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RESEARCH ARTICLE

Effect of Celery Powder as an Alternative Nitrite Source on Some Quality Properties and Nitrosamine Formation in Sucuk

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ABSTRACT

This study aimed to determine the effects of celery powder (nitrate converted to nitrite) and cooking time on the formation of nitrosamine in sucuk. The microbiological and physicochemical properties were also investigated. Four sucuk batters were prepared: T1 - 100 mg/kg synthetic sodium nitrite, T2 - 150 mg/kg synthetic sodium nitrite, T3 - celery powder equivalent to 100 mg/kg nitrite, T4 - celery powder equivalent to 150 mg/kg nitrite. After ripening (initial fermentation temperature: 24±1°C, ripening time: 7 days), the samples were subjected to the analyses. Lactic acid bacteria and *Micrococcus/Staphylococcus* were not affected by the treatment. T4 treatment showed higher pH values than T1 and T2. The celery powder groups (T3 and T4) showed lower a_w values than other groups (T1 and T2). No significant differences were observed between the treatments in terms of thiobarbituric acid reactive substance (TBARS) value, residual nitrite level, N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) contents. However, N-nitrosopiperidine (NPIP) content was found to have higher in T4 treatment. Cooking time, especially 3 min, caused a significant increase in nitrosamine content. The effect of cooking time on nitrosamines was also revealed in principal component analysis.

Keywords: Sucuk, Fermented sausage, Nitrosamine, Celery powder, NDMA, NDEA, NPIP

INTRODUCTION

Fermented sausages have an important place among meat products with their characteristic sensory properties. These products are classified according to such criteria as raw material, type and amount of fat, seasonings, moisture content, water activity (a_w), moisture:protein ratio, weight loss, the mincing size of meat and fat, casing diameter and geographical region. However, the most common classification is based on a_w value or moisture:protein ratio [1]. The maximum a_w value for dry fermented sausages is below 0.90. Heat treatment is generally not applied in this type of products. In semi-dry fermented sausages, the minimum and maximum a_w values are 0.90 and 0.95, respectively, and heat treatment/smoking may also take place in the process of these products [2]. Moreover, dry fermented sausages often have a moisture:protein ratio of less than 2.3:1. This ratio may go up to 3.7:1 in semi-dry fermented sausages [1].

Two different types of fermented sausages are produced in Türkiye: sucuk and heat-treated sucuk. While the history of sucuk production dates back to ancient times, heat-treated sausage started to be produced in the 1980s [2,3]. Sucuk has a final pH of 5.4, moisture:protein ratio lower than 2.5:1 and fat:protein ratio lower than 2.5:1. There are three main stages in the production of this type of fermented sausage: batter preparation, fermentation (fast or slow), and ripening/drying. In the heat-treated sausage, the moisture:protein ratio must be <3.6:1, the fat:protein ratio must be <2.5:1, and the pH value must be ≤5.6 [4].

Depending on the ripening time, nitrate and/or nitrite are used as curing agents in fermented sausages [5]. However, nitrate must be converted to nitrite in products using nitrate. Nitrite is a multifunctional additive and shows antimicrobial and antioxidant activity. It also contributes to the formation of color and the development of flavor. Yet, nitrite has an important effect on the formation of nitrosamines, which have carcinogenic, mutagenic, and teratogenic properties [6]. The level of nitrosamines in fermented meat products varies depending on the product type. Factors such as ingoing nitrite, residual

nitrite, catalysts and inhibitors, cooking method and time, pH and a_w play an important role in the formation of nitrosamines. However, the most important factors are the cooking degree, level of ingoing nitrite and residual nitrite [7].

Since nitrite poses a risk to consumer health, research has been conducted for a long time on the use of nitrate-rich vegetables such as celery, Swiss chard, and beetroot powder as an alternative curing agent, and the effects of these alternative additives on product properties have been investigated [8-12]. The number of studies on nitrosamine formation by vegetable extracts is quite limited, and the effects of Swiss chard and celery powder on nitrosamine formation in heat-treated sausage were investigated in these studies [13,14]. There is no study about the effect of vegetable extract on nitrosamine formation in dry fermented sausages such as sucuk. In the present study, the use of celery powder (nitrate converted to nitrite) as an alternative curing agent and the effect of cooking time on nitrosamine formation in sucuk were investigated. In addition, microbiological and physicochemical properties of the samples were determined at the end of ripening.

Material and Methods

Material

Beef and beef fat were used as raw materials in production. Celery powder (Veg stable® 506) (2.10% nitrite) (nitrate converted to nitrite) was obtained from a commercial company (Florida Food Products, USA). *Lactiplantibacillus plantarum* GM77 (at the level of about 10^7 cfu/g) and *Staphylococcus xylosus* GM92 (at the level of about 10^6 cfu/g) strains isolated and identified from sucuk by Kaban and Kaya [15] were used as starter cultures.

Sucuk Production

Four sucuk batters were prepared: T1 -100 mg/kg synthetic sodium nitrite, T2 - 150 mg/kg synthetic sodium nitrite, T3 - 100 mg/kg nitrite from CP, T4 - 150 mg/kg nitrite from CP. The basic formulation (meat, fat, spices, salt and saccharose) given by Akköse et al.[16] was used in the production. The productions were carried out at three different times using three different raw materials at each production.

Laboratory type cutter (Mado Typ MTK 662, Dornhan, Schwarzwald) was used to prepare the batter, and laboratory type filler (Mado Typ MTK 591, Dornhan, Schwarzwald) was used for the filling. Each sucuk had an approximate weight of 200 g. Ripening was carried out in the climate chamber (Reich, Urbach, Germany) as follows: 1 days at 24±1°C, 1 days at 22±1°C, 2 days at 20±1°C and 3 days at 18±1°C. The relative humidity was gradually decreased from 90±2% to 84±2%.

Cooking Procedure

The samples were sliced at a thickness of 0.5 cm, and the cooking was carried out at three different cooking times (0, 1 and 3 min) at 180°C on a hot plate. The surface temperature of the hot plate was accurately measured with a digital thermocouple (Testo 926, Testo, Titisee-Neustadt, Germany).

Microbiological Analyses

Twenty-five g samples were homogenized with 225 mL of sterile physiological water (0.85% NaCl) in a stomacher (Lab Blender 400-BA 7021, London, UK). For the enumeration of lactic acid bacteria and Enterobacteriaceae, de Man Rogosa Sharpe Agar (MRS, Merck, Darmstadt, Germany) and Violet Red Bile Agar (VRBD, Merck, Darmstadt, Germany) were used, respectively, and incubations were carried out under anaerobic conditions (at 30°C for 48 h); for *Micrococcus/Staphylococcus,* Mannitol Salt Phenol Red Agar (MSA, Merck, Darmstadt, Germany) was used, incubation was carried out for at 30°C 48 h [17].

Physicochemical Analyses

To determine the pH value of samples, 10 g samples were homogenized with 100 mL distilled water using an Ultra Turrax (IKA Werke, Breisgau, Germany), and the pH values were determined using a pH-meter (Mettler Toledo, Greifensee, Switzerland). The a_w value was determined using the a_w device (TH-500 a_w Sprint, Novasina, Pfaffikon, Switzerland) [18]. For the determination of thiobarbituric acid reactive substance (TBARS) value, the method by Lemon [19] was used, and the results were given as µmol malondialdehyde (MDA)/kg. For residual nitrite analysis, the method given by NMKL [20] was used. The residual nitrite was determined using high-performance liquid chromatography coupled with a diode array detector (Agilent 1100, USA). Hamilton PRP-X100 (5 µm × 150 × 4.6 mm, USA) was used as a column. In the system, UV wavelength and flow rate were set 220 nm and 2 mL/min, respectively. The LOD (limit of detection) and LOQ (limit of quantification) values were determined adding standard at different rates (1-20 mg/L). The LOD and LOQ values of nitrite were 1.05 mg/L and 3.00 mg/L, respectively. The coefficient of the regression line (R2) of standard curve was 0.9999. The results were expressed as mg/kg.

Nitrosamine Analysis

The extraction was carried out according to the method given by Wang et al. [21]. To determine the nitrosamine level of the extract, GC/MS (Agilent 6890 N/Agilent 5973, USA) was used. In the system, helium was used as carrier gas and DB-5MS (30 m × 0.25 mm × 0.25 µm) (Agilent, USA) was used as column, and selected ion monitoring mode was used for quantification. The oven temperature programme was gradually increased; first kept at 50°C for

2 min, increased to 100°C at a rate of 3°C/min and kept at this temperature for 5 min, then increased to 250°C at a rate of 20°C/min. Standard nitrosamine mix (EPA 521 Nitrosamine Mix, Sigma-Aldrich, USA) was used for identification and the results were given as µg/kg.

The validation of the analysis was carried out by adding standard at different rates (0.5-20 µg/L). The LOD (limit of detection) and LOQ (limit of quantification) values were determined for N-Nitrosodimethylamine (NDMA, LOD = 0.32, LOQ = 0.97), N-Nitrosodiethylamine (NDEA, LOD = 0.37, LOQ = 1.12), N-Nitrosomethylethylamine (NMEA, LOD = 0.39, LOQ = 1.21), N-Nitrosopyrrolidine (NPYR, LOD = 0.37, LOQ = 1.13), N-Nitrosodipropylamine (NDPA, LOD = 0.45, LOQ = 1.37), N-Nitrosopiperidine (NPIP, LOD = 0.32, LOQ = 0.98) and N-Nitrosodibutylamine (NDBA, LOD = 0.38, LOQ = 1.14). The coefficients of the regression line (R2) for nitrosamine standard curve were all >0.999.

Statistical Analyses

The treatment (T1:100 mg/kg synthetic sodium nitrite, T2: 150 mg/kg synthetic sodium nitrite, T3: celery powder equivalent to 100 mg/kg nitrite, T4: celery powder equivalent to 150 mg/kg nitrite) was evaluated as the main effect, and replications were evaluated as random effects. The experiment was carried out according to a completely randomized block design with three replicates (three batters for each treatment). For the evaluation of nitrosamines, treatment and cooking time were taken as factors. The analysis of variance was applied to the results, and the means of significant sources of variation were compared using Duncan's multiple range tests at the < 0.05 level. The statistical analysis was performed using the SPSS program (IBM SPSS Inc., Chicago, IL, USA). In addition, the relationship between treatment and cooking time factors and nitrosamines was evaluated by principal components analysis (Unscrambler, CAMO vs. 10.1, Oslo, Norway).

Results

The effect of using celery powder as curing agent on lactic acid bacteria (LAB), *Micrococcus/Staphylococcus*, pH, a_w, TBARS and residual nitrite of sucuk is given in *Table 1*. The treatment had no significant effect on LAB and *Micrococcus/Staphylococcus* (P>0.05). In all treatments, the numbers of LAB and *Micrococcus/Staphylococcus* were determined 10^8 cfu/g and 10^6 cfu/g, respectively. The number of Enterobacteriaceae was below the detectable limit (<2 log cfu/g) in all groups (data not shown). pH was affected by treatment, and the highest mean pH value was found in T4. However, the mean pH value of this treatment was not differ T3 group (P>0.05). The a_w value was ranged from 0.870 to 0.890, and the treatments with synthetic sodium nitrite showed higher a_w values compared to the T3 and T4 groups (*Table 1*) (P<0.05).

TBARS value indicating the extent of lipid oxidation was not affected by the treatment (P>0.05). Similarly, residual nitrite level was not also affected by treatment (P>0.05). In all groups, residual nitrite amount was found to have under 20 mg/kg *(Table 1)*.

Only three nitrosamines (N-Nitrosodimethylamine (NDMA), N-Nitrosodiethylamine (NDEA) and N-Nitrosopiperidine (NPIP)) were determined in sucuk samples. N-Nitrosomethylethylamin (NMEA), N- Nitrosopyrolidine (NPYR), N-Nitrosodipropylamine (NDPA) and N-Nitrosodibutylamine (NDBA) were not dedected in the samples. The effects of using celery powder and cooking time on NDMA, NDEA and NPIP of sucuk are shown in *Table 2*. The treatment had no significant effects on NDMA and NDEA (P>0.05). In contrast, NPIP was affected by treatment, and the highest mean NPIP level was found in T4 group (P<0.05). Cooking time had a significant effect on NDMA, NDEA and NPIP (P<0.01). The NDMA level increased as the cooking time progressed, but a statistical difference was observed in only 3 min of cooking. NDEA was found to be below the LOD value in raw samples. After 1 min of cooking, an average NDEA of 0.64±0.16 µg/kg was determined. The 3 min cooking process caused an increase in the amount of NDEA. The NPIP level increased as the cooking time progressed (P<0.05). However, treatment x cooking time interaction did not show any significant effect on nitrosamines (P>0.05).

Table 1. *The effect of using celery powder on lactic acid bacteria, Micrococcus/Staphylococcus, pH, a_w, TBARS and residual nitrite of sucuk*

Treatment	Lactic Acid Bacteria (log cfu/g)	*Micrococcus/ Staphylococcus* (log cfu/g)	pH	a_w	TBARS (µmol MDA/kg)	Residual Nitrite (mg/kg)
T1	8.68±0.19^a	6.38±0.22^a	4.81±0.06^b	0.890±0.008^a	7.02±1.94^a	15.96±4.52^a
T2	8.39±0.31^a	6.49±0.19^a	4.86±0.09^b	0.888±0.004^a	6.45±0.94^a	17.01±4.98^a
T3	8.46±0.40^a	6.64±0.28^a	4.94±0.05^{ab}	0.875±0.005^b	6.83±0.62^a	17.61±2.75^a
T4	8.26±0.25^a	6.50±0.31^a	5.01±0.05^a	0.870±0.008^b	6.78±0.58^a	19.69±2.88^a
P value	> 0.05	> 0.05	<0.05	<0.05	> 0.05	> 0.05

T1: *100 mg/kg synthetic nitrite,* ***T2:*** *150 mg/kg synthetic nitrite,* ***T3:*** *celery powder equivalent to 100 mg/kg nitrite,* ***T4:*** *celery powder equivalent to 150 mg/kg nitrite;* $^{a-b}$ *Means marked with different letters in the same column are statistically different (P<0.05)*

Table 2. *The effects of using celery powder and cooking time on NDMA, NDEA and NPIP levels of sucuk (µg/kg)*

Treatment (T)	NDMA	NDEA	NPIP
T1	0.82±0.36[a]	0.46±0.38[a]	1.39±0.49[b]
T2	0.98±0.20[a]	0.49±0.40[a]	1.34±0.50[b]
T3	0.96±0.25[a]	0.52±0.42[a]	1.50±0.53[b]
T4	1.04±0.26[a]	0.57±0.48[a]	1.74±0.68[a]
P value	> 0.05	> 0.05	<0.05
Cooking time (min) (CT)			
0	0.75±0.26[b]	< LOD	0.95±0.17[c]
1	0.87±0.13[b]	0.64±0.16[b]	1.49±0.41[b]
3	1.23±0.16[a]	0.89±0.17[a]	2.05±0.32[a]
P value	<0.01	<0.01	<0.01
The interaction of T x CT	>0.05	>0.05	>0.05

NDMA: *N-Nitrosodimethylamine,* ***NDEA:*** *N-Nitrosodiethylamine,* ***NPIP:*** *N-Nitrosopiperidine.* ***T1:*** *100 mg/kg synthetic nitrite,* ***T2:*** *150 mg/kg synthetic nitrite,* ***T3:*** *celery powder equivalent to 100 mg/kg nitrite, T4: celery powder equivalent to 150 mg/kg nitrite;* [a-c] *Means marked with different letters in the same column are statistically different (P<0.05). LOD: Limit of detection*

The relationship between the factors and nitrosamines was evaluated by Principal Components Analysis (PCA) and is shown in *Fig. 1*. The first two principal components (PC1: 95% and PC2: 4%) accounted for 99% of the variance. The groups that were not applied cooking (T1/0, T2/0, T3/0 and T4/0) and the groups of T1, T2 and T3, which were cooked for 1 min, were on the negative side of PC1. On the other hand, all groups cooked for 3 min and T4 (T4/1) group cooked for 1 min were on the positive side of PC1. In other words, the raw and 1 min cooked samples of T1, T2 and T3 showed close correlations and, at the same time, differed from the 1-min cooked group of the T4 group. The groups cooked for 3 min and the T4/1 group showed close correlation with nitrosamines.

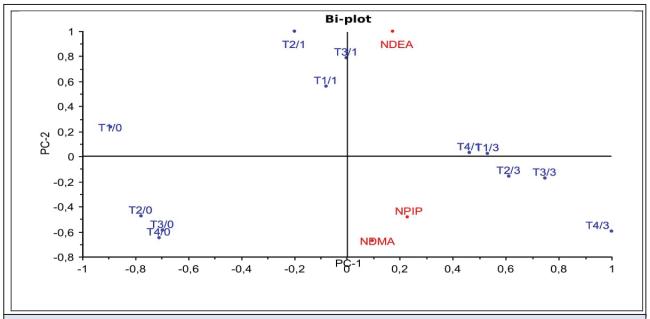

Fig 1. Principal component analysis of the relationship between the factors (treatment and cooking time) and nitrosamines (T/cooking time, NDMA: N-Nitrosodimethylamine, NDEA: N-Nitrosodiethylamine, NPIP: N-Nitrosopiperidine)

Discussion

In this study, autochthonous strains (*Lactiplantibacillus plantarum* GM77 and *Staphylococcus xylosus* GM92) were used as starter cultures. Both strains showed good adaptation to the sucuk environment and remained in high numbers. Similar results were also reported by Akköse et al. [16]. The use of celery powder in sucuk did not have a significant effect on the number of LAB. Similarly, Işıksal [22] reported that the use of celery in sucuk and heat-treated sucuk did not have a significant effect on the LAB count, while Pennisi et al.[23] reported a similar result for in Italian-type dry fermented sausage. Yılmaz Oral [13], on the other hand, reported that the use of celery powder equivalent to 150 mg/kg nitrite in heat-treated sucuk causes a decrease in LAB count, albeit slightly. Micrococci and staphylococci, another group of microorganisms that are technologically important in fermented sausages, show slow growth during fermentation since they are sensitive to acid [16-18]. Therefore, the number of *Micrococcus/Staphylococcus* in the final product increased slightly and did not exceed 10^7 cfu/g. The use of celery powder did not have an effect on these microorganisms *(Table 1)*. The members of the Enterobacteriacea family were found below the detectable limit in the final product due to their low a_w and pH values, which are in line with the results found in other studies [16,18].

pH is an important parameter in terms of both product safety and the development of sensory properties in sucuk and similar fermented sausages. LAB (spontaneous or starter culture) form lactic acid during fermentation, bringing the pH closer to the isoelectric point of muscle proteins, thus reducing the water holding capacity and facilitating drying [2]. In the present study, pH value increased slightly when using celery powder equivalent to 150 mg/kg nitrite. However, this increase was not at a level that would negatively affect product characteristics. On the other hand, Yılmaz Oral [13] reported that the pH value of the heat-treated sucuk increased as the proportion of celery powder use increased. It was also reported in a

study on ham that celery juice concentrate increased the pH value [9]. In terms of a_w, the groups containing synthetic nitrite gave higher a_w values than the groups containing celery powder. It is thought that this was resulting from the water holding capacity of celery powder. A similar result was also reported by Yılmaz Oral [13]. On the other hand, Işıksal [22] reported that the addition of different nitrate/nitrite sources did not affect the basic composition of the sucuk.

The TBARS value, which is a good indicator of the degree of lipid oxidation in meat products, was not affected by the use of celery powder. TBARS value was found below 1 mg MDA/kg in all groups. However, the use of celery powder in heat-treated sucuk slightly increased the TBARS value, but this value did not exceed 1 mg MDA/kg in any samples [13]. Sindelar et al.[8] also reported that the use of celery powder in ham production caused an increase in the TBARS value.

Residual nitrite level is an important factor in terms of nitrosamine formation in cured meat products [7,24]. In this study, residual nitrite levels were found below 20 mg/kg in all treatment groups and were not affected by the use of celery powder. In contrast, Işıksal [22] stated that residual nitrate level was higher in sucuk and heat-treated sucuk samples containing vegetal nitrate/nitrite both during production and storage stages. In this current study, it is thought that the low amount of residual nitrite is due to rapid acidification. The decrease in pH during fermentation accelerates the conversion of nitrite to nitric oxide and thus a significant decrease in the residual nitrite level occurs [25].

N-nitrosodimethylamine (NDMA) is one of the most commonly detected nitrosamines in fermented sausages [26,27]. This nitrosamine, which is formed as a result of the reaction between the nitrosating agent and dimethylamine, which is a secondary amine, is in the group of probably (Group 2A) carcinogenic compounds for humans [28]. Amines formed as a result of proteolysis during the ripening of fermented sausages can cause an increase in the level of this nitrosamine [29]. In this study, an increase in NDMA was not observed due to the use of celery powder. It was also reported in another study that the use of celery powder in heat-treated sucuk did not cause a significant change in NDMA content [13]. In addition, it was reported that the NDMA content of the Swiss chard powder was not affected in this fermented sausage type [14]. In the present study, NDMA content increased after 3 min of cooking. It was also determined in other studies that the NDMA content increased with cooking [24,27,30].

One of the probably (Group 2A) carcinogenic compounds for humans determined by the International Agency for Research on Cancer is NDEA [28]. This nitrosamine was unaffected by the use of celery. While it was below the LOD value in raw samples, it increased with cooking. The highest NDEA level (0.89±0.17) was obtained after 3 min of cooking. Ozel et al.[31] also determined that the NDEA content ranged between 0.10-0.95 µg/kg in sucuk.

In this study, it was determined that celery powder, when added at a level to match the nitrite level of 150 mg/kg, increased the NPIP level in the final product when added to the sucuk batter. A similar result was observed in heat-treated sucuk [13]. However, in a study using Swiss chard powder, no increase in NPIP was observed in heat-treated sucuk [14]. Black pepper, which is included in the formulation in sucuk and many other fermented sausages, plays a role in the formation of NPIP due to the piperine and piperidine it contains [7,32]. Piperidine is also found in celery [33]. In the present study, it is thought that the increase in NPIP level may be related to the piperidine content of celery powder. On the other hand, it was reported that lipid oxidation promotes the formation of NPIP [34]. In addition, as with other nitrosamines, cooking time caused an increase in NPIP, which is possible carcinogenic compounds (Group 2B) [28]. It has also been reported in other studies that the cooking time in sucuk and heat treated sucuk increases the NPIP content [13,24,27,32]. According to the PCA results, the increase in cooking time caused an increase in the level of nitrosamines. However, the effect of cooking on the formation of nitrosamines becomes more evident in the case of using celery powder at a level to meet the nitrite level of 150 mg/kg.

As a result, the use of celery powder as a curing agent in sucuk did not have a significant effect on the microbiological properties of the product. This vegetable product also had no significant effect on residual nitrite and lipid oxidation. The changes in pH and a_w values did not occur at a level that would affect the product properties. However, the addition of celery powder to the sucuk batter equivalent to 150 mg/kg nitrite caused an increase in the NPIP content in the final product. The cooking process also caused an increase in the content of nitrosamines, but especially the NDEA and NPIP content increased as the time progresses. Considering all these aspects, it was concluded that the use of celery powder as an alternative curing agent in sucuk is not effective in preventing the formation of nitrosamines and even slightly increases the NPIP content, and cooking is a more important factor in terms of nitrosamine.

Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author.

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Competing Interest

The author declared that there is no conflict of interest.

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