Reaction of nitrite with sarcoplasmic proteins

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In addition to the recent works[1-9] on the problems concerning the formation of cured meat color briefly reviewed in the previous paper[10], the results of investigations on the reaction of nitrite in meat products and its distribution in myofibrils and actomyosin fractions[11], non-enzymatic reduction and oxidation of heme pigments by nicotinamide adenine dinucleotide and flavins[12], and the relation between enzymatic activities and myoglobin content in bovine and porcine muscles[13] have also been reported quite recently.

In processing cooked sausage, a rapid emulsion curing process has come to be widely employed in our country of late, where the raw meat emulsion is cooked immediately after the comminuted raw meat has been mixed with curing ingredients and then stuffed into a casing.

In the previous work[10] it has been observed that of all the four fractions obtained from porcine skeletal muscle, viz., sarcoplasm, myofibrils, mitochondria and microsomes fractions, sarcoplasm fraction exhibited the most favorable effects on the decomposition of nitrite and the formation of cooked cured meat color in the above-mentioned rapid curing process under anaerobic condition.

The main purpose of the present work is to investigate the effects of sarcoplasm, in particular the low-molecular fraction of sarcoplasm obtained from porcine skeletal muscle on the behavior of nitrite and the formation of cooked cured meat color in the above-mentioned rapid curing process under anaerobic condition.

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Experimental

1. Sarcoplasm fraction was prepared from minced porcine skeletal muscle(M. adductores) just after slaughter following the procedure of Greaser et al.[14].

2. Whole, high- and low-molecular fractions of sarcoplasm were prepared by the procedure given in Fig. 1.

minced porcine skeletal muscle 100 g
(M. adductores)
[Greaser et al. (1969)]
sarcoplasm fraction 400 ml
concentrate to 80 ml in a rotary vacuum evaporator kept at 10°C
40 ml 40 ml

Reaction of Nitrite with Sarcoplasmic Protein.

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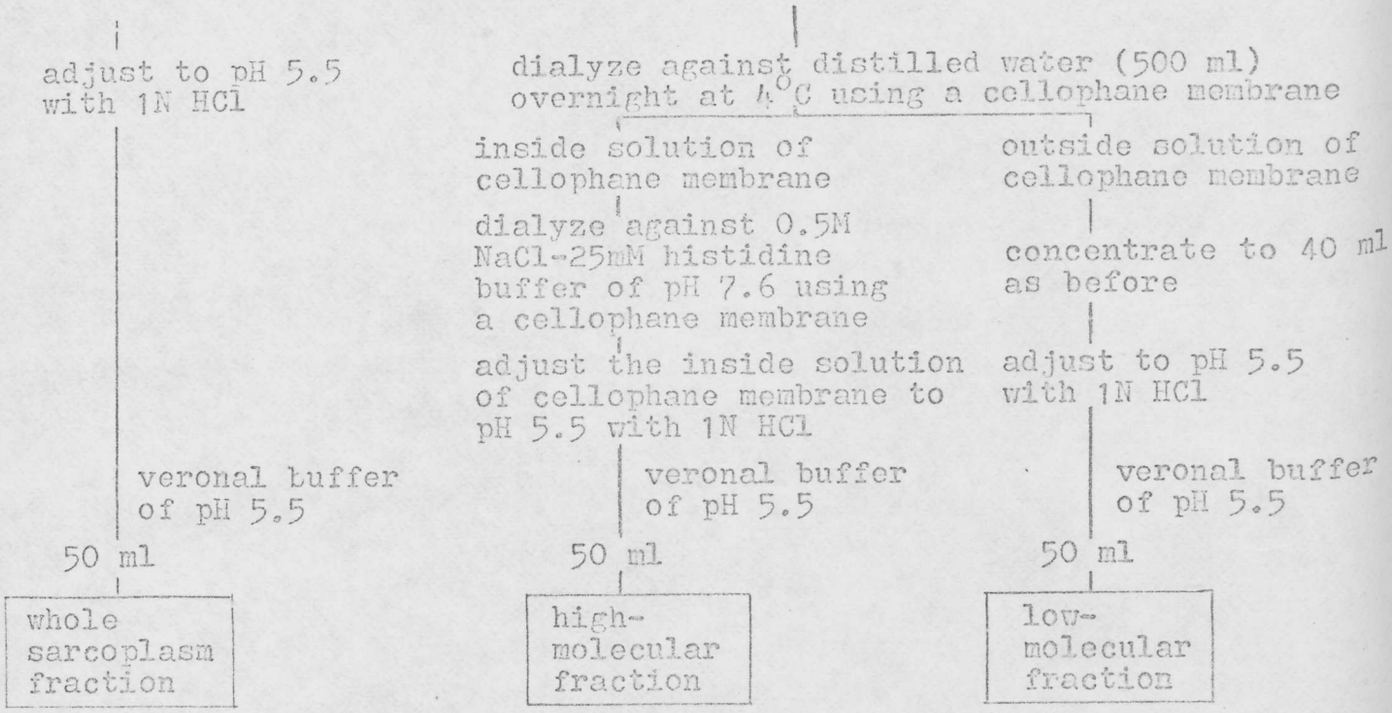

Fig. 1. Preparation of whole, high- and low-molecular fractions of sarcoplasm from porcine skeletal muscle

3. Purified porcine myoglobin(Mb) was prepared in the same manner as described in the previous paper[10].

4. Figures for color formation ability(CFA), nitrite, reducing ability(RA) and sulfhydryl groups were determined in the same manners as described in the previous paper[10].

5. Each test sample of whole, high- and low-molecular fractions of sarcoplasm was prepared according to the procedure given in Fig. 2, and then subjected to the determination of CFA, nitrite, RA and SH groups.

each sarcoplasm fraction 1 ml
(in a Thunberg tube)
veronal buffer of pH 5.5 1 ml
11% Mb aqueous solution 0.1 ml*
veronal buffer of pH 5.5 containing 0.22% $NaNO_2$ 0.1 ml*
cook at 75°C for one hour at a vacuum of 5 mm Hg
cool with running tap water for 10 min
determine CFA, $NaNO_2$, RA and SH groups

* final concentration { Mb 0.5%, $NaNO_2$ 0.01% }

Fig. 2. Preparation of each test sample of whole, high- and low-molecular fractions of sarcoplasm from porcine skeletal muscle

6. The low-molecular fraction of sarcoplasm from porcine skeletal muscle was further separated into fifty fractions of 5 ml each by Sephadex G-50 gel filtration following the procedure given in Fig. 3, and then each fraction of 5 ml was subjected to both the determination of ninhydrin-positive substance by the method of Yemm and Cocking[15] and the determination of carbohydrate by the method of Scott and Melvin[16].

Further, as shown in Fig. 4, it has been found that the low-molecular fraction of sarcoplasm has an absorption maximum at 248 mμ. This means that there is a possibility of the existence of some unknown substance(s) having an absorption maximum at 248 mμ. Therefore, each fraction of 5 ml was also subjected to the measurement of the absorbance at 248 mμ.

minced porcine skeletal muscle 50g
|
(M. adductores)
[Greaser et al. (1969)]
sarcoplasm 200 ml
|
concentrate to 40 ml in a rotary vacuum evaporator kept at 10°C
|
dialyze against distilled water(500 ml) overnight at 4°C using a cellophane membrane
|
outside solution of cellophane membrane
|
concentrate to 15 ml as before
|
separate into 50 fractions of 5 ml each by Sephadex G-50 gel filtration at 4°C

conditions
- column : 2.5 × 40 cm
- buffer : 0.1M NaCl-5mM histidine buffer (pH 7.6)
- flow rate : 0.5 ml/min

Fig. 3. Fractionation of the low-molecular fraction of sarcoplasm from porcine skeletal muscle by Sephadex G-50 gel filtration

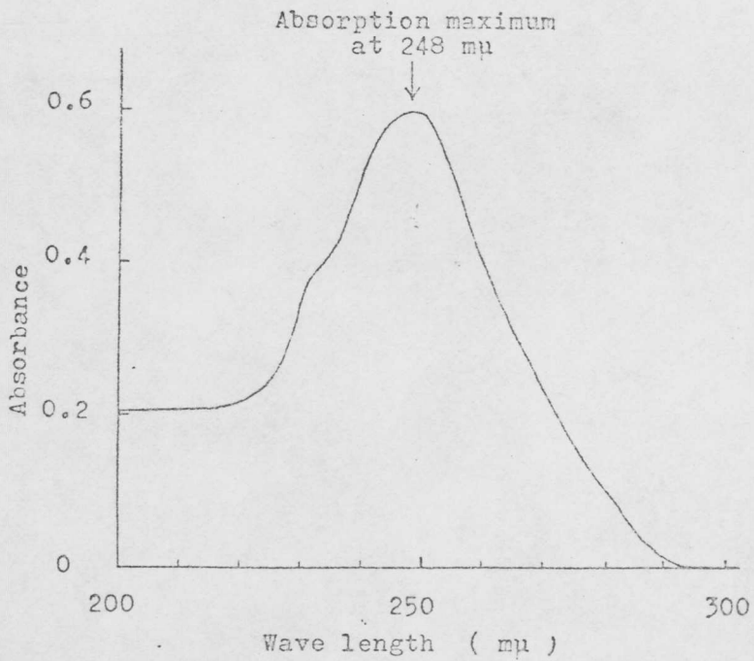

Fig. 4. Ultraviolet spectrum of the low-molecular fraction of sarcoplasm from porcine skeletal muscle

(In this case, the low-molecular fraction sample was prepared by diluting the outside solution of cellophane membrane obtained by the procedure given in Fig.3 10-fold with 0.1M NaCl-5mM histidine buffer of pH 7.6.)

7. Each test sample of the fractions of 5 ml each of the low-molecular fraction of sarcoplasm fractionated was prepared following the procedure given in Fig. 5, and then subjected to the determination of CFA, nitrite, RA and SH groups.

each fraction of 5 ml of the low-molecular fraction of sarcoplasm fractionated
↓
adjust to pH 5.5 with 0.1N HCl solution
↓
make up to 10 ml with veronal buffer of pH 5.5
↓
pipette 2 ml into a Thunberg tube
| 2.75% Mb aqueous solution 0.1 ml*
| veronal buffer of pH 5.5 containing 0.055% $NaNO_2$ 0.1 ml*
↓
cook at 75°C for one hour at a vacuum of 5 mm Hg
↓
cool with running tap water for 10 min
↓
determine CFA, $NaNO_2$, RA and SH groups

* final concentration { Mb 0.125% ; $NaNO_2$ 25 ppm }

(corresponding to 0.5% Mb and 0.01% $NaNO_2$ in meat sample)

Fig. 5. Preparation of each test sample of the fractions of 5 ml each of the low-molecular fraction of sarcoplasm fractionated

Results and Discussion

1. Effects of whole and fractionated sarcoplasm fractions of porcine skeletal muscle on the behavior of nitrite and the formation of cooked cured meat color

As shown in Table 1, the low-molecular fraction of sarcoplasm exerted evidently more favorable effects than the high-molecular fraction on the decomposition of nitrite and the formation of cooked cured meat color.

2. Distribution patterns of CFA, RA, and the amounts of SH groups, remaining nitrite, ninhydrin-positive substance(s), carbohydrate(s) and unknown substance(s) having an absorption maximum at 248 mμ in the low-molecular fraction of sarcoplasm on Sephadex G-50

According to the results given in Fig. 6, a main peak exhibiting the most favorable effect on the formation of cooked cured meat color was observed in the region between fraction

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Table 1. Effects of whole and fractionated sarcoplasm fractions of porcine skeletal 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 sarcoplasm fraction	68.0	68	32.0	100	0.625	100
High-molecular fraction	83.2	83	16.8	53	0.408	65
Low-molecular fraction	74.5	75	25.5	80	0.512	82

a) Cooked for one hour at 75°C immediately after 100 ppm of $NaNO_2$ had been added to each fraction.
b) Figures for index number were calculated on the basis of 100 ppm of $NaNO_2$ added as 100.
c) Figures for index number were calculated on the basis of the amount of $NaNO_2$ decomposed in whole sarcoplasm fraction as 100.
d) Figures for index number were calculated on the basis of the absorbance value for whole sarcoplasm fraction as 100.

numbers 35 and 40 or so, and in this region both the amounts of SH groups and nitrite decomposed were the largest, and the reducing ability was the most striking too.

In addition, as shown in Fig. 7, this region was found on the whole also rich in ninhydrin-positive substance(s), carbohydrate(s) and unknown substance(s) having an absorption maximum at 248 mμ.

It seems very interesting that in the rapid emulsion curing process under anaerobic condition, according to the results of the present investigation, in the whole muscle tissue the sarcoplasm fraction, in particular the low-molecular fraction of sarcoplasm was the most efficacious in promoting the formation of cooked cured meat color, and that the results of the experiment fractionating the low-molecular fraction of sarcoplasm by Sephadex G-50 gel filtration represented in Fig. 6 and Fig. 7 seem to support the suggestion that the substances which may have played most important roles in developing cooked cured meat color may probably be those having fairly low molecular weights.

With great interest further work is in progress to clarify the chemical properties of the substances existing in the low-molecular fraction of sarcoplasm and exhibiting the most favorable effect on the

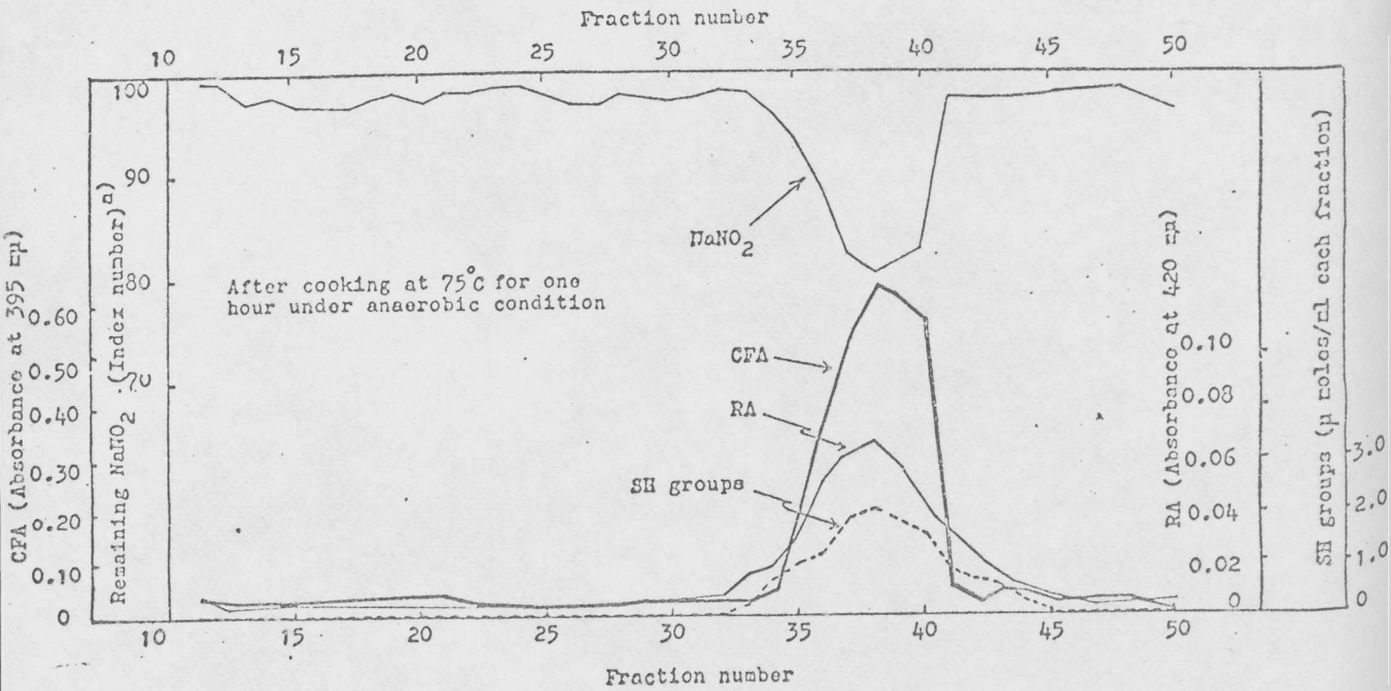

Fig. 6. Distribution patterns of CFA, RA and the amounts of SH groups and remaining $NaNO_2$ in the low-molecular fraction of sarcoplasm from porcine skeletal muscle on Sephadex G-50

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

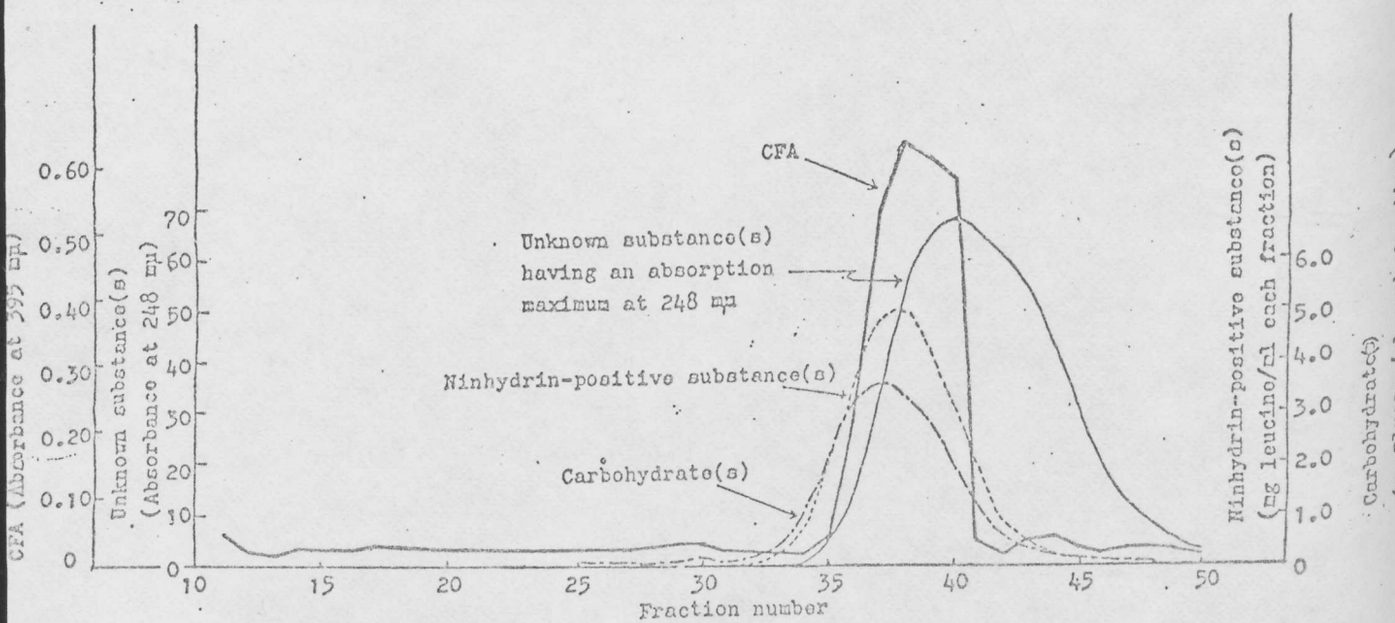

Fig.7. Distribution patterns of CFA, ninhydrin-positive substance(s), carbohydrate(s) and unknown substance(s) having an absorption maximum at 248 mμ in the low-molecular fraction of sarcoplasm from porcine muscle on Sephadex G-50

formation of cooked cured meat color in the rapid emulsion curing process under anaerobic condition.

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