Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1249523

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 6 - Issue 3: 304-313 / May 2023

THE INVESTIGATION OF THE USE RED BEET POWDER (*BETA VULGARIS*) AS A NATURAL COLORANT ON COLOR AND ANTIOXIDANT PROPERTIES OF HEAT TREATED SUCUK

Ömür SERTDEMİRCİ[1], Hüseyin GENÇCELEP[1]*

[1]*Ondokuz Mayis University, Faculty of Engineering, Department of Food Engineering, 55139, Samsun, Türkiye*

Abstract: The purpose of this study was to assess red beet as a natural colorant in heat treated sucuks and to investigate the effect of red beet on some quality characteristics of heat treated sucuks during 30 d of cold storage. Red beet was prepared as a powder and a substitute with sodium nitrite (nitrite 50 and 100 ppm) at 1% levels in heat treated sucuks. Both treatment and storage periods had significant (P<0.01) effects on moisture, pH, aw, residual nitrite, free fatty acids (FFA), 2-hiobabituric acid reactive substance (TBARS), total mesophilic aerobic bacteria (TMAB), *Enterobacteriaceae*, yeasts-moulds and color (L*, a* and b* values) of heat treated sucuks. The red beet powder has an antioxidant effects on sucuk samples. Red beet powder caused a decrease in L* value compared with the control sample with nitrite added. As a result, red beet powder can be used as an alternative to synthetic colorants in some meat products or as a reduction of nitrite.

Keywords: Sucuk, Red beet powder, Color properties, Lipid hydrolysis, Nitrite

***Corresponding author:** Ondokuz Mayis University, Faculty of Engineering, Department of Food Engineering, 55139, Samsun, Türkiye
E mail: hgenccelep@omu.edu.tr (H. GENÇCELEP)
Ömür SERTDEMİRCİ https://orcid.org/0000-0002-1307-1049
Hüseyin GENÇCELEP https://orcid.org/0000-0002-8689-7722

Received: February 10, 2023
Accepted: April 29, 2023
Published: May 01, 2023

Cite as: Sertdemirci Ö, Gençcelep H. 2023. The investigation of the use red beet powder (*beta vulgaris*) as a natural colorant on color and antioxidant properties of heat treated sucuk. BSJ Agri, 6(3): 304-313.

1. Introduction

The most important dried fermented meat product in Türkiye is sausage (sucuk). In the production of sucuk, red meat, water buffalo meat, veal, sheep tails and some chemical (nitrites, nitrate, salt, sugar) are used together with many different seasoning (black and red pepper, garlic, cumin). Starter culture is used depending on demand. In traditional sucuk production, heating process is not applied at the end of ripening process (Gökalp et al., 1999; Gençcelep et al., 2007). Heat treatment is preferred to ensure quality and microbial safety of meat products. Changes have been made in the sucuk production process over the last four decades and although heat application is not part of traditional production, it has begun to be applied to products at the end of ripening time (Ercoşkun et al., 2010). Heat treatment in sucuk production eliminates pathogens, prolongs shelf life and reduces training period and price (Ercoşkun et al., 2010).

Consumer concerns about artificial colorants used in meat products and the many side effects of these colorants on health have led to searches for the use of natural colorants in meat products. Owing to the powerful phenolic compounds contained in red beetroots, it has found use in many industrial and domestic food products (Sucu and Yıldız Turp, 2018; Posthuma et al., 2018 and Schopfer et al., 2022). The most effective factor for buyers to buy meat products is color. The bright red color of the meat products is regarded as an indication of freshness by the consumers (Barbut, 2001; Martinez et al., 2006a). The most important additive used in the production of meat products is nitrite salts. Nitrite restricts the development of certain microorganisms, *Clostridium botulinum* and *Listeria monocytogenes*, and contributes to the improvement of the product color and flavor. However, there is a growing concern about the use of nitrite in meat products because of the formation of N-nitroso carcinogens from amines and amides (Sang-Keun et al., 2014).

Long storage has a disadvantage in terms of stability of meat color. Red beet root (*Beta vulgaris*) is a very abundant source of betalain pigment group betacyanins (red) and betaxanthins (yellow). Betalaines are colorants containing water soluble nitrogen which are responsible for the red, violet (betacyanins class) and yellow (betaxanthins class) colors found in many flowers, fruits, vegetables and plants (Socaciu, 2008; Lee and Jin 2012). Red beet root is a good source of natural coloring matter and antioxidant substances. In addition, betalaines are more resistant to pH than anthocyanins used in food to give red purple color. Kujala et al. (2002) also determined many phenolic compounds in red beet root. Red beet root powder can be used to enhance the color of a lot of meat products (cooked, smoked, semidried and fermented sausages) (Martinez et al., 2006a; Lee and Jin 2012). In addition, red beet is used as coloring material instead of nitrite, but betalain does not have antimicrobial properties. Moreover, because of the presence of nitrite naturally in the red beet composition, it contributes to the production of more nitrite in the food as a source of nitrite when added to the products (Socaciu, 2008). Most researchers have focused on the purification of betalaines from red beet and the investigation of phenolic compounds. A small number of work have known about the stability of betalain in the ingredient of colored foods. There is a few research into the added of red beet powder in meat products (Martinez et al., 2006b; Kujala et al., 2002; Lee and Jin, 2012; Sang-Keun et al., 2014 and Hwang et al., 2017). The aim of this research was to identify red beet powder as a natural antioxidant and colorant in heat treated sucuk and to research the influence of red beet powder on microbiological quality and chemical properties during 30 days of refrigerator storage period.

2. Materials and Methods

2.1. Production of Heat-Treated Sucuk

The ground meat (the back of cattle) was separated into five batches, which were mixed with the subsequent formulations: (1) control (C) (no nitrite and red beet powder), (2) nitrite (N) (100 ppm), (3) red beet powder (P) (1%), (4) red beet powder and nitrite (NP1) (1% and 50ppm, respectively) and (5) red beet powder and nitrite (NP2) (1% and 100 ppm, respectively); all the batches contained 1500 g minced meat, 500 g fat (beef meat fat), 36 g NaCl, 50g garlic, 72 g red pepper, 36 g cummin, 2 g black pepper, 1 g dextrose, 7 g dipolyphosphate, 1.4 g ascorbic acid and 2.5 g glikono delta lakton. Each batter was stuffed into dried natural casing (Ø35 mm; Delarom Aroma ve Gıda Katkıları, İstanbul, Türkiye) using a stuffing machine (Seydelmann KG, Stuttgart, Germany). The sucuk samples were exposed to heat treatment (63°C in the core of the samples in 15 min.) and cooled directly to 5°C with chilly water shower. Sucuks were storage 4±1°C in refregerator for 30 days. Sucuks were produced by Köytaş meat product (Sungurlu, Çorum, and Türkiye). Sucuks were brought to the laboratory by storage 4±1°Cimmediately after production.

2.2. Preparation of Red Beet Powder

Roots of red beet (*Beta vulgaris*) were purchased in a local market in Samsun, Türkiye. The roots were washed, peeled, and chopped into small pieces. The minced roots were then frozen at -20 °C. Chopped roots was later put into a freeze dryer (Freezone 12 plus, Labconco, Kansas City, MO, USA). After about 24-30 hours freeze drying, the dried product was ground using a hammer mill (TEKPA, Laboratory mill, Ankara, Türkiye). The final moisture content of the red beet powder (RBP) was determined to be 10.90±0.5%.

2.3. Physical and Chemical Analysis

Proximate analysis of meat and sucuk were analyzed according to the AOAC (2009). The homogenized meat and sucuk compound were determined as follows: crude protein, using the Kjeldahl method (Barros et al., 2007); crude fat, using the Soxhlet method (SOXTEC System, NUVE, Ankara, Türkiye); moisture by oven-drying to constant weight at 105±2 0C. pH value was measured by using a pH meter (Lab Star pH; Schott LTD 6880, Germany). The free fatty acids (FFA) were determined according to AOAC (2009). Thiobarbituric acid reactive substances (TBARS) test was performed according to Lemon (1975). The amount of residual nitrite was found according to the method of Tauchmann (1987). The water activity was determined using Aqua LAB Water Activity (Dew Point Water Activity Meter 4TE, Pullman, WA, USA). All measurements were duplicated.

2.4. Color Measurement

The color of sucuk was found using a reflectance Hunter's Lab. (ColorFlex EZ User's Manual Reston, Virginia, USA). L*(lightness), a*(redness), b*(yellowness) color values were measured. Measurements were taken from six different points on the surface color of the samples during storage (0, 15 and 30 days) and central part of the cut surface of the two slices (3.6-cm diameter) of two sucuks. The mean of the six measurements were taken for each L*, a* and b* values (Soyer et al., 2005).

2.5. Microbiological Analysis

A sample of 25 grams of sucuk was taken under aseptic conditions and homogenized in a blender (Waring 80011S, Torrington, CT, USA) sterile physiological saline (0.85% NaCl). The number of total aerobic mesophilic bacteria was determined on Plate Count Agar (PCA; Merck) incubated at 37°C for 48 h, while moulds-yeasts on Potato Dextrose Agar (PDA; Merck) incubated at 25°Cfor 5 days. *Enterobacteriaceae* were cultured on Violet Red Bile Glucose Agar (VRBGA; Merck) incubated at 30°Cfor 24 h. (Rödel et al., 1975; Baumgart, 1986).

2.6. Statistical Analysis

The result of analyses, which depend on RBP and nitrite levels and storage time, were analyzed according to a completely randomized design with two replicates. Exemplification was made by selecting of sucuk randomly, 0, 15 and 30 days. All data were subjected to variance analyses and the differences between means were evaluated by Duncan's multiple range test (significance $P<0.01$) using the SPSS statistic program (Chicago, IL) (2011). The results of statistical analysis are shown as mean values ± standard deviation in tables (SPSS, 2011).

3. Results and Discussion

The compositions of fresh meat+fat mixture are pH, protein, fat, moisture, 6.40, 19.65%, 31.50%, 47.67%, respectively. Red beet powder (RBP) analysis results are pH, moisture, L*, a*, b*, 5.50, 10.90%, 27.26, 29.53 and -0.53, respectively. Kerr and Varner (2019) found that the freeze-dried beet powders had the moisture contents 8.10 %. Hwang et al. (2017) found that pH values fermented red beet extract 4.65. Antigo et al. (2018) showed that freeze-dried beet extracts had less degradation and longer shelf-life than spray-dried samples. Nemzer et al. (2011) found the lowest levels of total betalains in spray-dried extracts (0.24–0.46%) followed by air-dried extracts (0.56–0.59%), and with freeze-dried products having the most (0.89–1.26%). The lightness of sample values ranged from 24.80 to 29.33. The lightness of the raw beets prior to dehydration was much lower, at 19.79. This indicates that samples got darker from the removal of water during dehydration. The L* values did not differ based on the temperatures at which samples were dried at, with values of 25.74, 25.64, and 24.80 for samples dried at 75, 85, and 95 °C (Kerr and Varner, 2019). The results determined in the study are similar to the literature values.

The results of chemical composition of sucuk at the beginning of storage are control (C), 100 ppm nitrite (N), 1% red beet powder (P), 50 ppm nitrite+1% RBP (NP1) and 100 ppm nitrite+1% RBP (NP2), protein %, 17.38±0.50, 16.78±0.51, 15.98±0.45, 16.09±0.48, 16.49±0.39, respectively and fat %, 27.99±0.68, 27.95±0.75, 26.31±0.49, 26.39±0.64 and 26.47±0.54, respectively. It was determined that the treatment had no effect on the protein and fat content of the sucuks (P> 0.05).

3.1. Chemical Analysis

The chemical analysis results of the heat-treated sucuks in the study are given in Table 1. The application, storage time and interaction had very important effects on the pH (P<0.01) of samples (Table 1 and Figure 1). A significant (P<0.01) lower pH value characterized the sucuks with beetroot powder. This result may be explained by the low pH value of beetroot powder besides other components of beetroot.

Table 1. Overall affect of treatment and storage period on the pH, moisture, aw, FFA, TBARS, residual nitrite and microbiological counts of sucuk (values are means± SD)

	pH	Moisture %	a_w	Resudial Nitrite (ppm)	FFA g oleic acid/100 g	TBARS g MDA/kg	TMAB Counts (log CFU/g)	Enterobacteriae Counts (log CFU/g)	Moulds-Yeast Count (log CFU/g)
Treatment									
C	6.44±0.06^{a}	48.29±4.48^{b}	0.94±0.01^{b}	2.06±0.52^{c}	1.22±0.34^{b}	0.54±0.05^{b}	5.68±0.33^{b}	2.33±0.37^{b}	2.02±1.56^{b}
N	6.45±0.19^{a}	51.97±3.65^{a}	0.96±0.00^{a}	7.00±2.03^{b}	1.38±0.47^{a}	0.54±0.41^{b}	5.08±1.45^{c}	2.97±0.28^{a}	2.79±1.44^{a}
P	6.37±0.10^{b}	41.61±4.03^{d}	0.94±0.01^{b}	7.02±1.88^{b}	1.00±0.14^{c}	0.55±0.38^{b}	5.49±0.40^{b}	1.94±0.68^{c}	2.81±2.27^{a}
NP1	6.42±0.11^{a}	42.83±5.57^{c}	0.92±0.02^{c}	9.01±2.58^{a}	1.00±0.19^{c}	0.65±0.07^{a}	5.85±0.74^{a}	1.90±0.59^{c}	2.89±2.27^{a}
NP2	6.15±0.19^{c}	43.50±3.25^{c}	0.94±0.00^{b}	8.67±2.51^{a}	1.41±0.31^{a}	0.56±0.05^{b}	5.84±1.91^{a}	2.08±0.51^{bc}	2.76±1.70^{a}
Significance	**	**	**	**	**	**	**	**	**
Storage period									
0d	6.37±0.07^{b}	54.49±1.29^{a}	0.95±0.01^{a}	4.63±1.45^{b}	0.83±0.10^{b}	0.56±0,02^{ab}	4.33±1.71^{b}	2.11±0.65^{b}	0.78±1.01^{c}
15d	6.34±0.20^{b}	45.66±6.97^{b}	0.95±0.01^{a}	7.66±3.76^{a}	1.41±0.25^{a}	0.60±0,06^{a}	4.67±1.08^{b}	1.93±0.58^{b}	3.67±0.34^{b}
30d	6.39±0.21^{a}	40.97±6.69^{c}	0.92±0.01^{b}	7.96±2.89^{a}	1.37±0.26^{a}	0.54±0,08^{b}	5.97±0.54^{a}	2.69±0.34^{a}	4.72±0.98^{a}
Significance	**	**	**	**	**	**	**	**	**
TxS	**	**	**	**	**	**	**	**	**

C= control; N 100 ppm nitrite; P= % 1 red beet powder; NP= 150 ppm nitrite + %1 red beet powder; NP_2= 100 ppm nitrite + %1 red beet powder. $^{a–d}$ Any two means in the same column having the same letters in the same section are not significantly different at P>0.05, **P<0.01, NS= not significant; SD= standard deviation

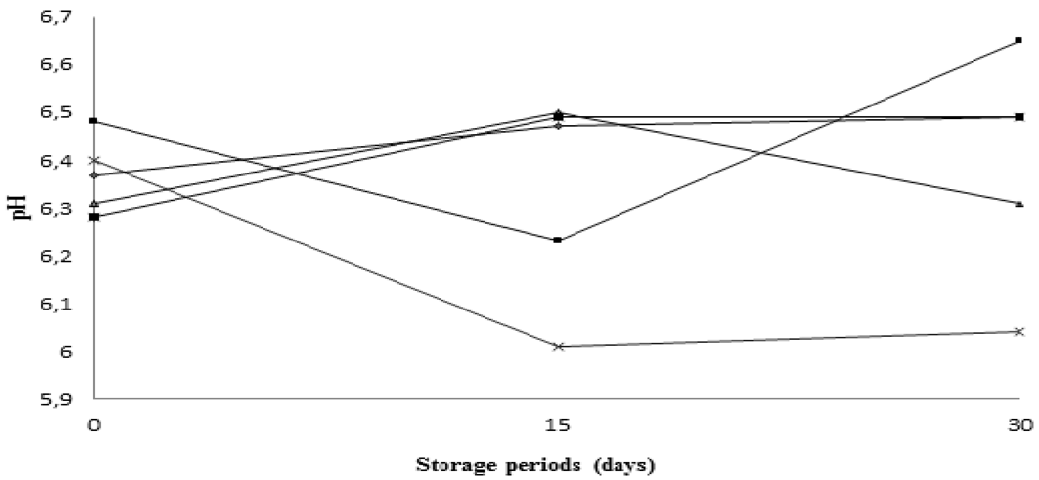

Figure 1. The effect of the interaction between treatments x storage period on pH values.
Control (–♦–); 100ppm nitrite (–■–); 1% RBP (–▲–); 50ppmNitrite+1% RBP (–☒–); 100ppm Nitrite+1% RBP (-x-).

The starting pH values of all sucuk tested was approximately determined 6.4. The pH values of all sucuk groups were not found below 6.0 during the storage period. pH is a significant factor in the control of microbiological growing in sausages (Buncic et al., 1993; Teodorovic et al., 1994). Therefore, the pH value of sucuks did not have a preventing effect on microbial growth (Table 1). Sang-Keun et al. (2014) pH of emulsified sausage of red beet added was found 6.31. Aykın-Dinçer et al. (2020) found the pH value of sucuk to be 6.49 after heat treatment. These researchers suggest that these pH results might be attributed to the greater degree of water loss and lactic acid formation in sausages during storage. The aw values decreased during the storage time; at the starting of the ripening, with a mean value of 0.95±0.01, and 0.92±0.01 at the end of storage period (Table 1). The moisture of sucuk decreased with the adding of red beet powder regardless of the added level (1.0%). The water content of red beet powder is much lower than that of sucuk, and as the beet ratio increases, the amount of water in the product decreases. Moisture decreased during the storage time depending of drying (Table 1).

The quantity of residual nitrite in the sucuks reduces after the heat treatment and it slightly increased during the storage time. Treatments with added red beet and nitrite had higher residual nitrite values at treatments of compared to control (P<0.01). As expected, all nitrite-added treatments were showed high values of residual nitrite, varying from 4.58 to 5.81 ppm at 0 d of storage. These values increased to 4.63-7.96 ppm to 0 and 30 days depending of drying. In this study, the joining of 1% red beet powder in sucuk contained residual nitrite (Table 1). It is accepted that vegetables, including red beet root, are well sources of nitrite because of their nitrate content (Sebranek and Bacus 2007). Socaciu (2008) reported that red beet has high nitrate levels. There are found almost the same residual nitrite level in the other groups except for control. The same increasing residual nitrite, as shown by Sindelar et al. (2007) and Sang-Keun et al. (2014) was determined in the present study; however, nitrite and red beet powder-added treatments (N and NP 2) did not demonstrated the similar trends. Residual nitrite contents of salami-type sausages containing different levels of nitrite reduced to similar levels at the beginning of the ripening period and detected as 1.4 and 1.9 mg/kg at the end of the ripening period (Sammet et al., 2006). According to Xi et al. (2012), the residual nitrite amount was about 75% of the initial concentrations after the production process and ranged between 4 mg/kg and 10 mg/kg by the end of the storage period. Depending on the type of processed meat, the processing conditions, the presence of sodium ascorbate, and other factors (myoglobin, nonheme proteins, and lipids), the added nitrite can react with many components in the matrix. Thus, the analytical detection of nitrite or nitrate content does not reflect the initially added preservative (Cassens, 1997; Jiménez-Colmenero and Solana, 2009).

Sucu and Yıldız-Turp (2018) As a result of their studies using nitrite and red beet powder in sucuk, found that there was no significant difference between the residual nitrite contents of the samples at the end of the storage period. Feifei et al. (2022) suggest that in the industrial application stage, it will be possible to reduce the amount of nitrite used when natural colorants are mixed with nitrite.

Lipid oxidation is one of the most important changes during meat products storage and production. It may change the color, aroma, flavor, texture, and nutritive values of meat products (Tarladgis et al., 1960). The results of the FFA amounts determined during the storage in the sucuk samples are given in Table 1. There was a significant (P<0.01) difference between the control group and the other groups in terms of FFA values determined in sucuks (Table 1). This level was determined to be at lower quantity in samples P and NP1 than in the control, N and NP2 samples. The FFA levels were significantly affected by storage period (P<0.01). The level of FFA in fat correlates with the lipolytic activity of lipases, the microbial metabolic process, and oxidative reactions that work on the FFA released in lipolysis. The prevention of hydroperoxide decomposition by antioxidants are critical in protect food quality (Maillard et al., 1996). These reactions are clearly related to both the crude material used to prepare sucuk and the production process (Toldra, 1994). In whole the groups, there was a developing increase of the FFA production over time, but some differences determined among the treatments (Figure 2).

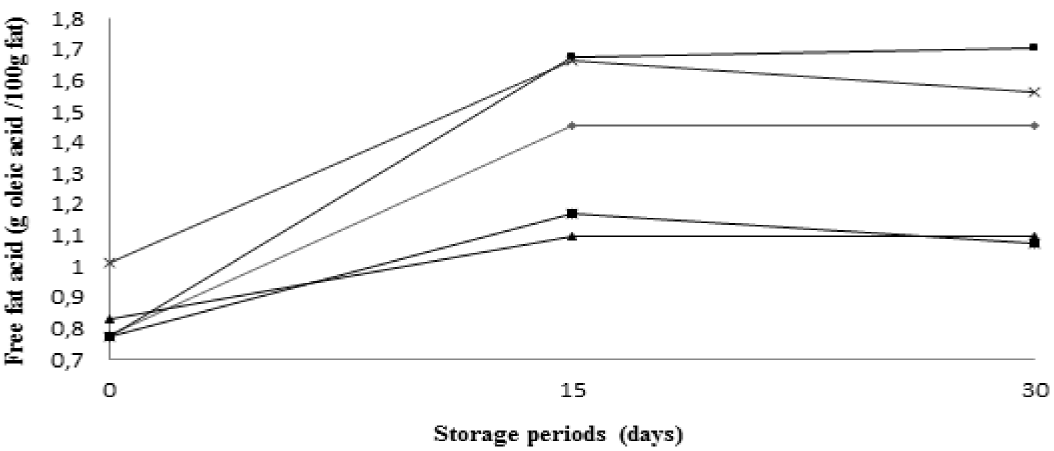

Figure 2. The effect of the interaction between treatments x storage period on ffa values. Control (–♦–); 100ppm nitrite (–■–); 1% RBP (–▲–); 50ppmNitrite+1% RBP (–☒–); 100ppm Nitrite+1% RBP (-x-).

In the sucuk with RBP had found lower FFA values. RBP is regarded as a well source of antioxidants and natural colorants because of the phenolic compounds and betalains that are existing in red beet (Sang-Keun et al., 2014; Ravichandran et al., 2012; Ravichandran et al., 2013). Betalains may exhibit strong antioxidant activity in biological environments (Socaciu, 2008). These results showed that the red beet powder may have an antioxidant effects on sucuk samples in this study.

The results of the thiobarbituric acid reagent (TBARS) analysis were not affected by the red beet powder added to the sucuks (P>0.05) but, TBARS was significantly affected by treatments and storage period (P<0.01). The utmost mean of TBARS level was found NP1 group (Table 1). An effect from red beet powder was not found big differences among treatments during the storage time except for 15 d when the addition of red beet powder increased TBARS levels (P<0.01).

According to Tarladgis et al. (1960), the acceptable limits of TBARS value of cooked meat products during storage is 0.5–1.0 mg MD/kg. Kohsaka (1975) have reported that malondialdehyde concentration of 0.5 mg MD/kg is a threshold value for rancidity perception by consumers. These results suggested that TBARS values less than 1.0 mg MD/kg does not indicate rancidity. The use of beetroot powder would have been expected to bigger exhibition an antioxidant activity due to betalain content. However, TBARS values of red beet powder were determined as almost the same results in comparison with control and during the storage days TBARS values were lower than the limit (< 1.0 mg/kg) (Tarladgis et al., 1960). As noted by many authors (Goulas and Kontominas 2007; Maqsood and Benjakul 2010), the TBARS value of meat products tending to decrease towards the end of storage time is attributed to the interaction of these unstable low molecular weight compounds with organic acids. These degradation products cannot be determined by the TBARS test. This effect of red beet supplementation on the TBARS value of sausage was expected because red beet contains betalains that show antioxidant and radical scavenging activities (Sang-Keun et al. 2014; Escribano et al., 1998; Georgiev et al., 2010).

However, the addition of 1.0% of red beet powder determined too small to influence of lipid oxidation of heat treatment sucuk. The amount of nitrite and beetroot powder used in the study affected the free fatty acid level and this amount increased during the storage period. Heat treatment caused oxidation of oxidizable fatty acids and increased the amount of TBARS. Based on this threshold value and our results were showing that TBARS values of treatments added with red beet powder did not exceed 0.65 mg MD/kg by the end of the storage period. These results were in accordance with study indicating that the TBARS values of fermented sausages with freeze-dried leek powder used as a nitrate source are higher than those of control group sausages with nitrite thus indicating the necessity of adding nitrites to sausages with freeze-dried leek powder (Tsoukalas et al., 2011).

More specifically, studies are needed to determine the chemical reactions between betalains and meat components and the effects of betalains on physical changes in meat product during processing.

3.2. Microbiological Analysis

The microbiological analysis of heat treatment sucuks with red beet powder and nitrite substituted are given in Table 1. The use of RBP and storage period and interaction had very important effects (P<0.01) on total mesophilic aerobic bacteria (TMAB), moulds/yeasts and *Enterobacteriaceae* counts (Table 1). While the total number of mesophilic aerobic bacteria (TMAB) decreased in the N group samples on the 30th day of storage, it increased in the samples from the other groups. On the 30th day of storage, *Enterobacteriaceae* and mold-yeast numbers in all samples increased compared to the initial numbers. The TMAB numbers in the N and control groups were lower than the RBP added groups. TMAB numbers reached almost 6 log CFU/g at the end of the storage period. The initial numbers of *Enterobacteriaceae* were 2.11 log CFU/g and continued to increase rapidly in all sucuk samples throughout the storage period. The initial number of *Enterobacteriaceae* were 2.11 log CFU/g and not fast much more still continued to grow in all sucuk samples during the storage time. Counts of microorganisms did not sufficiently reduce during the storage period with heat treatment (Table 1). High microorganism counts may affect the safety and shelf life of the sausage as they may contain spoilage and pathogenic microorganisms. It was known that the growth of the microorganisms, particularly *Enterobacteriaceae* was affected by pH to reduce, but in this study microorganisms were not affected by pH level. Because, the pH values of sucuks were not sufficiently decreased during the storage time. Also, starter culture was not used in sucuk manufacture and fermentation was not apply in this study. As a result, the pH value and water activity was not reduced and the microorganisms were not affected by these conditions of the sucuk. Effects of these microorganisms may be prevented at certain counts by heat treatment, pH and aw. In order to produce safer sucuk, starter culture must be used and heat treatment should be applied after fermentation. Our results are in deal with many other studies (Gençcelep et al., 2007; Ercoşkun et al., 2010).

3.3. Color Analysis

The effect of adding beetroot powder instead of sodium nitrite on the color of sausages at the beginning and end of storage the evolution of outside and cut surface color parameters is given in Table 2.

Table 2. Overall effect of treatment and storage period on color properties of sucuk (means± SD)

	Color of surface			Color of cut surface		
Treatment	L*	a*	b*	L*	a*	b*
C	30.59±4.25[b]	12.15±3.44[ab]	11.28±3.86[c]	37.26±3.09[a]	20.85±1.64[a]	23.97±3.15[a]
N	37.20±6.29[a]	11.92±4.90[ab]	15.34±1.93[a]	36.53±3.73[a]	19.45±3.17[b]	21.54±3.52[b]
P	29.08±3.53[b]	11.49±2.95[b]	10.27±2.27[d]	33.25±3.13[c]	17.35±2.79[d]	18.02±3.23[c]
NP1	30.36±3.03[b]	12.65±2.88[ab]	10.46±2.86[cd]	33.48±2.90[c]	17.77±2.91[cd]	16.43±3.71[d]
NP2	30.14±3.40[b]	12.76±3.38[a]	12.57±1.20[b]	34.93±1.45[b]	18.65±1.38[bc]	17.98±1.07[c]
Significance	**	**	**	**	**	**
Storage period						
0d	31.05±4.53[a]	15.95±2.24[a]	23.97±3.15[a]	36.81±1.67[a]	21.04±1.64[a]	21.66±3.06[a]
15d	31.65±6.73[a]	11.93±1.84[b]	21.54±3.52[b]	36.30±3.34[a]	19.14±1.82[b]	20.92±3.66[b]
30d	31.74±3.64[a]	8.70±1.51[c]	18.02±3.23[c]	32.17±2.48[b]	16.26±2.20[c]	16.18±3.20[c]
Significance	NS	**	**	**	**	**
TxS	**	**	**	**	**	**

C= control; N 100 ppm nitrite; P= % 1 red beet powder; NP= 150 ppm nitrite + %1 red beet powder; NP_2= 100 ppm nitrite + %1 red beet powder. [a–d] Any two means in the same column having the same letters in the same section are not significantly different at P>0.05, **P<0.01, NS= not significant; SD= standard deviation

All color measurements (outside and cut surface) of L*, a* and b* levels of the sucuk samples were importantly affected by treatment and storage period and its interaction (P<0.01; Tables 2 and Figures 3 and 4). Addition of red beet powder has an effect on the value of redness and the maximum levels have been monitored from the sample in group NP2 outside color of sucuks. Only nitrite added sucuk had the highest L* values in the samples and multiple comparisons of treatments in the processing time revealed that the lightness of red beet powder added groups were significantly different from the only nitrite samples. The lightness was not changed with the adding RBP (%1) and RBP (%1) + nitrite levels (50 and 100 ppm) in sucuk (Table 2). These can be attributed to the color effect on the L* values of the RBP causing the darker color. Likewise, Sucu and Turp (2018) reported that the addition of beetroot powder as a nitrate source into fermented sausages led to a decrease in the L* and b* values of sausages at the beginning of storage (P<0.05).

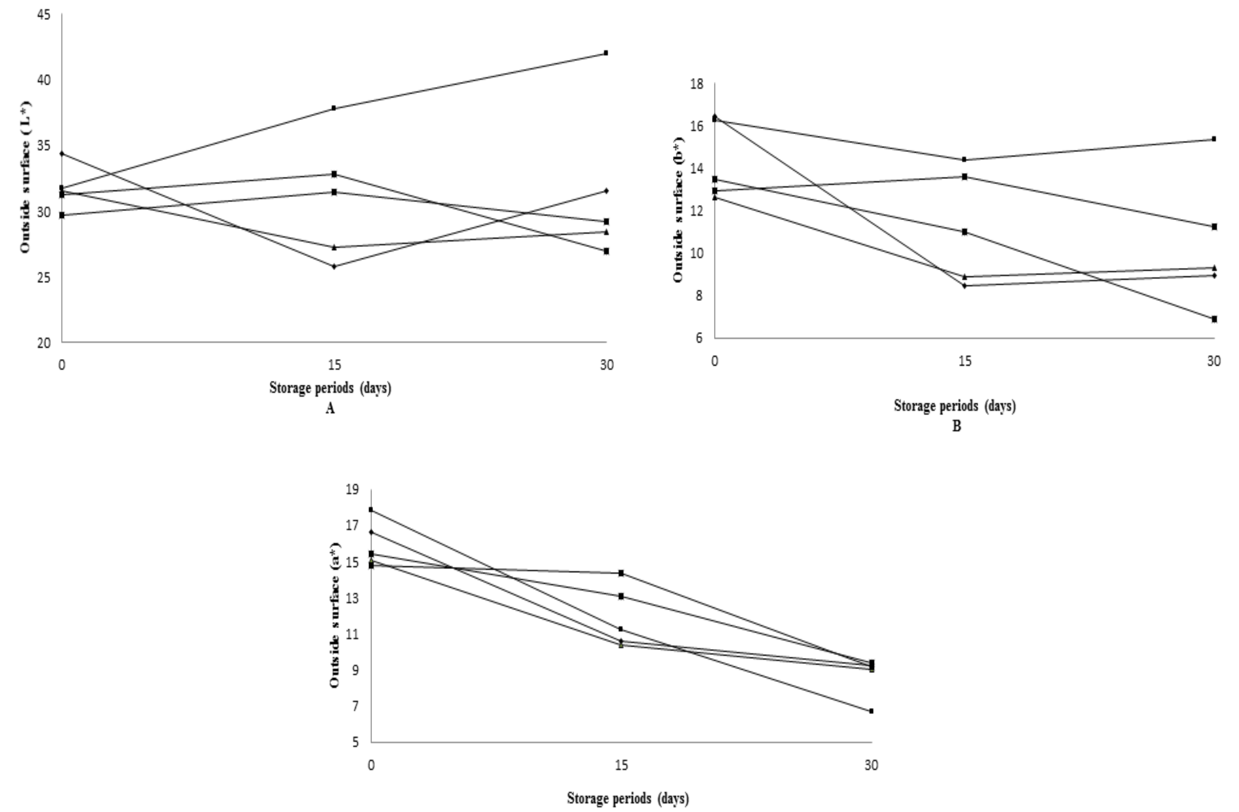

Figure 3. The effect of the interaction between treatments x storage period on colors (outside surface) values. Control (–♦–); 100ppm nitrite (–■–); 1% RBP (–▲–); 50ppmNitrite+1% RBP (–☒–); 100ppm Nitrite+1% RBP (-x-).

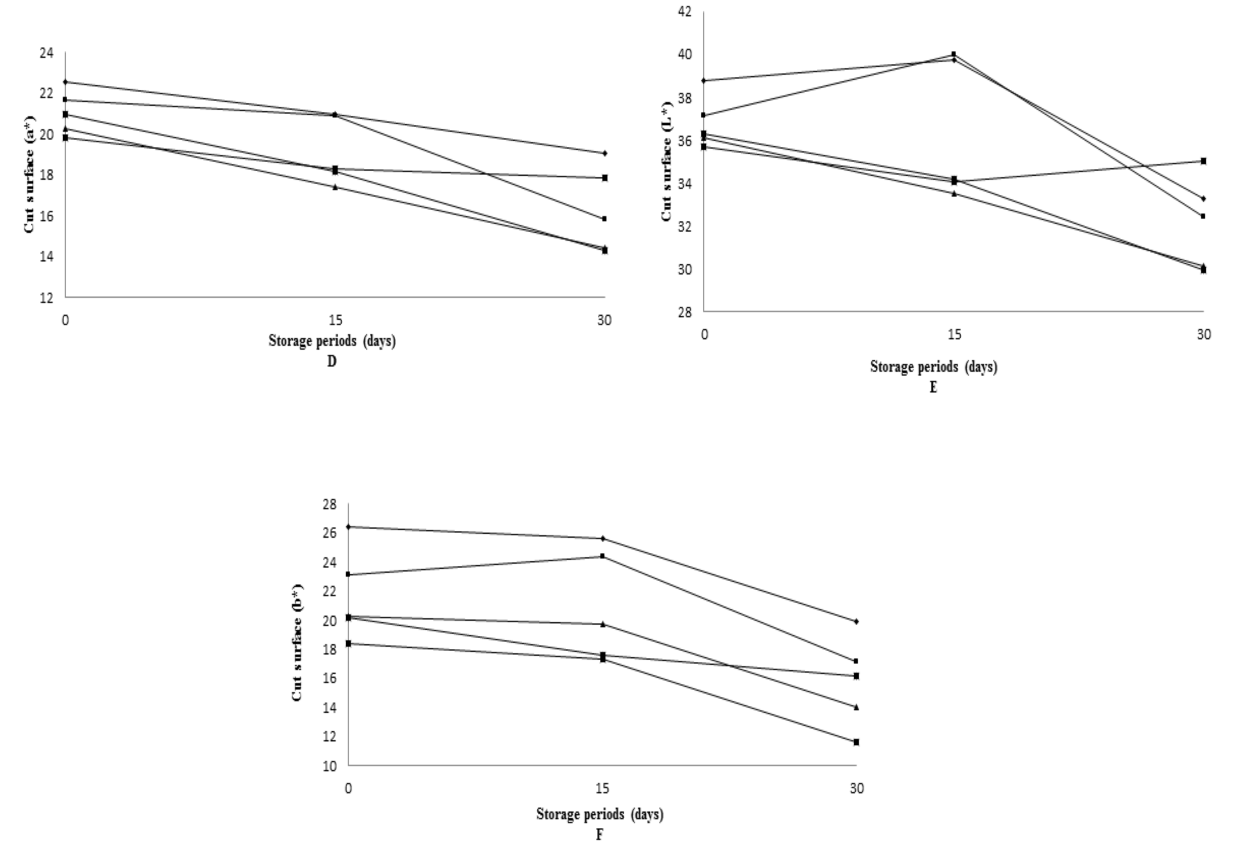

Figure 4. The effect of the interaction between treatments x storage period on colors (cut surface) values. Control(–♦–); 100ppm nitrite (–■–); 1% RBP(–▲–); 50ppmNitrite+1% RBP (–⊠–). 100ppm Nitrite+1% RBP (-x-).

The reducing in L* value are represented by the formation of dark color in the sucuk because of the browning reaction. Üren and Babayiğit (1996) determined that the lightness values of sucuk samples were between 35.87 and 45.92, whereas redness and yellowness values found between 6.87 and 14.14, and 10.04 and 17.62, respectively. Similarly, Kayaardı and Gök (2003) reported that L* values of sucuk usually reduced during the 15 days of ripening period. The lightness (L*) values of inside color of heat treated sucuk specimens decreased during storage time (Table 2). The decrease in L* levels of traditional sucuks were also determined by Bozkurt and Bayram (2006), Kayaardı and Gök (2003). The decrease in L* value indicate darkening due to drying (Üren and Babayiğit, 1996). However, heat treatment caused an increase in L* values of sausages ($P<0.01$). Denaturation of myoglobin can cause a light color in sausages (Chasco et al., 1996). Compared to the L* values of the heat-treated sample at different periods of fermentation, traditional sucuk had a lower L* value ($P<0.01$). During the initial days of storage time, nitrogenous compounds (such as nitrite) present in meat colour combined with myoglobin to perform the desired color pigment. This pigment has a red color, therefore, a* values would be different. The pigment formed during this period is denatured and so, it caused some decreases in a* values. Similar results were observed as stated by Kayaardı and Gök (2003) that a* values of sucuks increased during the first 5 days of ripening, but decreased during the later ripening period. Perez-Alvarez et al. (1999) reported that nitrosomyoglobin formation and moisture loss may be related to the reasons for the increase in a* values. A possible reason for the decrease in a* values may be the partial or complete breakdown of nitrosomyoglobin due to lactic acid production (Muguerza et al., 2002). Martínez et al. (2006a) indicated that betalains, natural colorant found in red beetroot, can be easily affected by effects such as light, oxygen, pH, temperature. These results indicate that red beet is effective in increasing the redness of heat treatment sucuk because of the red beet contained betalains (Ravichandran et al., 2013).

This result shows that beetroot powder is effective in providing the desired red color in sausages due to their betalain and also nitrate content. A similar result was reported for mortadella sausage by Baldin et al. (2018). Moreover, the use of beetroot powder caused an increase in the a* values of sausages compared to the control. Also, similar results have been reported by different authors for emulsified pork sausage (Jin et al., 2014), emulsified beef sausage (Turp et al., 2016) and Turkish fermented beef sausage (sucuk) (Sucu and Turp, 2018).

Although redness decreased over storage time, this is probably caused by pigment degradation as noted by Fernandez-Gines et al. (2003). Moreover, high water activity, high storage temperature, high luminosity and presence of oxygen and metal ions have been reported to have negative effects on the stability of betalains, natural colorant found in beetroot (Aykın-Dinçer et al., 2020); therefore, beetroot powder may not protect sausages from discoloration throughout the storage in spite of increasing redness. The decrease in b* value during storage indicates that the color of the sucuk changes from yellow to blue (nearing a negative b* value) over time. It can be said that the reason for this may be browning reactions because melanoidins have a brown color.

Betalains are considered substrates of peroxidase due to their chemical structure. In addition to oxygen, hydrogen peroxide was also reported to accelerate betanin degradation. As a result of this study, it is thought that the decrease in a* and b* values in sucuk during the storage period is due to the degradation of betalins by peroxidase enzymes produced by microorganisms (Pedreno and Escribano 2000; Herbach et al., 2006).

The color results determined in the study are similar to the results shown by Kayaardı and Gök (2003). Perez-Alvarez et al. (1999) determined that the b* values of Spanish sausages also decreased during fermentation and maturation periods. They explained the decrease in b* values as the decrease in oxymyoglobin, which contributes to the use of oxygen by microorganisms and therefore the yellow color. Jeong et al. (2010) also reported a decrease in redness of low-fat sausage, which added red powder homogenate during storage, but the smoked low-fat sausage with 75 ppm sodium nitrite and 0.5% red powder homogenate showed stable redness during storage. These results disagreed with this study due to differences between the type of red powder (powder and homogenate) and type of sausage (emulsified sausage and smoked low-fat sausage). The addition of red beet decreases lightness and yellowness of heat treatment sucuk; thus, the added level of red beet needs to be adjusted to control desirable properties.

4. Conclusion

The use of beetroot powder instead of nitrite in heat treated sucuk which is a popular traditional meat product affected some quality characteristics of this reformulated product depending on the used amount.

While there was statistically significant difference between the residual nitrite contents of the samples at the end of the storage period, containing only beetroot powder had a significantly higher residual nitrite in comparison with those of the control sample. Color change and lipid hydrolysis in heat treatment sucuk is a big problem during cold storage and the same problem continues in the marketplace. Redness value (a*) of the samples in surface and cut surface decreased and was no well protected during the storage when an increased amount of beetroot powder was used.

Instead of red beet, after pure betalain extraction from red beet, can be a suitable colorant for meat products as well as an additive substitute. In addition, beetroot powder can be considered as an alternative to synthetic colorants and nitrite in cooked, smoked, semi-dried and fermented sausages or less nitrite in all cured meats.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	Ö.S	H.G
C	30	70
D	50	50
S	10	90
DCP	20	80
DAI	40	60
L	80	20
W	10	90
CR	30	70
SR	20	80
PM	20	80
FA	20	80

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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