The shelf life of cooked sausages with reduced salt content

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Abstract

The aim of this study was to compare the microbiological quality of cooked sausages produced with a traditional salt content (2.1%) and reformulated batches with a salt content reduced to 1.7%. The reformulation was tested on two types of comminuted meat products – Špekáčky sausage with a diameter of up to 46 mm or Bologna-type sausages in diameter of 85 mm (Gothaj sausage) or 75 mm (Junior sausage). The total viable count (TVC) increased only slightly during the four-week storage (4 ± 1 °C) of all batches of Špekáčky sausage. Comparing batches 1.7 and 2.1, there is an evident difference in the number of CFU/g, with samples of Špekáčky 1.7 showing numbers of bacteria higher by approximately 1 logarithmic order throughout practically the entire storage period ($P = 0.001$). The population of lactic acid bacteria (LAB) remained well beneath a value of 5.0 log CFU/g even at the end of the experiment. For Bologna-type sausages, the TVC was either beneath the limit of detection or at its boundary in all samples. LAB were not detected during storage of Bologna-type sausages. The results confirmed that the proportion of salt in cooked sausages can be reduced to 1.7% without negatively affecting the shelf life or safety of the final products.

Bologna-type sausage, lactic acid bacteria, total viable count, reformulation

Salt serves a number of functions during meat processing (Feiner 2006; Gómez-Salazar et al. 2021). It has a crucial role in enhancing taste, for which a proportion of 1% NaCl is sufficient (Honikel 2008). Technologically speaking, salt is irreplaceable for dissolving myofibrillar proteins and thereby increasing the water binding capacity of meat; a minimum of 1.2% NaCl is required for this purpose (Honikel 2008). Salt has also been used since ancient times for preserving and thus extending the shelf life of meat (Honikel 2007; Sebranek 2009).

The principle of the preservative effect of salt is an increase in osmotic pressure in an environment of microorganisms (Gutierrez et al. 1995). The addition of salt also reduces water activity values (Inguglia et al. 2017). The value of the osmotic pressure of the environment increases as soon as salt is added to food, which is registered by the receptors of the bacteria present (Gutierrez et al. 1995). Cells evaluate this situation as hyperosmotic shock. The response of microorganisms to osmotic stress (known as osmoadaptation) includes both physiological changes and variations in gene expression (Gandhi and Chikindas 2007). For bacteria, osmoadaptation means mobilising forces to restore intracellular pressure (turgor) and temporarily ceases their growth (a bacteriostatic effect). In this way, salt acts as an obstacle to bacterial growth, although the effect of salt in this respect is only short-lived. It does, however, mean a delay in the onset of meat spoilage in the technology of preparing meat products before the application of heat treatment or fermentation and drying depending on the type of product.

A number of products with a reduced salt content have found their way onto the market in recent years as consumer demand for foods with a more favourable effect on

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health has increased significantly (Muñoz et al. 2020). Food reformulations aiming to reduce the proportion of salt, sugar or fat have become part of the nutritional action plans of many EU countries (Belc et al. 2019). Food reformulation is defined as "change to the nutrient content of processed foods with the effect of reducing the content of certain substances, such as sodium, saturated fatty acids or trans-fatty acids, or energy (kilojoules), or increasing the proportion of components with a positive effect on the human organism, such as fibre, fruit, vegetables, whole-grain cereals and unsaturated fatty acids" (Neacsu et al. 2015). In the area of processed meats, such reformulations mainly involve reduction to the salt content and/or fat content (Shan et al. 2017; Fraqueza et al. 2021). Since salt in foods exhibits the preservative effect described above by limiting bacterial growth, there is a theoretical consideration as to whether a reduction in the proportion of salt may have a negative impact on the safety of reformulated foods (Sleator and Hill 2007).

The aim of this study was to compare the microbiological quality of cooked sausages produced with a traditional salt content exceeding 2% and reformulated batches with a salt content reduced to 1.7%.

Materials and Methods

Špekáček sausages

Two batches of the comminuted cooked meat product Špekáčky were prepared in co-operation with producer A. One batch was a product from the regular range of producer A with a recipe consisting of fresh beef R4 and R5 (beef trimmings according to the GEHA system), S4 (lean pork trimmings), S8 and S11 (pork fat). Other ingredients in the recipe were skin emulsion, ice, and potato starch, along with other functional ingredients and a mixture of seasonings. These Špekáčky sausages will further be referred to as 'Špekáčky 2.1' in the text (referring to the salt proportion of 2.1%). The second batch was prepared from a recipe containing the main components R4, R5, S4 and S8. Ice, potato starch and functional ingredients were used in the same way as in the Špekáčky 2.1 batch. The proportion of salt in this second batch was set as 1.7%, and the samples in this batch will further be referred to as 'Špekáčky 1.7' in the text. Meat mixture was filled in beef casings of a diameter of up to 46 mm, with individual portions weighing 58–75 g being separated by tying. Heat treatment took place in accordance with the national legislation (Decree 2016) with the attainment of a thermal effect corresponding to the action of a temperature of 70 °C for a period of 10 min throughout the entire product. The products were vacuum packed immediately following cooling and sent to a microbiological laboratory for further analyses while observing the cold chain. The samples were stored in a cold room at a temperature of 4 ± 1 °C for the duration of the analyses. The preparation of samples and their analyses took place twice: in the months May–June 2019 and 2020. For the purposes of comparison, two batches of Špekáčky sausage (1.7 and 2.1) were prepared by producer B in 2023, and the same microbiological analyses were subsequently performed on them.

Bologna-type sausages

Batches of Gothaj sausage and Junior sausage (standard and reformulated batches) were prepared by producer A in the period 2021–2022; specifically, batches with a reduced salt content of 1.7% in 2021 and batches with a reduced salt content (1.7%) together with a reduced proportion of fat (Gothaj minus 33%; Junior minus 47%) in 2022. The recipe contained pork meat S3, S4, S5, S6, R5 and, for Gothaj sausage, also S8. The meat mixture was filled in plastic barrier casings of a diameter of 85 mm (Gothaj sausage) or 75 mm (Junior sausage). Heat treatment was performed in the same mode as for the Špekáčky sausages. Shelf life was tested for up to 3 months at 4 ± 1 °C. All samples were prepared as part of the QK1910100 project focused on food reformulation, including meat products. Reformulations of meat products are primarily aimed at reducing the proportion of salt, or to reduce the proportion of fat. As a higher proportion of fat is typical for the Špekáčky sausage, batches with a lower proportion of fat were prepared only in the case of Bologna-type sausages.

Microbiological testing

The total viable count (TVC), the number of lactic acid bacteria (LAB) and the number of bacteria of the family *Enterobacteriaceae* were determined in the samples. Twenty-five g of the inner part of the product (excluding the technological casing) were weighed into sterile homogenisation bags using sterile instruments. The samples were diluted 1:9 with buffered peptone water (BPW; OXOID, UK). Tenfold dilutions of the initial samples were subsequently prepared.

Determination of the TVC was performed in accordance with ISO 4833. Cultivation took place for 72 h at 30 °C under aerobic conditions. Agar with glucose, tryptone and yeast extract (TGYE; OXOID) was used as the growth medium.

Determination of the number of LAB was performed on a nutrient-rich medium under anaerobic conditions during incubation for 72 h at 30 °C in accordance with ISO 15214. MRS agar (OXOID) was used as the growth

medium. All colonies displaying different morphological characteristics were selected from each sample and tested for the presence of catalase and oxidase (JK Trading, Prague, Czech Republic). Determination of the family *Enterobacteriaceae* was performed on agar with crystal violet, neutral red, bile salts and glucose (VRBG; OXOID) under aerobic conditions with incubation for 24 h at 37 °C in accordance with ISO 21528-2.

Microbiological analysis was performed on the day of sample delivery (week 0) and after 1, 2, 3 and 4 weeks of storage (Špekáčky sausage) and, in the case of producer B, again after 2 months. Samples of Bologna-type sausages were tested on the day of sample delivery (month 0) and after 1, 2 and 3 months of storage at 4 ± 1 °C. Two samples from both batches were tested on each day of sampling. Intact samples of sausages were also subjected to an incubator test after 3 months of storage in accordance with the procedure described by Veselá et al. (2022).

Identification of bacterial species by MALDI-TOF mass spectrometry

Isolates with negative oxidase and catalase tests were further identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) following the procedure described by Dušková et al. (2012). The samples for MALDI-TOF MS analysis were prepared by protein extraction (ethanol/formic acid) according to a standard protocol (Freiwald and Sauer 2009). Mass spectrometry measurements were performed using an UltrafleXtreme instrument (Bruker Daltonik, Germany) operated in the linear positive ion mode using FlexControl 3.4 software. Mass spectra were processed using BioTyper software (version 3.0; Bruker Daltonik). The identification results were expressed by BioTyper log(scores) indicating the similarity of the unknown MALDI-TOF MS profile to BioTyper database entries (version 10.0; 9607 entries). A BioTyper log(score) exceeding 2.0 indicates a highly confident identification at the species level. A BioTyper log(score) between 1.7 and 2.0 means identification at the species level with lower confidence. Only isolates with a log(score) over than 1.7 were taken into account.

Determination of the salt proportion

The proportion of salt was determined on the basis of determination of the sodium content by the atomic absorption spectrophotometry (AAS) method according to the procedure described by Macharáčková et al. (2021). Analyses were performed on a ContrAA700 High-resolution Continuum Source Atomic Absorption Spectrophotometer (HR-CS AAS) (Analytik Jena AG, Jena, Germany). The instrument was connected to an AS 52 S autosampler (Analytik Jena AG). The oxidising flame was acetylene-air (Linde Gas a.s., Prague, Czech Republic).

The proportion of salt was determined according to the definition given in Annex I of Regulation (EU) No. 1169/2011 on the provision of food information to consumers according to the formula salt = sodium × 2.5. Determination of the proportion of salt was conducted in order to check the batches made.

Statistical analysis

All data were entered into spreadsheets (Microsoft Office Excel 2019). The obtained experimental data (CFU/g) were log transformed, and the mean values and standard deviations were calculated. The differences were compared using paired *t*-test because Shapiro-Wilks tests were not able to reject the normality of the data. Significance was accepted at $P < 0.05$.

Results

Špekáčky sausage

Tables 1 and 2 contain the results of the microbiological analysis of the samples of Špekáčky sausage with a salt content of 1.7% and 2.1% (the salt content was confirmed by AAS analysis). The results for the family *Enterobacteriaceae* are missing from the tables as they were beneath the limit of detection in all cases.

Two facts are evident from Table 1. Firstly, the TVC increased only slightly during the four-week storage of all 4 batches of Špekáčky from producer A. In the experiment conducted in 2019, the population of detected bacteria remained of the order of magnitude of the initial values throughout the shelf-life period. Similar results were also provided by the experiment conducted in 2020. Secondly, when comparing batches 1.7 and 2.1, there is an evident difference in the number of CFU/g, with samples of Špekáčky with a lower proportion of salt showing numbers of bacteria higher by approximately 1 logarithmic order throughout practically the entire storage period. This difference was statistically significant ($P = 0.001$). As can be seen further in Table 1, the LAB population remained well beneath a value of 5.0 log CFU/g even at the end of the experiment after four weeks of storage. The limit value for the spoilage of meat and meat products is, meanwhile, generally considered 7.0 log CFU/g.

The Špekáčky sausage prepared by producer B displayed perhaps even better microbial quality. The TVC did not exceed 2 log CFU/g even after 2 months. No LAB could be demonstrated in the samples at all (Table 2). Differences in microbial quality between batches 1.7 and 2.1 were statistically insignificant ($P > 0.05$).

Table 1. Results of the development of the total viable count (TVC) and number of lactic acid bacteria (LAB) in batches of Špekáčky from producer A with a proportion of 1.7% and 2.1% salt during four weeks of storage at 4 ± 1 °C (mean ± SD in log CFU/g).

Bacterial group	Year	Batch	Week					*P*
			0	1	2	3	4	
TVC	2019	1.7	2.69 ± 1.00	2.80 ± 2.22	2.84 ± 2.49	2.43 ± 2.22	2.30 ± 0.70	0.001
		2.1	1.60 ± 0.70	1.11 ± 0.40	2.08 ± 1.67	1.98 ± 1.16	1.76 ± 1.22	
	2020	1.7	3.11 ± 2.76	2.89 ± 2.37	2.77 ± 1.78	4.80 ± 4.76	3.15 ± 2.74	0.331
		2.1	1.75 ± 0.44	1.62 ± 0.37	1.54 ± 0.25	2.34 ± 0.68	4.54 ± 0.44	
LAB	2019	1.7	1.88 ± 1.40*	<1.70	<1.70	<1.70	<1.70	0.222
		2.1	<1.70	<1.70	1.40 ± 1.40[A]	2.48 ± 2.48 [B]	<1.70	
	2020	1.7	<1.70	<1.70	<1.70	4.89 ± 3.73[C]	3.11 ± 3.07[D]	0.190
		2.1	<1.70	<1.70	<1.70	2.08 ± 2.06[E]	3.54 ± 3.11[F]	

[A]*Leuconostoc carnosum*; [B]*Streptococcus vestibularis*; [C]*L. carnosum*, *Carnobacterium maltaromaticum*; [D]*L. carnosum*, *Latilactobacillus sakei*; [E]*L. sakei*; [F]*L. carnosum*, *L. sakei*; *without identification by MALDI-TOF MS

Table 2: The results of the development of the total viable count (TVC) and number of lactic acid bacteria (LAB) in batches of Špekáčky from producer B with a proportion of 1.7% and 2.1% salt during four weeks of storage at 4 ± 1 °C (mean ± SD in log CFU/g).

Bacterial group	Batch	Sampling				*P*
		0	After 1 week	After 1 month	After 2 months	
TVC	1.7	1.00 ± 0.00	1.45 ± 1.35	<1.00	<1.00	>0.05
	2.1	1.48 ± 1.30	1.48	1.26 ± 1.10	1.30	
LAB	1.7	<1.70	<1.70	<1.70	<1.70	>0.05
	2.1	<1.70	<1.70	<1.70	<1.70	

Bologna-type sausages

The TVC was either beneath the limit of detection or at its boundary in all samples. Occasionally (Table 3 and Table 4), it exceeded a value of 2 log CFU/g. In both experiments (sausages with a reduced proportion of salt and a standard proportion of fat or sausages with a reduced proportion of salt and a reduced proportion of fat; the salt content was confirmed by AAS analysis) LAB were not detected during storage. Not even the experiment with an incubator test with cooked sausages demonstrated the presence of LAB. This testifies to the extremely high-quality input raw material and the perfectly mastered heat treatment used by producer A. Bacteria of the family *Enterobacteriaceae* were not determined in the samples of cooked sausages.

The reformulated meat products were favourably evaluated in the sensory analysis; for some indicators they received a higher point score than standard sausages (data not shown). The proportions of salt in the prepared batches were confirmed by AAS analysis (data not shown).

Table 3. Values for the total viable count (TVC) in samples of reformulated Bologna-type cooked sausages with a reduced proportion of salt during a three-month storage period at 4±1 °C and after an incubator test (mean ± SD in log CFU/g).

Sample	Month of sampling				*P*	IT 15 °C/7 days
	0	1	2	3		
Junior standard	1.40 ± 0.70	<1.00	<1.00	<1.00	0.015	2.18 ± 1.81
Junior reformulated	2.23 ± 1.40	1.98 ± 0.65	2.08 ± 0.70	2.53 ± 2.42		3.04 ± 2.82
Gothaj standard	1.54 ± 1.18	1.63 ± 0.40	1.00	<1.00	0.309	2.11 ± 2.06
Gothaj reformulated	<1.00	1.26 ± 1.10	1.48 ± 1.00	1.30 ± 1.00		1.74 ± 0.98

IT - incubator test with Bologna-type sausages at 15 °C for 7 days.

Table 4. Values for the total viable count (TVC) in samples of Bologna-type cooked sausages with a reduced salt content and a reduced fat content (mean ± SD in log CFU/g).

Sample	Month of sampling				*P*	IT 15 °C/7 days
	0	1	2	3		
Junior standard	<1.00	<1.00	1.30 ± 1.00	1.30	0.002	<1.00
Junior reformulated	2.30 ± 1.74	2.08 ± 1.60	1.68 ± 1.63	2.08		1.81 ± 0.70[A]
Gothaj standard	1.68 ± 1.63	<1.00	1.11 ± 0.88	1.70 ± 1.60	0.328	1.32[B]
Gothaj reformulated	1.26 ± 1.10	1.36 ± 1.24	1.40 ± 0.70	1.32		1.30[C]

IT - incubator text with Bologna-type sausages at 15 °C for 7 days; [A]*Bacillus pumilus*; [B]*Bacillus* sp., *Paenibacillus* sp., *Micrococcus* sp.; [C]*B. pumilus*, *Micrococcus* sp.

Discussion

No LAB were detected during storage in the experiments with Bologna-type sausages with a reduced salt content and standard fat content or sausages with a reduced salt content and reduced fat content. Meanwhile, this abundant bacterial group represents the main proportion of the microbiota associated with the spoilage of cooked meat products (Samelis et al. 2000; Vermeiren et al. 2005; Martins et al. 2020). The study by Veselá et al. (2022) showed that LAB are capable of surviving the heat treatment of meat products. Their subsequent growth is encouraged by a combination of microaerophilic conditions in the product, the presence of sodium chloride and sodium nitrite, and a reduced water activity value (Audenaert et al. 2010). In frankfurters with an emulsified meat dough (pork meat, mechanically separated poultry meat, skin emulsion, ice) filled in collagen casings, the LAB population increased to a figure of 5.76 log CFU/g during three weeks of storage, while LAB not could be proven at all at the beginning of the experiment immediately after packing (Kameník et al. 2014). No LAB were detected in samples of cooked ham after heat treatment in the experiment conducted by Veselá et al. (2022). However, after they were kept in an incubator at 15 °C for 7 days, sub-lethally damaged LAB cells multiplied in the hams and were subsequently detected on agar at an average count of around 3.48 log CFU/g.

Excellent microbiological quality of Špekáčky sausage was also found from the same producer. Although it was possible to isolate LAB from a number of samples taken from inside the products, their numbers reached maximum values of the order of 4 log CFU/g after 3 weeks of cold storage, and their number did not increase further after another week. On the contrary, it fell to values 1 order lower. The question remains as to why the LAB demonstrated after three weeks of storage in Špekáčky sausage with both standard and

reformulated salt proportions were unable to multiply further during the following week. Meanwhile, the growth of LAB during the storage of meat products has been reliably documented in a number of studies (Kameník et al. 2015; Dušková et al. 2016; Veselá et al. 2022), in spite of the fact that no LAB were proven on culture media immediately after heat treatment. A possible explanation is the degree of sub-lethal damage during heat treatment and the excessively low storage temperature which did not allow significant development of the isolated LAB despite the fact that there were species normally isolated from the environment of meat products among them (*Latilactobacillus sakei* and *Leuconostoc carnosum*).

The fact that the microbiota remained at low levels throughout the entire experiment and the number of LAB was beneath the limit of detection in the majority of analyses raises the question as to which bacteria represented the predominance of the microbiome in the tested meat products. A proportion of the detected bacteria was classified as aerobic sporogenous bacteria (Table 4) on the basis of the morphology of the colonies found on the agar used and, first and foremost, the use of the MALDI-TOF MS method. According to Fraqueza et al. (2021), the heat treatment of meat products eliminates non-sporogenous bacteria, though not spores. However, unlike LAB, representatives of this bacterial group do not find favourable conditions for their growth in the environment inside meat products. Their population remains at a low level, though it persists in the products practically throughout their entire shelf life.

It can be stated in conclusion that even products with a salt content of around 1.7% (i.e. a reduction of 20–30% compared to the standard products) do not pose a risk from the point of view of supporting the growth of bacteria. The results confirmed the conclusions reached by Aaslyng et al. (2014) that the proportion of salt in cooked sausages can be reduced from 2.2% to 1.7% without negatively affecting the shelf life or safety of the final products.

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