EFFECTS OF SOME FRACTIONS OF PORCINE MUSCLE ON THE BEHAVIOR OF NITRITE AND THE FORMATION OF COOKED CURED MEAT COLOR

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Since the formation of cured meat color or cooked cured meat color is one of the most important problems in processing meat products, a lot of works have been performed on this problem.

In processing meat products as well as in fresh meat, the enzymatic reduction of metmyoglobin to myoglobin is of great importance. In this respect it has lately been shown that the addition of nicotinamide adenine dinucleotide and various substrates of the enzymatic pathways of glycolysis and respiration is effective in promoting the enzymatic reducing ability of meat.[1,2]

The possibility of the production of various nitrosoreductant intermediates[3,4] and nitrosothiole[5] during the course of curing has also been reported in recent papers. According to a suggested mechanism, under anaerobic condition nitric oxide ferricytochrome is produced as an nitric oxide-intermediate, the formation of nitric oxyde metmyoglobin follows it, and then nitric oxide metmyoglobin is reduced by mitochondria to nitric oxide myoglobin.[6,7]

As to the condition under which meat products are to be produced, both the formation and stability of cured meat color have been found to be evidently better under anaerobic condition than under aerobic condition.[4,8,9]

Most of the former works, however, seem to have dealt with the formation of cured meat color not in the modern rapid emulsion curing process but in the conventional common curing process.

Emulsion curing has widely been used in processing sausages of late, in particular in manufacturing cooked sausage meat emuls-

ion is often cooked immediately after the meat has been comminuted and mixed with curing ingredients in a silent cutter and then stuffed into a casing.

In this rapid emulsion curing process the mechanism of the formation of cured meat color may probably differ from that in the conventional curing process in many respects, because in rapid emulsion curing process as the meat emulsion is subjected to cooking treatment immediately after the meat has been mixed with curing ingredients the enzymatic activities of the enzymes existing in meat may hardly be able to exhibit any appreciable effects on the formation of cured meat color. But in this case too, not because of their enzymatic activities, but because of the increase in sulfhydryl groups of enzyme-proteins released by heat denaturation, the enzymes may be able to promote the formation of cooked cured meat color as was previously demonstrated.[10)]

The purpose of the present work is to investigate the effects of some fractions of porcine muscle on the behavior of nitrite and the formation of cooked cured meat color in the abovementioned modern rapid emulsion curing process.

Experimental

In all the present experiments the porcine muscle samples just after slaughter were used.

Experiment I

1. Preparation of purified myoglobin

The myoglobin (Mb) used in the present experiments was purified from porcine heart by the method of Walters and Taylor.[11)] The purified metmyoglobin (MMb) was freeze-dried, then stored at 4°C until use.

The absorption curves for Mb and its derivatives MMb, oxymyo-

globin (MbO_2) and nitric oxide myoglobin (MbNO) prepared by the procedure of Ginger and Schweigert[12] were also measured, and according to the results, the findings for the wavelength of maximum absorption of Mb and its derivatives were quite the same as those given by previous workers [3,12,13] with only one exception of that of MMb. Namely, MMb from porcine heart had two absorption peaks at 503 and 630 mu in the present experiment, and of which the higher peak at 503 mu was slightly different from the higher peaks at 500 and 505 mu of MMb from horse heart[13] and bovine muscle,[12] and bovine heart[3] observed by previous workers.

2. Preparation of meat sample

After fat and connective tissue had been removed from porcine skeletal muscle (M.adductores) as far as possible, the muscle was ground three times with a meat grinder and stored at 4°C until use.

3. Determination of Mb in meat sample

Since all the porcine muscle samples were those just after slaughter, the Mb content in meat sample was determined by the procedure given in Fig.1.

4. Preparation of meat homogenate

Fifty grams of meat sample was homogenized with 100 ml of veronal buffer of pH 5.5 in a Waring blendor for 3 minutes, cooling with ice water.

Since in studying the formation of cooked cured meat color the content of myoglobin is one of the most important factors, to keep the content of Mb in meat sample always at a constant level of 0.5%, the Mb content of each meat sample was determined first, and the necessary amount of Mb to reach 0.5% was added to each meat homogenate as a 10% Mb aqueous solution, and the pH of which was adjusted to 5.5 with 1 N HCl and then diluted to 200 ml with

distilled water.

5. Experiments under aerobic and anaerobic conditions

In aerobic experiment, 20 ml of meat homogenate was pipetted into a 100-ml Erlenmeyer flask and to which one ml of veronal buffer of pH 5.5 containing 0.05% $NaNO_2$ (corresponding to 0.01% $NaNO_2$ in meat sample) was added. For the purpose of comparison, another 20ml of meat homogenate was placed into another Erlenmeyer flask, to which added one ml of veronal buffer of pH 5.5 containing no nitrite.

In anaerobic experiment, the quite same procedure as that in aerobic experiment was carried out, except that in place of an Erlenmeyer flask a Thunberg tube was used.

In cooking treatment, in aerobic experiment meat homogenate sample was cooked in the Erlenmeyer flask in the presence of air, while in anaerobic experiment in the Thunberg tube at a vacuum of 5 mm Hg, just after preparation for 20, 40, 60 and 90 minutes at 75°C with continuous shaking, respectively.

6. Determinations of color formation ability (CFA), nitrite, reducing ability (RA) and sulfhydryl groups

Immediately after cooking treatment at 75°C each meat homogenate sample was cooled with running tap water for 10 minutes, and then homogenized for one minute in a Waring blendor. The pH of which was adjusted to 7,0 with 1 N NaOH solution and made up to 50 ml with distilled water, then the resulting each homogeneous sample was subjected to the following individual determinations.

1) Determination of CFA : Ten milliliters of each homogeneous sample (corresponding to 1 g of meat sample) was centrifuged at 3,500 rpm for 10 minutes. After the precipitate had been collected, the cooked cured meat pigment was extracted from the precipitate

and its absorbance was measured by the method previously reported.[14] The absorbance values at 395 mu measured in this way were expressed as CFA.

2) Determination of nitrite : Twenty milliliters of each homogeneous sample was taken, and its remaining amount of nitrite was determined by the method proposed previously.[15]

3) Determination of RA : RA of each homogeneous sample was determined according to the procedure outlined in Fig.2.

4) Determination of sulfhydryl groups : Five milliliters of each homogeneous sample was taken and the amount of sulfhydryl groups in which was determined by the method given in Fig.3 with N-ethylmaleimide (NEM) on reference to the procedure of Hamm and Hofmann.[16]

Experiment II

1. Preparation of whole muscle homogenate

After being freed from fat and connective tissue as far as possible, 50 g of porcine skeletal muscle (M.adductores) was homogenized with 100ml of veronal buffer of pH 5.5 in a Waring blendor for 3 minutes, to which 1.169 g of NaCl was added to give a final concentration of 0.1 M NaCl, the pH of which was adjusted to 5.5 with 1 N HCl solution and then diluted to 200 ml with distilled water.

2. Separation of some fractions from porcine muscle

On reference to the paper of Creaser et al.[17], from the same porcine skeletal muscle (M.adductores) that described just above, some fractions, i.e., sarcoplasm, myofibrils, mitochondria and microsomes, were separated by the procedure oulined in Fig.4.

Sarcoplasm fraction was concentrated to one third of its original volume (about 300 ml) by freeze-drying, the pH of which

was adjusted to 5.5 with 1 N HCl solution and then diluted to 200 ml with veronal buffer of pH 5.5.containing 0.1 M NaCl.

3. Determination of nitrite and CFA

Twenty milliliters of each of the whole muscle homogenate and the other fractions was pipetted into a Thunberg tube. To keep the content of Mb in the whole muscle homogenate and the other fractions always at a constant level of 0.125% (corresponding to 0.5% Mb in meat sample), necessary amount of Mb to reach 0.125% was added as a 10% Mb aqueous solution to each Thunberg tube. Then one ml of veronal buffer of pH 5.5 containing 0.05% $NaNO_2$ was added to each Thunberg tube.

Since it has been shown in Experiment I that the formation of cooked cured meat color is extremely better under anaerobic condition than under aerobic condition, every sample was cooked at 75°C for one hour under anaerobic condition in a Thunberg tube at a vacuum of 5 mm Hg in Experiment II.

The figures for nitrite before and after cooking and CFA of each sample were determined by the methods described in Experiment I.

Results and Discussion

Experiment I

1. Effect of cooking condition on the behavior of nitrite in meat homogenate and the formation of cooked cured meat color of meat homogenate

The results given in Fig.5 indicated that the amount of nitrite decomposed by cooking under anaerobic condition was significantly larger than that decomposed under aerobic condition.

According to the results given in Fig.6, the formation of cooked cured meat color was extremely better under anaerobic cook-

ing condition than under aerobic cooking condition. Some similar tendencies in this respect have been observed in previous works.[4,8,9]

The above fact indicates that in manufacturing cooked sausage by rapid curing process the treatment of cooking under anaerobic condition is very effective in promoting the formation of cooked cured meat color.

2. Effect of cooking condition on the behaviors of reducing ability (RA) and SH groups of meat homogenate

In the course of cooking at 75°C for 90 minutes, the figures for RA of meat homogenate samples cooked under aerobic and anaerobic conditions both increased rapidly until 20 minutes, but thereafter the figures for RA in aerobic condition decreased gradually until 90 minutes, whereas those in anaerobic condition exhibited a tendency to remain almost unchanged or to increase slightly until 90 minutes, and the difference in the figures for RA between the samples cooked under aerobic and anaerobic conditions increased significantly in both cases in the absence and presence of nitrite with cooking time as shown in Fig.7. A similar tendency was observed in the behavior of SH groups of meat homogenate as shown in Fig.8.

The results in Fig.9 indicated that the reducing ability of meat homogenate tended to increase slightly by the addition of nitrite, in particular from 20 till 90 minutes in cooking treatment at 75°C in both cases cooked under aerobic and anaerobic conditions. Such a tendency was more clearly observed by the addition of nitrite in the behavior of SH groups of meat homogenate as shown in Fig.10.

These observations seem to present some interesting problems. In cooking treatment at 75°C for 90 minutes, concurrently with the

rapid increases in the reducing ability and the amount of SH groups of meat homogenate the amount of nitrite decomposed and that of cooked cured meat heme pigment formed increased with cooking time until 20 minutes in both cases cooked under aerobic and anaerobic conditions.

Thereafter, however, in the absence of nitrite though the reducing ability and the amount of SH groups of meat homogenate decreased appreciably under aerobic condition and remained almost unchanged or slightly increased under anaerobic condition until 90 minutes respectively, yet the amount of nitrite and that of cooked cured meat heme pigment continued to increase nearly in their respective same manners as before with cooking time until 90 minutes in the presence of nitrite under both aerobic and anaerobic conditions (Fig.5,6,7,8), and in both cases cooked under aerobic and anaerobic conditions the reducing ability and the amount of SH groups of meat homogenate tended to increase to some extent until 90 minutes by the addition of nitrite (Fig.9,10), although after the addition of nitrite the amount of nitrite decomposed increased consistently and the formation of cooked cured meat color continued to progress continually throughout the course of cooking treatment.

The causes of these facts remain unexplained. Further detailed experiments are necessary to approach to throw light on them.

Experiment II

Effects of some fractions of porcine muscle on the behavior of nitrite and the formation of cooked cured meat color

According to the results given in Table I, the amount of nitrite decomposed in whole muscle homogenate by cooking for one hour at 75°C was the largest, and those in the other fractions decreas-

ed in the order decomposed in sarcoplasm, myofibrils, mitochondria and microsomes, while the color formation ability of whole muscle homogenate was the most excellent, and those of the other fractions were designated in descending order of color formation ability as sarcoplasm, mitochondria, microsomes and myofibrils.

In the present experiment the color formation ability of myofibrils was the wrost of all the fractions tested, although the amount of nitrite decomposed in myofibrils was larger than those decomposed in mitochondria and microsomes. The following fact may be considered as one of the reasons for this, namely, since the myofibrils fractionated in the present experiment was passably impure preparation containing many other components nitrite added to myofibrils may have presumably been decomposed by some other side reactions, and accordingly the formation of cooked cured meat color was the wrost, although the amount of nitrite decomposed was relatively large.

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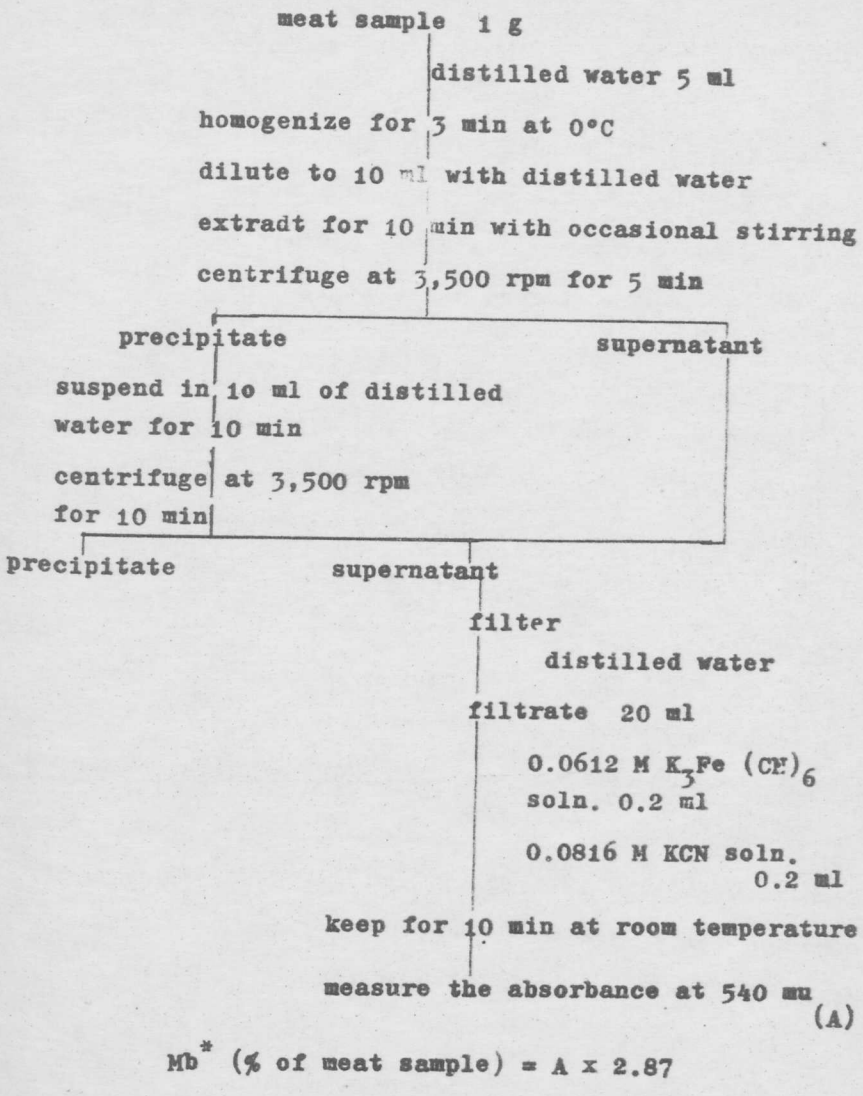

Fig. 1. Determination of Mb in meat sample

* The content of all heme pigments in meat sample was expressed as Mb.

Homogeneous sample 5 ml

| 5 mM $K_3Fe(CN)_6$ solution 2 ml

let stand for 60 min at 0°C with occasional stirring

| 0.5 % NH_4-sulfamate solution 0.1 ml

| 0.5 M Pb-acetate solution 0.2 ml

let stand for 10 min at room temperature

| 20 % TCA solution 2.5 ml

make up to 10 ml with distilled water

filter

filtrate

measure the absorbance at 420 mu

reducing ability (RA) = B - A

A : absorbance of sample observed

B : absorbance of 1 mM $K_3Fe(CN)_6$ solution observed

Fig.2. Determination of RA

homogeneous sample 5 ml

0.2 M phosphate buffer of pH 7.0 5 ml

2×10^{-3}M NEM solution 5 ml

let stand for 60 min at 25°C with occasional stirring

20 % TCA solution 5 ml

let stand for 10 min at room temperature

filter

filtrate

measure the absorbance at 300 mu

calculate the amount of SH groups in meat sample

(moles -SH/10^5g meat)

Fig. 3. Determination of SH groups with N-ethylmaleimide (NEM)

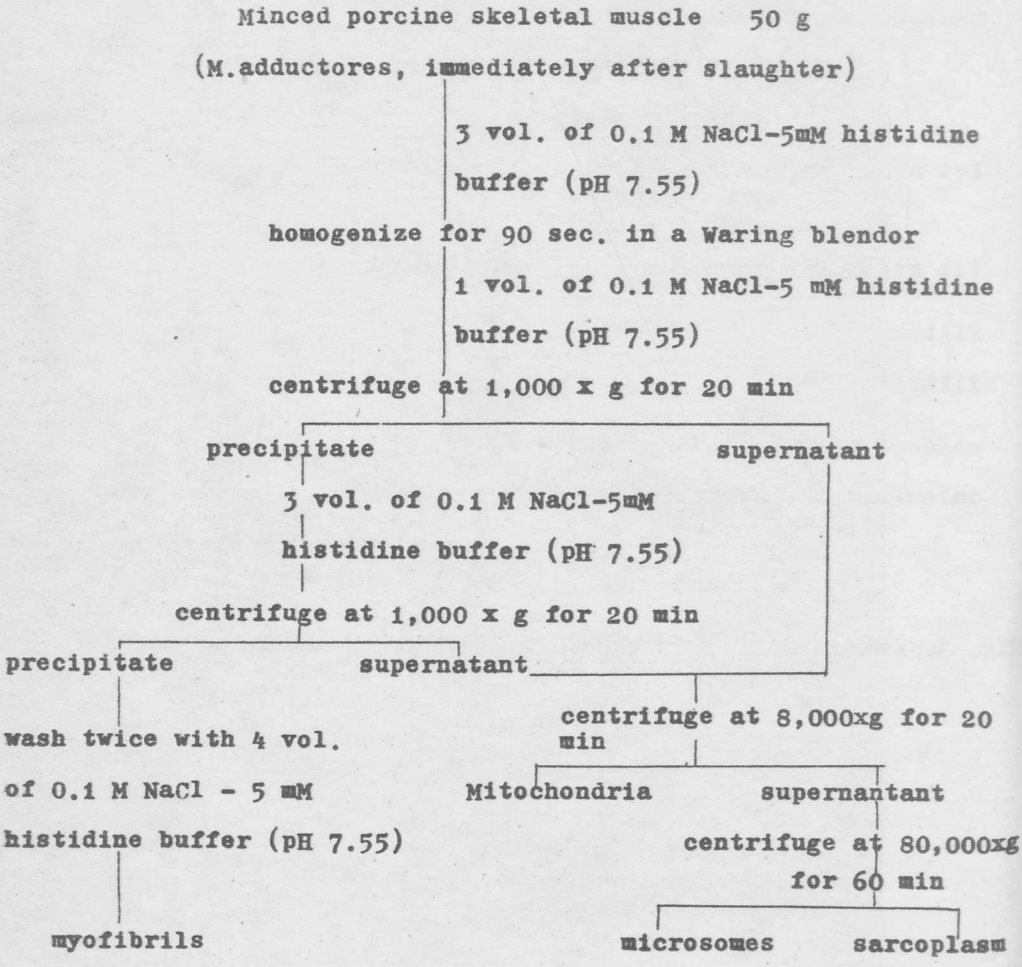

Fig. 4. Separation of some fractions from porcine skeletal muscle

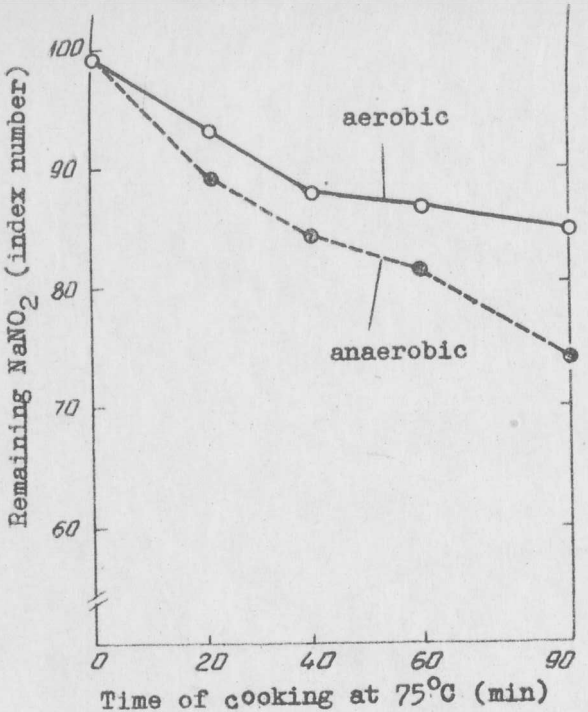

Fig.5. Effect of cooking condition on the behavior of nitrite in meat homogenate in rapid curing process

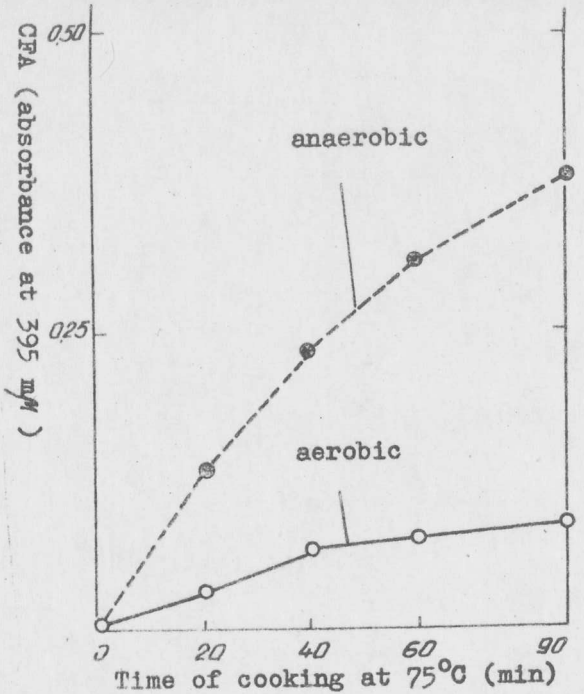

Fig.6. Effect of cooking condition on the behavior of CFA of meat homogenate in rapid curing process

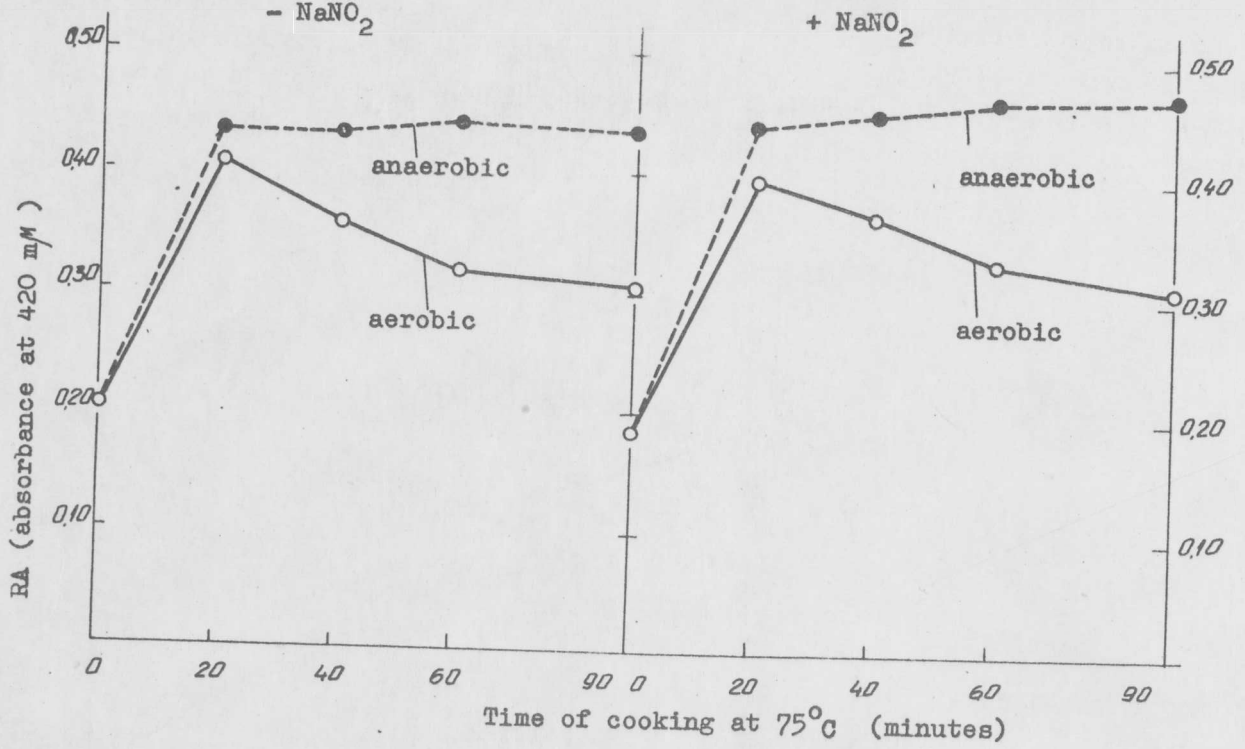

Fig.7. Effect of cooking condition on the behavior of RA of meat homogenate in rapid curing process

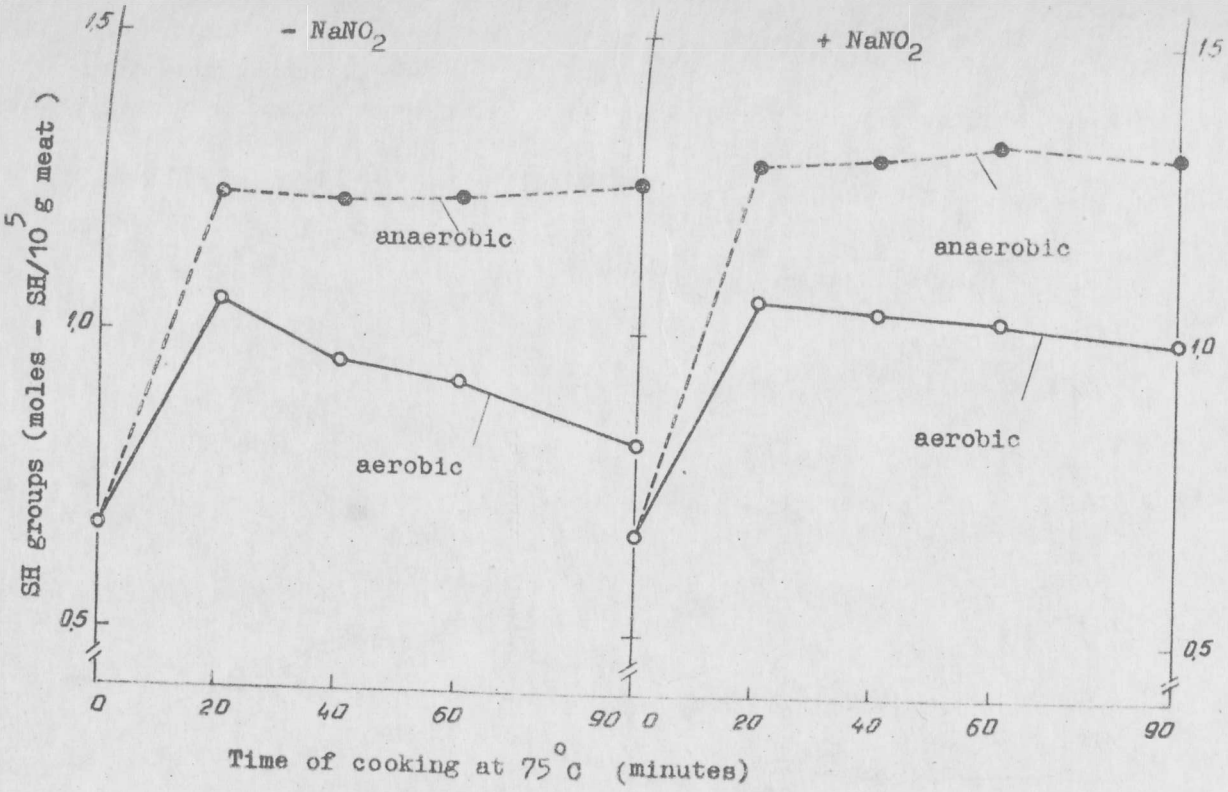

Fig.8. Effect of cooking condation on the behavior of SH groups of meat homogenate in rapid curing process

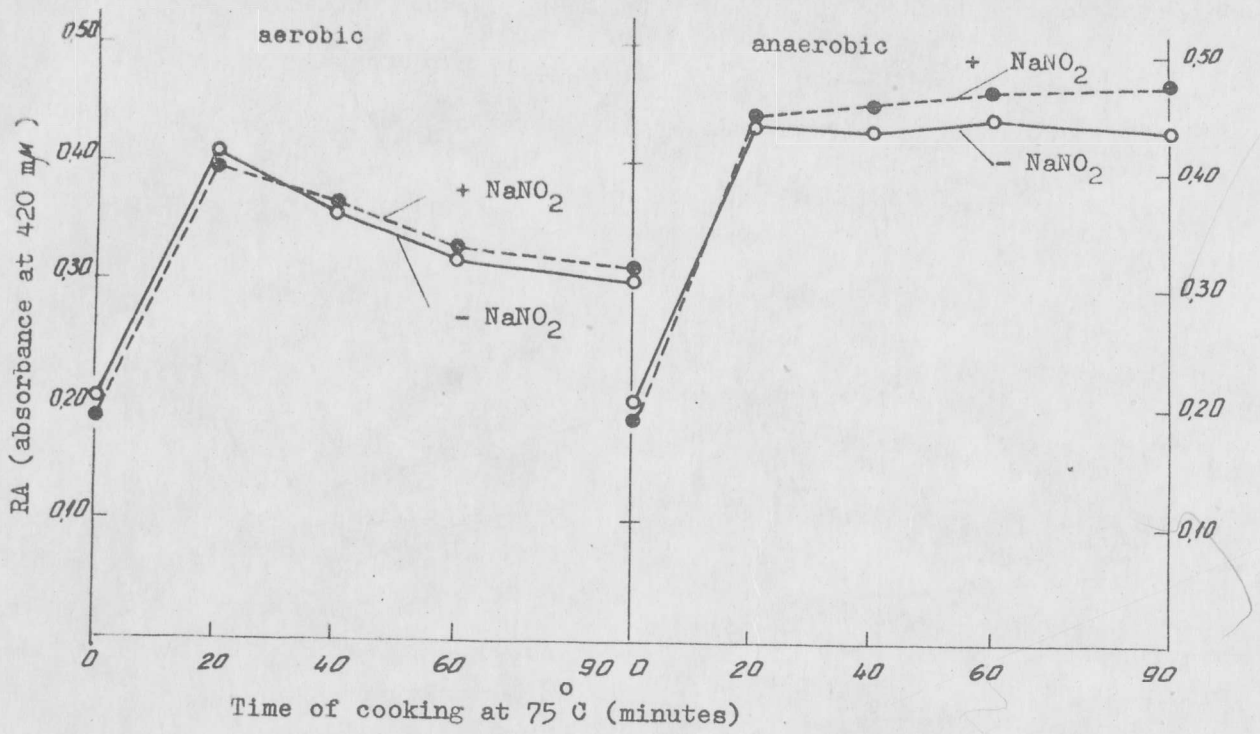

Fig.9. Effect of cooking condition on the behavior of RA of meat homogenate in rapid curing process

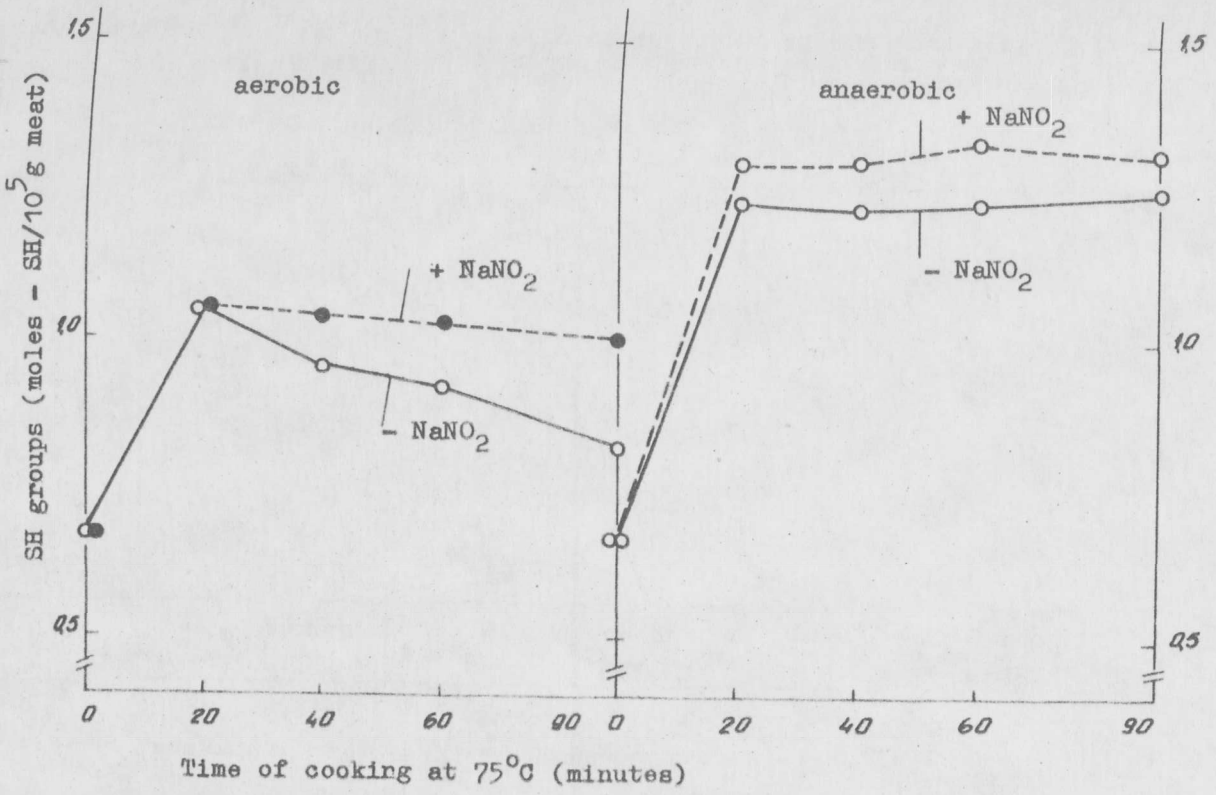

Fig.10. Effect of cooking condition on the behavior of SH groups of meat homogenate in rapid curing process

Table 1. Effects of some fractions of porcine muscle on the behavior of nitrite and the formation of cooked cured meat color in rapid curing process

Fraction	Remaining $NaNO_2$ after cooking a)		$NaNO_2$ decomposed after cooking a)		CFA	
	ppm	Index number b)	ppm	Index number c)	Absorbance at 395 mμ	Index number d)
Whole muscle homogenate	20.8	83	4.2	100	0.233	100
Sarcoplasm	21.2	85	3.8	90	0.167	72
Myofibrils	22.2	89	2.8	67	0.145	62
Mitochondria	22.5	90	2.5	60	0.155	67
Microsomes	23.2	93	1.8	43	0.149	64

a) Cooked for one hour at 75°C immediately after 25 ppm of $NaNO_2$ had been added to each fraction.

b) Figures for index number were calculated on the basis of 25 ppm of nitrite added as 100

c) Figures for index number were calculated on the basis of the amount of nitrite decompo sed in whole muscle homogenate as 100.

d) Figures for index number were calculated on the basis of the absorbance value for who le muscle homogenate as 100.