

## Effectiveness of natural plant extracts in the technology of combined meatcontaining breads

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### Abstract

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**Introduction.** The aim of this study was to analyze the effectiveness of rosemary and cranberry extracts in the technology of combined meatcontaining breads with freshwater fish and a high content of polyunsaturated fatty acids.

**Materials and methods.** A model for studying the effectiveness of rosemary and cranberry extracts was meatcontaining bread with freshwater fish. The acid value, peroxide value, thiobarbituric acid reactive species were determined during the storage of meatcontaining breads with extracts.

**Results and discussion.** Rosemary extract has a high antioxidant activity and effectively inhibits the process of lipid oxidation in meatcontaining combined breads with Muscovy duck meat and freshwater fish meat.

Cranberry extract does not inhibit the hydrolysis of fat during storage of meatcontaining combined breads, and has little positive effect on the formation of primary oxidation products and the accumulation of secondary lipid oxidation products.

The addition of rosemary extract in the amount of 0.02–0.06% allows slowing down the hydrolytic oxidation of minced lipids by 36.19–36.36%. The using of rosemary extract in concentrations of 0.02–0.06% by weight of minced meat helps to slow down the lipid peroxidation in meatcontaining breads with fish, reducing the amount of peroxides almost four times.

The rosemary extract at a concentration of 0.06% by weight of raw materials was the most stabilizing effect. PV in this sample at the end of the study period was  $0.013 \pm 0.001\%$  J<sub>2</sub>, whereas in the control this parameter was  $0.05 \pm 0.001\%$  J<sub>2</sub>, which is almost 4 times higher.

The stabilization of lipid peroxidation in meatcontaining breads with Muscovy duck meat and white carp has the effect of inhibiting the formation of secondary oxidation products, which is confirmed by the results obtained. The amount of aldehydes and ketones was the lowest at the end of the shelf life of breads with rosemary extract and was 0.74–0.76 mg MA/kg, which is lower than in the control sample by 17–20%. The products contain a significant amount of moisture in the active phase, which prevents long-term storage of products without the use of substances that slow down the oxidation process.

**Conclusions.** The addition of rosemary and cranberry extracts has been shown to inhibit lipid oxidation during storing meatcontaining breads with a combined raw material composition.

## Introduction

Meat and meatcontaining products technology involves such technological operations as grinding, mixing, heat treatment, during which the lipids of the raw material (pork, poultry meat, pork fat, fat emulsions, etc.) undergo various transformations. Changes also occur in the lipid complex of products during storage. All these factors affect their composition, nutritional and biological efficiency, and, as a result, the consumer appeal of the finished products [1, 2].

The oxidative spoilage of fat in meat products could be explained by three different reactions: enzymatic oxidation; non-enzymatic, free-radical (peroxide) oxidation of lipids; and non-enzymatic, non-radical oxidation. The main reason of oxidative spoilage of meat and meat products is lipid peroxidation caused by reactive oxygen [3, 4].

Lipid peroxidation is a chain reaction that provides extended reproduction of free radicals that initiate the further spread of peroxidation [5]. In the process of meat processing a balanced oxidation system is destroyed as a result of technological operations – grinding, salting and heat treatment [6, 7].

A mixing of lipids and oxidation catalysts, which can contact with oxygen occur when grinding meat there is [8]. The type and duration of heat treatment have a significant influence on the rate of oxidation processes. There are various technological methods that allow suppressing oxidation processes in fresh raw materials until the formation of oxidation products [9–11]. The use of antioxidants is one method of minimizing the oxidative spoilage problem in the meat industry [12].

Scientists suggest the addition of natural antioxidants, the main source of which is plants, in order to suppress the processes of oxidation in boiled and baked products [13].

Rosemary (*Rosmarinus officinalis*) has a high content of phenolic components, which include hydroxybutyric acids, including rosemary acid, flavonoids, including quercetin and rutin [14]. Rosemary extract and other antioxidant preparations from this plant have been successfully used to inhibit oxidative spoilage in the food industry [15–17].

Blackberries, strawberries, black currants and other berries can also be described as natural preparations of natural antioxidants [18, 19]. The high concentration of anthocyanins allows suggesting that the addition of berry processing products can be effective in preventing oxidative spoilage of foods with high fat content [20]. In particular, cranberry extract with a high content of phenolic components has a positive effect even on the physiological functions of laboratory animals and humans [21–23].

Moreover, preparations made from cranberries and their waste products can be used to inhibit the oxidation of food lipids in the production of semi-finished products [24–25], various types of cheese [26], cooked sausage products [27], etc.

On the other hand, the promising ways of development of meatcontaining products with the combined composition is the use of freshwater fish in their formulations. The addition of freshwater fish mince into the formulations as an ingredient allows obtaining products with high nutritional and biological value and consumer qualities [28–32]. However, the high lipid content of the unsaturated group in fish poses a risk of intensification of the oxidation processes in the minced meat during the processing of raw materials and storage of finished products. For this reason it is important to develop and implement technological techniques designed to prevent oxidative spoilage of such products.

Thus, the effect of natural origin antioxidants on the oxidation processes in meatcontaining systems of mixed composition, consisting of meat and fish raw materials, remains unexplored.

Therefore, the aim of our work was to evaluate the effectiveness of rosemary and cranberry extracts in the technology of meatcontaining combined breads with duck meat and freshwater fish during storing.

## Materials and methods

### Experimental design

Combined meatcontaining breads of duck meat and freshwater fish were studied, which included Muscovy duck meat, white carp minced meat, pork fat, dry demineralized whey, wheat flour and functional additives.

The rosemary extract (Food Ingredients Mega Trade, USA) and cranberry extract (CE) (Ukraine) were added to the minced meat. The extracts were added to the forcemeat samples according to the following scheme: № 1 – RE 0,02%; № 2 – RE 0,04%; № 3 – RE 0,06%; № 4 - CE 0,02%; № 5 – CE 0,04%; № 6 – CE 0,06% to the raw material mass, the forcemeat sample without antioxidants was the control one.

The technological concentration of antioxidants for use in the technology of meat products ranges from 0.01 to 0.1% [33–35]. In view of this, an appropriate concentration of rosemary and cranberry extracts was selected, taking into account the content of different groups of substances with antioxidant properties.

The finished breads were stored for 6 days at +4 °C. During the storage of meatcontaining combined breads, acid value (AV) and peroxide value (PV), thiobarbituric acid reactive substances (TBARS) were the controlled indicators. These indicators were determined according to the methods [36, 37].

### Manufacture of breads

Meatcontaining breads were prepared using the formulation: 30% duck meat, 45% white carp (*Hypophthalmichthys molitrix*) meat, 10% pork fat, 5% dry demineralized whey, 2% wheat flour and functional additives (Table 1).

Table 1

Formulation of meatcontaining bread

Raw materials	Amount,%
Muscovy duck meat	30
Minced white carp meat	45
Pork fat	10
Dry demineralized whey	5
wheat flour	2
Aprored	3
XB Fiber	2
Egg melange	3
<b>Total</b>	<b>100</b>
Salt	1,5
NaNO <sub>2</sub>	0,0075
Sugar	0,1
Pepper black	0,1
Coriander	0,05
Fresh garlic	0,2

The preparation of the samples was carried out in accordance with the technology for the preparation of minced meat bread with the addition of 20% water to the main raw material [38].

Minced duck meat is ground into a top with the grating orifices diameter 2-3 mm. After that minced meat of silver carp was added. At the same time, supporting materials are being

prepared. Coriander and ground black pepper are sifted to prevent large particles from being minced.

Raw meat is weighed according to the recipe and sent for processing on a cutter. All ingredients are mixed for 8-10 min. Firstly the Muscovy duck meat was added, then white carp meat, after some water and ice, salt, and processed for 3-5 minutes. Then the rest of the water and ice with fat was added, dry demineralized whey, rosemary or cranberry extract, spices and processed for another 5-6 minutes.

The molding of the products occurs in metallic forms. The formed breads are processed in a rotary oven at a temperature of 100-110°C for 2 hours to a temperature in the center of the product 68-72°C. After heat treatment, they are cooled to a temperature in the center of the product not higher than 8°C. The finished breads were stored for 6 days at + 4°C.

### **Lipid oxidation measurements (acid value, peroxide value, thiobarbituric acid reactive substances)**

The acid value was determined by the batch titration with sodium hydroxide in the concentration in the presence of fenolftalein alcohol solution [36]. 3-5 g of the investigated forcemeat was weighted in the conic retort with the ume of 150-200 cm<sup>3</sup> with the error of no more than 0,001 g. The batch was heated on the water bath and after the addition of 50 cm<sup>3</sup> of neutralized ether-alcohol mixture shaken. Then 3-5 drops of fenolftalein alcohol solution with the mass share of 1% were added. The received solution while shaking was titrated fast with potassium hydroxide solution with the molar concentration 0,1 mol/dm<sup>3</sup> till the distinct rose coloration appeared and kept for 1 min. The acid number was calculated by the formula:

$$X=(V \times K \times 5,61) / m, \quad (1)$$

where V – ume of potassium hydroxide solution, with the molar concentration 0,1 mol/dm<sup>3</sup>, used for titration; K – correction to alkali solution for recalculation on the distinct (0,1 mol/dm<sup>3</sup>) one; 5,61 – number of milligrams of potassium hydroxide, contained in 1 cm<sup>3</sup> (0,1 mol/dm<sup>3</sup>) of solution; m – forcemeat batch mass, g.

The method of PV determination is based on the batch extraction by the mixture of chloroform and icy acetic acid and further titration by the sodium hyposulfite solution with the previously added starch solution [36].

0,8–1 g of a batch, weighted with accuracy of no more than 0,0002 g were placed in the conic retort with the stopper, melt on the water bath and 10 cm<sup>3</sup> of chloroform and 10 cm<sup>3</sup> of icy acetic acid were gently poured on the retort sides. 0,5 cm<sup>3</sup> of saturated, freshly prepared potassium iodine solution was quickly added. The retort was closed with the stopper; the content was mixed by turning movements and put into the dark place for 3 minutes. Then 100 cm<sup>3</sup> of distilled water with the previously added 1 cm<sup>3</sup> of starch solution with the mass share of 1% was gently poured into the retort. After that it was titrated with sodium hyposulfite solution with the molar concentration of 0,01 mol/dm<sup>3</sup> until the blue coloration disappeared.

To verify the clearness of reagents the control determination without a batch was realized. The peroxide number was calculated by the formula:

$$X=[(V-V1) \times K \times 0,00127 \times 100] / m, \quad (2)$$

where V – ume of sodium hyposulfite solution with the molar concentration 0,01 mol/dm<sup>3</sup>, used for titration in the main experiment with the forcemeat batch, cm<sup>3</sup>; V1 – ume of sodium hyposulfite solution (0,01 mol/dm<sup>3</sup>), used for titration in the control experiment without a forcemeat batch, cm<sup>3</sup>; K – coefficient of correction to sodium hyposulfite for recalculation on the distinct (0,01 mol/dm<sup>3</sup>) solution; 0,00127 – number of grams of iodine, equivalent to 1 cm<sup>3</sup> (0,01 mol/dm<sup>3</sup>) of sodium hyposulfite; m – mass of the studied forcemeat batch, g.

TBARS was determined by measuring the coloration intensity of the mixture of the studied sample distillate and thiobarbituric acid solution (1:1) after 35 minutes on the water bath on the spectrophotometer “Spekol-11” (Germany) at the wave length 535 nm [37].

50 g of forcemeat batch were put into the porcelain mortar, 50 cm<sup>3</sup> of distilled water were measured by the glass cylinder, added to the mortar and ground with the pestle into the uniform mixture. The prepared sample was quantitatively transferred into Kjeldahl retort, remains were washed away from the mortar with 47,5 cm<sup>3</sup> of distilled water and then 2,5 cm<sup>3</sup> of hydrochloric acid were added. The distillation was carried out in Kjeldahl apparatus, collecting 50 cm<sup>3</sup> of distillate in the umetric flask. 5 cm<sup>3</sup> of distillate were taken, poured into the retort with the fitted stopper. After the addition of 5 cm<sup>3</sup> of thiobaturic acid, the retort was closed with the fitted stopper and heated on the boiling water bath for 35 min.

Simultaneously the control experiment was held, using 5 cm<sup>3</sup> of distilled water instead of the distillate. Then the solutions were cooled in the cold running water for 10 min, and the optic density at the wave length of 535±10 nm as to the control solution was measured.

The thiobarbituric acid reactive species, mg of MA (malonic aldehyde) / kg of the product, was calculated by the formula:

$$X=D\times 7,8, \quad (3)$$

where D – optic density of the solution; 7,8 – coefficient of proportional dependency of MA density on its concentration in the solution. This coefficient is a permanent value.

### Statistical analysis

The absolute error of measurements was determined by Student criterion, the reliable interval P=0,95, the number of repetitions in calculations – 3–4, the number of parallel tests of studied samples – 3.

Parallel to the determination of AV and PV in the test samples were determined values of activity of water  $a_w$ .

The definition of  $a_w$  was performed with a portable high-speed instrument of model AquaLab 3TE with measurement accuracy up to ±0,003 – according to the requirements [39].

## Results and discussion

### Study of the plant extracts effect on the hydrolytic lipid oxidation of the meatcontaining breads

The results of studies on the acid value dynamics during the storage of combined meatcontaining breads with the addition of plant extracts are shown in table 2.

Analysis of the table 2 shows that among the test samples the tendency to decrease the concentration of free fatty acids was observed on the first day of storage. At the end of the shelf life after 6 days AV in samples with rosemary extract reached from 1.05±0.04 mg KOH in sample 1 to 0.91±0.01 in the third sample, which is 36.19–36.36% less than the control. However, the samples containing CE had an acid value greater than the control. Thus, on day 6 of storage, the meatcontaining breads with the addition of cranberry extract samples 2 and 3 have an acid value level of 1.46-1.54, which is 2.1–7.7% higher than in the control.

Table 2

Dependence of the acid value on the concentration of the added extracts, mg KOH

Sample	Storage, days		
	1	3	6
Control	1,24±0,01	1,29±0,03	1,43±0,08
1 (Meatcontaining bread + RE 0,02 %)	0,85±0,07	0,96±0,08	1,05±0,04
2 (Meatcontaining bread + RE 0,04 %)	0,74±0,07	0,79±0,01	0,97±0,07
3 (Meatcontaining bread + RE 0,06 %)	0,74±0,07	0,74±0,07	0,91±0,01
4 (Meatcontaining bread + CE 0,02 %)	1,01±0,03	1,26±0,01	1,34±0,04
5 (Meatcontaining bread + CE 0,04 %)	1,02±0,04	1,40±0,03	1,46±0,02
6 (Meatcontaining bread + CE 0,06 %)	1,11±0,01	1,45±0,07	1,54±0,07

The obtained results indicate that the added rosemary extract inhibits fat hydrolysis in systems with combined lipid composition due to the high concentration of flavonoids. The most effectively rosemary extract inhibits the hydrolytic decomposition of acylglycerides at a concentration of 0.06%. Cranberry extract, does not have such effect.

**Study of the plant extracts effect on the lipid peroxidation of the investigated meatcontaining breads**

The results of the peroxide value dynamics in meatcontaining combined breads during the shelf life are shown in the Figure 1.

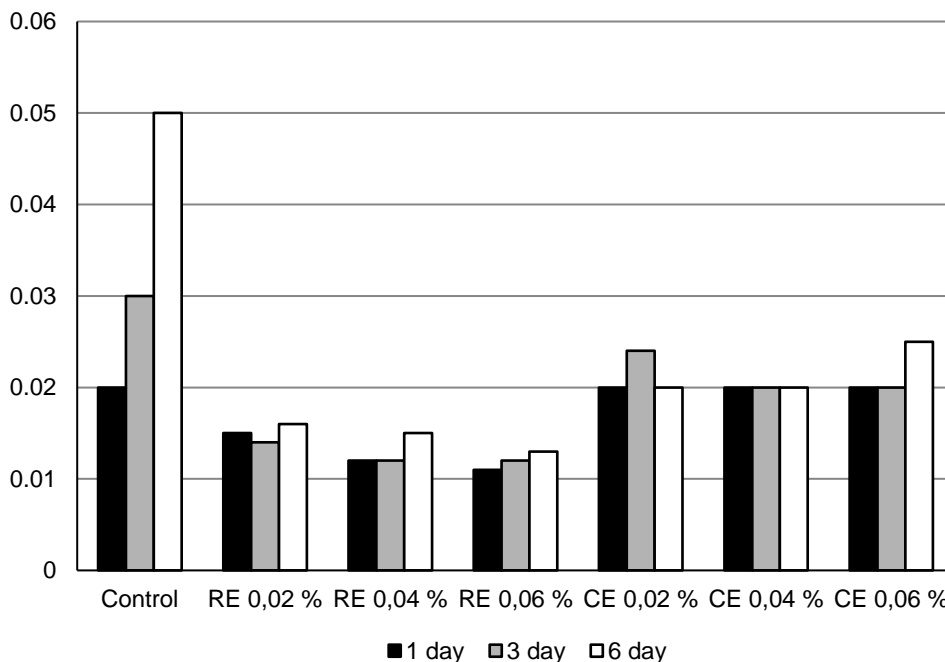


Figure 1. Dependence of the peroxide value on the concentration of the added rosemary and cranberry extract, % J<sub>2</sub>

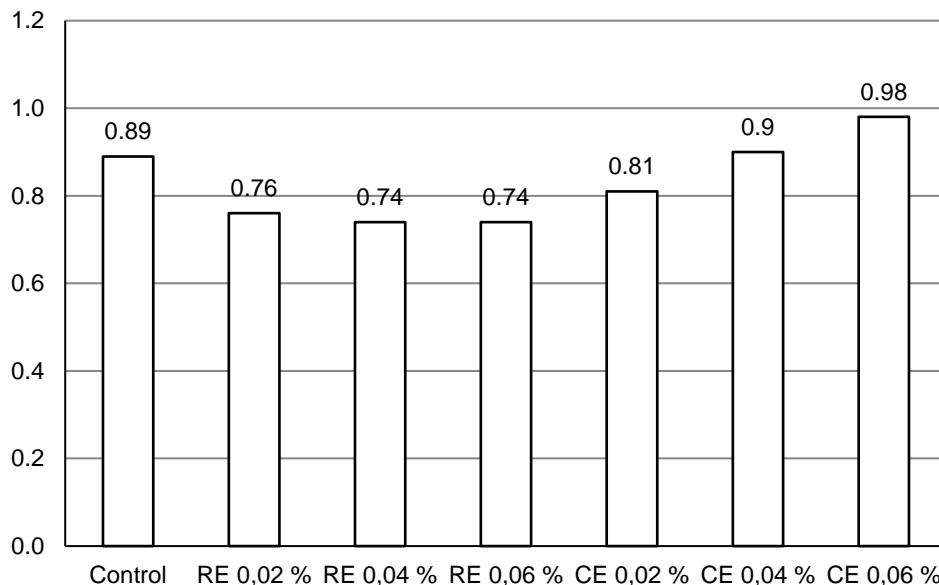
The addition of rosemary extract helps to slow down the oxidation processes, as evidenced by the Figure.

Among the experimental samples of combined bread PV increased more intensively in the control sample. The addition of rosemary extract at a concentration of 0.06% by weight of raw materials has the most stabilizing effect. PV in this sample at the end of the study period was  $0.013 \pm 0.001\%$  J<sub>2</sub>, whereas in the control this parameter was  $0.05 \pm 0.001\%$  J<sub>2</sub>, which is almost 4 times higher. This confirms the results of studies [40–42], which say that the most active components of rosemary extract are carnosol, carnosic acid, rosemary acid, are the powerful antioxidants.

The using of cranberry extract in the technology of combined bread also had a positive effect on the accumulation of primary oxidation products. Thus, at the beginning of the shelf life PV in the samples with CE was 0.02% J<sub>2</sub>, which is almost identical with the control sample, but at the end of the storage period there was a tendency to decrease this indicator in the experimental samples. Thus, PV on the 6th day of storage in samples 4-5 was 0.02-0.025% J<sub>2</sub>, which is 2-2.5 times less than the control. It can be argued that the flavonoids of cranberry extract bind to act as oxidant absorbers, preventing the accumulation of primary lipid oxidation products without participating in the inhibition of lipolysis.

#### Study of the plant extracts effect on the accumulation of secondary lipid oxidation products of the investigated meatcontaining breads

The antioxidant effect of the additives is observed in the accumulation of mono- and dialdehydes that react with 2-thiobarbituric acid [43]. To determine the concentration of secondary oxidation products on the last day of storage of bread samples, the thiobarbituric acid reactive substances was investigated, the results of the TBARS study are presented in Figure 2.



**Figure 2. The influence of bioflavonoids of the rosemary and cranberry extracts on the accumulation of secondary products of oxidizing the lipids of the meatcontaining loaves, mg MA/kg**

The thiobarbituric acid reactive substances are an indicator used to evaluate the extent of lipid oxidation during storage. Accumulation of TBARS is facilitated by the second stage of autooxidation, in which peroxides are oxidized to aldehydes and ketones. Lipid oxidation in meat systems, which have the main ingredient as pork, depends on time and temperature [44].

Analyzing the data in Figure 2, it can be argued that added rosemary extract inhibited the formation and accumulation of secondary lipid oxidation products during the storage of meatcontaining breads with duck meat and white carp.

Thus, at the end of the shelf life, TBARS in the samples with the addition of RE was 0.74-0.76 mg MA/kg of product, which is 17-20% lower than in the control sample of bread. The using of RE in the amount of 0.04 and 0.06% had the same effect. Adding of cranberry extract to the combined bread had an effect only in the sample with a concentration of 0.02% and TBARS in this case was 0.81 mg MA/kg, which is 9.88% lower than in the control sample.

In samples with a concentration of CE 0.04-0.06%, the accumulation of aldehydes and ketones as a result of lipid oxidation was at the same level as in the control sample. In the present study, TBARS is less than 1.0 during the entire study period, which is important because in large quantities the level of TBARS is toxic, carcinogenic and mutagenic [45].

Water activity studying of minced meat samples with cranberry extract and rosemary extract revealed that for all types of meat bread this Figure was within 0.966-0.973 units in minced meat and finished meat loaves. That is, these products contain a significant amount of moisture in the active phase, which prevents long-term storage of products without the use of substances that slow down the deterioration process.

Comparative results show that the effectiveness of rosemary extract is higher than of cranberry extract when added to duck and fish combined products. It should also be noted that the efficacy is directly proportional to the amount of extract applied.

## Conclusions

1. Studies have confirmed the high antioxidant activity of rosemary extract and the effective inhibition of the lipid oxidation process in meatcontaining combined breads with Muscovy duck meat and white carp (*Hypophthalmichthys molitrix*).
2. Cranberry extract does not inhibit the hydrolysis of fat during storage of meatcontaining combined breads, and has little positive effect on the formation of primary oxidation products and the accumulation of secondary lipid oxidation products.
3. Adding of RE in the amount of 0.02–0.06% allows to slow down the hydrolytic oxidation of lipids by 36.19–36.36%.
4. The using of rosemary extract in concentrations of 0.02–0.06% by weight of minced meat helps to slow down the lipid peroxidation in meatcontaining breads, reducing the amount of peroxides almost four times.
5. The stabilization of lipid peroxidation in meatcontaining breads with Muscovy duck meat and minced meat of white carp has the effect of inhibiting the formation of secondary oxidation products at high values of  $a_w$ . This fact is confirmed by the obtained results. The amount of aldehydes and ketones was the lowest at the end of the shelf life of bread with RE and was 0.74–0.76 mg MA/kg, which is 17–20% lower than in the control sample.



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