The influences of salt replacers on the antioxidative activity of pork

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Abstract. Lowering salt intake is of great importance for reducing blood pressure and cardiovascular diseases. The aim of this research was to study the effects of salt replacers on the antioxidative activity of meat. The treatments were formulated with minced pork muscles that were salted with 2.0% salt (control) or different salt mixtures with reduced sodium content (composition 1 – 1.0% NaCl + 1.2% KCl (experiment 1), composition 2 – 1.0% NaCl + 0.6% KCl + 0.8% CaCl₂ (experiment 2)). After 24 hours of curing, the total antioxidative activity of the antioxidant enzymes was measured. The use of potassium chloride instead of half the sodium chloride did not lead to a significant change in the total antioxidative activity, nor in the activities of glutathione peroxidase and catalase; however, it induced a decrease in the activity of superoxide dismutase (p<0.05). Addition of composition 2 led to a reduction in the total antioxidative activity by 17.7%, and inhibited the activity of superoxide dismutase by 23.8% (p<0.05). The obtained results of the negative effect of the compositions of sodium, potassium and calcium salts suggest the need to develop approaches that allow inhibition of the oxidative changes in meat products with reduced sodium content.

1. Introduction

Excessive sodium chloride consumption is associated with an increased risk of hypertension [1,2], which in turn is a crucial risk factor for stroke and other cardiovascular diseases as well as kidney diseases. The necessity to reduce table salt in meat products requires searching for innovative techniques and methods. Among them are a decrease in the amount of salt added in food processing [3,4], a change in the form of the salt, and size of the salt particles [5], the use of taste enhancers [6], and replacement of sodium chloride with mixtures having low sodium content [7, 8].

Taking into account the multifunctionality of table salt in production of meat products, including salt’s significant influence on the palatability of the finished product, reduction of sodium chloride will result in deterioration of organoleptic and functional properties of meat products. Thus, many studies assess the expediency of sodium chloride replacement with other chlorides (Ca, K, Mg), which affect taste, water binding capacity, and inhibition of microorganisms. It is known that table salt has many influences on oxidative changes in fat due to salt’s inhibitory effect on the activity of the antioxidative enzymes. The antioxidative activity indicates the total protection of the meat system from peroxidation. Reduced antioxidative activity suggests a decreased ability to withstand the oxidative changes. In this context, the aim of this research was to study the effects of salt replacers on the antioxidative activity of meat and on the activity of the antioxidative enzymes (catalase, superoxide dismutase, and glutathione peroxidase).
2. Materials and methods

Fresh and post-rigor pork muscles (Longissimus dorsi) were taken from female 2-year old Large White pigs in Russia. Meat was ground through a 2-3 mm meat grinder (Vitek, Russia) and salted with 2.0% edible table salt for the control group. The other treatments were prepared with the salt compositions as follows: Composition 1 – 1.0% NaCl + 1.2% KCl (experiment 1); composition 2 – 1.0% NaCl + 0.6% KCl + 0.8% CaCl\(_2\) (experiment 2). The prepared minced meat samples were held for 24 hours at 4-6°C.

The extraction was performed according to the method described by Hernandez et al. [9] with some modifications. The minced pork samples were subjected to extraction with laboratory dispersing equipment (LDE, Labotex, Russia), using 0.05 M phosphate buffer (pH 7) for 3 min at a 1:5 ratio of volume of extracted sample to extractant solution, at 4-5°C and 5000 rpm. The extract was separated by centrifugation at 15,000 rpm for 15 min at 4.0°C in a centrifuge Sigma 3K30 (Germany). The supernatant was filtered through glass wool Sigma-Aldrich (Germany).

Determination of antioxidative activity was based on the oxidation rate of reduced 2,6-dichlorophenolphindophenol (2,6-DCPIP) by oxygen dissolved in the reaction medium. This reaction turns the colorless leuco form of 2,6-DCPIP to the colored form with maximum absorption at 600 nm. The optical density was measured with a photometer (BioChem SA, HTI, USA). Inhibition coefficient (IC) of antioxidation 2,6-DCPIP by the supernatant was the indicator of antioxidative activity.

Determination of the catalase activity was performed according to the method described by Jin et al. [10]. Supernatant (0.1 mL) was mixed with 2.9 mL of 11 Mm hydrogen peroxide in phosphate buffer (pH 7.0) at room temperature (22±2°C). The resultant mixture was stirred thoroughly, transferred into the 1 cm cuvettes, and the optical density was measured immediately after the start of the reaction and after 3 min of incubation at a wavelength of 240 nm on a photometer (KFK-3-01 ZOMZ, Zagorsk, Russia). The calculation of the decrease in the hydrogen peroxide concentration took into account the extinction coefficient of 39.4 M\(^{-1}\)cm\(^{-1}\). To measure catalase activity, the amount (mmol) of hydrogen peroxide that was decomposed during 1 min by the supernatant extracted from 1 g of meat was calculated. The results were expressed in U/g of meat.

Determination of the superoxide dismutase activity was performed by the method described by Gatellier et al. [11], by measuring the pyrogallol autoxidation inhibition. Supernatant (75 µL) was mixed with 75 µL of 10 Mm pyrogallol solution in 2850 µL of 50 Mm phosphate buffer (pH 8.2). The resultant mixture was stirred thoroughly, transferred into 1 cm cuvettes, and the optical density was measured immediately after the start of the reaction and after 2 min of incubation at a wavelength of 340 nm on a photometer (KFK-3-01 ZOMZ, Zagorsk, Russia). Pyrogallol autoxidation was determined in a blank sample of a similar reaction mixture that contained the same volume of distilled water instead of the supernatant. One unit of superoxide dismutase activity is the ability of the sample to inhibit 50% of the reaction. The results were expressed in U/g of meat.

Determination of the glutathione peroxidase activity was performed according to the method described by Jin et al. [10]. The reaction mixture contained 1 mL of 75 mM phosphate buffer (pH 7.0), 10 µL of 150 mM reduced glutathione, 10 µL of 46 E/mL glutathione reductase, 30 µL of 25 mM EDTA, 30 µL of 5 mM NADPH, 200 µL of supernatant and 10 µL of 20%-TritonX-100. The volume of the prepared mixture was 1.5 mL. Addition of 50 µL of 7.5 mM H\(_2\)O\(_2\) initiated the reaction. Conversion of NADPH into NADP+ was registered on the photometer (KFK-3-01 ZOMZ, Zagorsk, Russia) at a wavelength of 340 nm during 3 min, taking into account the extinction coefficient of 6220 M\(^{-1}\)cm\(^{-1}\). One unit of glutathione peroxidase (E) activity is the amount (mol) of NADPH that is decomposed during 1 min by the supernatant extracted from 1 g of meat. The results were expressed in U/g of meat.

All experiments were carried out in triplicate. Statistical processing of the results by determination of the Pearson correlation coefficients and the approximation of reliability was performed using MS Excel for Windows. Assessment of the statistical significance of the differences between parameters was performed using Student’s t-test.

3. Results and discussion
The results of determination of the antioxidative activity of the extracts (Figure 1) obtained from the salted meat samples showed that replacement of table salt with potassium chloride did not significantly affect the total antioxidative activity (p>0.05). On the contrary, the use of composition 2, which contained sodium, potassium and calcium salts, led to a decrease in the antioxidative activity by 17.7% (p<0.05).

![Antioxidative activity graph](image)

**Figure 1.** The effect of the salt compositions on the antioxidative activity. Experiment 1 – 1.0% NaCl + 1.2% KCl; experiment 2 – 1.0% NaCl + 0.6% KCl + 0.8% CaCl₂ (*p<0.05 compared to the sample salted with sodium chloride).

Taking into account the significant role of the antioxidant enzymes as a natural form of antioxidative protection, the changes obtained in the meat antioxidative activity in the presence of salt replacers required a more detailed study of the main components of the meat antioxidative system. These are catalase, which catalyzes the breakdown of hydrogen peroxide into water and oxygen; superoxide dismutase, which initiates the conversion of superoxide to oxygen and hydrogen peroxide, and; glutathione peroxidase, which catalyzes the reduction of peroxide by tripeptideglutathione [12]. The results of the determination of the catalase activity in the salted meat (Figure 3) showed that replacement of table salt with the reduced sodium compositions did not significantly affect the activity of catalase (p>0.05).
Figure 2. The effect of the salt compositions on the catalase activity. Experiment 1 – 1.0% NaCl + 1.2% KCl; experiment 2 – 1.0% NaCl + 0.6% KCl + 0.8% CaCl$_2$ (* $p<0.05$ compared to the sample salted with sodium chloride)

This study also showed replacement of table salt with potassium and calcium salts did not influence the activity of glutathione peroxidase ($p>0.05$) (Figure 3).

Figure 3. The effect of the salt compositions on the glutathione peroxidase activity. Experiment 1 – 1.0% NaCl + 1.2% KCl; experiment 2 – 1.0% NaCl + 0.6% KCl + 0.8% CaCl$_2$ (* $p<0.05$ compared to the sample salted with sodium chloride)

However, the table salt replacers did affect superoxide dismutase activity (Figure 4). According to the data obtained, the use of potassium chloride and salts of potassium and calcium for partial replacement of sodium chloride led to a decrease in the activity of superoxide dismutase by 18.0 and 23.8 %, respectively ($p<0.05$), by the two salt compositions used.
Figure 4 The effect of the salt compositions on the superoxide dismutase activity. Experiment 1 – 1.0% NaCl + 1.2% KCl; experiment 2 – 1.0% NaCl + 0.6% KCl + 0.8% CaCl₂ (* p<0.05 compared to the sample salted with sodium chloride)

The increase in the products of oxidation in the meat salted with compositions of salts with potassium chloride and calcium chloride with reduced sodium content could be explained by the decrease in the activity of superoxide dismutase, which was responsible for the reduction in the total antioxidative activity of meat.

4. Conclusion
The results of this study contribute to confirmation of the effects of salt replacers on the oxidative changes in minced pork muscles. Replacement of potassium chloride with sodium chloride did not significantly influence the antioxidative activity of pork; however, it led to inhibition of the activity of superoxide dismutase.

The use of the mixture of potassium chloride and calcium chloride as a salt replacer led to a decrease in the activity of superoxide dismutase and was responsible for a decrease in the total antioxidative activity of meat. Therefore, the use of salt replacers weakened the natural protection of meat from oxidation and, hence, initiated oxidative changes. Since replacement of table salt in meat products will lead to a reduction of the antioxidative protection in the meat raw materials, it will be necessary to use suitable technological approaches that facilitate retardation of oxidation, for example, additional use of antioxidants.

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