



## EFFECT OF SODIUM CHLORIDE ON FAT OXIDATION IN THE PRESENCE OF HEME PIGMENTS

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<https://doi.org/10.34302/2019.11.4.10>

### Article history:

Received:

29 April 2019

Accepted:

29 November 2019

### Keywords:

*Back fat;*

*Fatty acid composition;*

*Heme iron;*

*Myoglobin;*

*Salt*

### ABSTRACT

The effects of salt on oxidative changes in fat and heme pigments were evaluated. The back fat was salted with sodium chloride in the presence of the hemoglobin. The indicators of the hydrolytic and oxidative spoilage were investigated in the samples of back fat from White Large pigs at two years of age. The proportions of heme and non-heme iron, as well as the content of metmyoglobin were determined in the samples of m. *Longissimus dorsi* from two-year-old female Large White pigs. Back fat salting did not significantly affect the acid value of back fat. Addition of salt in amounts of 3.5 % and 5.0 % initiated the oxidative changes. Addition of sodium chloride initiated fatty acid oxidation in the presence of the heme pigments. The content of unsaturated acids decreased by 22.8 % when 5.0 % of salt were added. The proportion of heme iron increased by 6.5 % relative to free iron when 5.0 % of sodium chloride were added. The positive correlation between the metmyoglobin content and the thiobarbituric acid value was found with the correlation coefficient of 0.98.

### 1. Introduction

For several decades, scientists have been studying the effect of meat salting on the oxidative changes. The data available from the scientific technical literature about the pro- and anti-oxidative effects of salt do not allow formulation of the unified view on the mechanism of the sodium chloride effect on fat oxidation. For example, according to some data, sodium chloride has the anti-oxidative effect (Andres *et al.*, 2004) or does not influence the fat chemical properties (Calligaris and Nicoli, 2006). On the contrary, several researchers found the pro-oxidative effect of sodium chloride (Gheisari *et al.*, 2010; Rhee and Ziprin, 2001; Jin *et al.*, 2012). Sarraga *et al.* (2002) established that addition of sodium chloride

inhibited the activity of the antioxidant enzyme, glutathione peroxidase, but on the other hand salting facilitated a decrease in the thiobarbituric acid value. There are data about interrelations between oxidation of lipids and myoglobin; oxidation of one of these components leads to formation of chemical compounds that can catalyze oxidation of another (Faustman *et al.*, 2010; Min and Ahn, 2005; Min *et al.*, 2010). Many hypotheses explain the chemistry of the oxidative processes under the impact of sodium chloride. Among the main the action of the reactive chloride ion on lipids or modification of protein heme that catalyzes lipid oxidation (Ladikos and Lougovois, 1990), interaction of the heme iron ions with the chlorine ion, displacement of the iron ion from

the protein macromolecule under an impact of the sodium ion (Zanardi *et al.*, 2010), *etc.* Incoherence of data on the salt effect on the chemical changes in fats, apparently, can be explained by differences in selection of research samples, when different meat types with different content of heme pigments as well as different types and parameters of meat technological processing were mainly used. With this experiment setting, the number of factors influencing the processes of hydrolysis and oxidation upon salting significantly increases and can be a consequence of only indirect effect of sodium chloride on fats. It is worth noting that among these factors are the dose of sodium chloride, the presence of the heme pigments, heat treatment and so on (Jin *et al.*, 2010; Cobos *et al.*, 2008).

Despite different explanations of the mechanism of salt action on chemical changes in fats, the majority of researches link the pro-oxidative effect of sodium chloride with its impact on heme iron. Nevertheless, an iron type and a mechanism of its influence on the oxidative processes still leave many questions unanswered. Therefore, the aim of this study was to investigate the relationship between of the fat and heme pigments oxidation under the impact of sodium chloride.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Back fat samples

The indicators of the hydrolytic and oxidative spoilage were determined in the samples of back fat from White Large pigs at two years of age. Taking into consideration the technological doses of sodium chloride in the recipes of sausages and products from meat, a range of sodium chloride concentrations from 2.0 to 5.0 % was chosen for the experiment. For homogeneous salt distribution, back fat was minced in a grinder through a plate with a hole diameter of 2-3 mm and salted with salt in amounts of 0.0, 2.0, 3.5 and 5.0 % in the presence of the hemoglobin solution (160g/l) in an amount of 1.25 % of back fat weight as this dose ensures the concentration of heme iron in

back fat equal to the concentration of heme iron in myoglobin contained in 100 g of pork. After salting, back fat was vacuumed and cooked in a water bath at a temperature of 80 °C, then chilled and stored at a temperature of 4±2 °C for 3 days.

#### 2.1.2 Meat samples

The proportions of heme and non-heme iron, as well as the content of metmyoglobin were determined in the samples of *m. Longissimus dorsi* from two-year-old female Large White pigs. Meat was minced in a grinder through a plate with a hole diameter of 2-3 mm and salted with table salt in amounts of 0.0, 2.0, 3.5 and 5.0 %. The prepared samples were held at a temperature of 4±2°C for 24 hours, after which they were packed under vacuum and subjected to heat treatment until reaching a temperature of 72±2°C.

## 2.2 Methods

The acid value was determined by the method based on titration of free fatty acids in the ether-alcohol solution of fat with the aqueous solution of alkaline. 20 g of the sample were ground in a mortar with 40 g of anhydrous sodium sulfate until smooth, the mixture was extracted for 5 min with 100 ml of chloroform in a laboratory shaker, then filtered through a paper filter. 10 ml of the filtrate was mixed with 10 ml of ethanol and 2 drops of a 1% aqueous-alcoholic solution of phenolphthalein and titrated with a 0.1 M sodium hydroxide solution until a faint pink color was observed that was stable for 30 sec. The acid number  $X$ , mg KOH / g of fat contained in the sample was calculated by the formula:  $X = 5.61 \cdot V \cdot K / m$ , where  $V$  is the volume of a 0.1 M solution of potassium hydroxide used for titration, ml;  $K$  is the correction factor to the potassium hydroxide solution; 5.61 – the amount of potassium hydroxide contained in 1 ml of a 0.1 M solution;  $m$  is the mass of fat in the sample, determined gravimetrically in the filtrate, g.

Peroxide value by the method based on oxidation of iodhydric acid with peroxides contained in fat with the following titration of

released iodine with sodium thiosulphate. For this, 50 g of the sample were ground in a porcelain mortar with 100 g of anhydrous sodium sulfate to a homogeneous mass, extracted with 150 ml of chloroform in a laboratory shaker for 5 min and filtered through a paper filter. 10 ml of a fat-containing extract was mixed with 10 ml of glacial acetic acid and 1 ml of a 50% freshly prepared potassium iodide solution, the flask was immediately closed, the contents were stirred, and left for 5 min in a dark place at room temperature 22 °C. Then, 100 ml of distilled water was poured into the flask, thoroughly mixed, 1 ml of a 1% starch solution was added. The liberated iodine was titrated with a 0.01 M sodium thiosulfate solution until a milky white color, stable for 5 s. The peroxide value  $X$ , mmol of active oxygen / kg of fat (mmol O<sub>2</sub>/kg) contained in the sample, was calculated by the formula  $X = (V_1 - V_2) \cdot C \cdot K \cdot 1000 / m$ , where  $V_1$  is the volume of sodium thiosulfate solution used in the analysis, ml;  $V_2$  is the volume of sodium thiosulfate solution used in the control determination, ml;  $C$  is the concentration of the used sodium thiosulfate solution, mol / L;  $K$  is the correction factor for the titer of sodium thiosulfate solution; 1000 – coefficient taking into account the conversion of the measurement result in mmol / kg;  $m$  is the mass of a portion of fat, determined gravimetrically in the extract, g.

Determination of the thiobarbituric acid value was carried out by the method based on the development of stained substances as a result of interaction of fat oxidation products with 2-thiobarbituric acid and measurement of the color intensity on a spectrophotometer (Zhuravskaya *et al.*, 1985). 50 g of the sample were homogenized with 50 ml of distilled water, 47.5 ml of water and 2.5 ml of a 3 M hydrochloric acid solution were added, the mixture was placed in a distillation apparatus, and distillation was distilled off to collect 50 ml of distillate. 5 ml of the obtained distillate was mixed with 5 ml of a 0.02 M solution of thiobarbituric acid and kept for 35 minutes at a temperature of 100 °C. In parallel, control was carried out with 5 ml of distilled water instead of distillate. The solutions

were cooled for 10 min to a temperature of 25 °C and the absorbance was measured at a wavelength of  $535 \pm 10$  nm. Thiobarbituric number  $X$ , mg malondialdehyde per kg of product, was calculated by the formula:  $X = D \cdot 7.8$ , where  $D$  is the optical density of the solution; 7.8 - empirical coefficient.

The fatty acid composition was determined using a flame ionization detector by the method of gas chromatography (Ivankin *et al.*, 2016). For this, a 10-g weighed portion of a sample was treated for 3–24 h with a mixture of 10 mL of chloroform and 10 mL of methanol by the modified Folch method in the presence of a 1% KCl solution to dissolve the lipid component; the extract was filtered through paper and evaporated to dryness. Then, 0.01 g of the residue was mixed with 3 mL of a 15% solution of acetyl chloride in methanol; the mixture was incubated for 2 h at 100°C; and the pH of the mixture was adjusted to 5.0–6.0 by adding a potassium hydroxide solution in methanol. Three milliliters of a saturated NaCl aqueous solution and 3 mL of hexane were added to the resulting mixture. The solution was allowed to stand for several minutes, and 0.2 mL of the clear hexane layer containing fatty acid methylesters was sampled for analysis. The fatty acid composition was determined using a 7890A gas chromatograph with a HP-Innowax capillary column (0.2 mm in diameter, 30 m in length, and with the thickness of the stationary phase layer of 0.33 μm) and a flame ionization detector (FID). Conditions of chromatography using a HP-Innowax capillary column with a FID: the temperature in the column oven was increased from 100 to 260 °C at a rate of 10°/min; the injector temperature was 250 °C; and the detector temperature was 300 °C. Nitrogen was used as a carrier gas with the flow rate of 20 mL/min; the hydrogen flow rate was 35 mL/min; the injection sample volume was 1 μL; and the split flow ratio was 1 : 100. The total analysis time was 30 min. The amount of an analyte was assessed by comparing its peak area with the peak area of an internal standard. Calculation of the content of individual fatty acids according to

an automatic program, taking into account graduation by standard substances.

Determination of iron in the samples was carried out by acid mineralization under pressure using 10 ml HNO<sub>3</sub> in the MARS 6 system. After mineralization, the solutions are transferred to a 50 ml flask and brought to the mark with distilled water. Analytical determination of iron concentration was carried out on the atomic absorption spectrophotometer with electrothermal atomization Agilent 280Z. Measurements were carried out using hollow cathode lamps at a wavelength of 248.3 nm, slit width 0.2 nm, with background correction. The measurement parameters were selected according to the specified parameters of the equipment used.

Heme iron was determined by the acidified acetone extraction method (Hornsey, 1956) with modifications. Meat (5 g) was transferred to a 50 ml Polypropylene centrifuge tube and 10.0 ml of acidified acetone (95.7 % acetone; 2.4% HCl) was added. The suspension was homogenized for 30 S at 13.500 rpm using Ultra Turrax T25, which was then washed 3 times with 3.0 ml of acidified acetone. The final concentration in the soluble phase of the suspension was 80% acetone and 2.0 % HCl. The suspension was quickly stirred and kept on ice for 1 hour. Insoluble substances were previously precipitated by centrifugation (1 hour, 0 °C, 10 000 g) and 5 ml of supernatant was filtered through a Minisart RC 15 filter. The filtrate absorption was measured at 640 nm and the iron heme content was calculated. All filtered samples were visually checked for turbidity before measurement. The turbid samples were filtered again before measurement.

Absorption spectra of MetMb, DeoMb and OxyMb solutions were obtained using a Varian Cary Bio-50 spectrophotometer (USA)

with a 1 cm path length cuvette from 650 nm to 475 nm.

### 2.3 Statistical analysis

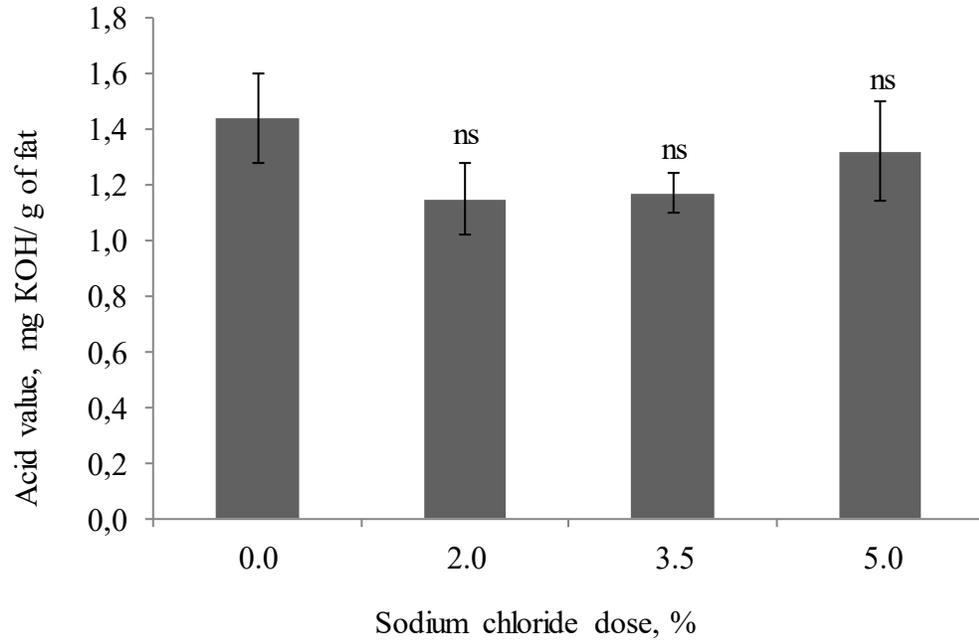
Each experiment was carried out in three replications. Data are reported as mean values with standard deviation. The statistical significance of differences between indicators was assessed using the Student's t-test. P values less than 0.05 were considered statistically significant.

## 3. Results and discussions

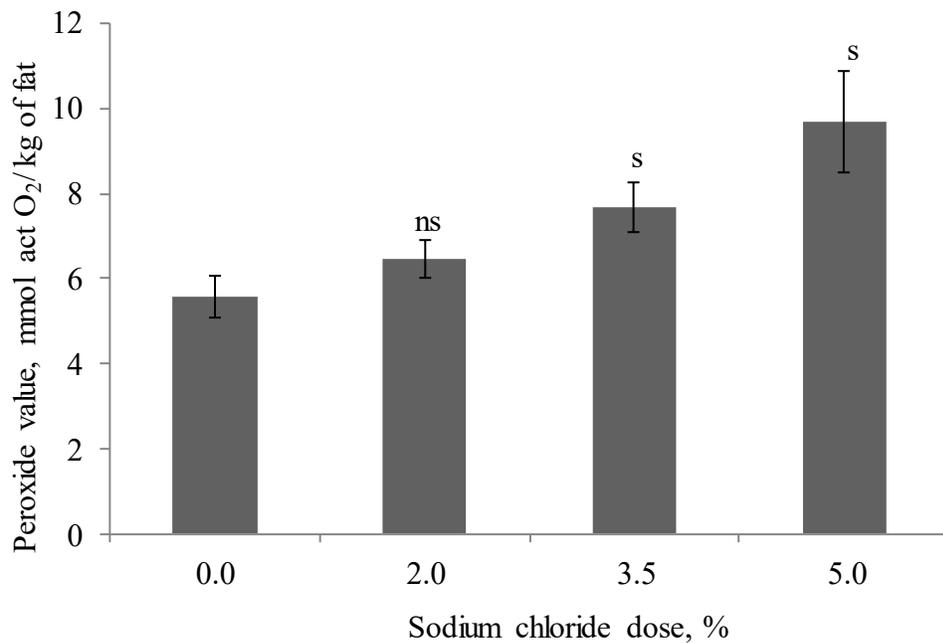
### 3.1. Fat hydrolysis and oxidation

Meat and meat products are a complex multi-component system, which significantly complicates acquisition of reliable data about an effect of sodium chloride on fat oxidation in the presence of many biochemical compounds of animal raw materials and different technological factors, among which are the heme pigments and temperature factor, which can affect the anti- and pro-oxidative activity of salt. In this connection, the indicators of the hydrolytic and oxidative spoilage of salted back fat in the absence and presence of the heme pigments after heat treatment were studied.

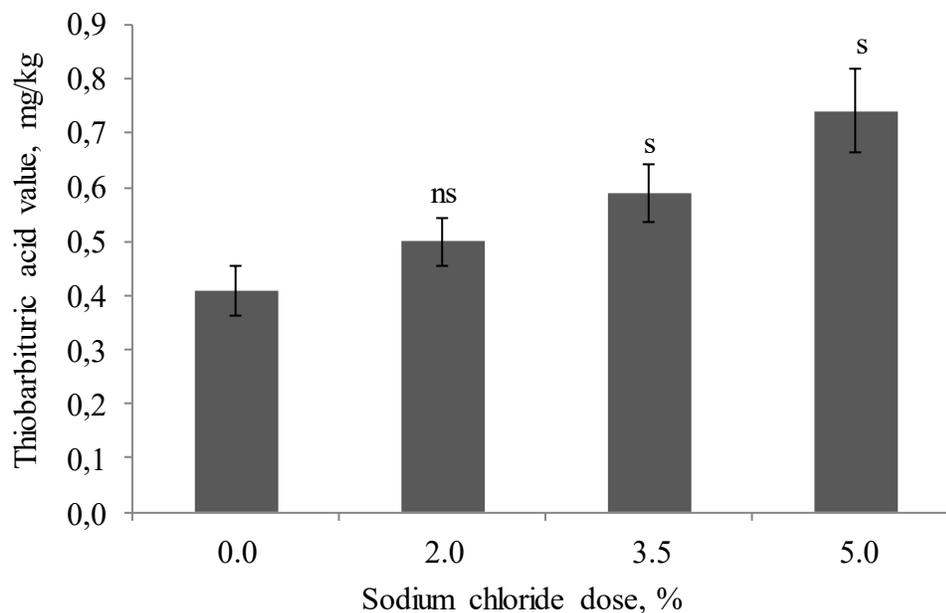
Addition of table salt in the presence of heme pigments did not significantly influence the hydrolytic changes in back fat – the acid value ( $p > 0.05$ ) (Fig. 1). Back fat salting with sodium chloride in an amount of 3.5 % facilitated an increase in the peroxide value by 37.6 % ( $p < 0.05$ ), the further increase in the salt dose up to 5.0 % initiated the growth of the peroxide value by 73.2 % ( $p < 0.05$ ) compared to unsalted back fat (Fig. 2). The similar trend was observed in the measurement of the thiobarbituric acid value. Addition of sodium chloride in amounts of 3.5 % and 5.0 % led to an increase in the thiobarbituric acid value by 43.9 % ( $p < 0.05$ ) and 80.7 % ( $p < 0.05$ ), respectively (Fig. 3).



**Figure 1.** Effect of sodium chloride on the acid value in back fat in the presence of heme pigments  
<sup>ns</sup>not significant at  $p \geq 0.05$  in comparison with unsalted sample.



**Figure 2.** Effect of sodium chloride on the peroxide value in back fat in the presence of heme pigments  
<sup>ns</sup>not significant at  $p \geq 0.05$ ; <sup>s</sup> significant at  $p < 0.05$  in comparison with unsalted sample.



**Figure 3.** Effect of sodium chloride on the thiobarbituric acid value in back fat in the presence of heme pigments

<sup>ns</sup>not significant at  $p \geq 0.05$ ; <sup>s</sup> significant at  $p < 0.05$  in comparison with unsalted sample.

The studies on the effect of different salt doses on back fat oxidation in the absence of hemoglobin demonstrated that addition of salt into the back fat composition did not result in an increase in the indicators of the hydrolytic and oxidative spoilage. On the contrary, addition of 2.0% of salt caused a decrease in the oxidation product (Tunieva *et al.*, 2017). The antioxidative effect of small doses of salt can be explained by an effect of sodium chloride on a decrease in the water activity, which retards fat oxidation, and by the absence of the heme pigments in back fat. Compared to these data, the presence of heme initiated the oxidative changes in unsalted back fat by 2.2 times ( $p < 0.05$ ) for the peroxide value and 1.5 times ( $p < 0.05$ ) for the thiobarbituric acid value.

Therefore, the presence of the heme pigments changes the character of the table salt effect on lipid oxidation initiating chemical changes in fat in the presence of sodium chloride. The obtained data correspond to the results of the study on the volatiles composition of salted back fat, which suggest that in the presence of the heme pigments, addition of even

small amounts of sodium chloride (2.0 %) initiates the oxidative processes compared to unsalted back fat (Tunieva and Ivankin, 2015).

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### 3.2. Fatty acid composition

Back fat salting did not significantly influence the changes in the fatty acid composition in the absence of heme pigments ( $p > 0.05$ ). Addition of the hemoglobin solution led to changes in the character of the table salt effect on the fatty acid composition of back fat after heat treatment (Fig. 4). Addition of sodium chloride initiated oxidation of fatty acids. The quantity of unsaturated acids in the sample salted with 5.0 % of table salt decreased by 22.8 % relative to their content in the sample of unsalted back fat ( $p < 0.05$ ).

Therefore, the presence of the heme pigments initiated the oxidative changes in lipids during salting. The acceleration of the oxidative processes in the presence of salt is a consequence of the indirect pro-oxidative effect of sodium chloride linked with its impact on the heme pigments.

### 3.3. Heme and non-heme iron

To establish the mechanism of the heme pigment involvement in the development of the oxidative processes within the framework of the experiment, an effect of salt on the ratio of heme

and non-heme iron was studied. The available data on the salt effect on the heme pigments during heat treatment are ambiguous. For example, the research of Kristensen and Purslow (2001) indicates an increase in the proportion of heme iron relative to free iron during heat treatment in the pork samples salted with sodium chloride. It is necessary to note that this positive trend was established only for heat treated salted meat as the studies on the effect of table salt on the meat heme pigments before cooking indicated the absence of significant differences in the content of heme and non-heme iron. On the contrary, Min *et al.* (2010) found that addition of sodium chloride to meat products from beef led to degradation of myoglobin, which facilitated an increase in the concentration of free iron in meat.

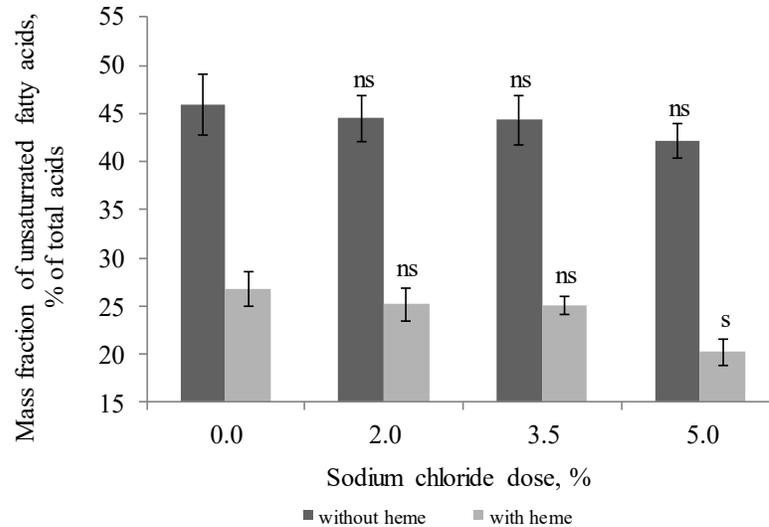
The results of our studies on determination of heme and non-heme iron indicate an increase in the proportion of heme iron relative to free iron by 6.5% as a result of salting upon addition of sodium chloride in an amount of 5.0 % ( $p < 0.05$ ) (Fig. 5). It is obvious that addition of sodium chloride stabilizes the heme pigments and prevents their destruction during heat treatment. Therefore, the negative correlation between heat denaturation of the heme pigments and oxidative changes was found. This effect can be explained differently: the lower catalyzing effect of free iron compared to heme iron and a role played by a form of iron oxidation, not by an iron type. The obtained results correspond to the study of Johns *et al.* (1989) who established that heme iron had a greater pro-oxidative effect compared to free iron.

Several studies give evidence of the relationship of heme and non-heme iron with fat oxidation. Rhee and Ziprin (1987) found that the heme iron concentration was more significant than an amount of non-heme iron when predicting lipid oxidation in meat. Other studies (Baron and Andersen, 2002; Tichivangana and Morrissey, 1985) demonstrated the stronger catalyzing effect of non-heme iron on meat product oxidation compared to heme iron.

Taking into consideration such ambiguous results of available studies and the fact that heme iron can also be present in the oxidized forms of myoglobin, we studied the changes in the proportion of metmyoglobin relative to the total content of the heme pigments depending on addition of sodium chloride (Fig.6).

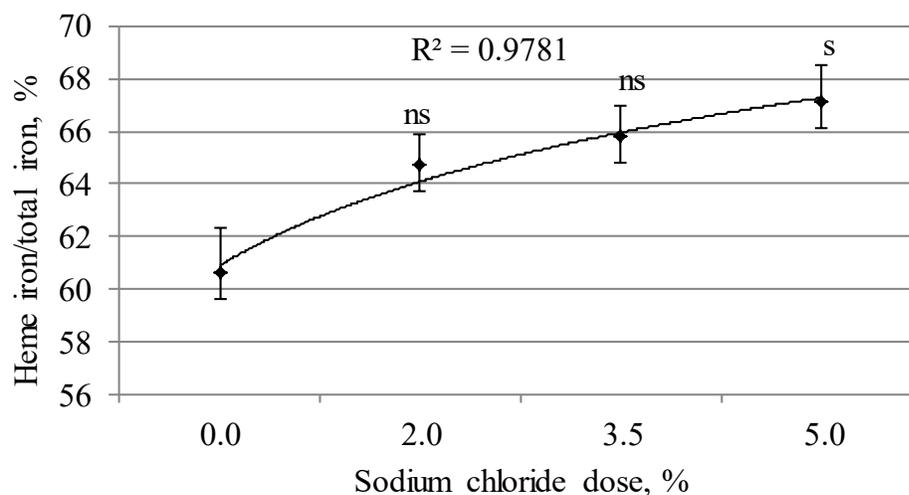
The results showed that the ratio of metmyoglobin to the total content of the myoglobin forms increased with an increase in

the salt dose. Therefore, the positive correlation was established between the metmyoglobin content and the thiobarbituric acid value (correlation coefficient 0.98%). The obtained results are consistent with other studies that established the relationship between oxidation of myoglobin and meat lipids (Rhee and Ziprin, 2001; Andersen and Skibsted, 1991; Yin and Faustman, 1993).



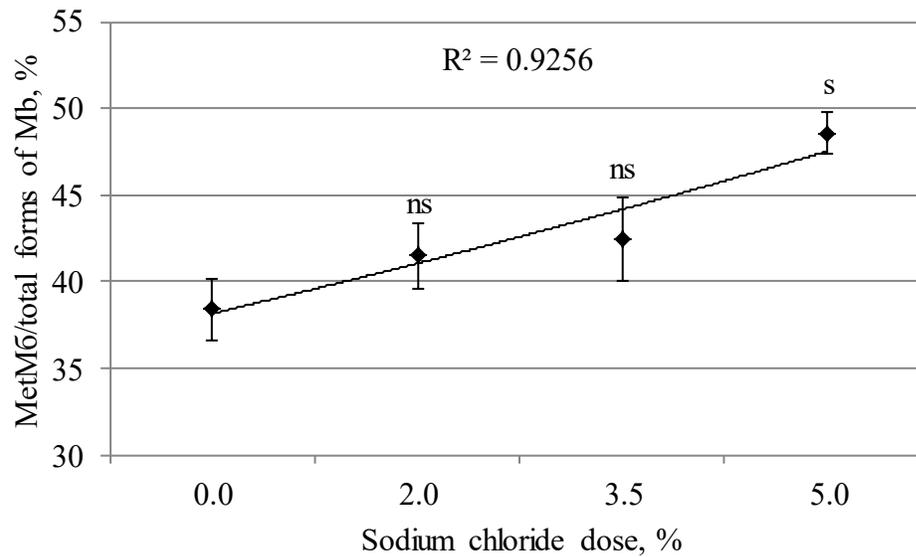
**Figure 4.** Dynamics of changes in the mass fraction of unsaturated fatty acids in back fat depending on the sodium chloride concentration

Averages in the same line do not differ significantly (ns) at  $p \geq 0.05$  or differ (s) at  $p < 0.05$  from unsalted sample.



**Figure 5.** Changes in the proportion of heme iron relative to total iron depending on the sodium chloride concentration

<sup>ns</sup> not significant at  $p \geq 0.05$ ; <sup>s</sup> significant at  $p < 0.05$  in comparison with unsalted sample



**Figure 6.** Changes in the myoglobin content depending on the sodium chloride concentration (Mb - myoglobin)

<sup>ns</sup> not significant at  $p \geq 0.05$ ; <sup>s</sup> significant at  $p < 0.05$  in comparison with unsalted sample.

#### 4. Conclusions

The results of the study make a contribution to substantiation of the sodium chloride effect on the oxidative changes. It was established that addition of sodium chloride increased the heme pigment stability to high temperature and concurrently initiated the oxidative changes in lipids. In this connection, it was suggested that heme iron had the higher catalyzing effect on fat oxidation compared to free iron. The obtained effect can be explained by the salt impact on the development of the myoglobin oxidized forms, which catalyzed lipid oxidation.

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