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Effects of Nisin and Nitrite on Some of The Chemical Characteristics of "Sucuk"-A Dry Fermented Turkish Sausage

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The effects of nitrite (0, 100 and 200 mg kg^{-1}) and nisin (0, 250 and 500 mg kg^{-1}) on pH, moisture, lipolysis, peroxide, proteolysis and residual nitrite values of Sucuk-a dry fermented Turkish Sausage were investigated by utilizing central composite design of response surface methodology. While additional nitrite levels linearly decreased peroxide values ($p < 0.05$), they increased residual nitrite values significantly ($p < 0.01$). Residual nitrite levels were found below 6 ppm at the end of the ripening period. The effects of nitrite and nisin were not found to be significant ($p > 0.05$) on pH, moisture, lipolysis and proteolysis values for the sucuk.

Key Words: Sucuk, Dry fermented sausage, Nitrite, Nisin, Oxidation.

INTRODUCTION

Sucuk is manufactured from beef and/or sheep meat with tail fat and is a typical dry fermented sausage. The fermentation process of sucuk is based on the interaction between meat, fat, bacterial growth, physico-chemical interactions and biochemical processes. Microorganisms are naturally present in the raw materials of sucuk and can result in contamination during the process or can be added as starter culture[1]. Metabolic activities of starter culture are required for the desirable changes which determine the particular characteristics of dry fermented sausages[2]. Lactic acid bacteria are well adapted to the meat fermentation environment and are involved in the multiple changes that occur during the ripening process. These bacteria produce lactic acid, which reduces the pH of dry fermented sausage[3].

New approaches, such as using bacteriocinogenic lactic acid bacteria cultures and/or the bacteriocins from these cultures, to control pathogenic and spoilage micro-organisms have been developed in order to increase food safety[4,5]. Among the bacteriocins, nisin is predominately used in food and particularly in dairy products. However, less is known regarding the effects of nisin in the processing of meat products[4]. Nisin is still the only bacteriocin approved as a food preservative (E234) in more than 50 countries worldwide, including the United State, European Union, Brazil and China[4].

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Proteolysis and lipolysis constitute the main biochemical reactions in the generation of flavour or flavour precursors. These groups of reactions are due to proteases and lipases, respectively[6,7]. Several muscle proteases and lipases play an important role in the biochemical mechanisms that occur during the ripening period, which are directly related to the final quality[6]. Increasing oxidation and proteolysis rates can increase secondary products, such as malondialdehyde and biogenic amine formation[8].

The aim of this study is to determine the effects of nitrite and nisin on the chemical and biochemical properties of sucuk were studied using response surface methodology.

EXPERIMENTAL

Meat (beef) were obtained from local markets. Nisin (Nisaplin MS-50; Danisco) was obtained from Germany. Nitrite (Merck, Darmstadt, Germany) was used as sodium nitrite.

Sucuk preparation: Sucuk was prepared according to the following recipe[8]: 84.6 % red 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 (*ca.* 4 cm^3 in size), spices, garlic, salt, sugar and phosphate were mixed and minced in a grinder (Cem, Turkey). Starter cultures (*Lactobacillus sake*, *Pediococcus pentosaceus*, *Staphylococcus carnosus* and *Staphylococcus xylosus*; Bactoferm™; Chr. Hansen, Denmark) were added to the sucuk dough and mixed in. Sucuk dough was divided in to 10 equal parts and varying amounts of nisin and nitrite, which were dissolved in 20 mL distilled water, were added to each part as given in Table-1. Each of resulting batches of dough was rested for 12 h at 4 °C and stuffed in to 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 during 13 days. For equilibration, the relative humidity was adjusted to 60 ± 3 % in the first 6 h of the ripening period and was then increased to 87 ± 3 % and decreased every day by 1 unit. After the ripening period, samples were stored at 2 ± 1 °C during analysis.

Determination of pH, moisture and residual nitrite values: The pH values were measured using a pH meter equipped with a temperature probe (Consort R735, Belgium), as described by Ockerman[9]. Moisture (%) and residual nitrite (mg/kg sucuk) were determined according to AOAC[10].

Determination of proteolysis value: Proteolysis was determined according to Anonymous[11], 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 samples were homogenized for 30 s and allowed for sedimentation for 15 min. After centrifugation at 2800 g for 20 min at 4 °C, the supernatant was filtered in a

TABLE-1
CENTRAL COMPOSITE DESIGN OF TWO INDEPENDENT VARIABLES

Run order	Codified levels		Actual levels	
	X_1 (Nitrite)	X_2 (Nisin)	Nitrite (mg kg^{-1})	Nisin (mg kg^{-1})
1	-1	-1	0	0
2	-1	0	0	250
3	-1	1	0	500
4	0	-1	100	0
5	0	0	100	250
6	0	0	100	250
7	0	1	100	500
8	1	-1	200	0
9	1	0	200	250
10	1	1	200	500

test tube and adjusted to 50 mL by the addition of trichloroacetic acid solution. Non-protein nitrogen components were analyzed in 20 mL of the extract solution using the Kjeldahl method. Proteolysis was calculated as:

$$\text{Proteolysis} = \text{NPN} \times 100/\text{TN}$$

where NPN = non-protein nitrogen, TN = total nitrogen

Determination of the amount of free fatty acids (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. The amount of free fatty acid was expressed as g oleic acid/100 g fat[12].

$$\text{Free fatty acid (\%)} = (S \times N \times F) \times 28.2/W$$

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 (kg) in the sample.

Determination of peroxide value: The peroxide value was determined according to the AOAC[13], 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:

$$\text{POV (meq } O_2\text{/kg fat)} = (S \times N) \times 1000/W$$

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

Experimental design and statistical analysis: The experimental design and statistical analysis were performed using Jump Software. The experiments were based on a central composite design with a total of 10 combinations, including two replicates of the centre point were carried out in random order. The codified and actual levels were given in Table-1. The variables were coded according to the following equation:

$$X_i = \frac{(x_i - \bar{x}_i)}{\Delta x_i}$$

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

The variance for each factor assessed was partitioned into linear, quadratic and interactive components and were 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_j, which are defined as the independent variables. The analysis was performed using uncoded units.

RESULTS AND DISCUSSION

pH, moisture, lipolysis and proteolysis: The effects of pH, moisture, lipolysis and proteolysis on sucuk were not found to be significant ($p > 0.05$; Table-2). Proteolytic enzymes in dry fermented sausages are mainly of endogenous origin and their enzymatic activities increase with decreasing pH values[14]. 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 free fatty acids released in the lipolysis[15]. Such lipases are mainly of endogenous origin and their activities increase with decreasing pH values in dry cured meat products[6,7,16]. Zanardi *et al.*[14] 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.

Peroxide: As summarised in Table-2, the linear effect of nitrite was found to be significant ($p < 0.05$) on peroxide values. As shown in Fig. 1, peroxide values decreased with increasing nitrite levels, which was in accordance with the results of Kurt[8]. The antioxidant effect of nitrite is most likely due to a chelating action of nitrite towards non-haem iron that is released during meat processing[17]. Nitrite was reported to stabilize myoglobine and prevent the increase of non-haem iron[18].

TABLE-2
ANALYSIS OF VARIANCE OF THE EFFECTS OF NITRITE AND NISIN ON SOME PARAMETERS OF SUCUK

Sources of variation	DF	pH	Moisture (%)	Lipolysis (g oleic acid/100 g fat)	Peroxide (meq O_2/kg fat)	Proteolysis (NPN × 100/TN)	Residual nitrite (mg/kg)
		$R^2 = 0.76$	$R^2 = 0.74$	$R^2 = 0.72$	$R^2 = 0.83$	$R^2 = 0.76$	$R^2 = 0.96$
		F-value	F-value	F-value	F-value	F-value	F-value
Model	5	2.593	2.304	2.018	3.900	2.468	17.677**
X_1 (Nitrite)	1	0.756	3.760	7.442	16.068*	1.145	72.084**
X_2 (Nisin)	1	5.968	5.717	0.068	2.704	7.687	7.656
$X_1 \times X_2$	1	4.979	1.716	0.228	0.015	1.656	6.986
$X_1 \times X_1$	1	0.216	0.298	0.901	0.586	0.553	1.638
$X_2 \times X_2$	1	1.176	0.065	1.061	0.050	0.998	0.128
Lack of fit	3	10.036	1.778	0.078	0.109	0.127	1.346
C. total	9	–	–	–	–	–	–

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

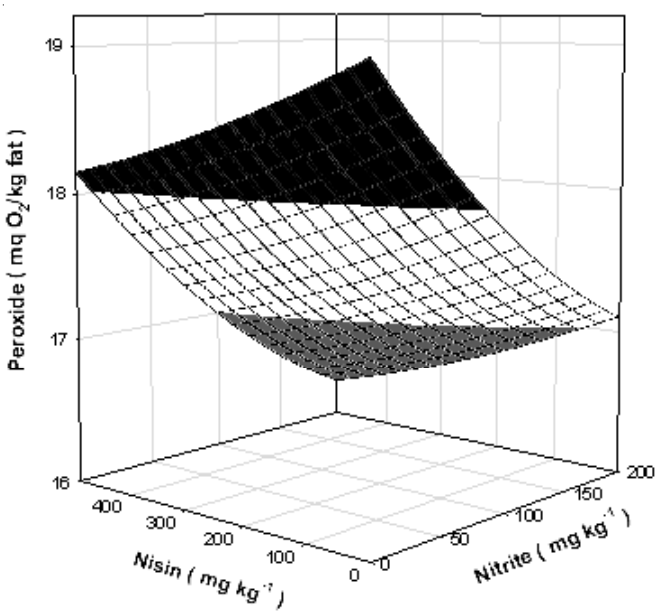

Fig. 1. Effects of nitrite and nisin on peroxide values

Lipids and phospholipids are hydrolyzed by lipase and phospholipase during the ripening/drying period, yielding free fatty acids, which are oxidized into peroxides[19]. Zanardi *et al.*[14] reported that some technological parameters, such as starter cultures, additives and spices, did not seem to have perceivable effects on lipolysis and lipid oxidation. Franco *et al.*[20] reported that peroxide values were found at 16 meq O_2/kg and 28 meq O_2/kg in Spanish fermented sausage at 0 and 14 days, respectively.

Residual nitrite: The linear effects of nitrite were found to be significant ($p < 0.01$) on residual nitrite (Table-2). The increasing levels of nitrite increased residual nitrite values. As shown in Fig. 2, addition of nitrite decreased the residual nitrite value below 6 ppm at the end of the ripening period. Kurt[8] reported that a significant portion of additional nitrite levels was reduced during processing and the first day of ripening period. After the addition of nitrite, this chemical is known to react with water and is affected by reducing bacteria[21]. Nitrite reduction was also found to be related to decreasing pH values of sucuk[21]. Furthermore, nitrite might also be transformed into nitrate and react with meat proteins during the ripening period[22].

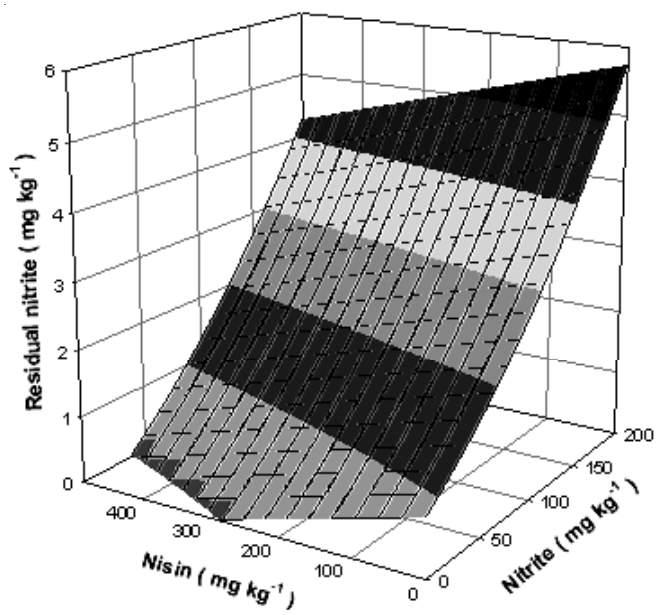

Fig. 2. Effects of nitrite and nisin on the residual nitrite values

The effects of ripening period, nitrite level and heat treatment on lipolysis, peroxide, TBA, proteolysis and residual nitrite values on sucuk are also expressed mathematically in Table-3. These predicted model equations are useful for understanding the significance of some chemical properties of sucuk and the interactions between studied factors. Hence, the performance of many levels of these factors in the studied range of factor levels can be evaluated using predicted model equations for the studied parameters.

Conclusion

Nitrite and nisin did not significantly affect lipolysis and proteolysis in sucuk. This result indicates that lipolytic and proteolytic enzymes are mainly of endogenous origins. Additional nitrite levels can be decreased with addition of nisin, which needs further investigations for determining microbial quality of sucuk.

TABLE-3
PREDICTED MODEL EQUATIONS FOR THE EFFECTS OF NITRITE (X_1) AND NISIN (X_2) ON SOME PARAMETERS OF SUCUK

Parameters	Predicted model
pH	$Y = 5.715 - 0.035X_1 + 0.098X_2 + 0.03X_1^2 - 0.07X_2^2 - 0.11X_1X_2$
Moisture	$Y = 33.584 + 0.536X_1 - 0.661X_2 - 0.242X_1^2 + 0.113X_2^2 + 0.444X_1X_2$
Lipolysis	$Y = 2.589 - 0.105X_1 + 0.01X_2 - 0.059X_1^2 - 0.064X_2^2 - 0.023X_1X_2$
Peroxide	$Y = 17.25 - 0.947X_1 - 0.388X_2 + 0.29X_1^2 + 0.085X_2^2 + 0.035X_1X_2$
Proteolysis	$Y = 9.98 - 0.248X_1 - 0.643X_2 + 0.277X_1^2 + 0.372X_2^2 + 0.366X_1X_2$
Residual nitrite	$Y = 1.976 + 2.393X_1 - 0.780X_2 + 0.579X_1^2 - 0.161X_2^2 - 0.913X_1X_2$

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