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Original scientific paper

Sensory quality, oxidative stability, textural and fatty acid profile of nitrite-reduced kulen fermented sausage during ripening

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A R T I C L E I N F O

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A B S T R A C T

The aim of the study was to investigate the effect of nitrite reduction on oxidative stability, chemical composition, textural and sensory properties of traditional kulen sausage. In total, three batches of kulen were made. The control batch (C) contained 110.0 mg/kg of sodium nitrite ($NaNO_2$), the second (R1) batch contained 55 mg/kg of $NaNO_2$, while the third (R2) was produced without nitrites. Nitrite removal from the sausage formulation significantly affected oxidative stability, while the reduction of nitrite from 110 mg/kg to 55 mg/kg did not affect the oxidative stability of the product. When it comes to texture, complete removal of nitrite from kulen resulted in significantly lower values of hardness, gumminess and chewiness. Sensory scores for colour were similar between all analysed batches. However, scores for aroma, taste, consistency and overall acceptability were significantly lower in R2 batch sausages. At the same time, scores of all investigated sensory parameters were similar for sausages formulated with 110 mg/kg and 55 mg/kg of $NaNO_2$.

1. Introduction

Fermented sausages are products with long tradition of manufacturing in the Europe. Traditionally, they are produced using a combination of hurdles which all together contribute to the safety of the product. These include salting, drying, fermentation, sometimes smoking and addition of different additives and spices. One of the most commonly used additives in industrial processed meat production is nitrite salts. Nitrite salts are used in the form of sodium nitrite ($NaNO_2$) or potassium nitrite (KNO_2). Their use is very important in terms of development of bright red colour of meat and antimicrobial activity against *Clostridium botulinum (Djordjevic et al.,* 2019). In addition, nitrites are powerful antioxidants and may improve taste of the product (*Simunovic et al.*, 2022). Hence, nitrites and nitrates are recognized by the meat industry as irreplaceable additives, especially due to their effect on the development of the characteristic bright red colour of cured meat.

Consumers are more interested in products produced without additives because they are more aware of their negative impact on human health (*Simunovic et al.*, 2022). Therefore, food companies, in addition to initiatives aimed at reducing greenhouse gas emissions, are changing their marketing

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approach and launching new products with modified nutritional composition (*Rajic, et al.,* 2021). When it comes to nitrites, their use is considered dangerous because they participate in the formation of N-nitrosamines, which are found to induce cancer (*Flores & Toldrá*, 2021). Besides their carcinogenic effect, these compounds were also found to cause insulin resistance diseases and Alzheimer's disease (*Tong et al.*, 2009). Therefore, in the last few decades, there has been a great number of studies examining the possibility of reducing or replacing nitrite in processed meat (*Christieans et al.,* 2018; *Hospital et al.,* 2016; *Ozaki et al.*, 2021; *Simunovic et al.*, 2022). The main potential hazard regarding nitrite reduction in processed meat is growth of *C. botulinum* and formation of botulinum neurotoxins, some of which can cause botulism in humans. However, *Hospital et al.* (2016) found that reduction and removal of nitrites and nitrates from formulation of fermented sausages did not compromise safety regarding *C. botulinum* in tested conditions.

During the production of fermented sausages, nitrates can be reduced to nitrites, which is why many studies have proposed foods naturally rich in nitrates as nitrite alternatives (*Gassara et al.,* 2016; *Pennis et al.,* 2020; *Pini et al.,* 2020; *Sucu & Turp*, 2018; *Tang et al.*, 2021). Nitrates are found in high concentrations in green, leafy vegetables like spinach and also in vegetables like celery, radish, beetroot and others. Paprika (*Capsicum* spp.) is also reported to contain high amounts of nitrates (*Colavita et al.,* 2014). According to (*Vuković et al.,* 2012), paprika can contain up to around 500 mg/kg of nitrates. On the other hand, paprika is an irreplaceable ingredient in every type of kulen and its content varies in the formulation from 1 to 3% (*Tomasevic et al.,* 2022).

The aim of this study was to investigate the effect of nitrite reduction on oxidative stability, chemical composition, textural and sensory properties of traditional kulen sausage.

2. Materials and methods

2.1. Production of kulen

Three batches of traditional kulen were made using 80% of pork ham and 20% of pork firm fatty tissue. The first (C) and the second (R1) batch were produced with addition of 110 mg/kg of nitrites and 55 mg/kg of nitrites, respectively. The third (R2) batch was produced without addition of nitrites. After stuffing in casings, sausages were traditionally smoked and transferred to ripening chamber for a total of 40 days. Sampling was performed on the manufacturing day and then on each 8 days of ripening. Shelf life of sausages was assessed after 50 and 100 days of storage at 2°C.

2.2. Physicochemical analysis

Protein, fat, ash and thiobarbituric acid-reactive substances (TBARS) contents were measured as described in our previous study (*Simunovic et al.*, 2021). TBARS were expressed as mg of malondialdehyde (MDA) per kg of sample. Peroxide number and acid value were determined in compliance with ISO 3960:2017 and ISO 660:2020, respectively.

2.3. Texture analysis

Texture profile analysis (TPA) was performed according to (*Simunović, Đorđević, Rašeta, et al.*, 2022), with some modifications. Prior to compression of each sample, exact dimensions of sausage cuboids were obtained using digital calliper and were entered into the processing software.

2.4. Sensory analysis

An experienced twelve-member panel evaluated following attributes of sausages at the end of the ripening: colour, aroma, taste, consistency and overall acceptability. The panel was trained during three months according to ISO 8586:2012 (*ISO*, 2012). Participants were served tap water and apples in order to clean the palate between the samples. In addition, a total of 43 consumers who regularly consume kulen took part in a triangle test. Three samples marked with a random three-digit numbers were served to each participant, one of which was different. They were asked to choose only one sample which they thought was different. In order to reduce the number of trials needed, sequential analysis was performed according to ISO 16820:2004 (*ISO*, 2004).

2.5. Statistical analysis

Statistical analysis was performed using SPSS software (IBM, Armonk, NY, USA). This statistical software was used to calculate mean values and significant differences. The results of the survey were analysed using MS Excel (Microsoft, Redmond, WA, USA).

3. Results

3.1. Chemical content and oxidative stability

At the end of ripening and storage, peroxide values were found to be the significantly ($P < 0.01$) different among all batches, being highest in sausages of the control batch, followed by R1 and R2 (Figure 1). When it comes to secondary products of lipid oxidation, significantly lower levels of TBARS were found in sausages formulated with sodium nitrite (C and R1) than those found in nitrite-free sausages (Figure 2). This is because nitric oxide binds and inhibit activity of ferrous and ferric ions, which act as catalysts of hydroperoxide decomposition to hydroxyl, peroxy and alkoxy radicals (*Domínguez et al.*, 2019; *Wanjala et al.,* 2021). The results of our study are in accordance with those reported by *Berardo et al.* (2016) and *Karwowska et al.,* (2019), who studied the effect of nitrite reduction on lipid oxidation of fermented and cooked sausages, respectively. The authors found significantly higher TBARS levels in nitrite-free sausages than in that produced with addition of 150 mg/kg of sodium nitrite, indicating the strong antioxidative effect of nitrites. However, results of our study did not align with those reported by *Karwowska et al.* (2019), who found significant differences between TBARS values of sausages formulated with 50 and 100 mg/kg of sodium nitrite. In present study, no significant ($P < 0.01$) differences were observed between sausages treated with 110 mg/kg of sodium nitrite (C) and those containing 55 mg/kg of sodium nitrite (R1) in terms of TBARS levels. It is important to point out the importance of reduction of nitrates from paprika to nitrites during ripening which, coupled with antioxidative activity of carotenoids, provides an additional antioxidant effect in kulen (*Sebranek & Bacus*, 2007). The study of *Revilla and Vivar-Quintana* (2004) revealed that by increasing levels of paprika in sausage formulation, TBARS values are reduced. To support this, TBARS values found in this study for kulen are lower than those reported by a number of authors for different dry sausages produced without red paprika powder (*Berardo et al.*, 2016; *Ozaki, Santos, et al.*, 2021; *Tang et al.*, 2021).

3.2. Texture profile analysis

Hardness, gumminess and chewiness increased significantly throughout the ripening in all analysed batches (Table 1). Regardless of concentration, nitrite addition influenced significantly ($P < 0.01$) higher values of hardness and chewiness. This outcome is in accordance with the study of *Tang et al.* (2021), who reported significantly higher values of hardness and chewiness of sausages made with 150 mg/kg of sodium nitrite compared to those found in nitrite-free sausages. In the present study, gumminess was found to be significantly lower in nitrite-free sausages to that found in the control batch but similar to that found in sausages produced using 55 mg/kg of nitrites. On the other hand, nitrite reduction showed no significant differences in cohesiveness and springiness between the batches. Effects of nitrite on texture parameters could be explained by the formation of carbonyls, which may lead to cross-linking of muscle proteins and affect their net charge, which consequently results in their changed spatial arrangement (*Bao & Ertbjerg*, 2019).

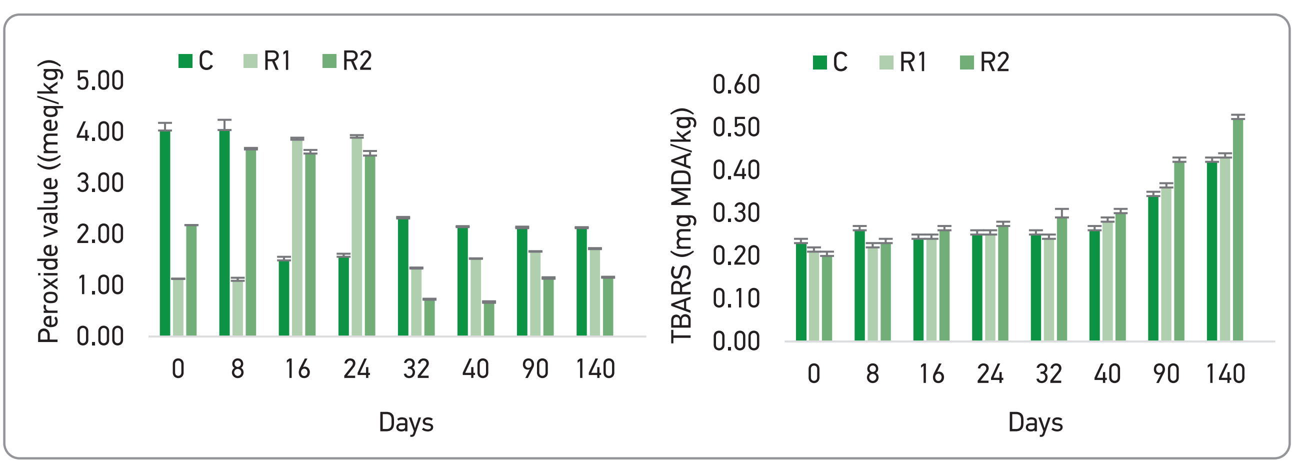

Figure 1. Evolution of peroxide values (meq/kg) and TBARS (mg MDA/kg) during production and storage of *kulen* produced with different levels of sodium nitrite (mean ± standard deviation).

Table 1. Chemical and textural changes during the first 16 days of production of nitrite reduced kulen

	Processing time (days)											
	0			SEM	8			SEM	16			SEM
	C	R1	R2		C	R1	R2		C	R1	R2	
Chemical parameters												
Fat content (%)	21.481^{a}	23.712^{a}	22.723^{a}	0.32	24.261^{b}	25.732^{b}	25.242^{b}	0.22	29.071^{c}	26.652^{c}	28.151^{c}	0.36
Protein content (%)	16.201^{a}	15.701^{a}	15.821^{a}	0.10	17.561^{b}	17.561^{b}	18.372^{b}	0.14	21.011^{c}	21.011^{c}	21.282^{c}	0.05
Ash content (%)	3.091^{a}	3.041^{a}	2.981^{a}	0.02	3.351^{b}	3.281^{a}	3.331^{b}	0.01	3.961^{c}	4.221^{b}	4.032^{c}	0.04
Texture												
Hardness (N)	7.131^{a}	7.351^{a}	6.661^{a}	0.21	20.641^{b}	20.1212^{b}	17.142^{b}	0.51	37.291^{c}	36.541^{c}	31.322^{c}	0.72
Springiness	0.771^{a}	0.692^{a}	0.7212^{a}	0.01	0.901^{b}	0.8412^{b}	0.792^{ab}	0.01	0.911^{b}	0.861^{b}	0.831^{b}	0.02
Cohesiveness	0.5312^{a}	0.521^{ac}	0.562^{a}	0.00	0.471^{b}	0.481^{b}	0.451^{b}	0.01	0.501^{abc}	0.501^{ab}	0.471^{b}	0.01
Gumminess (N)	4.441^{a}	3.362^{a}	3.611^{a}	0.13	9.411^{b}	11.431^{b}	9.331^{b}	0.33	16.531^{b}	18.492^{c}	15.421^{c}	0.34
Chewiness (N)	3.351^{a}	2.422^{a}	2.432^{a}	0.13	8.8612^{b}	10.201^{b}	7.642^{b}	0.29	16.741^{c}	15.541^{c}	11.522^{bc}	0.49

[1] Abbreviations: C = control (110 mg/kg of sodium nitrite); R1 – 55 mg/kg of sodium nitrite; R2 = without sodium nitrite

[2] Values are displayed as arithmetic means ± standard deviation (mean ± SD). Mean values in the same row (corresponding to the same day of ripening) not followed by a common number differ significantly (P<0.01) Mean values in the same row (corresponding to the same batch) not followed by a common letter differ significantly (P<0.01).

Table 2. Chemical and textural changes during the last 16 days of production of nitrite reduced kulen

	Processing time (days)											
	24			SEM	32			SEM	40			SEM
	C	R1	R2		C	R1	R2		C	R1	R2	
Chemical parameters												
Fat content (%)	29.38^{1c}	32.12^{2d}	28.82^{1c}	0.51	32.12^{1d}	33.81^{2e}	32.81^{3d}	0.25	31.28^{1e}	34.10^{1e}	32.47^{1d}	0.41
Protein content (%)	22.15^{1d}	22.57^{1d}	22.27^{1d}	0.11	23.93^{12e}	23.72^{1e}	25.19^{2e}	0.25	23.91^{1e}	24.21^{12e}	24.82^{1e}	0.15
Ash content (%)	4.05^{1c}	4.29^{2b}	4.04^{1c}	0.04	4.52^{1d}	4.38^{2b}	4.43^{12d}	0.02	4.90^{1d}	4.95^{1b}	4.96^{1e}	0.07
Texture												
Hardness (N)	41.66^{1c}	41.85^{1d}	39.15^{1d}	0.67	48.06^{1d}	47.71^{1e}	43.70^{2e}	0.61	53.57^{1d}	52.71^{1e}	49.09^{2f}	0.42
Springiness	0.79^{1a}	0.83^{1b}	0.74^{1ab}	0.02	0.71^{1a}	0.70^{1a}	0.69^{1a}	0.01	0.73^{1a}	0.78^{1ab}	0.70^{1a}	0.01
Cohesiveness	0.52^{1abc}	0.48^{2b}	0.48^{2b}	0.01	0.59^{1ad}	0.56^{1acd}	0.54^{1a}	0.01	0.61^{1d}	0.60^{1d}	0.56^{1a}	0.01
Gumminess (N)	18.18^{1c}	21.01^{2c}	17.85^{1c}	0.60	26.43^{1d}	26.96^{1d}	23.22^{2d}	0.50	28.94^{1d}	27.36^{12d}	24.15^{2d}	0.59
Chewiness (N)	16.79^{1c}	16.87^{1c}	14.50^{1cd}	0.47	17.73^{1c}	17.30^{1c}	15.98^{1d}	0.43	19.22^{1c}	19.40^{1c}	16.49^{2d}	0.52

[1] Abbreviations: C = control (110 mg/kg of sodium nitrite); R1 – 55 mg/kg of sodium nitrite; R2 = without sodium nitrite

[2] Values are displayed as arithmetic means ± standard deviation (mean ± SD). Mean values in the same row (corresponding to the same day of ripening) not followed by a common number differ significantly (P<0.01) Mean values in the same row (corresponding to the same batch) not followed by a common letter differ significantly (P<0.01).

3.3. Sensory evaluation

Sensory evaluation conducted by an experienced sensory panel showed significant ($P < 0.01$) differences between nitrite free sausages and sausages formulated with nitrite (C and R1) in terms of scores obtained for aroma, taste, consistency and overall acceptability (Table 3). However, scores of these sensory parameters were found to be similar for sausages of C and R1 batch. In terms of colour, no significant differences were observed between the three batches.

Table 3. Sensory evaluation of kulen formulated with different levels of sodium nitrite (mean±standard deviation)

Attributes	C	R1	R2
Colour	4.33±0.65[a]	4.42±0.79[a]	4.33±0.65[a]
Aroma	4.42±0.79[a]	4.25±0.96[a]	3.33±0.65[b]
Taste	4.42±0.51[a]	4.08±0.99[a]	3.42±0.67[b]
Consistency	4.17±0.58[a]	4.17±0.72[a]	3.50±0.90[b]
Overall acceptability	4.50±0.67[a]	4.42±0.51[a]	3.58±0.90[b]

[1] Abbreviations: C = control (110 mg/kg of sodium nitrite); R1 – 55 mg/kg of sodium nitrite; R2 = without sodium nitrite

[2] Values are displayed as arithmetic means ± standard deviation (mean ± SD). Values with different lowercase letters (a-b) in the same row differ significantly ($P < 0.01$).

4. Conclusion

The results showed significant effects of nitrite on the oxidative stability of kulen. However, reduction of nitrites from 110 mg/kg of $NaNO_2$ to 55 mg/kg of $NaNO_2$ did not compromise oxidative stability of kulen. Removal of nitrites from kulen formulation resulted in significantly lower values of hardness, gumminess and chewiness than in sausages with nitrites. In addition, complete removal of nitrites from the sausage formulation resulted in lower scores obtained for overall acceptability. To sum up, content of $NaNO_2$ can be reduced from 110 mg/kg of $NaNO_2$ to 55 mg/kg of $NaNO_2$ without compromising oxidative stability, chemical quality, texture or sensory parameters of traditional kulen.

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