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Fixing of Shelf Life of Sausages of Gerodietetic Application of Quail Meat

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Running title: **Storage of Sausages Made of Quail Meat**

Abstract
Ukraine has a poorly developed market of meat production, which can meet the physiological demand of the population of older age groups. The authors have developed a recipe for sausages from the meat of quail with herbal supplements and partial replacement of animal fat with vegetable oil.
The work is devoted to the study of the dynamics of changes in the physico-chemical and microbiological parameters in the process of storage, as well as the fixing of the shelf life of a new product.
The investigators studied the changes in acid value, peroxide value, thiobarbituric value and active acidity index, as well as the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM), coliform bacteria (*E. coli*), sulphite-reducing clostridia, coagulase-positive staphylococcus (*St. aureus*), pathogenic microorganisms, including bacteria of *Salmonella genus* and *L. Monocytogenes* within 12-day storage period.
The investigators fixed that adding the meat flakes and walnut oil to wheat germ has no significant impact on the quality indices of the ready-made product. All the studied indices meet the requirements of regulatory documents. The results revealed that the developed product can be stored under the same parameters as the sausages of conventional product range – temperature 0 – 6 °C, shelf life – up to 12 days, relative humidity 75 – 78 % with the application of vacuum packaging in a thermoforming film.

Practical applications
The practical significance of the research is the option to expand the range of meat products for human consumption for older age groups and the research proves that adding of herbal supplements (cereal wheat germ and walnut oil) has no significant effect on the quality indicators of the ready-made product and ensures storage of a new product under the same conditions as the conventional poultry sausages.

Key words: sausages, fat-quality index, microbiological parameters

Introduction

The successful resolution of the issue of a healthy nutrition for people of different determined groups shall be based on the use of specialized products of high nutritional value and guaranteed safety of their industrial production.

Given the requirements of gerodieteric nutrition, Odessa National Academy of Food Technologies developed a recipe of sausages from the meat of quail to feed the elderly people (Agunova & Kuzelov, 2016).

In this regard, our study was aimed investigation of the development of oxidative processes and changes of microbiological parameters during storage developed meat product and the establishment of an acceptable shelf life while maintaining high quality and safety of the finished product.

One of the key tasks of the industry is to meet the needs of the market, including chain stores, in products with regulatory and increased shelf-life. While resolving these issues, manufacturers must comply with the optimum ratio of organoleptic characteristics, freshness, and safety of our products.

Deep transformations in the lipids structure may occur under the influence of the heat treatment, the hydrolytic lipolysis can take place under the effect of the lipolytic enzymes of microorganisms; these processes contribute to the accumulation of free fatty acids which increase the possibility of oxidative damage of fat in the product, which is defined by the rate of change of lipids' acid value (Antipova at al., 2001).

Peroxide compounds are primary products of the fats oxidized in food systems, they are formed both during processing and during storage, their accumulation is not accompanied by a change in the organoleptic characteristics of the product (Zhuravska et al, 1985).

Investigators also made a useful study of the dynamics of change in thiobarbituric value. It helps to assess the level of accumulation of secondary products of oxidative damage of fat (dialdehydes).

Increased attention is to the processes of oxidative changes of fat in the present study due to the fact that they affect not only the quality and safety of the finished product, but also cause a reduction in the biological, nutritional value and organoleptic characteristics (Nunez de Gonzalez at al., 2008).

The composition and viability of the product microflora depend on the value of pH active acidity. The study of this index is especially important in relation to the use of non-traditional raw materials.

The microbiological stability of the product is the main factor in fixing food safety and shelf life of new types of meat products. While making the study, it is important to fix not only the qualitative and quantitative composition of microflora but also the changes of the total microbial contamination during storage.

Materials and Methods

Materials

Chicken sausages

In the industrial environment of VEKKA meat processing plant, the village of Blogadatnoe, Kominternovsky district, Odessa region, the producer made a batch of sausages from the meat of quail, in a natural casing. Formulation of sausages: meat of quails – 60 %, bacon – 21 %, wheat germ flakes – 10 %, walnut oil – 9 %, salt – 1.5 %, a mixture of spices – 0.1 %, sodium nitrite – 7 mg per 100 g, ice water – 15 %. Flakes of wheat germ were put into the cutter prior to raw fat, while vegetable walnut oil was added at the last stage of cutting.

The developed sausages have a significant fat content (up to 30%), including fat with highly unsaturated fatty acids, due to the adding of vegetable oils, so their transformations during storage can have a significant impact on the quality parameters of the finished product.

These sausages were used for the study. The sample was packaged under vacuum in thermoformed film.

These sausages were stored with the parameters regulated by the normative and technical documentation. According to the Ukraine's current DSTU 4529:2006 "Chicken and rabbit cooked sausage. General specifications"; shelf life of the products is 12 days at a temperature of 0 – 6 °C and a relative humidity of 75 – 78 %.

Methods of analysis

Samples preparation

During the analysis, investigators sampled in the produced batches the products weighing 400 – 500 g, without violating the integrity of casing.

Samples were subjected to investigation immediately after production, and then every 48 hours, i.e. on the 2nd, 4th, 6th, 8th, 10th and 12th day of storage.

Before the study, samples were passed twice through a meat grinder with a diameter of grid holes from 2 to 3 mm and were mixed thoroughly.

Next, the sample was placed in 400 cm^3 glass bottle stoppered and sealed with a lid. The sample was stored in the refrigerator at a temperature (4±2) °C for up to 24 hours after grinding.

Extraction of lipids

First, the investigators made a preliminary extraction of lipids (Antipova at al., 2001). For this purpose, 40 g of ground sample was placed in the flask with hermetically closed stopper, added 130 cm^3 of methanol, stirred and milled in a homogenizer for 1 – 2 minutes until homogeneous mass. Then 65 cm^3 of chloroform was added to homogenate and shook for 10 minutes, afterward the mixture was added another 65 cm^3 of chloroform and shaken again, but this time for 5 minutes. 65 cm^3 of distilled water was poured to the resulting mixture and shook for 30 seconds. The flask contents are filtered through a paper filter under a slight vacuum on a Buchner funnel.

Together with the filter the residue and a small piece of filter paper used to clean the funnel were transferred to the same mixing flask and re-extracted 100 cm^3 of chloroform for 10 min. The mixture was filtered into a common flask. The flask and residue were washed with 50 cm^3 of chloroform, and the entire filtrate was collected in 500 cm^3 graduated cylinder. The layers were separated in a separatory funnel, the lower chloroform layer was selected, evaporated on a rotary evaporator to obtain fat to be further analyzed.

Acid value

Acid value (AV, mg KOH/g of studied fat) was fixed by titration of free fatty acids (DSTU ISO 660:2009).

Peroxide value

Peroxide value (PV, $\%J_2$) was fixed by iodometric method (ISO 3960-2001).

Thiobarbituric value

Thiobarbituric value (TBV mg/kg) was fixed by the thiobarbituric acid reaction with malonic dialdehyde formed upon oxidation of unsaturated fatty acids contained in the product, followed absorbance measurements of the formed color with the spectrophotometer (Antipova at al., 2001).

Active acidity (pH)

The pH of the meat products is determined by the potentiometric method using a pH meter, in accordance with (ISO 2917-2001).

The number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) (GOST 10444.15)

The method is based on the product seeding or product sample dilution in the culture medium, incubation of crops, calculation of all the visible colonies.

Coliform bacteria (coliforms), pathogenic microorganisms, including bacteria of the *Salmonella* genus and sulphite-reducing clostridia in accordance with (GOST 9958).

The essence of the method lies in the ability of coliform bacteria to decompose glucose and lactose. In CODE environment acidic products are formed, changing the color of the indicator.

In course of fixing sulphite-deoxidate clostridia in EDN environment by reduction with sodium sulfite to sodium sulfate, there is an interaction with the iron chloride, there is blackening of the environment due to ferrous sulfide.

The essence of the method of fixing of bacteria of the *Salmonella* germ is to determine the characteristic growth of *Salmonella* on the selection environments and fixing of biochemical and serological properties.

Detection of Staphylococcus aureus in accordance with (ISO 6888-1:2003)

Horizontal method for the detection and quantification of Staphylococcus aureus by showing on Baird-Parker agar medium, incubating of crops and calculating the number of typical colonies.

Detection of L. Monocytogenes in accordance with (MI, 2006)

L. Monocytogenes detection method involves detection of the presence/absence with the use of special selective media and confirmation of microorganism by blackening of the medium due to esculin hydrolysis with ions Fe^+.

Statistical analysis of test results

Experimental data processing was performed by using the method of variation statistics. The research results were processed using software package of *Microsoft, Inc. (USA), MathCAD 2000 Professional*. (Ostapchuk & Stankevich, 2006, Zaidel, 1985).

Results

Study of the depth of hydrolytic changes in the fat of sausages of experimental sample proves that the initial processes of biochemical rancidity occur at

an early stage of storage, ie, immediately after the end of the manufacturing process (Fig. 1, a). Immediately after the process, AV value was 1.18 mg KOH/g. Such changes may be explained by the impact of lipolytic enzymes of both the raw meat and microorganisms contained in it at the storage stage before the start of the manufacturing process and during production. Another important factor is the high moisture content of the final product (75 %), and sufficiently high thermal treatment temperature which leads on the one hand to destroying of enzymes, but at the same time changes the lipid structure. The growth of AV on the 12th day of storage is 11 % (0.14 mg KOH/g).

The primary products of fats oxidation in the investigated sausages during storage were not rapidly formed, in our opinion due to the barrier packaging film and evacuation process. Thus, at the initial stage of the study the accumulation of peroxides and hydro peroxides, ie, PV was at 0.01 % J_2; this is easily explained by the presence of walnut oil among the ingredients of sausages of gerodieteric application. On the 12th day of storage, PV reached 0.02 %J_2 for the experimental sample (Fig. 1, b).

TBV change indicates the accumulation of secondary products of lipid decomposition (alcohols, aldehydes, ketones) in the value.

TBV on the first day of storage, which is measured at a wave $\lambda = (535 \pm 10)$ nm, is insignificant and reach 0.021 mg/kg for the experimental sample. The increase of this indicator value in the storage process takes slightly, and on the 12th day of storage is only and 0.024 mg/kg (Fig. 2, c). The findings suggest a low dynamic accumulation of secondary products of lipid decomposition in sausage during storage.

Studies of changes in the acidity of the experimental sample of sausages fixed that during storage the pH slightly decreased from 6.3 to 5.8. (Fig. 1, c). In our opinion, a slight shift of value to the acid factor (on 0.5) is due to the activity of microorganisms, accumulation in the food of compounds formed by the oxidation of lipids and the content of plant raw materials (cereals of wheat germ).

Research of MAFAnM number, CFU / 1 g of the product, confirms the effectiveness of heat treatment. The results indicate that after the heat treatment the total microbial load test sample is within the regulated parameters. A number of mesophilic aerobic and facultative anaerobic microorganisms is 680 ± 4 CFU/1 g. During storage, the total number of microflora increases and reaches 890 ± 4 CFU / 1 g.

On the surface of the meat-and-peptone agar, there are mucous capsule- and spore-forming colonies, of $Gram^+$ color staining (Fig. 2).

The results of comprehensive microbiological studies are summarized in Table 1.

Discussion

Changes that occur during storage of the sausage meat of quails demonstrate that the introduction of formulated herbal supplements (cereal wheat germ and walnut oil) does not lead to a sharp increase in the oxidation and microbiological spoilage. Thus, the introduction of additives allows enriching the product of linolenic acid (n−3 fatty acid), which has a property to reduce the level of serum triglycerides, reduce the risk of blood clots in blood vessels, promotes the synthesis of prostaglandins. Deficiency of linolenic acid dramatically manifests itself in older age groups and leads to impaired mental capacity and deterioration of visual acuity (Yazeva et al., 1989). However, excessive consumption of oils is impractical due to their high caloric and possible accumulation in the body of unsaturated fatty acid oxidation products. The diet of the elderly for at least 1/3 of the fat should be of plant origin (Guidelines 2.3.1.2432-08 MR, 2008). Unfortunately, significantly reducing the total fat content of the product is impossible, since fat is needed in the manufacturing process for forming the structure of the sausage, and also participates in the formation of flavor and juiciness of the finished product. In addition to high biological value, gerodieteric meat should be soft and easy to nibble ingest (Japan Meat Information Centre, 2004). However, the fat in the product have a high biological value (Agunova & Kuzelov, 2016) and comply with the requirements of gerodieteric.

In the production of sausage meat stuffing quails provided in a natural casing (casings lamb), which has a capacity of oxygen relative to the air, which is at the minimum level is in the middle of the package after evacuation products. Thus, the vacuum does not guarantee an absolute protection against oxidative changes of fat during storage of finished products.

The data obtained in the study of the dynamics of the accumulation of free fatty acids, primary and secondary products of oxidation of fats on the dynamics of change in CN, IF TBCH indicate that the use of quail meat and the presence in the product easily oxidized lipids walnut oil leads to a slight increase in these indicators.

The dynamics of changes in the storage thiobarbituric process correlates with increasing values of the peroxide numbers.

Oxidative changes in sausages from the meat of quail shifted toward the accumulation of lipid peroxidation products, but are still within an acceptable range for the entire storage period.

In the study of microbiological criteria it is found that an experimental model of sausage does not contain pathogenic and opportunistic pathogenic microorganisms, and the total colonization of the product, even at the end of the storage process, on the 12th day, is regulated in the range - the number of MAFAnM $0{,}89 \cdot 10^3$, CFU / 1 city

The absence of the test products coliform bacteria (*E. coli*) indicates an efficient mode of heat treatment and high sanitary and hygienic conditions of production.

The next step is to develop a hardware-technological scheme and normative-technical documentation in order to implement the developed product in mass industrial production.

Conclusions

Sausages for gerodieteric power based on quail meat, cereals containing wheat germ and walnut oil can be stored under the same conditions as the meat and sausages from the traditional assortment of birds, made in accordance with the State Standard 4529: 2006, which are present in the Ukrainian market.

Provided thermoformed using vacuum film materials and shelf life of 12 days at a temperature of 0 – 6 °C and a relative humidity of 75 – 78 %.

References

Agunova, L. V., Kuzelov, A. (2016). Eriatric dietary meat-based products. *Macedonian journal of animal science. International Scientific Journal,* **6**(1): 51-57.

Antipova, L. V., Glotova, I. A., Rogov, I. A. (2001). Methods of research of meat and meat products: the textbook for high schools: 571. (in Russian)

Nunez de Gonzalez, M. T., Boleman, R. M., Miller, R. K., Keeton, J. T., Rhee, K. S. (2008). Antioxidant properties of dried plum ingredients in raw and precooked pork sausage. *Journal of Food Science,* **73**: 63-71.

GOST 10444.15-94 Foodstuffs methods for determining the number of mesophilic aerobic and facultative anaerobic microorganisms. (in Ukrainian)

GOST 9958-81 Sausages and meat products. Methods of bacteriological analysis.

ISO 660: 2009 Animal fats and vegetable and oil. The method of determining the acid number and acidity (ISO 660: 1996, ITD). (in Ukrainian)

ISO 3960-2001 Fats and oils of animal and vegetable. Determination of the peroxide numbers (ISO 3960: 1998, ITD). (in Ukrainian)

ISO 2917-2001 Meat and meat products. Determination of pH (reference method) (ISO 2917: 1999, ITD). (in Ukrainian)

ISO 6888-1: 2003 Microbiology of food and animal feed. The horizontal method of calculation coagulase-positive staphylococci (Staphylococcus aureus and other species). Part 1. Method using agar Baird-Parker medium (ISO 6888-1: 1999, IDT). (in Ukrainian)

Zhuravskaya, N. K. , Alekhina, L. T., Otryashenkova, L. M. (1985). Research of meat and meat products quality control: 296. (in Russian)

Methodical instructions MI 10.10.2.2.132-2006 «Organization and control methods for detection of bacteria Listeria monocytogenes in foodstuffs and food raw materials», approved by the Ministry of Health of 11.08.2006 number 559. (in Ukrainian)

Ostapchuk, M. V., Stankiewicz, G. M. (2006) Mathematical modeling on the computer: a textbook: 313. (in Russian)

Seidel, A. (1985) The errors of measurement of physical quantities: 112. (in Russian)

Yazeva, L. I, Filippova, G. I, Fedina, N. I. (1989). On the biological properties of vegetable oils containing linoleic acid (18: 3 ω-3). *Problems of nutrition*, **3**: 45-50. (in Russian)

Guidelines 2.3.1.2432-08 MR. (2008). The norms of physiological requirements in energy and nutrients for different groups of the Russian population. Federal Centre of Sanitary Inspection Ministry of Health of Russia, Moscow. (in Russian)

Japan Meat Information Centre. (2004). *Shukunikuy no Chisiki*, 51-53. (in Japanese)

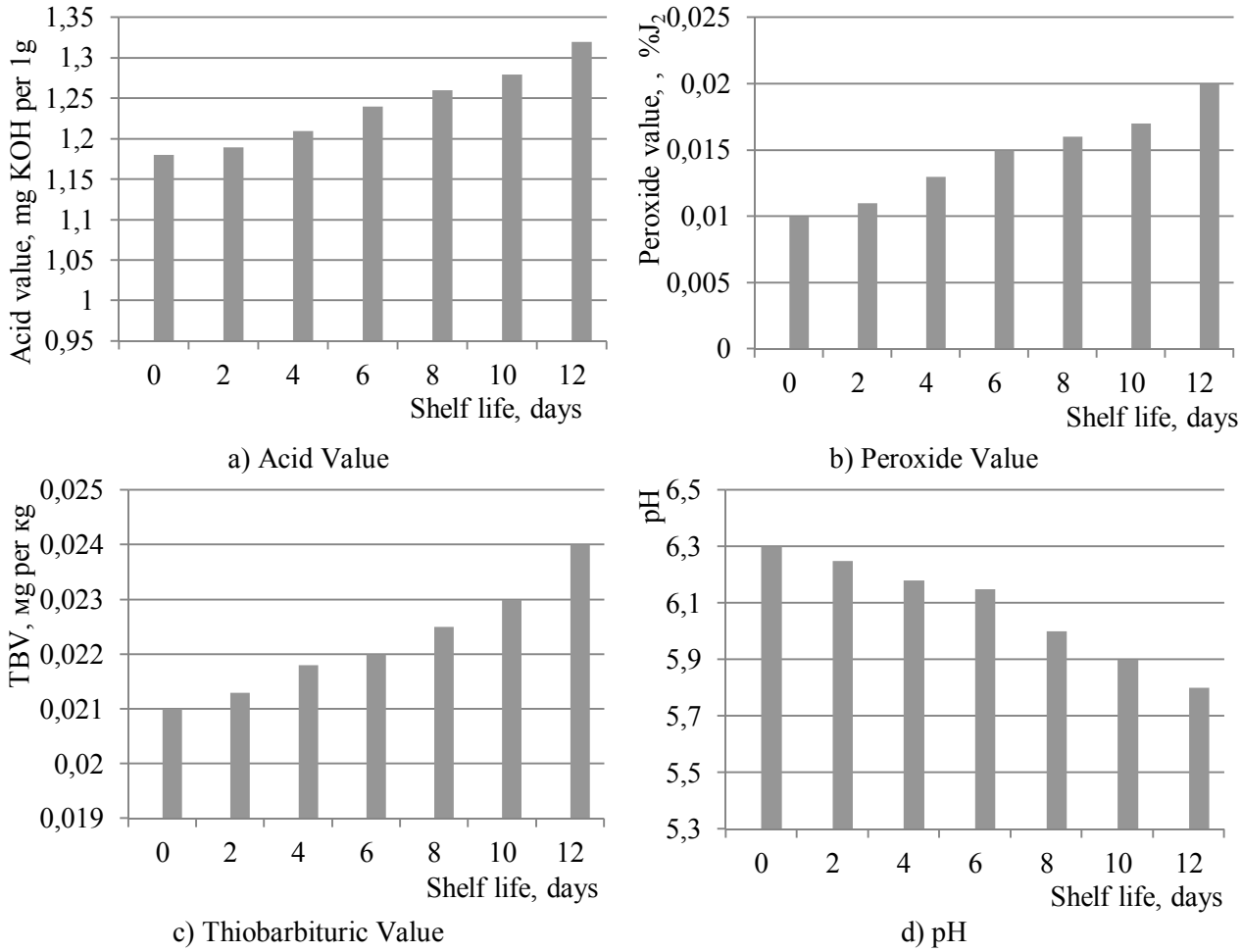

Figure 1. Changes in Acid Value during, Peroxide Value, Thiobarbituric Value and pH during storage (*n=5; p≥95*)

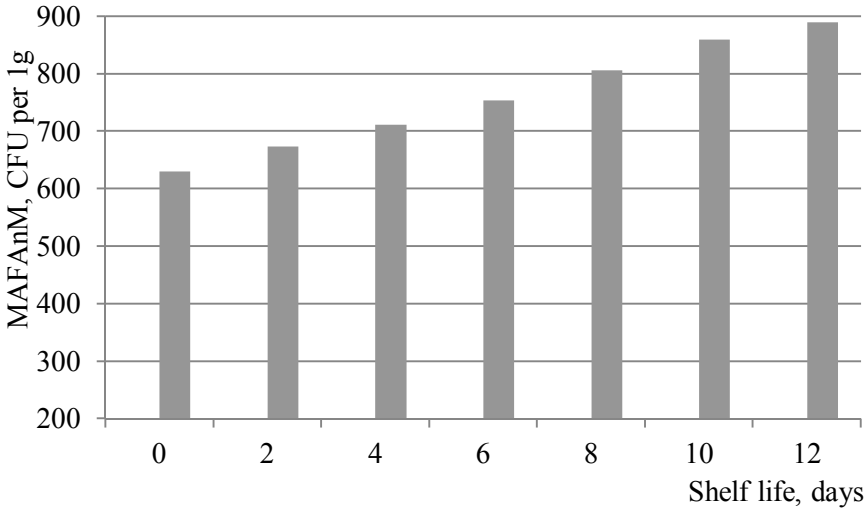

Figure 2. Changes in MAFAnM during storage (*n = 5; p≥95*)

Table 1. Bacteriological indices of the ready-made sausages

Item	In accordance with DSTU 4529:2006	Sausages of quail meat
Number of MAFAnM, CFU, in 1 g of product, not more	$1 \cdot 10^3$	$0.63 \cdot 10^3$
Pathogenic microorganisms, including bacteria of *Salmonella,* in 25 g of product	Should be absent	N/D
Coliform bacteria (*E. coli*), in 1 g of product	Should be absent	N/D
Sulphite-reducing clostridia, in 1,0 g of product	Should be absent	N/D
Staphylococcus aureus, in 1,0 g of product	Should be absent	N/D
L. Monocytogenes, in 25 g of product	Should be absent	N/D