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Effect of Ripening Period, Nitrite Level and Heat Treatment on the Chemical Characteristics of Turkish Dry Fermented Sausage (Sucuk)

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ABSTRACT : In order to determine the potential for reduction of nitrite levels and ripening period with heat treatment, the effects of ripening period (1-13 days), nitrite level (45-195 ppm), and heat treatment (30-90°C) on lipolysis, peroxide, TBA, proteolysis, and residual nitrite values of sucuk were investigated using response surface methodology. The ripening period significantly ($p<0.01$) increased lipolysis, peroxide, TBA, and proteolysis values and decreased residual nitrite values. The effects of additional nitrite levels were found to significantly affect peroxide and residual nitrite values. Significant amounts of the additional nitrite levels were reduced during processing and on the first day of ripening periods. (**Key Words :** Sucuk, Dry Fermented Sausage, Nitrite, Heat Treatment, Oxidation)

INTRODUCTION

Sucuk is a dry fermented Turkish sausage that is manufactured from beef or sheep meat with tail fat. Changes in the fermentation process of sucuk are based on the interaction between meat, fat, bacterial growth, physico-chemical interactions and biochemical processes (Chizzolini et al., 1998). Microorganisms are naturally present in raw materials, and can result in contamination during the process or can be added as starter culture (Bover-Cid et al., 2001a). Metabolic activities of starter culture are required for the desirable changes that determine the particular characteristics of dry fermented sausages (Erkkilä, 2001). Lactic acid bacteria (LAB) is particularly well adapted to the meat fermentation and are involved in all the alterations occurring during the ripening process (Bover-Cid et al., 2001b). They produce lactic acid, which reduces the pH of dry fermented sausage (Bover-Cid et al., 2001b).

Proteolysis and lipolysis constitute the main biochemical reactions in the generation of flavour or flavour precursors, where these process are mediated by proteases and lipases, respectively (Chizzolini et al., 1998; Toldrá, 1998). Several muscle proteases and lipases play an important role in the biochemical mechanisms that occur during the ripening period, where these are directly related to the final quality of sucuk (Toldrá, 1998). A significant percentage of the generated free fatty acids occur as a result of phospholipid hydrolysis in dry cured meat products (Toldrá, 1998). Typical breakdown products, such as aldehydes, are derived from lipid oxidation (Chizzolini et al., 1998). Denaturation and the partial fragmentation of proteins improve the sensorial quality characteristics of sausages (Ordóñez et al., 1999). Mottram (1998) reportad that a complex series of thermally induced reactions leading to a wide range of products, that determine the aroma attributes and contribute most the characteristic flavours of meat. Some products of proteolysis, such as biogenic amines, form carcinogenic nitrosamines by reacting with nitrite. In addition to this effect, nitrite may also cause hemoglobinemia (Gökalp, 1984). Therefore the decreasing the nitrite level is critical in sucuk production.

The ripening period determines the physical, chemical and sensorial characteristics of sucuk (Kurt, 2006). Kurt (2006) reported that the ripening period can be shortened from 13 days to 7-8 days using a heat treatment. A shortened ripening period may positively affect oxidation of sucuk. Kurt (2009) reported that shortened ripening period with heat treatment prevented biogenic amines formation in dry fermented sausage. In order to determine the possibilities for the reduction of nitrite level and ripening period with heat treatment, the effects of the these factors

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on some of the chemical characteristics of sucuk were examined using response surface methodology.

MATERIALS AND METHODS

Sucuk preparation

Sucuk was prepared as follows: 84.6% meat (beef), 9.4% lamb tail fat, 1.9% salt, 0.94% garlic, 0.66% red pepper, 0.47% black pepper, 0.85% cumin, 0.24% allspice, 0.47% sugar and 0.47% phosphate (K_2HPO_4; Merck, Darmstadt, Germany). The meat and fat pieces (~4 cm^3 in size), spices, garlic, salt, sugar, and phosphate were mixed and minced in a grinder (Cem 32, Cem, İstanbul, Turkey). Starter cultures (*Lactobacillus sakei, Pediococcus pentosaceus, Staphylococcus carnosus* and *Staphylococcus xylosus*; Bactoferm™ F-RM-52 and T-SPX; Chr. Hansen, Denmark) were added to the sucuk batter and mixed in. Sucuk batter was divided into 18 equal parts and varying amounts of nitrite, which was dissolved in 20 ml distilled water, were added to each part as shown in Table 1. Each of resulting batches of batter was rested for 12 h at 4°C and stuffed into collagen casings (Naturin Darm, Germany) of 35 mm diameter using a filling machine (Cem, Turkey). Each sample was washed under running water, and then a 10% potassium sorbate solution was sprayed on it. Samples were ripened at 20±1°C. For equilibration, the relative humidity was adjusted to 60% in the first 6 h of the ripening period and was then increased to 87±3% and decreased every day by 1 unit. At the end of the each ripening period (Table 1), sucuk samples were heated by steam. The temperature difference between the ambient and core temperatures was adjusted to 5±1°C. Ambient and core temperatures were controlled with PID (Proportional with Integral and Derivative) temperature controllers (Emko, Turkey) equipped with probes (Pt-100, Emko, Turkey). Sucuk samples were heated from 20±1°C to the required core temperatures (Table 1) and immediately cooled to 25±1°C with a cold water spray. Samples were maintained at 20±1°C for 2.5 h for equilibration and were stored at 2±1°C during analysis.

Determination of residual nitrite and TBA values

TBA (mg malondialdehyde/kg sucuk) was determined according to the distillation method, as described by Tarladgis et al. (1960). Residual nitrite (mg/kg sucuk) was determined according to AOAC (2000).

Determination of proteolysis value

Proteolysis was determined according to Anonymous (1989), with some modifications. A 4 g minced sample was weighed into a test tube and 40 g trichloroacetic acid solution (20 g/100 ml) was added. The sample was homogenised with a homogenizer (Pro260, Pro, The United States) for 30 sec and 15 min were allowed for sedimentation. After centrifugation (Hettich Universal 32R, Germany) at 2,800 g for 20 min at 4°C, then the supernatant was filtered (Schleicher and Schuel No: 2095) in a test tube and adjusted to 50 ml by the addition of trichloroacetic acid

Table 1. Central composite rotatable design of three independent variables

Run order	Codified levels			Actual levels		
	X_1	X_2	X_3	Ripening periods (day)	Heat levels (°C)	Nitrite levels (mg kg^{-1})
1	-1.5	0	0	1	60	120
2	-1	-1	-1	3	40	70
3	-1	-1	1	3	40	170
4	-1	1	-1	3	80	70
5	-1	1	1	3	80	170
6	0	-1.5	0	7	30	120
7	0	0	-1.5	7	60	45
8	0	0	0	7	60	120
9	0	0	0	7	60	120
10	0	0	0	7	60	120
11	0	0	0	7	60	120
12	0	0	1.5	7	60	195
13	0	1.5	0	7	90	120
14	1	-1	-1	11	40	70
15	1	-1	1	11	40	170
16	1	1	-1	11	80	70
17	1	1	1	11	80	170
18	1.5	0	0	13	60	120

solution. Non-protein nitrogen components were analysed in 20 ml of the extract solution using the Kjeldahl method. Proteolysis was calculated as:

Proteolysis = NPN×100/TN

Where NPN = Non-protein nitrogen,
TN = Total nitrogen

Determination of free fatty acid (FFA) - lipolysis

A 10 g ground sample was mixed with 25 ml of chloroform and 0.5 g of sodium sulfate for 5 min, and then filtered with filter paper. The free fatty acids in the 25 ml of filtrate were titrated with 0.1 N NaOH. Free fatty acid content was expressed as g oleic acid/100 g fat (Egan et al., 1981).

Free fatty acid (%) = (S×N×F)×28.2/W

where S is the volume of titration (ml), N is the normality of the sodium hydroxide solution, F is the factor of the sodium hydroxide solution and W is the fat weight (g) in the sample.

Determination of peroxide value

The peroxide value was determined according to the AOAC (1999), with some modifications. The 5 g sample was weighed in a 250-ml glass stoppered Erlenmeyer flask and heated in a water bath at 60°C for 3 min to melt the fat, then thoroughly agitated for 5 min with 30 ml acetic acid-chloroform solution (3:2 v/v) to dissolve the fat. The sample was filtered under vacuum through Whatman filter paper to remove meat particles. Saturated potassium iodide solution (0.5 ml) was added to the filtrate and transferred to dark medium for 5 min. After addition of 30 ml water, the filtrate was titrated against a standard solution of sodium thiosulfate (25 g/L). The peroxide value (POV) was calculated and expressed as milliequivalents peroxide per kg of sample:

POV (meq O_2/kg fat) = (S×N)×1,000/W

where S is the volume of titration (ml), N is the normality of sodium thiosulfate solution (N = 0.01), and W is the fat weight (g) in the sample.

Statistical analysis

The experimental design and statistical analysis were performed using JMP 4 Software (SAS Institute Inc.). The experiments were based on a central composite rotatable design with a total of 18 combinations, including four replicates of the centre point were carried out in random order. The codified and actual levels are given in Table 1. The variables were coded according to the following equation:

$$X_i = (x_i - \overline{x_i}) / \Delta x_i$$

where X_i is the coded value of an independent variable, x_i is the real value of an independent variable, $\overline{x_i}$ is the real value of an independent variable at the centre point, and Δx_i is the step change.

Two replicates were performed for this study. The variance for each factor assessed was partitioned into linear, quadratic and interactive components and was represented using a second order polynomial equation. The equation is:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_{ii}^2 + \sum_{\substack{i=1 \\ i<j}}^{k} \sum_{j=1}^{k} \beta_{ij} x_i x_j$$

where Y is the estimated response, β_0, β_i, β_{ii}, and β_{ij} are constant coefficients, k is the number of factor variables, and X_i, X_{ii}, and X_{ij} represent the linear, quadratic and interactive effects of the independent variables (ripening period, heat treatment and nitrite level), respectively. The analysis was performed using uncoded units.

RESULTS AND DISCUSSION

Lipolysis

The analysis of results that indicate lipolysis are summarised in Table 2. The linear effect of the ripening period was found to be significant ($p<0.01$) for lipolysis. As shown in Figure 1, lipolysis was augmented with the increasing ripening period. During the ripening period, the level of free fatty acids in the fat of dry fermented sausage depends on the hydrolytic activity of the lipases, the microbial metabolic processes, and the oxidative reactions that alter the free fatty acids released in lipolysis (Soriano et al., 2006). Such lipases are mainly of endogenous origin and these enzymatic activities increase with decreasing pH value in dry cured meat products (Chizzolini et al., 1998; Toldrá, 1998; Vestergaard et al., 2000). Long processes with mild ripening conditions allow for relatively higher enzyme activity, and therefore, a greater generation of free fatty acids (Toldrá, 2006). A significant percentage of the generated free fatty acids in dry cured meat products as a result of phospholipid hydrolysis (Toldrá, 1998).

Peroxide

The linear effect of the ripening period was found to effect peroxide values ($p<0.01$, Table 2). As shown in Figure 2, the ripening period increased peroxide values. Toldrá (2006) reported that lipids and phospholipids are

Table 2. Analysis of variance of the effects of ripening period, nitrite and heat treatment on chemical properties of sucuk

Sources of variation		Lipolysis (g oleic acid/100 g fat)		Peroxide (meq O_2/kg fat)		TBA (mg kg^{-1})		Proteolysis (NPN×100/TN)		Residual nitrite (mg kg^{-1})	
	DF	F-value		F-value		F-value		F-value		F-value	
Model	9	18.104	**	18.371	**	20.433	**	17.412	**	144.690	**
X_1 (Ripening period)	1	156.923	**	155.787	**	176.823	**	140.682	**	49.268	**
X_2 (Heat treatment)	1	0.165		3.380		0.183		0.144		4.816	*
X_3 (Nitrite)	1	2.250		4.880	*	2.468		0.875		1,193.928	**
$X_1 \times X_1$	1	2.043		0.062		3.779		14.474	**	6.981	*
$X_2 \times X_2$	1	0.012		0.446		0.104		0.369		3.899	
$X_3 \times X_3$	1	0.721		0.654		0.016		0.661		11.252	**
$X_1 \times X_2$	1	0.428		0.029		0.129		0.144		0.436	
$X_1 \times X_3$	1	0.570		0.004		0.286		0.048		33.966	**
$X_2 \times X_3$	1	0.001		0.013		0.003		0.000		0.436	
Lack of fit	5	0.297		0.384		0.679		0.641		1.483	
C. total	35										

** p<0.01 significance level, * p<0.05 significance level. DF = Degrees of freedom.

hydrolyzed by lipase and phospholipase, yielding free fatty acids, which are oxidised into peroxides. A significant (p<0.01) positive correlation (0.978) was found between peroxide and lipolysis. Zanardi et al. (2004) reported that technology appeared to affect lipolysis indirectly in fermented sausage, as differences in the rate of pH decrease and final pH might affect endogenous lipases. They also reported that other parameters, such as starter cultures, additives, and spices, did not seem to have perceivable effects on lipolysis and lipid oxidation. Franco et al. (2002) reported that peroxide values were found to be 16 meq O_2/kg and 28 meq O_2/kg in Spanish fermented sausage at 0 and 14 days, respectively.

Peroxide values decreased significantly (p<0.05) with increasing nitrite levels (Table 2, Figure 2). The antioxidant effect of nitrite is most likely due to the chelating action of nitrite towards non-haem iron, which is released during meat processing (Morrissey and Tichivangana, 1985). Nitrite was previously reported to stabilise myoglobin and prevent the increase of non-haem iron (Ertaş, 1998).

TBA

Changes of in the TBA values of sucuk are given in Figure 3. TBA values were found to be affected (p<0.01) by the ripening period, which resulted in an increase of TBA values. TBA values may represent the content of secondary lipid oxidation products. However, a significant (p<0.01) positive correlation was found between TBA and peroxide

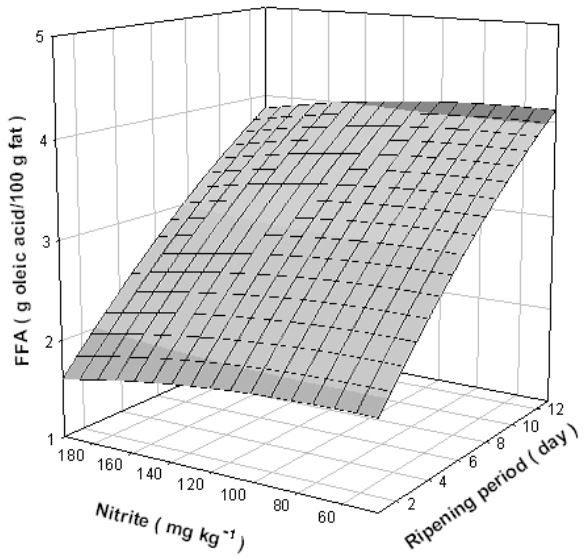

Figure 1. The effects of ripening period and nitrite level on lipolysis.

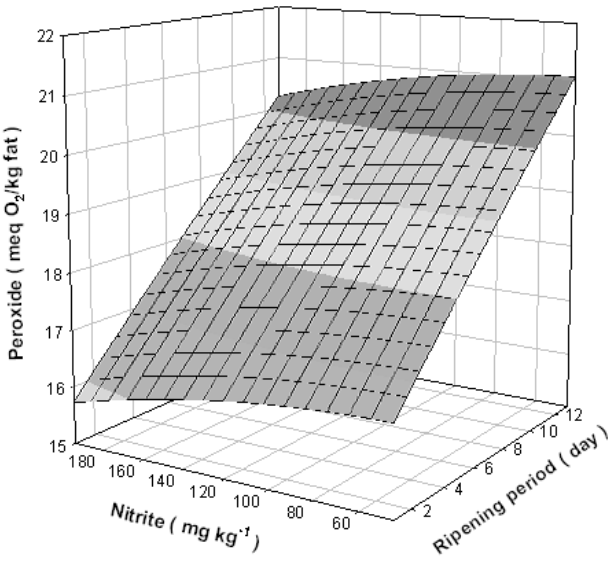

Figure 2. The effects of ripening period and nitrite level on peroxide.

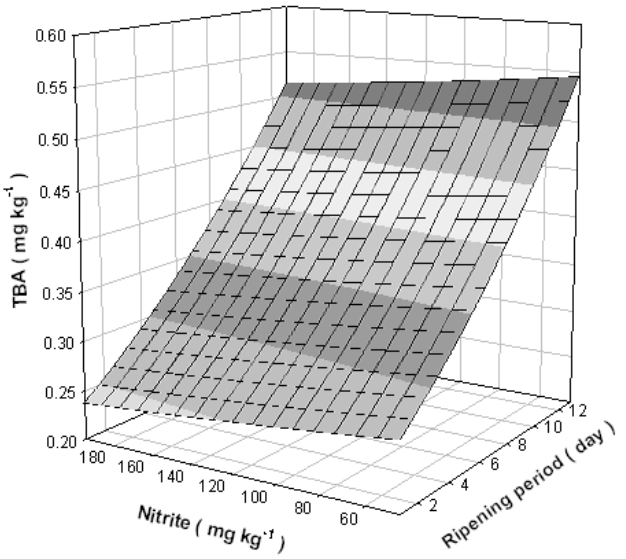

Figure 3. The effects of ripening period and nitrite level on TBA.

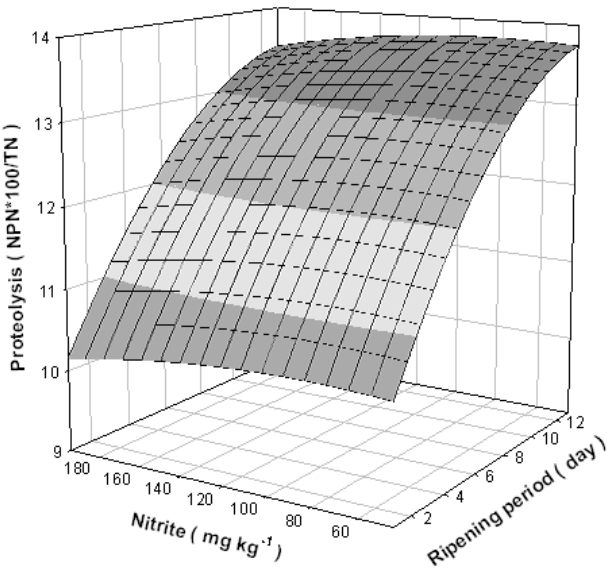

Figure 4. The effects of ripening period and nitrite level on proteolysis.

values. Increasing oxidation and proteolysis rates can increase secondary products, such as malondialdehyde and biogenic amine formation (Kurt, 2006). The effects of ripening period on TBA values were in accordance with the results of Bozkurt and Erkmen (2004), who reported that TBA values were significantly related to an increased ripening period for sucuk. In general, nitrite is known to have anti-oxidant effects on lipids. However, the differences in additional nitrite levels were not found to significantly ($p>0.05$) affect TBA values of sucuk (Table 2).

Proteolysis

As summarised in Table 2, the linear and quadratic effects of the ripening period were found to significantly alter proteolysis. As shown in Figure 4, proteolysis values increased linearly with increasing ripening time during the first days of ripening period, and the proteolysis rate then began to decrease in a a curve form until the end of the ripening time. Generally, proteolysis values increased in the first days of the ripening period (Díaz et al., 1997; Hughes et al., 2002). One of the characteristic properties of sucuk is a decline in pH value during the first days of the ripening period (Kurt, 2006). Proteolytic enzymes in dry fermented sausages are mainly of endogenous origin, and exhibit activities that increase with decreasing pH value (Zanardi et al., 2004). Zanardi et al. (2004) reported that technology appeared to affect proteolysis indirectly, and that differences in the rate of pH decline and final pH may affect the activity endogenous proteolytic enzymes. In addition to endogenous enzymes, microbial enzymes, mainly originated from *Micrococcus-Staphylococcus*, have been indicated to have a proteolytic effect in sucuk (Gökalp et al., 1998; Montel et al., 1998).

Residual nitrite

The linear and quadratic effects of nitrite were found to be significant ($p<0.01$) on residual nitrite (Table 2). The increasing levels of additional nitrite increased residual nitrite values. However, higher levels of additional nitrite decreased augmented rate of residual nitrite. The linear effect of the ripening period was found to significantly ($p<0.01$) effect residual nitrite levels, and the quadratic effect was also found to be significant ($p<0.05$). As shown in Figure 5, additional nitrite levels decreased below 14 ppm until the end of the first day of the ripening period. This decline indicated that a significant portion of

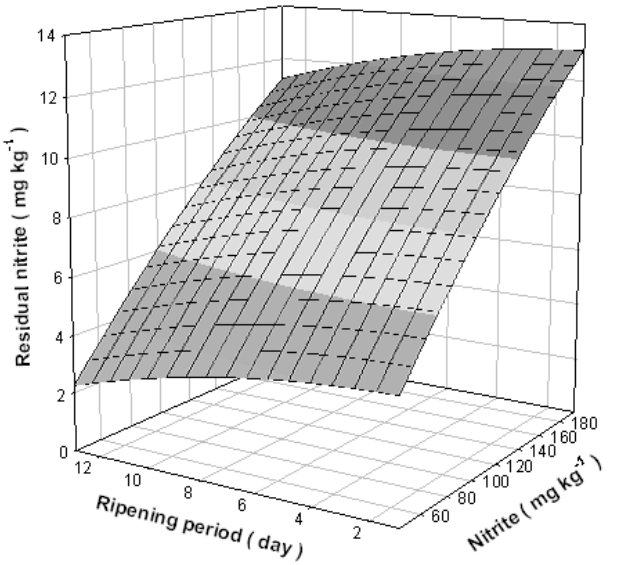

Figure 5. The effects of ripening period and nitrite level on residual nitrite.

Table 3. Predicted model equations for the effects of ripening period (X_1), nitrite level (X_2) and heat treatment (X_3) on chemical properties of sucuk

Parameters	Equations
Proteolysis	$Y = 12.948+1.108X_1+0.035X_2-0.087X_3-0.411X_1^2-0.066X_2^2-0.088X_3^2+0.044X_1X_2-0.026X_1X_3+0.001X_2X_3$
Lipolysis	$Y = 3.162+0.747X_1+0.024X_2-0.089X_3-0.099X_1^2-0.007X_2^2-0.059X_3^2+0.049X_1X_2+0.056X_1X_3-0.003X_2X_3$
Peroxide	$Y = 18.619+1.502X_1+0.221X_2-0.266X_3-0.035X_1^2+0.093X_2^2-0.113X_3^2-0.026X_1X_2-0.009X_1X_3-0.017X_2X_3$
TBA	$Y = 0.362+0.091X_1-0.003X_2-0.011X_3+0.015X_1^2-0.003X_2^2+0.001X_3^2-0.003X_1X_2-0.005X_1X_3-0.0004X_2X_3$
Residual nitrite	$Y = 8.929-0.606X_1-0.189X_2+2.982X_3-0.264X_1^2-0.197X_2^2-0.335X_3^2+0.071X_1X_2-0.629X_1X_3-0.071X_2X_3$

additional nitrite levels was reduced during processing and the first day of ripening period. This result was in accordance with that of Bozkurt and Erkmen (2004) and Pérez-Alvarez et al. (1999). After the addition of nitrite, this chemical is known to react with water and is affected by reducing bacteria (Gökalp, 1983). The reduction nitrite was also noted that be in relation to the decreasing pH value of sucuk (Gökalp, 1983). After the first day of ripening period, the rest of the nitrite level continued to decrease with increasing ripening time. The interactive effect of ripening period and nitrite was found to significant ($p<0.01$) alter residual nitrite levels. Furthermore, nitrite might be transformed into nitrate and react with muscle proteins during the ripening period (Pérez-Alvarez et al., 1999).

The linear effect of heat treatment was found to be significant ($p<0.05$) for residual nitrite levels, which decreased with higher temperatures, particularly above 65°C (Figure 6). As shown in Figure 6, the decreased residual nitrite was less than 1 mg kg^{-1}. However, Soyutemiz et al. (2004) reported that nitrite level decreased from 44.55 ppm to 24.75 ppm in sucuk during 30 minutes at 60°C.

The effects of ripening period, nitrite level, and heat treatment on lipolysis, peroxide, TBA, proteolysis, and residual nitrite values were also expressed mathematically in Table 3. These predicted model equations are useful for understanding the significance of proteolysis, lipolysis, and lipid oxidation levels and the interactions between studied factors.

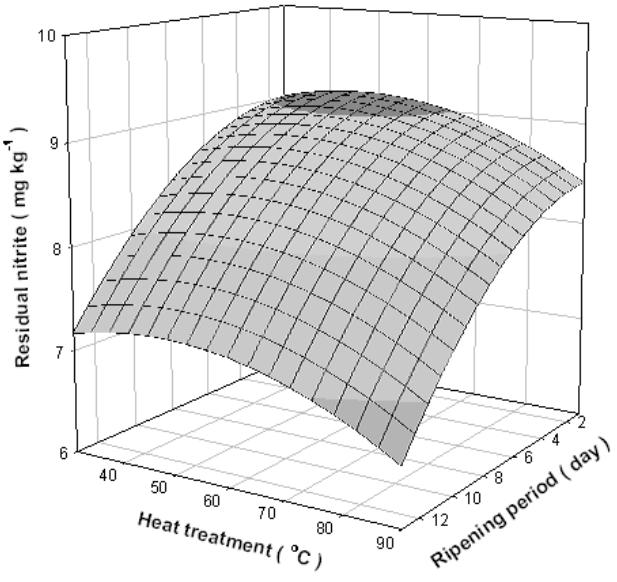

Figure 6. The effects of ripening period and heat treatment on residual nitrite.

CONCLUSION

The ripening period was determined to decrease with certain levels of heat treatment. Furthermore, additional nitrite level can be effectively decreased to lower levels. Traditional dry fermented Turkish sausage is produced without any heating process at the end of the ripening period. Decreasing the ripening period and nitrite level with certain levels of heat treatment can provide healthier sucuk, as well as provide standards for the production of commercial sucuk.

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