serdaroğlu, M. (2020). Pazı tozu ilavesi ve üretim faktörlerinin ısıl işlem görmüş fermente sucuklarda kalıntı nitrit miktarı üzerine etkisi. *GIDA* (2020) 45(4) 825-835 doi: 10.15237/gida.GD20064

ABSTRACT
This study was conducted to evaluate the utilization of chard (*Beta vulgaris* L. var. *cicla*) powder (CP) as a natural nitrate source in heat-treated sucuk (fermented Turkish sausage). Control (CN) sample was formulated with 150 ppm sodium nitrate, whereas CP1 sample was formulated with 75 ppm sodium nitrate plus 75 ppm-nitrate containing CP, and CP2 sample was formulated with 150 ppm-nitrate containing CP. No differences were obtained in chemical composition (P >0.05). Increased CP level resulted in increased pH and decreased acidity and L^*, a^*, and b^* values (P <0.05). The highest residual nitrite concentrations were recorded in CN samples in the dough and after fermentation (P <0.05). Heat treatment affected residual nitrite levels of CP1 and CP2 samples, and in final products, CP2 samples had the highest residual nitrite content (P <0.05). Consequently, utilization of additional ingredients and/or innovative applications would be necessary in combination with naturally nitrate-containing sources.
Keywords: Sodium nitrate, residual nitrite, healthier meat products, heat-treated sucuk, chard

PAZI TOZU İLAVESİ VE ÜRETİM FAKTÖRLERİNİN ISIL İŞLEM GÖRMÜŞ FERMENTE SUCUKLARDA KALINTI NİTRİT MİKTARI ÜZERİNE ETKİSİ

ÖZ
Bu çalışma, ısıl işlem görmüş sucuklarda doğal bir nitrat kaynağı olarak pazı (*Beta vulgaris* L. var. *cicla*) tozunun (PT) kullanımını değerlendirmek amacıyla gerçekleştirilmiştir. Kontrol (KN) örneği 150 ppm sodyum nitrat; PT1 örneği 75 ppm sodyum nitrat ve 75 ppm-nitrat içeren PT; PT2 örneği ise 150 ppm-nitrat içeren PT ile hazırlanmıştır. Örneklerin kimyasal kompozisyonu arasında farklılık tespit edilmemiştir (P >0.05). PT konsantrasyonlarının artması; pH değerinin yükselmesi, asitliğin azalması ve L^*, a^* ve b^* değerlerinin düşmesine neden olmuştur (P <0.05). Sucuk hamuru ve fermentasyon sonrasında en yüksek kalıntı nitrit konsantrasyonları KN örneklerinde kaydedilmiştir (P <0.05). Isıl işlem, PT1 ve PT2 örneklerinin kalıntı nitrit düzeylerini önemli ölçüde etkilemiş; son ürün örnekleri arasında PT2 grubunun en yüksek kalıntı nitrit miktarına sahip olduğu belirlenmiştir (P <0.05). Doğal nitrat içeren kaynaklarla birlikte ek bileşenlerin ve/veya yenilikçi uygulamaların kullanılması gerektiği sonucuna varılmıştır.
Anahtar kelimeler: Sodyum nitrat, kalıntı nitrit, sağlıklı et ürünleri, ısıl işlem görmüş sucuk, pazı

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INTRODUCTION

Sucuk is a Turkish type dry-fermented meat product that is an important part of the cultural food heritage. The traditional manufacture of sucuk mainly covers mixing the meat source and fat with curing agents and characteristic spices, stuffing the dough into casings, and thereafter a ripening process until a dry/semi-dry product is obtained (Kaban, 2013). On the other hand, latterly heat treatment is also applied as a common process in sucuk manufacture which eliminates the foodborne pathogens, shortens production time, and reduces product costs (Öztürk-Kerimoğlu et al., 2019).

One of the most widely used food additives in the production of muscle foods is sodium nitrate/nitrite that has multi-functional impacts on product quality. Sodium or potassium salts of nitrate and nitrites are incorporated into meat products to provide characteristics cured color and flavor, to inhibit the growth of pathogen bacteria (especially *Clostridium* spp. and *C. botulinum* in particular) and to retard oxidative deterioration (Bázan-Lugo et al., 2012; Hayes et al*.,* 2013; Alahakoon et al., 2015; Öztürk et al*.,* 2015). Despite these favorable contributions, since these additives are strongly correlated with potential health risks, international food safety authorities have recommended the reduction of nitrates and nitrites used in food products (Bakhtiary et al., 2018). International Agency for Research on Cancer (IARC) emphasized that the daily consumption of nitrates increases colorectal cancer risk by 18% (Hamdi et al., 2018). The formation of carcinogenic nitrosamines which are formed by the reactions of nitrite and amines under low pH and high temperature is also a serious risk for meat products (Bázan-Lugo et al., 2012; Hayes et al., 2013).

In recent years, the development of "clean-label" meat product formulations has been needed due to increased consumer demands for healthier foods. In this context, several studies have been carried out regarding the reduction of synthetic additives used in meat products by the inclusion of natural ingredients. Natural compounds extracted from fruits and vegetables are essential sources of phenolic compounds and nitrates (Martínez et al., 2019). Thus, an effective reformulation strategy for healthier meat products is the utilization of natural nitrate sources to reduce the addition of synthetic nitrite levels. Alahakoon et al. (2015) stated that leafy green vegetables such as celery, spinach, radish, and lettuce could be potential alternatives in various meat product formulations to replace synthetic nitrate and nitrites.

Chard (*Beta vulgaris* L. var. *cicla*) is a highly nutritive vegetable from Chenopodiaceae family that is a rich source of nitrate, sodium, ascorbic acid, vitamin A, fatty acids, folic acid, flavonoids (apigenin), phenolic acids, and betalains (Alibas and Okursoy, 2012; Shin et al., 2017; Mzoughi et al., 2019). Thereby this plant has a considerable role in the Mediterranean diet and has widespread use in many traditional dishes (Mzoughi et al., 2019). Chard is widely spread in Turkey and used as an antidiabetic in traditional medicine (Sacan and Yanardag, 2010), besides both in-vivo and in-vitro studies have indicated that chard extracts have high antioxidant capacity (Oztay et al., 2015; Mzoughi et al., 2019). A recent study has addressed the use of Swiss chard powder as a pre-converted nitrite source and mentioned this ingredient as a potential nitrite replacer in pork patties (Shin et al., 2017). To our knowledge, no study has evaluated the impacts of using chard powder to substitute nitrate in heat-treated sucuk (fermented Turkish sausage). The present study targeted to report the quality parameters of heat-treated sucuk containing chard powder as synthetic nitrate substitutes with regard to the changes in the residual nitrite content during processing, as well as the changes in the physical and chemical quality parameters.

MATERIALS AND METHODS

Materials

Fresh and non-damaged chard leaves were obtained from a local producer in Izmir that were harvested in winter season. Post-rigor beef (*Musculus semitendinosus*) as boneless rounds and beef fat were purchased from Migros Integrated Meat Co. (Izmir). The cold chain was maintained during the transport of the materials to the laboratory. Food additives, natural casings (D:36 mm, air-dried bovine small intestine), and other spices were supplied from the local market of Izmir. Freeze-dried starter culture mixture consisted of *Pediococcus acidilactici*, *Lactobacillus plantarum*, and *Staphylococcus carnosus* was obtained from Frutarom Co. (Istanbul).

Production and Characterization of Chard Powder

Chard leaves were washed under tap water to eliminate course dirt and foreign matters. The leaves were then separated from the stalks and cut into 3-cm lengths. The pieces were initially sun-dried on wire trays for 24 h to remove the free water on the surface and thereafter they were dried in a drying oven (Memmert, Germany) operated at 55±5 °C for 8 h (the pieces had 5.4% moisture after drying operation). The dried chard pieces were ground through a mini-grinder (Arçelik, Turkey) and then were sieved through 0.5 mm to obtain fine chard powder (CP). Total nitrate concentration of CP was determined as 9007.32 ppm. CP was stored in glass jars prior to the manufacture.

Production of Heat-Treated Sucuk

Three different sucuk formulations were prepared with different nitrate sources as given in Table 1. For the production of control (CN) treatment, the minced meat was mixed with beef fat (5:1) and 2% salt (NaCl), 0.4% sugar, 0.025% ascorbic acid, 0.015% sodium nitrate, 1.25% sweet red pepper, 1% cumin, 1% garlic powder, and 0.5% black pepper per kg of meat. According to Turkish Food Codex (2019a), 150 mg nitrate/kg (E 251-252) is allowed to add for the meat products during manufacturing. Taking this limit into consideration; CN treatment was formulated with 150 ppm sodium nitrate, whereas CP1 treatment was formulated with 75 ppm sodium nitrate plus 75 ppm-nitrate containing CP (8.33 g), and CP2 treatment was formulated with 150 ppm-nitrate containing CP (16.66 g) (thus, the amount of CP added to the formulation was calculated according to the nitrate concentration of CP). After preparing the sucuk dough, it was stuffed into synthetic collagen casings (Viscofan, Spain) by using a filling machine (Alpina-SG, Switzerland). The samples were then fermented at 23°C and 87% relative humidity (RH). When the pH value reached to 5.6, the samples were heat-treated in an oven (AFOS Mini Kiln, UK) operated at 80°C (up to core temperature reached to 68 °C). As soon as the heat treatment completed, the samples were immediately cooled using a cold water spray. The samples were finally ripened at 18°C and 72% RH until the moisture content dropped below 50%. Analyses of the final products were carried out within 48 h after production, while sampling for residual nitrite content analysis was conducted before stuffing (dough), after initial fermentation (pH = 5.6), and after heat treatment and ripening (final product) to assess the changes during production.

Analysis

Chemical Composition

Total moisture and ash contents of the samples were determined according to AOAC (2012). Lipid content was analyzed by the extraction method described by Flynn and Bramblett (1975). Protein content was measured by using a nitrogen determinator (LECO FP528, USA).

pH Value and Titratable Acidity

A portable pH-meter (WTW 330i/SET, Germany) equipped with a penetration probe was used to measure pH values of the samples. The probe was dipped straight into three different points of the sample and the value was recorded after the numerical value was fixed. Titratable acidity was calculated as a percentage of lactic acid by performing titration of the sample slurries with 0.1 N NaOH (AOAC, 2012).

Color

The surface color of the slices obtained from the samples was measured from four different points with a portable colorimeter (Minolta CR-200, Japan). Color parameters were expressed in terms of L^* (luminosity), a^* (redness), and b^* (yellowness).

Residual Nitrite

Residual nitrite concentration was determined according to AOAC (2012). The homogenized sample (5 g) was mixed with 40 ml of pure water at 80°C. The mixture was transferred to a volumetric flask (500 ml) and water was added to approximately 300 ml, then it was kept at a boiling water bath for 2 h, after that it was cooled to room temperature and filtered. The filtrate (45 ml) was transferred to a 50 ml flask and 2.5 ml of sulphanilamide was added. After 5 min, 2.5 ml of N-1 Naphtyl Ethylendiamine Dehydrochloride (NED) was included in the mixture and hold for 15 min for color development. The absorbance was read at 540 nm against the blank sample consisted of 45 ml of water, 2.5 ml of sulphanilamide, and 2.5 ml of NED. Residual nitrite concentration of the sample was calculated as mg nitrite per kg sample utilizing the standard curve plotted by different concentrations of the standard solution ($NaNO_2$).

Table 1. Formulation of heat-treated sucuk

		Treatments		
Ingredients (g)		CN	CP1	CP2
Meat		1500.0	1500.0	1500.0
Beef fat		300.0	300.0	300.0
Salt (Sodium chloride)		30.0	30.0	30.0
Sugar (Saccharose)		6.0	6.0	6.0
Ascorbic acid		0.375	0.375	0.375
Nitrate source	Sodium nitrate ($NaNO_3$)	0.225	0.112	0.0
	Chard powder (CP)	0.0	8.33	16.66
Spice mix		56.25	56.25	56.25

Heat-treated sucuk samples were formulated with CN: 150 ppm sodium nitrate (control), CP1: 75 ppm sodium nitrate plus 75 ppm nitrate-containing chard powder (CP), CP2: 150 ppm nitrate-containing CP.
Spice mix consisted of 18.75 g sweet red pepper, 15 g cumin, 15 g garlic powder, 7.5 g black pepper.

Statistical Analysis

The whole trial was replicated twice (two independent batches), with each replication corresponding to a different production day. For each batch, measurements of related traits were carried out in triplicate. Data were statistically analyzed by the Statistical Package for Social Science (SPSS) version 20.0 (IBM, USA). One-Way Analysis of Variance (ANOVA) was used to evaluate the significant differences between product formulations, while two-way ANOVA was used to assess the significance depending on the formulations and processing for residual nitrite analysis. Least-square differences (LSD) test was applied to compare the means and Duncan's multiple range test was utilized for posthoc comparisons at a 95% confidence interval.

RESULTS AND DISCUSSION

Chemical Composition, pH Value, and Titratable Acidity

The proximate composition of heat-treated sucuk samples is presented in Table 2. Total moisture, lipid, protein, and ash contents were between 48.12-50.02%, 27.44-28.27%, 18.59-18.90%, and 2.91-3.12%, respectively. No statistical differences were recorded in any of the chemical parameters among treatments (P >0.05). Thus, it was concluded that the incorporation of CP as partial/total replacer of synthetic nitrate did not lead to any changes in chemical components. Turkish Food Codex (2019b) states that in heat-treated sucuk, total meat protein shall be a minimum of 14% on mass, while the ratio of moisture to protein shall be lower than 3.6, and the ratio of fat to protein shall be lower than 2.5. Evaluating these legislations, all the samples had a protein content higher than 18%, the ratio of moisture to protein was 2.6 on average, and the ratio of fat to protein was 1.48 on average. Thus, our data showed that all the samples met the requirements given by those standards. The proximate composition of the sausages was also in agreement with the composition of heat-treated sausages previously reported by Öztürk-Kerimoğlu et al. (2019).

pH value and acidity are two interrelated critical indicators for fermented meat products. Acidification processes occur in the sausages during the fermentation period by the lactic acid bacteria, well-adapted microorganisms to the meat fermentation, that decompose carbohydrates and produce lactic acid, which then reduce the pH of the fermented sausages (Kurt and Zorba, 2010; De Maere et al., 2016). pH values and titratable acidity of the samples are presented in Table 2. The range of pH values was in accordance with the limit given in the communiqué (Turkish Food Codex, 2019b), in other words, all the samples had a pH value lower than or equal to 5.6 which confirmed adequate development of acidity. On the other hand, it was found that the lowest pH value belonged to CN samples with synthetic nitrate, and increased concentrations of CP resulted in a significant increment in pH values of the samples (P <0.05). Since the drop in pH is highly associated with the increase of lactic acid bacteria (Djeri and Williams, 2014), it was noted that in CN samples the production rate of lactic acid was higher than the other samples with CP. Similar results were obtained by Jackson et al. (2011) who observed increments in pH values of ham samples containing celery powder compared with ham samples that were conventionally cured. On the contrary, Riel et al. (2017) found that parsley extract added to the formulation of mortadella-type sausages resulted in lower pH values compared to the sodium nitrite added and the uncured variants. Djeri and Williams (2014) reported that pH values of bologna sausages did not change by the use of celery juice powder as nitrite substitutes. In another study, it was reported that the reduction in nitrite concentration had no effect on pH values of pork luncheon roll samples, whilst increased levels of tomato pulp powder significantly reduced pH values (Hayes et al., 2013). These differences might be related to the different characteristics of the ingredients added to the formulation, as well as to the type of the product. Total acidity of the samples ranged between 1.22-1.37%. This range was in accordance with Soyer (2005), who reported that total acidity of naturally fermented sucuk after ripening was between 0.82-1.71%. In line with pH results, the lowest acidity belonged to CP2 samples with 150 ppm nitrate-containing CP (P <0.05), due to the reduced rate of lactic acid formation.

Table 2. Proximate composition, pH, and titratable acidity of heat-treated sucuk samples

Treatments	Moisture (%)	Lipid (%)	Protein (%)	Ash (%)	pH	Titratable acidity (%)
C	48.78 ± 2.05	28.27 ± 1.97	18.89 ± 0.02	2.91 ± 0.50	5.40^{c} ± 0.02	1.37^{a} ± 0.05
CP1	50.02 ± 1.48	27.44 ± 1.33	18.59 ± 0.04	3.00 ± 0.67	5.48^{b} ± 0.01	1.33^{a} ± 0.01
CP2	48.12 ± 2.84	27.81 ± 0.86	18.90 ± 0.06	3.12 ± 0.66	5.60^{a} ± 0.03	1.22^{b} ± 0.05

Heat-treated sucuk samples were formulated with CN: 150 ppm sodium nitrate (control), CP1: 75 ppm sodium nitrate plus 75 ppm nitrate-containing chard powder (CP), CP2: 150 ppm nitrate-containing CP.
Data were presented as the mean values ±standard deviation.
a, b, c: Means with a different letter in the same column are significantly different (α=0.05 level).

Color

The color of muscle-based foods is the most important visual quality factor that largely affects consumer choice. Myoglobin (Mb) is mainly responsible for the color of meat, besides hemoglobin and other heme molecules may also contribute to it (De Maere et al., 2018). Nitrate and nitrites in meat products are the key additives that determine the color. Nitrite added in the formulation is reduced to nitric oxide (NO) that reacts with myoglobin iron to form nitrosylmyoglobin (NOMb) or nitrosylhemochrome, which gives the cured red (if uncooked) or pink (if cooked) color, respectively (De Maere et al., 2016; De Oliveira et al., 2012; Hayes et al., 2013).

Color attributes of heat-treated sucuk samples are presented in Figure 1. Luminosity (*L**) values of the samples formulated with CP were similar to that of CN samples formulated with synthetic nitrate ($P > 0.05$). Thus, the inclusion of CP did not affect the lightness of the sample compared with control. However, *L** value of CP2 samples was significantly lower than *L** value of CP1 samples ($P < 0.05$), meaning that higher concentrations of CP could lead to a darker color. In a similar study, it was reported that *L** values of bologna sausages were lower when they were formulated with a mixture of celery juice and cherry juice powders as nitrite replacers (Djeri and Williams, 2014). On the other hand, Riel et al. (2017) reported that parsley extract as a sodium nitrite replacer had no significant effect on *L** values of sausages. Redness values of our samples were highly affected by the formulation, where decreasing the concentration of synthetic nitrate significantly decreased *a** values of the samples ($P < 0.05$). This data confirmed the key role of nitrite in the formation of characteristic cured color. Similarly, De Oliveira et al. (2012) reported that mortadella samples with added nitrite had higher redness than samples without nitrite, and 100 mg/kg of sodium nitrite was essential for the development of the desired cured color. On the other hand, Bázan-Lugo et al. (2012) incorporated paprika or tomato paste into nitrite-reduced meat batter and found that this incorporation resulted in red coloration by the natural color pigments of those ingredients. Development of red color was also reported by Riel et al. (2017) in mortadella samples with added parsley extract by conversion of nitrate to nitrite and also by Shin et al. (2017) in pork patties with added pre-converted Swiss chard as nitrite replacer. In this manner, the characteristics of the pigments and/or the nitrite formation rate in the natural nitrate source seem to be deterministic factors for the development of cured color.

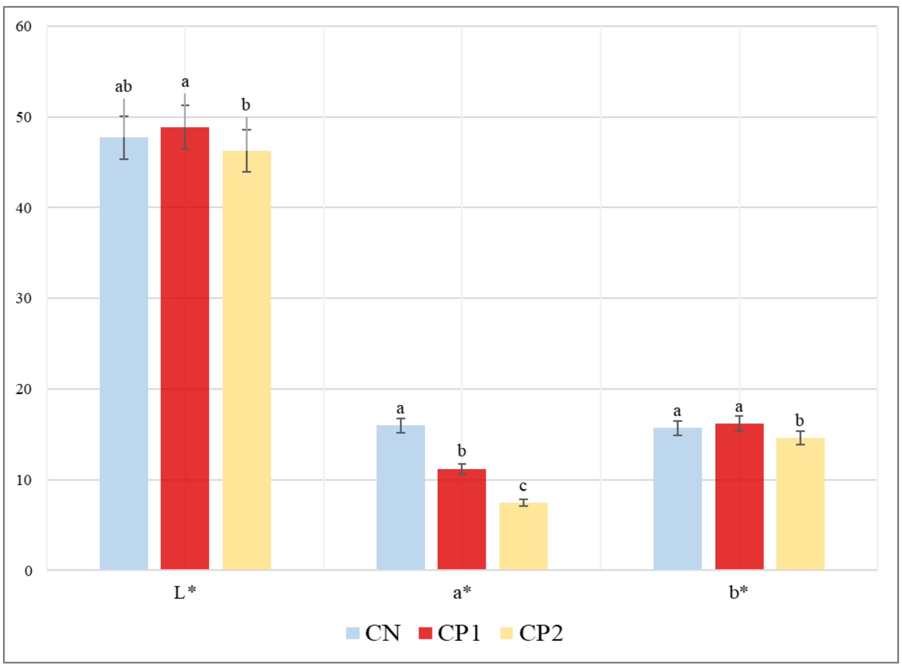

Figure 1. Color parameters of heat-treated sucuk samples
Heat-treated sucuk samples were formulated with CN: 150 ppm sodium nitrate (control), CP1: 75 ppm sodium nitrate plus 75 ppm nitrate-containing chard powder (CP), CP2: 150 ppm nitrate-containing CP.
a, b, c: Means with different letters in different columns within the same parameter are significantly different (α=0.05 level)

Although the combined use of synthetic nitrate and CP was effective to keep yellowness (*b**) similar to control, CP2 samples without synthetic nitrate had lower b* values compared to that samples (*P* <0.05). De Oliveira et al. (2012) emphasized that not only decreased *a** values but also increased *b** values are associated with fading of the cured color. However, our results were in contrast with this data. Therefore, the presence of natural bluish pigments in CP may be responsible for the decrease in *b** values rather than the inadequate formation of the cured color.

In spite of the characteristic cured product color formed by the inclusion of nitrites, De Maere et al. (2016) not long ago demonstrated that naturally occurring pigments (zinc protoporphyrin IX) could be formed that are promising for the development of color in dry fermented sausages. By this way, red-colored dry fermented sausages could be produced without the addition of nitrite-nitrates. For this reason, products with acceptable visual properties can be produced without using nitrite-nitrates, but further studies are still needed to ensure food safety.

Residual Nitrite

In meat products, a natural curing operation covers the utilization of naturally occurring nitrates, that are then reduced to nitrite by the function of specific microflora (Djeri and Williams, 2014). Nitrite is a highly reactive substance that undergoes several reactions in meat products and thus its utilization has to be under control (Honikel, 2008). The residual nitrite (RN) refers to the nitrite amount that has not reacted with myoglobin and is already available in the meat matrix (Hayes et al*.*, 2013).

Concentrations of RN in the samples during processing are shown in Figure 2. In sucuk dough, RN levels were between 22.60-73.09 mg/kg. The highest RN content was recorded in control (CN) samples with 150 ppm sodium nitrate (*P* <0.05), while the samples with CP had similar nitrite content. Higher nitrite amount of CN samples is presumably due to the rapid reduction ability of synthetic nitrate to nitrite. Likewise, Magrinyà et al. (2016) stated that sausages including vegetable concentrates had much lower RN concentrations compared with the sausages including with pure sodium nitrite, due to the slower reduction reaction from nitrate to nitrite. After the initial fermentation (pH = 5.6) and prior to heat treatment, RN concentrations ranged between 14.07-23.23 mg/kg. CN samples still had the highest amount of RN among treatments (*P* <0.05). Compared with the initial values, significant decreases in RN levels of all the treatments were observed by fermentation (*P* <0.05). This decline could be associated with the ongoing curing reactions and binding of reduced nitrite with meat proteins to develop the characteristic color compounds. On the other hand, an interesting trend in the samples was observed after the heat treatment and ripening processes. In the end products, RN concentrations ranged between 33.76-121.13 mg/kg. Although RN concentration of CN samples did not show a significant alteration with heat treatment and ripening, CP1 and CP2 samples had significantly higher RN levels compared with their RN levels before heat treatment and ripening (*P* <0.05). This increment could be related to the rapid reduction of nitrate still present in those samples by the impact of heat treatment. Conversely, Kurt and Zorba (2010) reported that RN levels decreased with higher temperatures, particularly above 65°C in heat-treated sucuk. Similarly, Li et al. (2013) found that nitrite concentrations of dry-cured sausages declined significantly during the ripening period. The reason for these opposite data found in the present study could be the use of natural sources in nitrate form that delayed the formation of nitrite. In this case, in our study, it was indicated that the reduction process of nitrate in chard was much slower than in synthetic nitrate. In addition, CP2 samples had the highest RN content among final products (*P* <0.05), indicating that increased concentrations of CP also resulted in an increment in RN level. Consequently, it could be said that the curing reactions could not be completed throughout the production period since it takes a long time to reduce the nitrate obtained from natural sources. As stated by Honikel (2008), the largest decrease in nitrite is seen during the manufacturing of meat products up to the end of the heating process. On the other hand, within 20 days of cold storage, the levels decreased further to a third of the level after heating. This information points out that following the concentration of RN during the storage is also of importance and the levels measured at different storage days would differ from the levels measured in the final products.

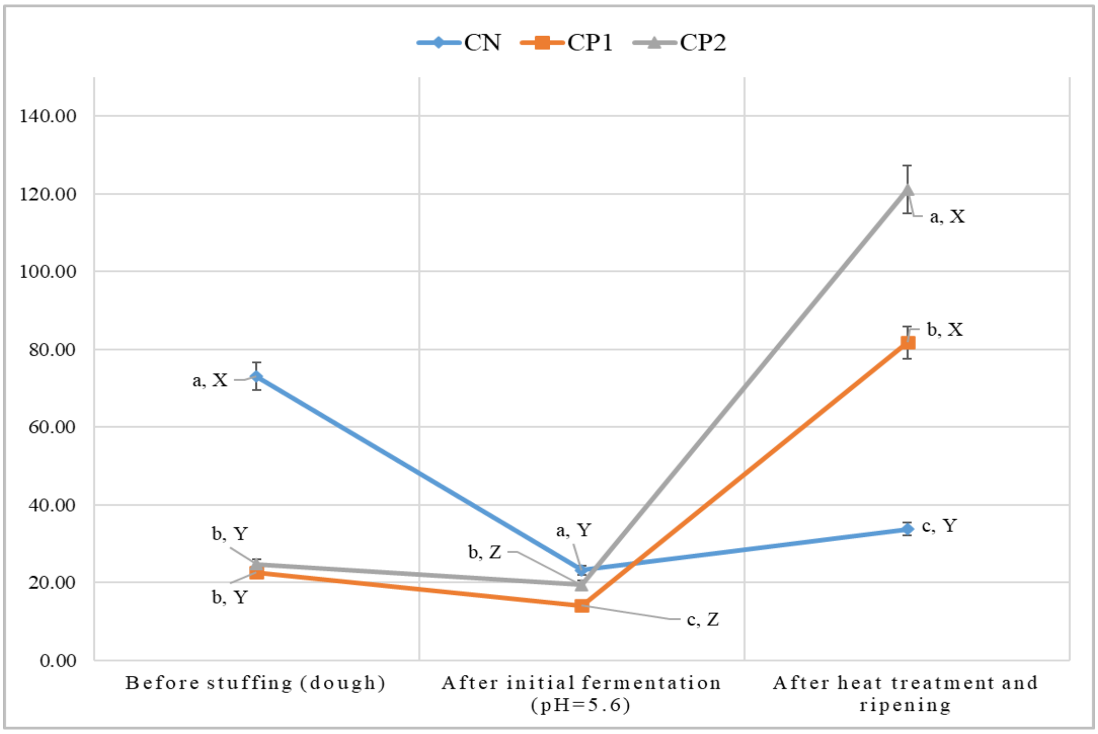

Figure 2. Residual nitrite concentrations (mg/kg) of heat-treated sucuk samples during production

Heat-treated sucuk samples were formulated with CN: 150 ppm sodium nitrate (control), CP1: 75 ppm sodium nitrate plus 75 ppm nitrate-containing chard powder (CP), CP2: 150 ppm nitrate-containing CP.

a, b, c: Means with different letters in different treatments within the same production step are significantly different (α=0.05 level).
X, Y, Z: Means with different letters in different production steps within the same treatment are significantly different (α=0.05 level).

European Union defined the limits for nitrates and nitrites for various meat products. Besides the limits for addition during manufacturing, the legal European limits (No 601/2014) for RN in final products range between 50-180 mg/kg in traditionally cured meat products (Govari and Pexara, 2015). Likewise, the maximum residue levels of nitrite in the end products are also the same in Turkish legislation (Turkish Food Codex, 2019a). Evaluating these limits, the RN concentrations of all the heat-treated sucuk samples were within these limits during the production steps, since the maximum level was recorded as 121.13 mg/kg. On the other hand, the RN content in a finished meat product is generally only a small fraction of the level added (Li et al., 2013). Considering this, in the present study the levels of RN in CP treatments were still high compared with the added amount, indicating that the curing reactions were not fully completed.

Nitrite is a chemical that is known to react with water and is affected by reducing bacteria (Kurt and Zorba, 2010). Besides, Hayes et al. (2013) underlined that the reactivity of nitrite increases with decreasing pH. Previously Kurt and Zorba (2010) found that nitrite level of dry fermented sucuk continued to decrease after the first day of ripening due to the drop in pH values. Therefore, in the present study, CN treatment might also had an accelerated nitrite reduction rate due to its lower pH compared with CP1 and CP2 treatments. Here it could be suggested to include additional natural pH enhancers to the formulations with CP to provide rapid acidification in these treatments. So far, promising results have been obtained by the utilization of natural nitrite alternatives on RN concentrations in various studies conducted with different meat products. In a study regarding the use of cleaner ingredients to reduce synthetic nitrite in a cured meat model, it was indicated that sodium nitrite groups had the highest RN levels, while the samples with celery juice powder and cherry powder had the lowest levels (Posthuma et al., 2018). Similary, Shin et al. (2017) reported that RN concentrations in pork patties containing synthetic nitrite were higher than those containing vegetable sources (Swiss chard and celery). The authors stressed that the additional antioxidant compounds from those natural sources provided reducing conditions for the formation of nitric oxide and thus lower residual nitrite contents were recorded. In a different research, it was observed that lower RN concentrations could be achieved by the inclusion of parsley extract powder as synthetic nitrite substitutes in sausages (Riel et al., 2017). Hayes et al. (2013) incorporated different levels of nitrite and tomato pulp powder into pork luncheon rolls and observed that the combination of the increased amount of the powder and the presence of nitrite was effective to reduce the RN levels. Pinsirodom et al. (2019) found that roselle anthocyanin extract enhanced the RN reduction in Nham, a fermented Thai meat product. Those promising data obtained from natural nitrite sources highlighted the importance of the reduction rate of nitrate to nitrite to provide proper curing and to reduce residual nitrite concentrations.

A new approach for benefiting from natural nitrate sources is to obtain “pre-generated” nitrite by converting nitrate to nitrite by microorganisms prior to the manufacture of the cured meat products; by this way it is possible to reduce the curing period (Djeri and Williams, 2014). Considering this process, in our study, due to the delayed converting time of nitrate to nitrite by the use of CP, a pre-generation step could be suggested to accelerate the reduction reactions and thereby obtain optimum curing.

CONCLUSION

The findings of the present study pointed out that the incorporation of chard powder as a natural nitrate source led to significant changes in pH value, total acidity, instrumental color parameters, and residual nitrite concentrations, but the chemical composition remained stable among samples. Residual nitrite levels of the sucuk samples with chard powder were highly affected by different steps of the production. Although the levels were almost similar at the beginning of stuffing, residual nitrite content of the samples with chard powder sharply increased after heat treatment due to the delayed conversion of nitrate. Therefore, additional research is necessary to improve the reduction rate of natural nitrate to nitrite present in chard by novel pre-conversation techniques. By this means an effective solution would be obtained to reduce the residual nitrite concentrations in heat-treated sucuk. Moreover, since the proposed natural ingredients have not yet been proven to control the microbial growth (especially *Clostridium* strains), further studies should be carried out to assess the use of the natural plant sources regarding covering the multi-functional impacts of nitrite-nitrates.

ACKNOWLEDGMENTS

The authors would like to thank Sinem Alev, Dilara Mehri, and Tuğçe Cengiz from Ege University Food Engineering Department for their assistance in the laboratory.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

AUTHOR CONTRIBUTION

Dr. Öztürk Kerimoğlu was responsible for methodology and formal analysis, investigation, visualization and writing, while Dr. Serdaroğlu supervised the study and carried out conceptualization, review and editing.

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