Sensory and functional parameters of sausages with reduced sodium nitrite content

Oksana Savinok¹*, Sergey Patyukov¹, Aco Kuzelov², Dmitrij Shepelenko¹

¹Department of Meat, Fish and Seafood Technology, Faculty of Food Technology, Parfumes and Cosmetics, Expertise and Commodity Studies, Odessa National Academy of Food Technologies, Odessa, Ukraine
²Faculty of Agriculture, Goce Delchev University, Stip, Macedonia

*Corresponding author: Assoc. Prof. Oksana Savinok, PhD; Department of Technology of Meat, Fish and Seafood, Faculty of Food Technology, Parfumes and Cosmetics, Expertise and Commodity Studies, Odessa National Academy of Food Technologies, 112 Kanatnaya St., Odessa, Ukraine, tel.: ++380 487 124 250, mobile: ++38097 343 34 77; E-mail: savoksamit12@gmail.com

Running title: Sausages with reduced nitrite content

Abstract
The effect of nicotinic acid, nicotinic acid amide on sensory and functional-technological properties of sausages is studied in this work. The use of additives makes it possible to stabilize the color of minced meat on the cut and to ensure its stability under the influence of external factors. Use of 0.006% of additives to the mass of the main raw material, allows to reduce residual content of sodium nitrite by 19…25% in comparison with control. Sausages are characterized by a juicy consistency and pleasant taste and stable pink color. When stored unpackaged, sensory properties are remains unchanged for 7…14 d, depending on type of sausages. Reducing the residual nitrite content will provide environmental friendliness of this product and can be used for Children's nutrition.

Practical applications
The use of nicotinic acid and nicotinamide permits to reduce the residual content of nitrites in sausage production. This reducing decrease probability of nitrosamine formation during the storage of sausages and increase the eco-level of production. The use of vitamins provides stabilizing of color of the product during the long storage and exclude necessity of the dye usage.

Key words: nicotinic acid amide, nicotinic acid, functional properties, sensory indices, sausages
Introduction

Quality and safety of meat products is very important and actual problem at present time. Introduction of new technologies, essential changes of functional properties of raw materials, use of different additives allows to obtain reasonable organoleptic properties of meat products. The safety of meat products in this case became doubtful. Use of sodium nitrite and its residual content remains significant problem because it leads to formation of nitrosamines, which are ecotoxicants.

The issue of the production of sausages with a low content of sodium nitrite was raised in different countries around the world. Scientists use natural or synthetic additives, microbial cultures for this purpose. However, the use of sodium nitrite is not limited to stabilizing the color of the products. Sodium nitrite serves as an antioxidant, an inhibitor of pathogenic microflora, in particular *Clostridium botulinum*. The full replacement of sodium nitrite with plant extracts will not allow the fulfillment of a set of functions performed by this product, which will cause need for additional use of antioxidants and antimicrobial substances of selective action.

To reduce the residual content of nitrites in sausages is possible in different ways, which are offered by scientists. The most popular are starter cultures of microorganism, for example *Staphylococcus carnosus* and *Staphylococcus utilis* (Bal-Pilipko & Leonova, 2015), *Staphylococcus* M3 and *Lactobacillus curvatus* HJ5 (Sergeeva, 2011), active bifidobacterial starter cultures containing *B. bifidum* strains No. 791 / BAG and *B. longum* (Merenkova & Lukin, 2014), *Staphylococcus carnosus*, *Lactobacillus plantarum* and *Bifidobacterium longum* subsp. animalis (ratio of 1:1:1) (Vinnikova & Asaulyak, 2010). The use of bacterial cultures leads to a decrease in the amount of residual sodium nitrite in the finished sausage products, as noted by numerous authors. Microbiological researches of samples with lactic acid, probiotics, and starter cultures indicate their good quality and safety. The proposed compositions of microorganisms break down sugar into lactic acid, which leads to a decrease in pH, inhibition of growth of undesirable microflora at the very beginning of sausage making, acceleration of the process of denitrification and stabilization of color formation.

An equally interesting way to reduce the residual sodium nitrite content, or to completely replace it, is the use of complex organic compounds, often of phenolic nature. Functional food ingredients - lycopene, possessing antioxidant and antimicrobial activity (Situn et al., 2016), black currant extracts (Latin Ribes nigra), cherries (Latin Prunus subg Cerasus) and grapes (Latin Vitis labrusca) (Kozlova, 2012), dry dyes from dogwood (Latin Corrinus) and thorn (Latin Prunus spinosa) (Melokanova & Kvasnikov, 2012), anthocyanin dye obtained from the American pacifier (Phytolacca Americana) (Kulikov & Dadyan, 2008), crushed fruits of barberry (Latin Berberis) (Dumcheva, 2015). The use of these ingredients is assumed in boiled and smoked sausages. In accordance with the recommendations of the authors, dry dogwood dyes allow completely eliminate the use of sodium nitrite 20, and provide a characteristic stable pink color to boiled sausages. Other additives help to reduce its residual concentration by half (Situn et al., 2016). The use of crushed fruits of barberry (Dumcheva, 2015) provides a stable colorat of the product during maturation and storage and its enrichment with vitamin C by 50 mg.100g⁻¹ of product.

Use of various vitamins in sausage production is unconventional but it also helps to reduce the residual sodium nitrite content. Vitamins are offered for use in pure form, modified and in various combinations. As a rule, the objects of research were vitamins, which can perform the function of the reducing ageing of sodium nitrite. Ascorbic acid and ascorbates have good reducing properties, therefore they are widely used in the meat industry. Vitamins B1, B2 and PP are often used to stabilize the coloring. Vitamin B1 is an antioxidant for ascorbic acid, so this mixture of vitamins in meat recipes contributes to their stability and safety. Vitamin B2 is often used as a food coloring (Shulyakovskaya et al., 2012).
The authors found that the highest content of nitrosopigments and stable coloration is provided in cooked sausages when compositions containing sodium nitrite, nicotinamide, ascorbic acid, succinic acid, B-group vitamins, calcium lactate, citric acid, ascorbic acid and sodium ascorbate are used. The analyzed indicators remain stable during products shelf-life.

Nasonova & Veretov (2012) used ascorbic acid and micellated water-soluble and fat-soluble solubilized of 10% ascorbic acid to increase sausage safety by reducing the amount of sodium nitrite introduced. The additives used allowed not only to provide high quality and safety indicators of sausages, but also improve the functional properties of minced meat.

In order to increase the biological value and reduce the dosage of sodium nitrite introduced, the possibility of using the vitamin-mineral supplement "Biomax" (Vichrov et al., 2016) in the technology of boiled sausage was investigated.

It contained such water-soluble vitamins as thiamine hydrochloride (1 mg), riboflavin (1.27 mg), ascorbic acid (50.00 mg) and nicotinamide (7.50 mg). Sodium nitrite was used in the interval 3.5...7.5 g/100kg of raw materials. The optimal dosages of the studied ingredients were 5.8 g of sodium nitrite and 370.9 g of the vitamin complex "Biomax" per 100kg. This combination made it possible not only to stabilize a color, but also to ensure the human need in vitamin B1 (thiamine) by 42.2%, in B2 (riboflavin) by 55.9%, in PP (nicotinamide) - by 30.3% and in vitamin C (ascorbic acid) - by 47.7% when using 100 g of sausage per day.

A significant change in the diet of farm animals, uncontrolled living conditions do not allow to obtain a standardized quality of raw materials. Manufacturers of sausages in Ukraine are faced with a problem of poor quality meat daily. Therefore, to produce quality sausages, in which the values of controlled chemical indicators are difficult, and sometimes simply impossible. In addition to chemical parameters, the sensory properties also change. The products obtained have a light, unnatural coloring on the cut, a water consistency.

Therefore, modern technology uses additives, whose action is aimed to stabilize the hydrogen index, water retention capacity, the color of minced meat on the cut and which often have a negative impact on human health. Therefore, the search for additives that improve the quality of sausages and their biological value will always be relevant.

Materials and methods

Materials

Meat raw materials

The subjects of the study were boiled and semi-smoked sausages developed according to a model recipe, consisting of 42.5% beef, 42.5% pork and 15% bacon; spices - black pepper 0.2%, nutmeg 0.05%; salt 2.5%, sodium nitrite 0.0075%. The choice of the ratio of the components of the formulation is justified by the need to study the influence of both beef and pork, equally on the quality of the finished product. Beef obtained from Simmental animals aged 10-12 months and pork obtained from 10-12 month old animals of the Landras breed were used. The slaughter was carried out according to the classical technology with electro damping, cooling - one-stage at an air temperature of 0-2°C to a temperature in the thigh thickness not exceeding 4°C. Further ripening of meat was carried out at a temperature of 0-4°C. The raw materials were used for processing 24h after slaughter.

Additives


Nutmeg (Myristica fragrans L.) produced by DFT Ecotechnika-K, Kiev, Ukraine

Vitamins

Vitamin PP in the form of nicotinic acid and amide of nicotinic acid. Nicotinic acid (Acidumnicotinicum) E375, gross formula C6H5NO3, produced in the USA, purchased from LABSNAB, a division of Trubosnab Ltd.,
Kharkov, Ukraine. Nicotinic acid amide (Nicotinamide), gross formula: C\textsubscript{6}H\textsubscript{6}N\textsubscript{2}O, manufactured in the USA and purchased from FOP Shelik, Kharkov, Ukraine.

**Chemicals and reagents**

**Devices**

pH-meter pH-420 purchased from LLC "MEDLAB", Kiev, Ukraine. The device used has a combined pH electrode with a knife cap, the limit of the permissible basic absolute error is 0.01. Photoelectric calorimeter KFK-2, purchased from the company "Medtekhnika", Kharkov, Ukraine, used wavelength of 538nm. The main absolute error of the calorimeter when measuring transmittance is not more than ± 1%. Planimeter PP-M-UHL 4.2 according to TU 25-04-1613-79.

**Experimental design**

The technology of production of semi-smoked sausages included the following operations: grinding of meat on a meat grinder with a 3 mm orifices in the grate, preparation of minced meat (temperature of minced meat - 2-2.5°C), forming sausages, exposition (temperature 0-2°C for 2-4h), short time smoking, cooking, smoking and drying (for 24h at a temperature of 12°C) using classical technology (DSTU 4436:2005. Boiled sausages).

The technology of production of boiled sausages includes the following operations: grinding of meat on a meat grinder with 3mm orifices in the grate, preparation of minced meat on a microcutter of Braun company (Germany) (temperature of minced meat is 2-2.5°C), forming sausages, exposition (temperature 0-2°C for 1-2h), short time smoking, cooking, cooling to the temperature in the center of the product no more than 8°C according to the classical technology (DSTU 4435:2005 Semi-smoked sausages (33975). Additives were introduced into minced meat at the end of the preparation of minced meat 1 minute before the end of the process. Concentration of additives – 0.006 % to the mass of the main raw material.

**Methods**

Changes in the properties were evaluated for the following parameters: pH, moisture content, and mass fraction of bound moisture for meat and water retention capacity for finished products, sodium nitrite content, determination of the number of colony forming units of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM), sensory indices.

**Samples’ preparation**

The ground samples were stored in glass boxes with ground-in glass stoppers. Studies were conducted using standard international methods as well as using original techniques. Sample preparation was carried out by double grinding on a laboratory grinder with aperture diameter of 3mm. The ground samples were thoroughly mixed. Samples for analysis were taken from the obtained averaged samples.

**Determination of moisture content**

Determination of moisture content in raw materials and products was carried out according to the standard procedure of DSTU ISO 1442:2005 Meat and Meat products. Method of moisture determination (control method). The determination was carried out with triple repetition.

**Determination of the hydrogen index**

The determination of the hydrogen index in raw materials and products was carried out according to the standard method Meat and Meat products. Determination of pH (control method) (DSTU ISO 2917:2001). The determination was carried out with triple repetition.

**Determination of sodium nitrite content**

The determination of the sodium nitrite content in sausages was carried out according to the standard method Meat and Meat products. The determination of total quantity of nitrite method (control method) (DSTU ISO 2918: 2005).
The essence of the method: the test sample is extracted with hot water, proteins are precipitated and filtered; sulfamide and \(N\)-1-naphthylethylenediamine dihydrochloride are added to the filtrate. In the presence of sodium nitrite, a red color appears. The determination was carried out with triple repetition.

**Determination of the mass fraction of bound moisture (water-retaining capacity)**

Determination of the mass fraction of bound moisture was carried out by pressing-out the free moisture contained in the sample. A portion of ground beef weighing 0.3g is weighed on a torsion balance on the polyethylene film with a diameter of 100mm, then specimen is transferred to an ash less filter placed on a glass or plexiglass plate so that the sample is under the polyethylene film. On top, the sample is covered with the same plate as the lower one, a weight of 1 kg is placed on it and it is kept for 10 min. After this, the filter with the sample is released from the load and the lower plate, then the contour of the stain is outlined with a pencil around the compressed meat. The areas of spots formed by compressed meat and adsorbed moisture are measured with a planimeter. The size of the wet spot is calculated from the difference between the area of the total spot and spot formed by the meat. It was experimentally established that 1 cm\(^2\) of wet filter spot area corresponds to 8.4 mg of moisture. Mass fraction of bound moisture (W) in minced meat, in% to meat weight was calculated according the next formula:

\[
W = (M - 8.4 S) \times 100. m^{-1}
\]  

(1)

Determination of water-holding capacity was carried out in a similar way (Antypova et al., 2004).

**Determination of the number of colony-forming units of mesophilic aerobic and facultative anaerobic microorganisms**

The method is based on counting all the colonies of mesophilic aerobic and facultative-anaerobic microorganisms that grow on a dense nutrient medium.

Preparation of samples and ten-fold dilutions for microbiological studies was carried out according to DSTU ISO 6887-1:2003 Microbiology of food and animal feed. The recalculation of the number of colony forming units per 1g of product was carried out in accordance with DSTU ISO 4833: 2006 Microbiology of food and animal feed. Horizontal method of counting microorganisms.

**Definition of sensory indices**


**Statistical methods**

Processing and analysis of the obtained results was carried out by calculating the arithmetic mean of the results obtained and determining the deviation from the obtained result-the confidence interval of the obtained values of P≤0.95 for the degree of freedom f = n - 1 (Ostapchuk & Stankiewich, 2006). The permissible value of the relative error was considered its values, which did not exceed 5%.

**Results**

**Determination of the hydrogen index**

For researches, raw meat was used after 24h from the moment of slaughter, which corresponds to the resolution of post mortem rigor. However, too low pH values at the analyzed stage of meat research indicate a violation of autolysis. The data obtained correspond to the quality of the average meat raw material obtained from animals grown using accelerated zoo technologies. The produced sausages from this raw material also had low pH values within the range of 5.52-5.60 regardless of the type of product.
With prolonged storage of sausages, the pH slightly increases due to the development of microflora.

**Determination of total moisture content**

The total moisture content (Table 1) is much higher than the average for beef and pork and ranges from 75.8% for beef to 77.2% for pork. Excessively high moisture content in raw materials adversely affected the total moisture content of the finished product. For boiled sausages the values were in the range from 75.2% to 74.8%, depending on the additive used, for semi-smoked sausages - from 65.7% to 64.1%. When storing products packed in a polymer film, without the use of vacuum and a modified gas medium, the moisture content decreases from 0.8% to 1.5%. The greatest mass losses were founding sausages with nicotinic acid. A similar trend is observed for semi-smoked sausages.

**Determination of the mass fraction of bound moisture (water retention capacity)**

The mass fraction of bound moisture is an important technological indicator characterizing the degree of moisture bound by proteins (Tables 1 and 2). Low pH of the feedstock resulted in a decrease in the hydration activity of muscle proteins, both beef and pork, and, correspondingly, a decrease in the mass fraction of bound moisture. Such properties of the raw materials did not allow to obtain sausages with high functional characteristics. The values of the mass fraction of bound moisture (water retention capacity) for cooked sausages were from 49.1% to 50.7% 24h after production and slightly increased after 168h of storage. A similar trend is observed for semi-smoked sausages. Absence of phosphates in the formulation, low pH value allowed to objectively assess the effect of additives added to meat raw materials.

**Determination of sodium nitrite content**

The use of nicotinic acid and nicotinamide to stabilize the color of sausages has reduced the residual nitrite content. In boiled sausages the residual nitrite content in sausages with nicotinic acid decreases by 25%, with nicotinamide by 19%. For semi-smoked sausages, a similar pattern is observed. It should be noted that the content of nitrite is increased during storage time. In boiled sausages, the increase is less intense than in semi-smoked sausages, in comparison with the initial nitrite content.

**Determination of the number of colony-forming units of mesophilic aerobic and facultative anaerobic microorganisms**

The introduction of vitamins in minced meat at the final stage of its preparation had no significant effect on the total number of microorganisms in sausages after 24h from the end of the heat treatment process. The difference in the values is within the margin of error. During the storage of the boiled sausages at a temperature of 2°C for 168h (7d), the amount of MAFNaM increases by 1.5 times in the sausage with nicotinic acid. In sausage with nicotinamide, the amount of MAFNaM increases by 1.7 times. In equal storage conditions in semi-smoked sausages the analyzed indicator increases by 1.2 and 1.4 times respectively.

**Determination of sensory indicators**

Sausages produced from the raw materials with low water binding capacity have a juicy and simultaneously watery consistency. Added supplements did not have sufficient influence on their sensory indicators. All examples had sourish aftertaste and friable consistency after pressing. Sausages with supplements had more homogeneous consistency and also had less fibrousness (Fig 1). All examples of semi-smoked sausages had elastic consistency after pressing and in oral cavity. The color of sausages with vitamins on the cut had more saturated nuances, light pink for boiled sausages and dark pink for semi-smoked (Fig. 2); the indicator was estimated by the degree of appearance of the descriptor.

**Discussion**

A significant change in the diet of farm animals, uncontrolled living conditions do not allow to obtain a standardized quality of raw materials. Manufacturers of sausages in Ukraine are faced with a problem of poor quality meat.
Therefore, it is difficult or even impossible to produce quality sausages in which the values of controlled chemical indicators are conformed to state standards. In addition to chemical parameters, the sensory properties are also changed. The products obtained have a light, unnatural coloring on the cut, a water consistency. Therefore, modern technology uses additives, whose action is aimed to stabilize the hydrogen index, water retention capacity, the color of minced meat on the cut and which often have a negative impact on human health. Therefore, the search for additives that improve the quality of sausages and their biological value is actual. During the series of studies, the main indicators affecting the quality of the finished product were analyzed. It should be noted that low values of the pH of the feedstock caused low functional characteristics of the finished product. The pH values in the interval of the isoelectric point of the main fibrillar proteins of meat - actin and myosin promoted the formation of an actomyosin complex, a decrease in their hydration activity, and a state of water in molecular form. This explains the high values of the total moisture content in beef and pork. Different types of sausages produced from such raw materials have excessively high values of the total moisture content, which should be not more than 72% for boiled sausages and not more than 55% for semi-smoked sausages according to state standards. And if for boiled sausages the figure is higher than normalized by 2-3%, then for semi-smoked sausages - by 7-8% and carrying out drying for 24h does not provide normative quality indicators. Despite the high content of moisture in the products, low pH inhibits the growth of putrefactive microflora, which increases the hydrogen index during storage. It can be noted as a positive moment. Comparing the effect of nicotinamide and nicotinic acid on the growth rate of microorganisms, it should be noted that nicotinamide shows great vitamin activity, although its functional effect is less active. The sensory indices of all samples do not have the maximum score for any of the indicators studied. The sausages had a watery and friable consistency and a slightly sour aftertaste.

Sourish taste was felt in all the samples, regardless of the additives introduced. It should be noted that the sourish taste is not so pronounced in cooked sausages, compared with semi-smoked sausages. Accordingly, the low value of the hydrogen index of raw materials causes the sour taste in the finished product. The color of the products on the cut in samples with nicotinamide and nicotinic acid is more intense than in the control sausages without additives. This is due to the reducing properties of vitamin PP compounds. This is confirmed by the residual sodium nitrite content. In sausages with vitamins, the values are lower, compared with the control recipe. A negative fact is that when storing all samples of sausages, an increase in the sodium nitrite content is observed. According to preliminary analysis of meat raw materials, it contains sodium nitrate, which accumulates in muscle tissue due to intake with feed. Sometimes the content of nitrates can exceed 2-3 times the threshold limit value. When storing sausages, nitrates are reduced by the microflora in the products to nitrites. In sausages with vitamin additives, the rate of accumulation of nitrite during storage is twice lower in comparison with the speed in the control formulation, which is also justified by their reducing function.

**Conclusion**

The use of vitamins in the form of nicotinic acid and nicotinamide makes it possible to reduce the residual sodium nitrite content to 25%, depending on the type of sausages. Proposed additives allow to reduce the rate of accumulation of nitrates during storage twice in compares on with the control sausages, and, therefore, to reduce the probability of formation of nitrosamines. The concentration of the added substances of 0.006% to the mass of the raw material will not adversely affect the physiological processes in the human body when consumed, but, on the contrary, enrich the food with vitamins. This technology can be recommended in the production of a children's product group.
References


### Table 1. Changes of properties of the meat during storage in a refrigerated state (n= 10, p<0.05)

<table>
<thead>
<tr>
<th>Raw materials</th>
<th>Moisture, %</th>
<th>pH</th>
<th>Mass fraction of bounded moisture, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>75.8±0.5</td>
<td>5.39±0.01</td>
<td>54.95±0.4</td>
</tr>
<tr>
<td>Pork</td>
<td>77.2±0.3</td>
<td>5.28±0.01</td>
<td>56.40±0.3</td>
</tr>
</tbody>
</table>

### Table 2. Changes of properties of sausages during storage (n=10, p <0.05)

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>Moisture, %</th>
<th>pH</th>
<th>Water-retaining capacity, %</th>
<th>Sodium nitrite content, mg.kg⁻¹</th>
<th>MAFAnMCFU, mg.g⁻¹ of product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration of storage, hours</td>
<td>Duration of storage, h</td>
<td>Duration of storage, h</td>
<td>Duration of storage, h</td>
<td>Duration of storage, h</td>
</tr>
<tr>
<td>Boiled sausages</td>
<td>24</td>
<td>168</td>
<td>24</td>
<td>168</td>
<td>24</td>
</tr>
<tr>
<td>Control sample</td>
<td>75.2±0.4</td>
<td>74.6±0.3</td>
<td>5.60±0.01</td>
<td>5.65±0.01</td>
<td>50.7±0.3</td>
</tr>
<tr>
<td>Sausage with nicotinamide</td>
<td>74.5±0.5</td>
<td>73.5±0.5</td>
<td>5.58±0.01</td>
<td>5.66±0.01</td>
<td>49.0±0.3</td>
</tr>
<tr>
<td>Sausage with nicotinic acid</td>
<td>74.8±0.4</td>
<td>73.3±0.4</td>
<td>5.52±0.01</td>
<td>5.63±0.01</td>
<td>49.1±0.5</td>
</tr>
<tr>
<td>Semi-smoked sausages</td>
<td>55.7±0.5</td>
<td>64.7±0.4</td>
<td>5.61±0.01</td>
<td>5.71±0.01</td>
<td>52.0±0.4</td>
</tr>
<tr>
<td>Control sample</td>
<td>64.6±0.3</td>
<td>64.2±0.4</td>
<td>5.59±0.01</td>
<td>5.67±0.01</td>
<td>50.1±0.5</td>
</tr>
<tr>
<td>Sausage with nicotinamide</td>
<td>64.1±0.5</td>
<td>63.4±0.5</td>
<td>5.60±0.01</td>
<td>5.67±0.01</td>
<td>51.8±0.4</td>
</tr>
</tbody>
</table>
Figure 1. Sensory indicators of boiled sausages

Figure 2. Sensory indicators of semi-smoked sausages