ORIGINAL SCIENTIFIC PAPER

Validation of meat products without added nitrites, nitrates or phosphates

Tea Agić¹*, Dorotea Piškor¹, Marina Posavec¹, Katarina Medak Čukelj², Iva Ladan¹, Maja Rečić¹, Dubravka Marija Kreković¹

Abstract:

The challenge for the meat industry is developing meat products that contain no additives, have similar sensory properties as traditional nitrite-cured products and will persist throughout their shelf life with the same level of quality and safety. In this study, new recipes with no additives or with food additive substitutes of natural origin were compared with commercially available products. It was concluded that the new meat products – cooked ham (as representative of thermally processed meat products) and fermented sausage had concentrations of nitrite and nitrate below the method's limit of quantification during the entire shelf life period. Acerola has successfully replaced sodium ascorbate as a natural source of ascorbic acid in meat products. Furthermore, it was proved that no phosphates, comparable to those from raw meat, were added to meat products. Using sensory evaluation, cooked ham and fermented sausage were classified into the highest quality category, in terms of color, flavor, odor and texture during the entire shelf life, and were evaluated as microbiologically safe, according to the Guide for Microbiological Criteria for Foodstuffs (Ministry of Agriculture, March 2011).

Keywords: nitrites, nitrates, phosphates, acerola, meat products

Introduction

Sodium nitrite and nitrate are among the most commonly used food additives in the meat industry. Nitrite has several roles in meat curing: inhibiting the growth of a variety of aerobic and anaerobic microorganisms, controlling pathogens such as *Bacillus cereus*, *Staphylococcus aureus*, and *Clostridium perfringens*, and, especially, suppressing the outgrowth of *Clostridium botulinum* spores, retarding lipid oxidation and rancidity, developing the cured meat flavor and producing the characteristic reddish-pink cured color after its reaction with myoglobin (Cobos and Diaz, 2014; Skibsted, 2011). At the optimum pH of meat (5.6 to 5.8), nitrites decrease by conversion to the compounds that can act as oxidizing, reducing or nitrosylating agents. They can produce carcinogenic nitroso compounds, such as *N*-nitrosamines, which are formed by the reaction of nitrosating agents with a substance having an amino group during food processing (heat treatments) and preser-

¹ Tea Agić,mag.chem., Dorotea Piškor,mag.ing.cheming., Marina Posavec,mag.sanit.ing., Iva Ladan,univ.mag.san.ing., Maja Rečić,mag.nutr., Dubravka Marija Kreković,dipl.ing.; Croatian Institute of Public Health, Rockefellerova 7, Zagreb, Croatia ² Katarina Medak Čukelj,dipl.ing.; PIK Vrbovec plus d.o.o., Zagrebačka ulica 148, Vrbovec *Corresponding author: tea.agic@hzjz.hr vation (Domanska-Blicharz et al., 2004,; Flores and Toldra, 2020; Herrmann et al., 2014). Several reviews suggested alternatives to nitrite, with a role of avoiding oxidation in meat products, such as sulfur dioxide, butylated hydroxyanisole, α-tocopherol, organic acids, spices etc. (Gassara et al., 2013; Alahakoon et al., 2015.; Oswell et al., 2018). Bacterial cultures have an important role in meat products with no added nitrite or nitrate salts. They can be divided into acidifying bacteria, microorganisms with color and/or flavor forming activities, microorganisms for surface coverage and bacteria for bio-protection. Starter cultures commonly used in the fermentation of sausages include the lactic cultures of Lactobacillus plantarum, L. pentosus, L. curvatus, L. sake, Pediococcus pentosaceus and P. acidilactici. They affect the technological properties and microbial stability of the final product. By inhibiting meat-borne pathogenic bacteria and coagulating soluble meat proteins, they reduce the water binding capacity and facilitate product drying. (Drosinos et al., 2007; Hugas and Monfort, 1997; Luecke, 2000). The Micrococcaceae species most often used in a large number of meat products are different species of Staphylococcus, more specifically, S. xylosus, S. saprophyticus and S. Carnosus, which are responsible for pigmentation, the synthesis of aromas and the degradation of excess nitrates. The Staphylococcus species has a high salt tolerance and lower oxygen requirements, and possesses diverse enzymatic activities of major importance for flavor formation in meat products, such as catalase, lipolytic and proteolytic activity, and high capacity for degrading amino and fatty acids into a wide variety of aroma compounds. Yeast extracts, especially from the Debaryomyces and Saccharomyces genera, give flavor to meat products through carbohydrate fermentation and lactate oxidation, proteolysis and degradation of amino acids and lipolysis (Drosinos et al., 2007). On the other hand, molds (most often Penicillium nalviogense, P.chrysogenum or P. camemberti) can oxidize lactic and other acids, and produce ammonia, thereby increasing pH. Additionally, they can affect flavor formation due to diverse metabolic activities, such as lipolytic and proteolytic activity (Robinson et al., 2000; Löfblom et al., 2017). Water activity, a,, (ranging from 0 to 1) affects different chemical reactions in the meat product and the resistance of microorganisms. Many ways of preserving food reduce the availability of moisture to microorganisms in the product, by increasing osmotic pressure in food, hence lowering water activity (Fontana et al., 2000). Processors today use vegetable products as natural sources of nitrate in processed meats. Vegetables, such as celery, lettuce, spinach, turnip and chard, in the form of powder or juice, are sources of high concentrations of nitrates. One of the advantages of Swiss chard (Beta vulgaris var. cicla) is that it contains no allergens (Sebranek et al., 2012). Researchers reported that 2 % pre-converted nitrite from Swiss chard powder positively affected the formation of nitrosoheme pigments in cooked pork patties. The acidic pH of Swiss chard powder also showed a reduced residual nitrite concentration in cooked pork patties (Shin et al., 2017). Moreover, Swiss chard powder prevented lipid oxidation in cooked pork patties and improved flavor and high acceptability ratings (Sebranek et al., 2012; Pyo et al., 2004). Also, vegetables contain various types of antioxidant compounds, which suppress the formation of harmful chemicals, i.e., nitrosamines (Correia et al., 2010). The oxidation-reduction property of antioxidants, such as ascorbic acid and α -tocopherol, helps reduce nitrosating agents to NO (Lidder and Webb, 2012; Bryan and van Grinsven, 2013). Phosphates are included in many curing solutions and cured meat formulations because of numerous beneficial effects that they bring to cured meat products, such as water holding, color protection, slowing down of oxidation, extension of shelf life, stabilizing and enhancing the structure of final products. Phosphates also encourage the binding of water in meat products, but excessive amounts of phosphorus in a meat product can negatively affect product safety (Bach Son Long et al., 2011). However, the exact minimal amount of phosphate needed to obtain good product quality probably depends on the product composition. Studies show that the current amount of phosphate added to emulsified meat products can be significantly reduced with a minimal loss in product quality (Glorieux et al., 2017).

Materials and methods

Materials for the study were obtained from the Croatian PIK VRBOVEC plus d.o.o. meat industry, and samples used were raw pork meat, cooked ham and fermented sausage. Focus was placed on alternative recipes: the first one with no added nitrite or nitrate salts (REC1), and the second one containing nitrate from a natural source, Swiss chard (REC2). In REC1, for cooked ham, nitrite salts, responsible for preservation, color and taste, have been replaced by sea salt, yeast extract and bacterial culture, and in the fermented sausage, the preservation role was assigned to sea salt in combination with bacterial culture. The nitrite, nitrate, phosphate and ascorbic acid content, together with microbiological and sensory properties, were monitored in the samples. Also, other parameters were determined such as pH, protein, fat, salt, dry matter and water activity.

Chemical and physical parameters

Approximately 5g of homogenized meat sample was mixed with hot water at 70-80°C, thermostated for 15 minutes, cooled down, purified with Carrez solutions, adjusted to pH 9.6-9.7 with orthophosphoric acid and filtered. For nitrate determination, the filtrate was analyzed using a HPLC-DAD instrument (Shimadzu Prominence LC 20), and instrument parameters are shown in Table 1. The final result is expressed as mg/kg of sodium nitrate. The determination of nitrite is based on the colorimetric reaction of the filtrate with sulfanilamide in an acidic medium, which forms a diazonium complex, and, subsequently, a purple azo dye with *N*-(1-naphthyl)-ethylenediamine dihydrochloride (McLoughlin, 1968). The content was measured on a Shimadzu UV-1601 spectrophotometer in photometric mode at 538 nm, and the final result is expressed as mg/kg of sodium nitrite.

For phosphate determination, a homogenized meat sample was burned in a muffle furnace for 2 hours at 550°C. The next step was acid hydrolysis of ash, with subsequent ammonium heptamolybdate, hydroquinone and sodium sulfite reactions (Bell and Doisy, 1920). A blue colored complex was generated and its intensity was measured by a spectrophotometer (Shimadzu UV-1601) at a wavelength of 650 nm. The final result is expressed as g/ kg of polyphosphate (P_2O_5).

For the determination of ascorbic acid, the homogenized sample was dissolved in 2 % meta-phosphoric acid; L-cysteine was added and the pH was adjusted, firstly to 7.0-7.2 and then to 2.5-2.8. The sample was measured on the HPLC-DAD instrument (Shimadzu Prominence LC 20), and instrument parameters are shown in Table 1 (Anonim., 2005).

	Nitrates	Ascorbic acid
Column	Zorbax C18 Eclipse XDB, 150mm x 4.6mm	Zorbax, Agilent 5 HC-C18, 250mm x 4.6mm
Particle size column, μm	5	5
Flow rate, mL/min	1	1.5
Mobile phase	10 mM <i>n</i> -octylamine in 20 % MeOH, pH= 6.5	0.01M monopotassium phosphate in H2O, pH=2.25
Run time, min	10	5.0
Wavelength, nm	201	243
Temperature of column, °C	30	25
Injection volume, µL	10	20
Sample solvent	water	2 % metaphosphoric acid

Table 1 HPLC conditions for determination of nitrates and ascorbic acid

Total fat content is determined using the method of M. Weibll and W. Stoldt (AOAC 991.36,1999 a). The principle of the method is destroying the sample with hydrochloric acid, which results in the hydrolysis of proteins and starch. The separated fat is filtered and extracted in a Soxhlet apparatus with petroleum ether. After the extraction is completed, the extraction vessel is dried in a dryer for half an hour at 105°C, cooled in a desiccator to room temperature and weighed. The proteins in the product are obtained by analyzing the total nitrogen, which is determined by the Dumas method on a software-controlled Primacs100 instrument (Skalar). The Dumas method is based on the difference in thermal conductivity of the reference gas (helium) and the mixture of the reference gas and nitrogen (AOAC 990.03, 2002). There are three stages of analysis: purification, combustion and analysis, and the result is firstly expressed as a percentage of the total nitrogen in the sample and then converted to g/100g of protein content (Anonim., 2018). The mass fraction of sodium was analyzed by ICP-MS 7900, (Agilent technologies) after microwave-assisted decomposition, expressed as sodium chloride (Anonim., 2018). The dry matter in the sample is determined by a halogen moisture analyzer Mettler Tolledo HX204 (AOAC 950.46, 1999). For pH determination, 1 % aqueous solution of homogenized sample is prepared and measured with a pH meter (Mettler Tolledo MP220).

Microbiological parameters

Detection of *Salmonella* **spp.:** according to standard HRN ISO 6579-1:2017 (Anonim., 2017 a).

Enumeration of *Escherichia coli***:** according to standard HRN ISO 16649-2:2001 (Anonim., 2001).

Enumeration of sulfite-reducing clostridia: according to standard HRN EN ISO 15213:2004 (Anonim., 2004).

Enumeration of *Staphylococcus aureus*: according to standard HRN EN ISO 6888-1:2021 (Anonim., 2021).

Detection of *Listeria monocytogenes*: according to standard HRN EN ISO 11290-1:2017 (Anonim., 2017).

Enumeration of yeasts and molds: according to standard HRN ISO 21527-1:2012 (Anonim., 2012).

Determination of water activity: Reference method HRN ISO 18787:2020 was used. Measurements were performed on a LabMaster-aw neo device (Novasina) (Anonim., 2020).

Sensory parameters

Product formulation and performance were experimentally carried out in the producti-

on plant making fermented sausages and cooked ham, whose appearance/color, texture/consistency, odor and flavor were determined by sensory evaluation (Bamidele and Feng, 2023; Miller, 2023). Sensory evaluation was carried out by a group of analysts ("panel") of 22 members. Firstly, the sensory evaluation was carried out using the test of difference, whose goal was to determine if there is a difference in the sensory properties between the prototype samples and an identical product made with standard additives (phosphates, nitrite and nitrate salt, sodium ascorbate): appearance/color, texture/consistency, odor and flavor, and the size of the recognized difference. The standard or the reference sample was specially marked in the test and the size of the difference was evaluated with respect to the deviation from the reference sample. The evaluation consisted of counting the responses in each difference size. For substitutes for emulsifiers/stabilizers and thickeners, texture/ consistency was evaluated. Also, for substitutes of preservatives and antioxidants, color was evaluated. Secondly, a scoring method with a sum of 20 weighted points was applied on cooked ham and fermented sausage. Each sample was presented by one individual sample for each storage time, and each sample was evaluated separately. The necessary evaluation sheets were created for each sample, in which quality requirements were expressed by appropriate assessments, and significance factors were entered. All samples were evaluated for 4 quality parameters, shown in Table 2, using grades from 1 to 5, and significance factors were applied for each individual parameter.

The obtained grades multiplied by the signi-

Quality parameter	Point	Sensory properties of meat product				
	5	In relation to the observed property, the product has extremely positive properties. The general impression is complete harmony, no defects or shortcomings can be observed.				
1.Appearance/color	4	A certain observed property shows barely noticeable defects or deficiencies. The prod- uct is almost full-value in the tested capacity.				
2. Odor 3. Flavor 4.Texture/consistency	3	In relation to the observed property, the positive characteristics of the product are of reduced intensity. The product has noticeable defects or deficiencies, but its use value still retains an acceptable level.				
	2	In relation to the observed property, the product contains defects and shortcomings and is, therefore rated as inadequate. The product has a reduced use value, but it can still be consumed.				
	1	In relation to the observed property, the product has significant shortcomings and defects and is, therefore, not for consumption.				

Table 2 Quality requirements of sensory properties for both types of meat products

Parameter	Max points	Significance factor	Max weighted points
Appearance/ color	5	0.8	4
Odor	5	1.0	5
Flavor	5	1.2	6
Texture/consistency	5	1.0	5
Sum	20	x	20

Table 3 The values of significance factors for each individual parameter

ficance factor gave the corresponding number of weighted points, as presented in Table 3.

The results were statistically interpreted and the samples were classified into quality categories based on the points achieved: <11.2 not acceptable; 11.2-13.1 still acceptable; 13.2-15.1 mediocre; 15.2-17.5 good and 17.6-20.0 excellent.

Results and discussion

The new recipes, REC1 and REC2, were compared with commercially available products using a standard recipe (added nitrite or nitrate salts). Table 4 shows the concentrations of sodium nitrate and sodium nitrite determined in 55 samples of fermented sausage and 32 samples of cooked ham using new recipes, compared with commercially available products on the Croatian market (Kovačević et al., 2016). Although their detection was not requested by the regulation (Anonim., 2011), nitrite and nitrate contents were also checked in 10 samples of unprocessed pork meat.

It was confirmed that concentrations of nitrite and nitrate salts in samples of unprocessed pork meat were below the limit of quantification, namely 27.4 mg/kg and 6 mg/kg, for sodium nitrate and sodium nitrite, respectively. By comparison with commercially available products, it was evident that new recipes had lower concentrations of nitrite and nitrate. By statistical analysis, as presented in our previous work (Agić et al., 2023), it was concluded that there is a significant difference between the standard and new REC1 and REC2 recipes. Also, a post-hoc analysis showed a statistically

FERMENTED SAUSAGE								
		Unprocessed pork meat	Commercially available products (CAP)	Recipe without nitrates and nitrites (REC1)	Recipe with nitrates from a natural source (REC2)			
Sodium pitrato	Number of samples	3	72	32	23			
Soulummitate	Mean concentration (mg/kg)	<loq< td=""><td>130±72</td><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	130±72	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>			
Sodium pitrito	Number of samples	3	48	32	23			
Mean concentration (mg/kg)		<loq 7<="" td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq>		<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>			
		COOKED	НАМ					
	Commercially Recipe without Recipe with Unprocessed available nitrates and nitrates from a pork meat products (CAP) nitrites (REC1) (REC2)							
Sodium pitrato	Number of samples	7	ND	17	15			
Souluin Intrate	Mean concentration (mg/kg)	<loq< td=""><td>ND</td><td><loq< td=""><td>22.9±18.1</td></loq<></td></loq<>	ND	<loq< td=""><td>22.9±18.1</td></loq<>	22.9±18.1			
Sodium nitrito	Number of samples	7	300	17	15			
Souluin mithle	Mean concentration (mg/kg)	<loq< td=""><td>42±21</td><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	42±21	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>			

Table 4 Concentrations of sodium nitrite, sodium nitrate and polyphosphate in the two recipes and unprocessed pork meat, compared to commercially available meat products (mean ± standard deviation)

ND – no data, LOQ – limit of quantification

significant difference in the content of sodium nitrate and sodium nitrite (p < 0.05) between thermally processed products using the new REC1 and REC2 recipes, while, in the case of fermented sausages, there were no significant differences between the two recipes. Furthermore, the content of sodium nitrate and nitrite was monitored during the shelf life period: for 36 days in the case of cooked ham, and for 144 days in the case of fermented sausage. The content of sodium nitrite in the cooked ham and the fermented sausage, as well as the content of sodium nitrate in the fermented sausage using both recipes, was below the quantification limit throughout the shelf life period (27.4 mg/kg and 6 mg/kg, for sodium nitrate and sodium nitrite, respectively). The content of sodium nitrate in the cooked ham with a natural source of nitrate decreased with time of storage, which, according to literature, could be explained by its tendency to reduce

to nitrites in certain conditions (Domanska-Blicharz et al., 2004).

Phosphates were not added in the samples of cooked ham using new recipes, which is evident in comparison with the results of the unprocessed pork meat, from which the obtained samples were prepared (Table 4). The result for raw pork meat was comparable with the pork ham from literature (4.22±0.93 g/kg) (Prica et al., 2015).

In meat products with added ascorbic acid from an acerola, a lower concentration of ascorbic acid was found than in products containing sodium ascorbate salt (Table 5). The microbiological and sensory properties of the products remained unchanged. Hence, it can be concluded that ascorbic acid from acerola is a good substitute for standard antioxidants, in accordance with the literature (Suchoparova et al., 2022).

Meat product	Decine	Ascorbic acid, mg/100g		
	кестре	Na-ascorbate	Acerola	
Fermented sausage	REC 1	62.43±22.77	35.54±21.42	
	REC 2	80.42±21.25	36.65±14.29	
Cooked ham	REC 1	75.27±12.66	36.80±4.53	
	REC 2	95.97±20.26	45.35±1.48	

Table 5 Average concentration of ascorbic acid in both new recipes of cooked ham and fermented sausage, in the form of sodium ascorbate and acerola (mean ± standard deviation)

The chemical and physical properties of the new products are shown in Table 6. No significant changes were observed in the chemical composition of samples. All products had a high protein content, depending on the content of dry matter. Water activity was stable through every recipe and type of product. Both recipes had a lower salt content than the commercially available products, approximately 10 to 20 % lower, compared to products from the same manufacturer available in supermarkets (Pleadin et al., 2009).

Table 6 Chemical and physical properties of products and recipes (mean ± standard deviation)

Most	Chemical and physical property						
product	Recipe	рН	a _w	Fat g/100g	Protein g/100g	Dry matter g/100g	NaCl g/100g
Fermented	REC 1	4.93 ± 0.31	0.89 ± 0.015	36.44 ± 3.12	26.24 ± 1.80	67.66 ± 2.22	3.00 ± 0.32
sausage	REC 2	4.82 ± 0.32	0.90 ± 0.018	36.18 ± 2.90	26.64 ± 1.66	68.54 ± 2.29	2.34 ± 0.27
Cooked	REC 1	6.01 ± 0.22	0.97 ± 0.009	1.89 ± 0.55	19.00 ± 0.71	28.43 ± 1.09	1.85 ± 0.43
ham	REC 2	6.00 ± 0.18	0.97 ± 0.008	2.23 ± 0.48	18.15 ± 1.07	27.47 ± 2.33	2.09 ± 0.75

Tables 7 showes microbiological test results and water activity values of fermented sausage and cooked ham. The test for each sample was carried out on 5 elementary units. From the obtained a_w results for fermented sausages, it could be concluded that the results were under 0.90, which confirmed that an increase in Enterobacteria or *Clostridia botulinum* was not to

Table 7 Microbiological results of packaged and sliced fermented sausage and cooked ham

	Meat product			
Microbiological parameter	Fermented sausage	Cooked ham		
Sulfite-reducing clostridia, cfu/g	<10	<10		
Salmonella spp., cfu/25g	Not isolated in 25g	Not isolated in 25g		
Escherichia coli, cfu/g	<10	<10		
Listeria monocytogenes, cfu/25g	Not isolated in 25g	Not isolated in 25g		
Coagulase positive staphylococci/Staphylococcus aureus, cfu/g	<10	<10		
Water activity	0.847-0.882	0.969-0.982		
Mold, cfu/g	NA	<10		

NA – not applicable

be expected. Water activity values for cooked ham were in accordance with the literature (Barbosa-Canovas et al., 2020). Changes in the recipes had no impact on the microbiological safety of the product. The test results for all tested samples/elementary units complied with the recommended microbiological criteria (Ministry of Agriculture, 2011). Also, the values of aw were in accordance with the current regulation (Anonim., 2018).

In the sensory evaluation by test of difference, the cooked ham and fermented sausage prototypes based on new REC1 and REC2 recipes were compared against the standard sample, in terms of sensory properties: appearance/color, texture/ consistency, odor and flavor. The 'cooked ham' product, as a standard, was compact, had firm elastic consistency and was easy to slice. It had a homogeneous texture and did not disintegrate in muscle parts. At product cross-section, parts of natural light pink and darker pink color were noticed, well brined. Also, no larger inclusions of fatty tissue could be seen. The product had a characteristic and delicate smell and taste/aroma of cooked pork meat and was mildly salty. The REC1 and REC2 prototypes were evaluated with positive marks, compared to the standard sample, and were considered as acceptable prototypes (Picture 1).



Picture 1 a) sample of cooked ham, REC1; b) sample of cooked ham, REC2; c) standard sample

At t cross-section of the product, fermented sausage, as a standard sample, the stuffing appeared mosaic, composed of pieces of red muscle tissue and white fat tissue, and the stuffing ingredients were evenly distributed and firmly interconnected. There were no hollows or cracks, and the sausage was easy to slice. The product had a characteristic taste, smell and aroma of ripe meat, infused with the aroma of smoke. The REC1 and REC2 prototypes were evaluated with positive marks, compared to the standard sample, and were considered as acceptable prototypes (Picture 2).

The sensory evaluation of the accepted prototypes of cooked ham and fermented sausage produced with no added nitrite salts (REC1) was carried out in order to determine the sensory properties during the shelf life period of 36 and 144 days, respectively. According to the evaluation results of each sensory property during the shelf life, as shown in Table 8, for cooked ham, it was evident that, within

ZNANSTVENO STRUČNI DIO



Picture 2 a) sample of fermented sausage, REC1; b) sample of fermented sausage, REC2; c) standard sample

Table 8 Weighted point values of the evaluation of each sensory property for cooked ham during theshelf life period

Sensory property/day	0	14	30	36
Appearance/color	4.0	4.0	4.0	3.7
Odor	5.0	5.0	5.0	5.0
Flavor	6.0	5.9	6.0	5.8
Texture/consistency	5.0	5.0	4.9	4.9
Sum of weighted points	20	19.9	19.9	19.4

the given period of 30 days, the product was awarded maximum points for each evaluated sensory proper-

ty, with a slight degradation thereof recorded on the 36th day of the shelf life.

Table 9 Weighted point values of the evaluation of each sensory property for fermented sausage duringthe shelf life period

Sensory property/day	0	30	60	90	120	144
Appearance/color	4.0	4.0	4.0	4.0	4.0	4.0
Odor	5.0	5.0	5.0	5.0	4.8	4.8
Flavor	6.0	5.9	6.0	5.9	5.3	5.3
Texture/consistency	5.0	5.0	5.0	5.0	5.0	5.0
Sum of weighted points	20.0	19.9	20.0	19.9	19.1	19.1

The average number of weighted points by all evaluators was calculated and the sample of cooked ham was classified into quality category "excellent". It could be concluded that the evaluated sensory and acceptance properties of the product have been retained. Also, according to the evaluation results of each sensory property during the shelf life, as shown in Table 9, for fermented sausage, the product was awarded maximum points for each evaluated sensory property over 90 days, with a slight degradation thereof recorded on the 120th and 144th day of the shelf life period.

According to the same principle as for cooked ham, the fermented sausage sample was classified

into quality category "excellent", with unchanged evaluated sensory and acceptance properties.

Conclusion

Nitrite and nitrate content was lower in the recipes with no added salts or with nitrate from a natural source than in the standard sample containing additives. Cooked ham and fermented sausage samples with no added nitrite or nitrate salts were evaluated through a test of difference for both meat products with positive marks, compared to the standard sample, and were considered as acceptable prototypes for further analysis. By monitoring nitrite

SCIENTIFIC AND PROFESSIONAL SECTION

and nitrate content over 36 days for cooked ham, and 144 days for fermented sausage, it was established that the concentrations of nitrites and nitrates in both meat products are below the limit of quantification, as corroborated by microbiological tests that proved the products are microbiologically safe throughout the entire shelf life period. Phosphates were not added to the new recipe samples, which is evident in comparison with the results of raw pork meat. By comparing the results of the concentration of ascorbic acid from the added sodium ascorbate and the natural source of acerola, it can be concluded that acerola can successfully replace sodium ascorbate as a source of ascorbic acid in meat products. Sensory evaluation confirmed the desirable sensory properties of the novel products, similar to those of conventional products containing additives.

References

- [1] AOAC (1999): Official Method 950.46. Moisture in Meat.. V: Official methods of analysis of AOAC International. Vol. 2, Cunniff P. (ed.) 16th ed. Washington, Chapter 39:1-1
- [2] AOAC (1999 a): Official Method 991.36. Fat (Crude) in Meat and Meat Products Solvent, 1999. V: Official methods of analysis of AOAC International. Vol. 2, Cunniff P. (ed.) 16th ed. Washington, AOAC International, Chapter 39:3-4
- [3] AOAC (2006): Official Method 990.03. Protein (Crude) in Animal Feed, Combustion Method Official methods of analysis of AOAC International. 18th ed. Gaithersburg, MD, AOAC International, Chapter 4:30-31
- [4] Agić, T., M. Rečić, D. Piškor, M. Posavec, D.M. Kreković (2023): Analysis of nitrate and nitrite content in meat products without added additives. MESO, XXV (4), 300-309. https://hrcak.srce.hr/306578.
- [5] Alahakoon, A.U., D.D. Jayasena, S. Ramachandra, C. Jo (2015): Alternatives to nitrite in processed meat: Up to date. Trends Food Sci Tech, 45(2015), 37-49 doi: 10.1016/j.tifs.2015.05.008
- [6] Anonymous (2001): HRN ISO 16649-2:2001 (ISO 16649-2:2001) Microbiology of food and animal feeding stuffs Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.
- [7] Anonymous (2004): HRN EN ISO 15213:2004 (ISO 15213:2003) Microbiology of food and animal feeding stuffs Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions.
- [8] Anonymous (2005): Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives, preuzeto: 27.09.2023.
- [9] Anonymous (2011): Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, preuzeto: 27.09.2023.
- [10] Anonymous (2012): HRN ISO 21527-1:2012 (ISO 21527-1:2008) Microbiology of food and animal feeding stuffs Horizontal method for the enumeration of yeasts and moulds Part 1: Colony count technique in products with water activity greater than 0,95.
- [11] Anonymous (2017): HRN EN ISO 11290-1:2017 (EN ISO 11290-1:2017) Microbiology of the food chain Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. Part 1: Detection method.
- [12] Anonymous (2017 a): HRN ISO 6579-1:2017 Microbiology of the food chain Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp.
- [13] Anonymous (2018): Pravilnik o mesnim proizvodima. Narodne novine NN 62/2018. https://narodne-novine.nn.hr/clanci/sluzbeni/2018_07_62_1292,preuzeto:27.09.2023.
- [14] Anonymous (2020): HRN ISO 18787:2020 (ISO 18787:2017) Foodstuffs -- Determination of water activity.
- [15] Anonymous (2021): HRN EN ISO 6888-1:2021 (EN ISO 6888-1:2021) Microbiology of the food chain Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 1: Method using Baird-Parker agar medium.
- [16] Bach, Son Long, NH, R.Gal, F.Bunka (2011): Use of phosphates in meat products. Afr J Biotechnol, 10, 19874-82, doi: 10.5897/ ajbx11.023
- [17] Bamidele, O.P., O.J. Adebowale, X. Feng (2023): Chapter 29 Sensory perspectives into indigenous fermented foods in the tropics: challenges and opportunities. Indigenous Fermented Foods for the Tropics, 483-502 doi:10.1016/B978-0-323-98341-9.00023-2
- [18] Barbosa-Canovas, G.V., Fontana jr. A.J., Schmidt S.J., Labuza T.P. (2020): Water Activity in Foods: Fundamentals and Applications. John Wiley & Sons, Inc., doi: 10.1002/9781118765982
- [19] Bell, R.D., E.A. Doisy (1920): Rapid colorimetric methods for the determination of phosphorus in urine and blood. J Biol Chem, 55-67 doi:10.1016/s0021-9258(18)86271-8
- [20] Bryan, N.S., H. Van Grinsven (2013): The role of nitrate in human health. In advances in Agronomy/Donald, L.(ed), Sparks, Elsevier Inc., USA, 2013., 153-182, doi:10.1016/B978-0-12-407247-3.00003-2
- [21] Cobos, A., O. Diaz (2014): Chemical Composition of Meat and Meat Products. Handbook of Food Chemistry , 1-32, doi:10.1007/978-3-642-41609-5_6-1

ZNANSTVENO STRUČNI DIO

- [22] Correia, M., A. Barroso, M.F. Barroso, D. Soares, M.B.P.P. Oliveira, C. Delerue-Matos (2010): Contribution of different vegetable types to exogenous nitrate and nitrite exposure. Food Chem, 120, 960-966, doi:10.1016/j.foodchem.2009.11.030
- [23] Domanska-Blicharz, K., M. Michalski, B. Kowalski (2004): Effect of different storage conditions on nitrates and nitrites in polish edible offals processed meat products. Influence on n-nitrosamine content. Bull Vet Inst Pulawy, 48, 63-68.
- [24] Drosinos, E.H., S. Paramithiotis, G. Kolovos, I.Tsikouras, I. Metaxopoulos (2007): Phenotypic and technological diversity of lactic acid bacteria and staphylococci isolated from traditionally fermented sausages in Southern Greece. Food Microbiol, 24, 260-270 doi: 10.1016/j.fm.2006.05.001
- [25] Flores, M., F. Toldra (2021): Chemistry, safety and regulatory considerations in the use of nitrite and nitrate from natural origin in meat products Invited review. Meat Sci, 171, 108272 doi: 10.1016/j.meatsci.2020.108272
- [26] Fontana, A.J. (2000): Understanding the Importance of Water Activity in Food. JR. Decagon Devices, Inc. Pullman, 2000.
- [27] Gassara, F., A.P. Kouassi, S.K. Brar, K. Belkacemi (2016): Green alternatives to nitrates and nitrites in meat-based products-a review. Crit Rev Food Sci, 56, 2133-2148 doi: 10.1080/10408398.2013.812610
- [28] Glorieux, S., O. Goemaere, L. Steen, I. Fraeye (2017): Phosphate Reduction in Emulsified Meat Products. Food Technol Biotechnol, 55, 390-397, doi: 10.17113/ftb.55.03.17.5089.
- [29] Herrmann, S.S., K.Granby, L. Duedahl-Olesen (2015): Formation and mitigation of N-nitrosamines in nitrite preserved cooked sausages. Food Chem, 174, 516-526 doi:10.1016/j.foodchem.2014.11.101
- [30] Hugas, M., J.M. Monfort (1997): Bacterial starter cultures for meat fermentation. Food Chem, 59 (4), 547-554. doi: :10.1016/ S0308-8146(97)00005-8
- [31] Kovačević, D., K. Mastanjević, K. Ćosić, J. Pleadin (2016): Količina nitrita i nitrata u mesnim proizvodima s hrvatskog tržišta. MESO XVIII, 40-46, https://hrcak.srce.hr/158322
- [32] Lidder, S., A.J. Webb (2012): Vascular effects of dietary nitrate (as found in green leafy vegetables and beetroot) via the nitrate-nitrite-nitric oxide pathway. Br.J.Clin.Pharmacol, 75(2012), 677-696, doi: 10.1111/j.1365-2125.2012.04420.x
- [33] Luecke, F.K. (2000): Utilization of microbes to process and preserve meat. Meat Sci, 56, 105-115 doi: 10.1016/s0309-1740(00)00029-2
- [34] Löfblom, J., R. Rosenstein, M.T. Nguyen, S. Stahl, F. Gotz (2017): Staphylococcus carnosus: from starter culture to protein engineering platform. Appl Microbiol Biotechnol, 101(2017), 8293-8307, doi: 10.1007/s00253-017-8528-6.
- [35] McLoughlin, J.V. (1968): Use of Sulphanilamide and N-(1-Naphthyl)-Ethylenediamine in the Determination of Nitrite in Bacon-Curing Brines. Irish J Agr Res, 7, 375-377
- [36] Miller, R.K. (2023): Chapter 15 The eating quality of meat: V Sensory evaluation of meat. Lawrie's Meat Science (Ninth Edition), Woodhead Publishing Series in Food Science, Technology and Nutrition, 509-548. doi.org/10.1016/B978-0-323-85408-5.00020-0
- [37] Ministry of Agriculture (2011): Vodič za mikrobiološke kriterije za hranu, 3. izmijenjeno izdanje.
- [38] Oswell, N.J., H. Thippareddi, R.B. Pegg (2018): Practical use of natural antioxidants in meat products in the U.S.:A review. Meat Sci, 145, 469-479 doi: 10.1016/j.meatsci.2018.07.020
- [39] Pleadin, J., N. Perši, A. Vulić, J. Đugum (2009): Quality of Fermented, Semi-dry and Pasteurised Sausages on Croatian Market. Hrvatski časopis za prehrambenu tehnologiju, biotehnologiju i nutricionizam 4, 104-108 https://hrcak.srce.hr/49982
- [40] Prica, N., M. Zivkov-Balos, S. Jaksic, Z. Mihaljev, B. Kartalovic, D. Ljubojevic, S. Savic (2015): Total phosphorus content in technologically unprocessed meat. Procedia Food Science, 5, 243 246, doi: 10.1016/j.profoo.2015.09.062
- [41] Pyo, Y.H.,T.C. Lee, L. Logendra, R.T. Rosen (2004): Antioxidant activity and phenolic compounds of Swiss chard (Beta vulgaris subspecies cycla) extracts. Food Chem, 85, 19-26, doi:10.1016/S0308-8146(03)00294-2
- [42] Robinson, R.K., C.A. Batt, P.D. Patel (2000): Encyclopedia of Food Microbiology. Academic Press, San Diego, 2000.
- [43] Sebranek, J.G., A.L. Jackson-Davis, K.L. Myers, N.A. Lavieri (2012): Beyond celery and starter culture: Advances in natural/ organic curing processes in the United States. Meat Sci, 92, 267-273 doi: 10.1016/j.meatsci.2012.03.002
- [44] Shin, D.M., K.E. Hwang, C.W. Lee, T.K. Kim, Y.S. Park, S.G. Han (2017): Effect of Swiss Chard (Beta vulgaris var. Cicla) as nitrite replacement on color stability and shelf-life of cooked pork patties during refrigerated storage. Korean J. Food Sci. Anim. Resour, 37, 418-428 doi: 10.5851/kosfa.2017.37.3.418
- [45] Skibsted, L.H. (2011): Nitric oxide and quality and safety of muscle based foods. Nato Sci S A Lif Sci, 24, 176-183 doi: 10.1016/j. niox.2011.03.307
- [46] Suchoparova, M., Š. Janoud, L. Rydlova, F. Beno, V. Pohunek, R. Ševčik (2022): Effect of acerola (Malpighia emarginata DC) fruit extract on the quality of soft salami. J Food Nutr Research, 61, 368-379.

Dostavljeno/Received: 13.09.2028. Prihvaćeno/Accepted: 13.10.2023.

Validacija mesnih proizvoda bez dodanih nitrita, nitrata i fosfata

Sažetak

Izazov je mesne industrije razvoj novih mesnih proizvoda bez dodanih aditiva sa senzorskim i mikrobiološkim svojstvima sličnim onima tradicionalnih proizvoda s nitritnom soli kroz cijeli rok trajanja. U ovom radu, novi recepti bez aditiva ili sa zamjenom aditiva iz prirodnog izvora uspoređeni su s komercijalno dostupnim proizvodima. Zaključeno je da novi mesni proizvodi – kuhana šunka (kao predstavnik termički obrađenih proizvoda) i trajna kobasica imaju koncentraciju nitrita i nitrata ispod granice kvantifikacije za cijelog roka trajanja. Acerola je uspješno zamijenila natrijev askorbat kao prirodni izvor askorbinske kiseline u mesnim proizvodima. Nadalje, dokazano je da fosfati, uspoređujući rezultate s onima iz neobrađenog mesa, nisu dodani u mesne proizvode. Kuhana šunka i trajna kobasica senzorskom su evaluacijom svrstane u najvišu kategoriju kvalitete u pogledu boje, okusa, mirisa i teksture za cijelog roka trajanja te su ocijenjene mikrobiološki ispravnima prema Vodiču za mikrobiološke kriterije za hranu (Ministarstvo poljoprivrede, ožujak 2011.).

Ključne riječi: nitriti, nitrati, fosfati, acerola, mesni proizvodi

Validierung von Fleischerzeugnissen ohne Zusatz von Nitriten, Nitraten oder Phosphaten

Zusammenfassung

Die Herausforderung für die Fleischindustrie besteht in der Entwicklung von Fleischerzeugnissen, die keine Zusatzstoffe enthalten und während ihrer gesamten Haltbarkeitsdauer ähnliche sensorische Eigenschaften wie herkömmliche nitritgepökelte Produkte aufweisen. In dieser Studie wurden neue Rezepturen ohne Zusatzstoffe oder mit Ersatzstoffen natürlichen Ursprungs mit handelsüblichen Produkten verglichen. Es wurde festgestellt, dass die neuen Fleischerzeugnisse - gekochter Schinken (stellvertretend für thermisch verarbeitete Fleischerzeugnisse) und fermentierte Wurst - während der gesamten Haltbarkeitsdauer Nitrit- und Nitratkonzentrationen unterhalb der Bestimmungsgrenze der Methode aufwiesen. Acerola hat erfolgreich Natriumascorbat als natürliche Quelle für Ascorbinsäure in Fleischerzeugnissen ersetzt. Außerdem wurde nachgewiesen, dass den Fleischerzeugnissen keine Phosphate zugesetzt wurden, die mit denen aus rohem Fleisch vergleichbar sind. Bei der sensorischen Bewertung wurden Kochschinken und fermentierte Wurst in die höchste Qualitätskategorie eingestuft, was Farbe, Geschmack, Geruch und Textur während der gesamten Haltbarkeitsdauer betrifft, und wurden als mikrobiologisch sicher bewertet, gemäß dem Leitfaden für mikrobiologische Kriterien für Lebensmittel (Landwirtschaftsministerium, März 2011).

Schlüsselwörter: Nitrite, Nitrate, Phosphate, Acerola, Fleischprodukte

Validación de productos cárnicos sin la adición de nitritos, nitratos y fosfatos

Resumen

El desafío de la industria cárnica es el desarrollo de nuevos productos cárnicos sin aditivos con propiedades sensoriales y microbiológicas similares a los productos tradicionales con sal de nitrito a lo largo de su vida útil. En este trabajo se compararon nuevas recetas sin aditivos o con la sustitución de aditivos de origen natural con productos disponibles comercialmente. Se concluyó que los nuevos productos cárnicos, como el jamón cocido (como representante de productos procesados térmicamente) y la salchicha curada, tienen concentraciones de nitritos y nitratos por debajo del límite de cuantificación durante toda su vida útil. Acerola ha reemplazado con éxito al ácido ascórbico sódico como fuente natural de ácido ascórbico en productos cárnicos. Además, se demostró que los fosfatos no se añaden a los productos cárnicos, comparando los resultados con los de la carne no procesada. El jamón cocido y la salchicha curada se clasificaron en la categoría más alta de calidad en términos de color, sabor, aroma y textura durante toda su vida útil según los criterios sensoriales y se evaluaron como microbiológicamente seguros según la Guía de criterios microbiológicos para alimentos (Ministerio de Agricultura, marzo de 2011).

Palabras claves: nitritos, nitratos, fosfatos, acerola, productos cárnicos

Validazione di prodotti a base di carne senza aggiunta di nitriti, nitrati e fosfati

Riassunto

Lo sviluppo di nuovi prodotti a base di carne senza additivi aggiunti, che abbiano per tutta la loro durata di conservazione proprietà sensoriali e microbiologiche simili a quelle dei prodotti tradizionali con nitrito di sodio, è una vera e propria sfida per l'industria della carne. In questo lavoro, nuove ricette senza additivi o con sostituzione di additivi di origine naturale vengono confrontate con prodotti disponibili in commercio. Si è concluso che i nuovi prodotti a base di carne - prosciutto cotto (come rappresentante dei prodotti trasformati termicamente) e la salsiccia secca stagionata - presentano una concentrazione di nitriti e nitrati inferiore al limite di quantificazione per l'intera durata di conservazione. L'acerola ha sostituito con successo l'ascorbato di sodio come fonte naturale di acido ascorbico nei prodotti a base di carne. Inoltre, è stato dimostrato che i fosfati, confrontando i risultati con quelli della carne non lavorata, non sono stati aggiunti ai prodotti a base di carne. Il prosciutto cotto e la salsiccia secca stagionata sono stati classificati mediante valutazione sensoriale nella categoria di massima qualità in termini di colore, sapore, odore e consistenza per tutta la loro durata di conservazione e sono stati valutati microbiologicamente idonei secondo la Guida ai criteri microbiologici per gli alimenti (Ministero dell'Agricoltura, marzo 2011).

Parole chiave: nitriti, nitrati, fosfati, acerola, prodotti a base di carne