

Analysis of nitrate and nitrite content in meat products without added additives

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Abstract

A food additive is any substance not consumed as a food ingredient but added to food for a specific purpose, for example inhibiting microorganism growth and maintaining sensory quality. Potassium and sodium nitrates and nitrites are among the most commonly used food additives in the meat industry due to their antimicrobial effect, in particular to prevent the growth of pathogenic bacteria *Clostridium botulinum* and its spores. Additionally, they provide specific sensory attributes, primarily the inherent pink color of meat products. Studies showed that excessive intake of nitrates and nitrites may pose a risk to human health. The object of this research was determination of nitrite and nitrate content in meat products without added additives or with those additives from natural sources. Samples were mortadella, cooked ham and bologna sausage as thermally processed (cured) meat products and fermented sausage. The statistical analysis of the results for mortadella, cooked ham and bologna sausage as thermally processed meat products showed a p -value <0.05 between recipes with added nitrite salt and new recipes. The values of the median mass concentration of sodium nitrite in the commercial recipes were from 3.50-40.50 mg/kg, whereas this value was below the limit of detection in new recipes. Also, for fermented sausage, the p -value below 0.05 between commercial recipe with nitrite and nitrate salt and new recipes confirmed the hypothesis for replacement of mentioned salts with new concepts. The value of the median mass concentration of sodium nitrate in the recipe with added nitrate salt was 268.52 mg/kg, whereas this value was below the limit of detection in other recipes.

Keywords: additives; antimicrobial effect; health risk; natural sources of nitrates

Introduction

Nitrite and nitrate salts of sodium and potassium are permitted preservatives that may be added to cured meat products. Their role is protecting these products primarily against pathogenic bacteria *Clostridium botulinum*, through inhibiting the outgrowth of spores. Nitrite has been reported to contribute to controlling the growth of several other pathogens, such as *Listeria mono-*

cytogenes, *Bacillus cereus*, *Staphylococcus aureus* and *Clostridium perfringens*. The effectiveness of nitrite as an antitoxigenic agent depends on pH, concentration of sodium chloride, reductants, iron content, etc. (Ferreira and Silva, 2008). Another contribution of nitrite to meat curing includes color development, antioxidant properties to retard lipid oxidation, flavor production and

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texture improvement (Merino et al., 2000). Nitrite reacts with the meat to form nitric oxide, which then reacts with oxymyoglobin in the meat to form an extremely unstable pink colored compound called nitrosylmyoglobin, MbFe(II)NO. During thermal processing, it is converted to a stable reddish compound called nitrosohemochrome, because of the denaturation of the protein moiety of the myoglobin pigment (Skibsted, 2011). Also, a low level of nitrite (50 ppm) is sufficient to develop the unique flavor of cured meat. The principle mechanism is still insufficiently known and one of the possible explanations is that specific flavor could be the result of a combination of nitrite-related flavors and aromas. Nitrate may also be used as a source of nitrite through the action of bacterial nitrate reductase. By oxidation, about 20 % of added nitrites turn into nitrates two hours after processing, and the process continues during storage (Pavličić Prokurica, 2010).

Nitrite is a highly reactive compound and can be an oxidizing, reducing or nitrosylating agent. As an oxidizing agent, it converts blood hemoglobin to methemoglobin, resulting in a reduced oxygen supply to body tissues. In addition, as a nitrosylating agent, it can produce carcinogenic nitroso compounds, such as *N*-nitrosamines. *N*-nitrosamines are formed in the reaction of nitrosating agents (nitrates, nitrites, oxides of nitrogen) with primary, secondary and tertiary amines or amides, proteins, peptides and amino-acids during food processing and preservation, e.g. frying, baking or other heat treatments (Wang et al., 2016; Domska-Blicharz et al., 2004; Flores and Toldra, 2021). In order to reduce the use of nitrates and nitrites, thereby reducing the risk of harmful and carcinogenic nitroso compounds, numerous studies suggest substitutes for nitrites and nitrates in meat products (Sebranek and Bacus, 2007, 2007b). Vegetables, such as celery, lettuce, spinach and turnip, in the form of powder or juice, and spices like parsley are natural sources of nitrates with concentrations of 1500 to 2800 ppm. Recently, Swiss chard powder (*Beta vulgaris* var. *cicla*) was also used as a natural source of nitrate. It contains 3-3.5 % nitrate and is recommended to be used at a concentration of 0.15-0.3 %. One of the advantages of Swiss chard is that it has no allergens, but high concentrations may negatively affect the sensory attributes (Sebranek et al., 2012). Mediterranean sea salt, which is used as a natural source of nitrates and

nitrites, contains 1.1 ppm nitrate and 1.2 ppm nitrite. Several reviews suggest alternatives to nitrite, with a role of avoiding oxidation in meat products, such as sulfur dioxide, butylated hydroxyanisole, α -tocopherol, organic acids etc. However, the expiration date of such products is significantly shorter than that of similar products with added nitrite salt (Gassara et al., 2016; Alahakoon et al., 2015; Oswell et al., 2018).

The manufacture of meat products without commonly used curing agents, such as nitrite and nitrate salts, includes mixing of meat and fat with salts, aromas, acidity regulators, sugar, antioxidants, spices, yeast extract (in case of thermally processed meat products) and well-defined starter culture under controlled conditions. Starter cultures for meat products normally contain lactic acid bacteria and staphylococci (such as *Staphylococcus*, *Kocuria* etc.), and, in some cases, micrococci with nitrate reductase activity. Lactic acid bacteria (e.g. *Pediococcus* spp.) affect the technological properties, as well as the microbial stability, of the final product. They inhibit meat-borne pathogenic bacteria and coagulate soluble meat proteins, thereby reducing water binding capacity and facilitating the drying of the product. The dominance of lactic acid bacteria is usually favored by anaerobic conditions, added curing salt and sugars, and by pH lower than 5.8 (Drosinos et al., 2007; Hugas and Monfort, 1997; Luecke, 2000). *Staphylococcus carnosus* and *Staphylococcus xylosus* are nitrate-reducing bacterial cultures that can optimally reduce nitrate at a temperature above 30 °C, often at 38-42 °C (Casaburi et al., 2005). They affect color stability, reduce spoilage and contribute to flavor development. Yeast extracts, most often derived from primary grown yeast of the genus *Saccharomyces cerevisiae*, and molds give flavor to meat products through carbohydrate fermentation and lactate oxidation, proteolysis and degradation of amino-acids and lipolysis (Drosinos et al., 2007). The challenge for the meat industry is developing meat products that have similar sensory properties, and will persist throughout a product's shelf life and provide the same level of safety as traditional nitrite-cured products. In this context, a representative of the Croatian meat industry launched a R&D project with aim to develop a new line of products without nitrite and nitrate commonly used in industrial production or products with nitrite and nitrate substitutes from natural origin.

Materials and Methods

Definition of recipes of meat products

Meat products were obtained from the Croatian PIK VRBOVEC plus d.o.o. meat industry, as part of the “CLEAN LABEL - Development of a line of meat products without additives” research project. In the industrial phase of project, four different types of meat products were analyzed: mortadella, cooked ham, bologna sausage and fermented sausage. Four recipes in thermally processed meat products – cooked ham, mortadella and bologna sausage were used as follows: recipe 1 had no added nitrites or nitrates (control sample); recipe 2 was the standard recipe with added nitrite salt; recipe 3 had yeast extract with bacterial culture, while recipe 4 contained added nitrates coming from two natural sources of nitrates – Swiss chard and parsley. Four recipes containing fermented sausage were the following: recipe 1 had no added nitrites or nitrates; recipe 2 was the standard recipe with added nitrite and nitrate salts; recipe 3 had sea salt with bacterial culture, and recipe 4 contained added nitrates coming from the same natural sources as mentioned above.

Chemicals and reagents

Chemicals and solvents were of HPLC or analytical grade. Nitrate and nitrite standard solutions with concentrations of 1000 mg/L were purchased from Sigma–Aldrich, Dermstadt, Germany. Working standard solutions were prepared by further dilution of standard. For the purification of the sample extract Carrez I (potassium hexacyanoferrate(II) trihydrate; CARLO ERBA reagents, Emmerdingen, Germany; 15 g dissolved in 100 ml water) and Carrez II (zinc sulfate heptahydrate; Gram – mol, Zagreb, Croatia; 23 g dissolved in 100 ml water) were used. Buffer solution was prepared in a 100 ml flask, by mixing 5 mL of concentrated hydrochloric acid (35 – 38 %, Macron, Gliwice, Poland) with 60 mL of water and 10 mL of ammonia solution (25 %, Gram – mol, Zagreb, Croatia). The flask was filled up to the set volume, and pH value of the solution was adjusted to 9.6–9.7. Reagents for color development were reagent I (0.5 g of sulfanilamide (CARLO ERBA reagents, Emmerdingen, Germany), 95 mL of water and 5 mL of concentrated hydrochloric acid), reagent II (0.1 g N-(1-naphthyl) ethylene diamine dihydrochloride (ACROS ORGANICS, New Jersey, USA) in 100 mL water) and 17 %

hydrochloric acid solution (445 mL concentrated hydrochloric acid was diluted with water up to 1000 mL). All chemical materials were weighed using PH 802L/A, Mettler Toledo. Milli Q purified water was used in the experiments.

Sample preparation

5 g of homogenized processed meat sample was weighed into a 100 mL volumetric flask, dissolved in approximately 50 mL Milli Q water at 70–80°C, thermostated for 15 minutes and cooled to room temperature (25°C). Buffer solution, Carrez solution I and II were added and the flask was filled up to volume. The sample was filtered through 110 mm filter paper (pore size 12–15 µm) and used for HPLC determination of nitrate. For nitrite determination, 5 ml of the filtered sample was added to a 50 ml flask containing 3 ml of 17% hydrochloric acid, 2.5 ml of reagent I and 1 ml of reagent II. After adding sample aliquot, the content was lightly mixed and left in the dark for 30 minutes, allowing color development, filled up to volume and measured on a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) in photometric mode at 538 nm.

Method validation

According to European Analytical Chemistry (Eurachem) guidelines (Magnusson and Örnemark, 2014), the validation process includes linearity, limits of detection (LOD), the limit of quantification (LOQ), precision, repeatability and accuracy. The calibration curves were calculated by analyzing different concentrations of standard solutions. Five calibration points, ranging from 0.2 to 2 mg/L for nitrite and from 1 to 81 mg/L for nitrate, were prepared by appropriate dilution of standard solutions with Milli Q water. Linear calibration curves were obtained by instrument response measurement for each concentration. The detection limit (LOD) is defined as lowest concentration of the analyte that can be detected by the method at a specified level of confidence, while the limit of quantitation (LOQ) refers to the lowest level at which the performance is acceptable for a typical application. The limit of quantification (LOQ) can determine as the lowest amount of analyte that can determine in a real sample, in such a way that repeatability and accuracy are calculated in ten replicates at that point utilization, which meet the criteria for individual analyte. The detection limit was calculated by regression parameters, respectively as $3.3 \cdot s/a$, where the symbol ‘s’ was the standard deviation of the response of the curve and ‘a’

was the slope of the calibration curve. The precision for both analytes was determined by calculating the percent relative standard deviation (%RSD) of repeatability. %RSD was calculated for six replicates of spiked solutions at three different concentrations (Magnusson and Örnemark, 2014). According to AOAC (2002), the trueness of the procedures was assessed by performing recovery studies. Processed meat samples were spiked six times with nitrate and nitrite standard solutions of three known concentrations, respectively. Recovery was calculated by determining the percent of added amount detected after subtracting sample analyte concentration.

Instrumentation and chromatographic conditions for determination of nitrate

The determination of nitrate was performed using a Prominence LC 20 high-performance liquid chromatography system (Shimadzu, Kyoto, Japan) with a diode array detector. The data processing of the standard and test samples were carried out using Labsolution software (Shimadzu, Kyoto, Japan). A Zorbax C18 Eclipse XDB; 4,6 mm x 150 mm, with 5 µm particle size column, was used for chromatographic separation thermostated at 30 °C. Isocratic elution was carried out at a flow rate of 1 mL/min, with a mobile phase consisting of 10 mM solution of n-octylamine (ACROS ORGANICS, New Jersey, USA) in 20 % MeOH, pH adjusted with concentrated o-phosphoric acid to 6.5. Nitrate ion was detected at 201 nm.

Instrumentation and conditions for spectrophotometric determination of nitrite

The determination of nitrite was performed using a Shimadzu UV-160 spectrophotometer, equipped with a 1.0 cm quartz cell. Absorbance measurement was carried out at 538 nm. The principle of the applied method is based on the ability of nitrite to react with sulfanilic acid in acidic medium to

form a diazonium salt, then coupling the diazotized sulfanilamide with 1-naphthylamine, forming an intensely pink azo compound that can be determined by spectrophotometric measurement.

Statistical Analysis

The data reported were analyzed by a Kruskal-Wallis test or one-way ANOVA analysis of variance test and, additionally, with a post-hoc Dunn's test, using IBM SPSS Statistics for Windows software, version 27.0.1. Concentrations of sodium nitrate and sodium nitrite between different recipes of meat products were compared, and significance of differences between concentrations was determined. Differences at $p \leq 0.05$ were considered as significant. The results were expressed as medians and interquartile ranges because of their non-parametric distribution.

Results and discussion

Results of method validation

Table 1 shows the validation results: linearity, precision and trueness. The regression equations were calculated as $y = ax + b$, where y is peak area and x is the concentration in ppm of the five standard solutions of nitrites and nitrates, respectively. The correlation coefficient (R^2) of the standard solutions in the prescribed ranges were 0.9993 for nitrites and 0.99996 for nitrates. The detection limit of spectrophotometric method for determination of nitrite content was 0.02 mg/L and quantification limit was 0.2 mg/L. The detection limit for determination of nitrate content was 0.1mg/L and quantification limit was 1mg/L. The %RSD for repeatability ranged between 1.74 % and 6.37 % for nitrite and 0.050 % to 0.53 % for nitrate. The nitrite recovery ranged from 95.31 % to 109.6 %, while the nitrate

Table 1 Validation results of spectrophotometric method for nitrite and chromatographic method for nitrate determination

Validation parameters		Analyte	
		Nitrite	Nitrate
Linearity	Calibration range (mg/L)	0.2-2	1-81
	Regression equation, $y=ax+b$	$y=0.0981x-0.0039$	$y=87903x+13490$
	Correlation coefficient (R^2)	0.9993	0.99996
Precision	Repeatability, %RSD	1.74-6.37	0.050-0.53
Trueness	Percentage recovery, %R	95.31-109.6	93.68-95.42
	LOD (mg/L)	0.02	0.1
	LOQ (mg/L)	0.2	1.0

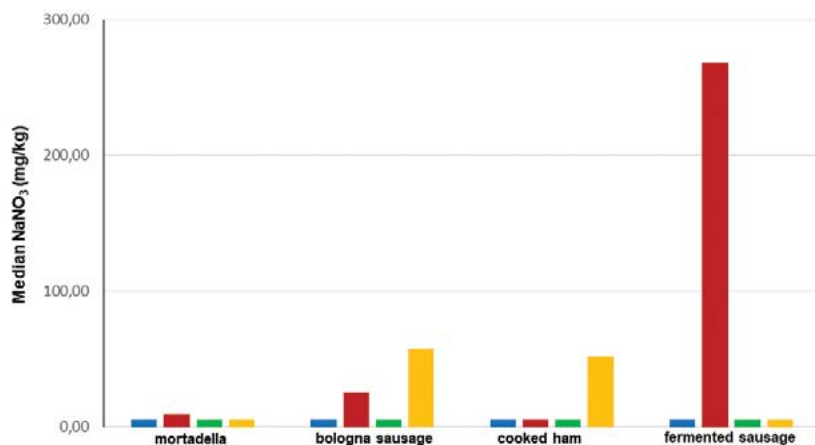


Figure 1 Sodium nitrate values in different type of meat products and recipes (■ recipe 1 (no added nitrate or nitrite salts), ■ recipe 2 (thermally processed meat products - added nitrite salt; fermented sausage - added nitrate and nitrite salt), ■ recipe 3 (thermally processed meat products - yeast extract with bacterial culture; fermented sausage - sea salt with bacterial culture), ■ recipe 4 (added nitrate from natural source))

recovery values ranged from 93.68 % to 95.42 %.

When compared to literature results, there was good agreement in minimal losses, analytical system accuracy and good alignment between spiking and calibration solution (Nerdy and De Lux Putra, 2018; Chamandust et al., 2016).

Nitrate and nitrite content

All results of measurements of nitrite content in meat products – mortadella, bologna sausage, cooked ham and fermented sausage, shown in Figure 1., were expressed as concentration of sodium nitrate in mg/kg. Medians and interquartile ranges proved better as values for statistical data processing, because of the non-parametric distribution of results.

Table 2 shows a detailed post-hoc Dunn analysis of statistical differences between individual recipes in the same product groups. Statistical differences in sodium nitrate values were visible in

every product type and, additionally, among different recipes within the same product type. In the mortadella group, the highest nitrate value was associated with recipe 2, which represents the commercially available version, while across all the other experimental recipes, there was no statistical difference. The cooked ham and bologna sausage groups had the highest sodium nitrate values in recipe 4, where nitrates were added from natural sources. Additionally, within the bologna sausage group, recipes 1 versus 3, and 2 versus 4 showed no statistical difference, and within the cooked ham group, only recipe 3 versus 4 showed statistical difference. All recipes showed significant difference in the fermented sausage group in comparison with recipe 2, and none compared to each other. This can be explained by the fact that sodium nitrate is added along with sodium nitrite in the commercial version of the product, represented in recipe 2. In mortadella,

Table 2 Post-hoc Dunn’s test for recipe differences in relation to each other for sodium nitrate

Post-hoc test: Dunn	p-value			
	Mortadella	Bologna sausage	Cooked ham	Fermented sausage
REC1 vs. REC2	0.036	0.029	0.705	<0.001
REC1 vs. REC3	0.204	0.446	0.763	1.000
REC1 vs. REC4	1.000	0.034	0.353	1.000
REC2 vs. REC3	0.514	0.009	0.804	<0.001
REC2 vs. REC4	0.017	0.330	0.057	<0.001
REC3 vs. REC4	0.144	0.004	0.015	1.000

REC 1- recipe 1 (no added nitrate or nitrite salts), REC 2- recipe 2 (added nitrite salt in thermally processed products or nitrate and nitrite salts in fermented sausage), REC 3- recipe 3 (yeast extract in thermally processed products or sea salt in fermented sausage, with bacterial culture), REC 4- recipe 4 (added nitrate from natural source)

bologna sausage and fermented sausage group there is no statistical difference between recipe 1 as control sample and recipe 3, the new concept without added nitrites or nitrites, and for cooked ham group there is not enough data for recipe 1.

Similar results were obtained for sodium nitrite values, expressed as the concentration of sodium nitrite in mg/kg, shown in Figure 2.

Table 3 shows a detailed post-hoc Dunn analysis of statistical differences between individual recipes in the same product groups for sodium nitrite. There were statistical differences across all product type groups, except for the fermented sausage group, which had values of $p > 0.05$, regardless of the recipes compared. The mortadella group had statistical differences when comparing recipe 2 with all other

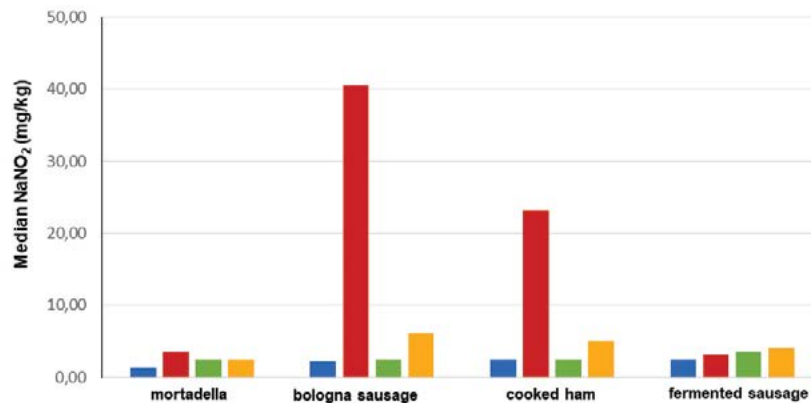


Figure 2 Sodium nitrite values in different type of meat products and recipes

(■ recipe 1 (no added nitrate or nitrite salts), ■ recipe 2 (thermally processed meat products - added nitrite salt; fermented sausage - added nitrate and nitrite salt), ■ recipe 3 (thermally processed meat products - yeast extract with bacterial culture; fermented sausage - sea salt with bacterial culture), ■ recipe 4 (added nitrate from natural source))

recipes, but compared to each other, no significant difference was observed. Contrary to the fermented sausage, the bologna sausage group showed differences across all recipes. Within the cooked ham group, recipe 1 (control sample) values had to be ignored because of insufficient data (only one data set) and all other recipes had p -values < 0.05 compared to each other, while recipe 2 had the highest value of sodium nitrite.

Conclusion

Two concepts of replacing sodium nitrite and nitrate in meat products were studied: firstly, without addition of nitrites and nitrates and, secondly, with the addition of nitrates from a natural source. From the obtained results, it can be concluded that the new recipes for all meat products can be applied to the final products that will be placed on the market in the future.

Table 3 Post-hoc Dunn’s test for recipe differences in relation to each other for sodium nitrite

Post-hoc test: Dunn	p-value			
	Mortadella	Bologna sausage	Cooked ham	Fermented sausage
REC1 vs. REC2	<0.001	<0.001	0.113	0.317
REC1 vs. REC3	0.171	0.003	0.782	0.334
REC1 vs. REC4	0.134	0.001	0.252	0.190
REC2 vs. REC3	0.003	<0.001	<0.001	0.332
REC2 vs. REC4	0.001	0.013	<0.001	0.249
REC3 vs. REC4	0.716	0.013	<0.001	0.583

REC 1- recipe 1 (no added nitrate or nitrite salts), REC 2- recipe 2 (added nitrite salt in thermally processed products or nitrate and nitrite salts in fermented sausage), REC 3- recipe 3 (yeast extract in thermally processed products or sea salt in fermented sausage, with bacterial culture), REC 4- recipe 4 (added nitrate from natural source)

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Conflicts of Interest: The authors declare no conflict of interest.

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Određivanje sadržaja nitrata i nitrita u mesnim proizvodima bez dodanih aditiva

Sažetak

Prehrambeni aditiv je svaka tvar koja se sama po sebi ne konzumira kao hrana, niti je prepoznatljiv sastojak hrane već se dodaje zbog svoje važne uloge. Najčešće se dodaje u hranu u svrhu konzerviranja, održavanja svježine, očuvanja konzistencije, poboljšanja teksture te usporavanja rasta mikroorganizama. Najčešće korišteni aditivi u mesnoj industriji su kalijevi ili natrijevi nitrati i nitriti. Tradicionalno se koriste za konzerviranje mesnih proizvoda zbog svoje učinkovitosti antimikrobnog djelovanja nitrita protiv patogene bakterije *Clostridium botulinum*, rasta njenih spora kao i protiv drugih bakterija. Osim konzervirajućeg učinka odgovorni su za karakteristična organoleptička svojstva, prvenstveno svojstvenu ružičastu boju mesnih proizvoda. Istraživanja ukazuju da prevelik unos nitrata i nitrita može predstavljati rizik za ljudsko zdravlje. Cilj ovog znanstvenog rada bio je određivanje sadržaja nitrita i nitrata u mesnim proizvodima bez dodanih aditiva. Također, istraživao se i učinak dodanih nitrata i nitrita na mesne proizvode iz prirodnih izvora, kao što su sastojci na bazi povrća – blitve i peršina. Mesni proizvodi u kojima se određivao sadržaj navedenih aditiva bili su termički obrađeni proizvodi poput mortadele, kuhane šunke i parizera i trajna kobasica. Statističkom obradom rezultata termički obrađenih mesnih proizvoda mortadele, kuhane šunke i parizera dobivene su p -vrijednosti <0.05 između recepture s nitritnom soli i novih receptura. Srednje vrijednosti masenih koncentracija natrijevog nitrita u komercijalnim recepturama bile su od 3.50-40.50 mg/kg dok su za nove recepture te vrijednosti bile ispod granice detekcije. U slučaju fermentirane kobasice, p -vrijednost ispod 0.05 između komercijalno dostupne recepture s nitratom i nitritnom soli i novih receptura je potvrdila hipotezu o zamjeni navedenih soli s novim konceptima. Srednja vrijednost masene koncentracije natrijevog nitrata u komercijalnoj recepturi bila je 268.52 mg/kg dok je ta vrijednost ispod granice detekcije kod novih receptura.

Glavne riječi: aditivi, nitrati, nitriti, mesni proizvodi, prirodni izvori nitrata

Analyse des Nitrat- und Nitritgehalts in Fleischerzeugnissen ohne Zusatzstoffe

Zusammenfassung

Ein Lebensmittelzusatzstoff ist eine Substanz, die nicht als Lebensmittelzutat verzehrt, sondern dem Lebensmittel zu einem bestimmten Zweck zugesetzt wird, z. B. zur Konservierung, Erhaltung der Frische und Konsistenz, Verbesserung der Textur und Hemmung des Wachstums von Mikroorganismen. Kalium- und Natriumnitrate und -nitrite gehören aufgrund ihrer antimikrobiellen Wirkung zu den am häufigsten verwendeten Lebensmittelzusatzstoffen in der Fleischindustrie, insbesondere zur Verhinderung des Wachstums von pathogenen Bakterien *Clostridium botulinum* und deren Sporen. Außerdem sorgen sie für bestimmte sensorische Eigenschaften, vor allem für die rosa Farbe von Fleischerzeugnissen. Studien haben gezeigt, dass eine übermäßige Aufnahme von Nitraten und Nitriten ein Risiko für die menschliche Gesundheit darstellen kann. Gegenstand dieser Untersuchung war die Bestimmung des Nitrit- und Nitratgehalts in Fleischerzeugnissen ohne Zusatzstoffe oder mit diesen Zusatzstoffen aus natürlichen Quellen, wie z.B. Inhaltsstoffe auf Basis von Gemüse – Mangold und Petersilie. Bei den Proben handelte es sich um Mortadella, Kochschinken und Bolognesewurst als thermisch verarbeitete (gepökelte) Fleischerzeugnisse und fermentierte Wurst. Die statistische Analyse der Ergebnisse für Mortadella, Kochschinken und Bolognesewurst als thermisch verarbeitete Fleischerzeugnisse ergab einen p -Wert $<0,05$ zwischen Rezepten mit zugesetztem Nitritsalz und neuen Rezepten. Die Werte der mittleren Massenkonzentration von Natriumnitrit in den Handelsrezepturen lagen zwischen 3,50 und 40,50 mg/kg, während dieser Wert in den

neuen Rezepturen unter der Nachweisgrenze lag. Auch bei fermentierter Wurst bestätigte der p -Wert unter 0,05 zwischen der Handelsrezeptur mit Nitrit- und Nitratsalz und den neuen Rezepturen die Hypothese für den Ersatz der genannten Salze durch neue Konzepte. Der Wert der mittleren Massenkonzentration von Natriumnitrat in der Rezeptur mit zugesetztem Nitratsalz betrug 268,52 mg/kg, während dieser Wert in den anderen Rezepturen unter der Nachweisgrenze lag.

Schlüsselwörter: Zusatzstoffe, Nitrate, Nitrite, Fleischprodukte, natürliche Nitratquellen

Análisis del contenido de nitratos y nitritos en productos cárnicos sin aditivos añadidos

Resumen

Un aditivo alimentario es cualquier sustancia que no se consume como ingrediente alimentario, pero se añade a los alimentos con un propósito específico, por ejemplo, para inhibir el crecimiento de microorganismos y mantener la calidad sensorial. Los nitratos y nitritos de potasio y sodio se encuentran entre los aditivos alimentarios más comúnmente utilizados en la industria cárnica debido a su efecto antimicrobiano, especialmente para prevenir el crecimiento de bacterias patógenas como el *Clostridium botulinum*, sus esporas y otras bacterias. Además, proporcionan atributos organolépticos específicos, principalmente el color rosa inherente de los productos cárnicos. Estudios han demostrado que la ingesta excesiva de nitratos y nitritos puede representar un riesgo para la salud humana. El objetivo de esta investigación fue determinar el contenido de nitritos y nitratos en productos cárnicos sin aditivos añadidos. También fue investigado el efecto de los nitratos y nitritos agregados en productos cárnicos de fuentes naturales, como ingredientes de origen vegetal: acelgas y perejil. Las muestras fueron mortadela, jamón cocido y salchicha de bolonia como productos cárnicos procesados térmicamente (curados) y salchicha fermentada. El análisis estadístico de los resultados para la mortadela, el jamón cocido y la salchicha de bolonia como productos cárnicos procesados térmicamente mostraron un valor $p < 0.05$ entre las recetas con sal de nitrito añadida y las nuevas recetas. Los valores de la concentración media de masa de nitrito de sodio en las recetas comerciales fueron de 3.50-40.50 mg/kg, mientras que este valor fue inferior al límite de detección en recetas nuevas. Además, para la salchicha fermentada, el valor p por debajo del límite de 0.05 entre la receta comercial con sal de nitrito y nitrato y las nuevas recetas confirmó la hipótesis de reemplazo de dichas sales con nuevos conceptos. El valor de la concentración media de masa de nitrato de sodio en la receta con sal de nitrato añadida fue de 268.52 mg/kg, mientras que este valor fue por debajo del límite de detección en las demás recetas.

Palabras claves: aditivos, nitratos, nitritos, productos cárnicos, efecto antimicrobiano, riesgo para la salud, fuentes naturales de nitratos

Determinazione del contenuto di nitrati e nitriti nei prodotti a base di carne senza additivi aggiunti

Riassunto

Un additivo alimentare è qualsiasi sostanza che non viene consumata come alimento di per sé, né è un ingrediente alimentare riconoscibile, ma viene aggiunta agli alimenti con una funzione specifica. Viene spesso aggiunto al cibo allo scopo di conservarlo, mantenerne la freschezza, preservarne la consistenza, migliorarne la texture e rallentare la crescita dei microrganismi. Gli additivi più comunemente usati nell'industria della carne sono i nitrati e i nitriti di potassio o di sodio. Sono tradizionalmente utilizzati per conservare i prodotti a base di carne grazie all'efficacia dell'azione antimicrobica del nitrito con-

tro il batterio patogeno *Clostridium botulinum*, la crescita delle sue spore, nonché contro altri batteri. Oltre all'effetto conservante, sono responsabili di alcune proprietà organolettiche caratteristiche, in primis del tipico colore rosa dei prodotti a base di carne. La ricerca mostra che l'eccessiva assunzione di nitrati e nitriti può rappresentare un rischio per la salute umana. Questo studio scientifico aveva lo scopo di determinare il contenuto di nitriti e nitrati nei prodotti a base di carne senza additivi aggiunti. Inoltre, è stato studiato anche l'effetto dei nitrati e nitriti aggiunti su prodotti a base di carne di origine naturale, come alcuni ingredienti a base vegetale - bietole e prezzemolo. I prodotti a base di carne in cui è stato stabilito il contenuto dei suddetti additivi sono prodotti termicamente trattati quali la mortadella, il prosciutto cotto, il parizer (una specie di grosso wurstel: n.d.t.) e la salsiccia stagionata. Mediante elaborazione statistica dei risultati dei prodotti a base di carne lavorati termicamente (mortadella, prosciutto cotto e parizer), tra la ricetta con sale nitrito e le nuove ricette è stato ottenuto un valore- $p < 0,05$. I valori medi delle concentrazioni in massa di nitrito di sodio nelle ricette commerciali erano di 3,50-40,50 mg/kg, mentre per le nuove ricette questi valori sono risultati inferiori al limite di rilevabilità. Nel caso della salsiccia fermentata, il valore- p inferiore a 0,05 tra la ricetta disponibile in commercio con sale nitrato e nitrito e le nuove ricette ha confermato l'ipotesi di sostituire i sali citati con nuovi prodotti. Il valore medio della concentrazione in massa di nitrato di sodio nella ricetta commerciale è risultato di 268,52 mg/kg, mentre nelle nuove ricette questo valore è risultato inferiore al limite di rilevabilità.

Parole chiave: additivi, nitrati, nitriti, prodotti a base di carne, fonti naturali di nitrati