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UTILIZATION OF NITROSOHEMOGLOBIN AS A COLORANT OF PROCESSED MEAT PRODUCTS

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INTRODUCTION

The colour of meat products is determined by the content of nitrite added as a colour forming agent in curing. Its use, however, has decreased out of concern for possible carcinogenic nitrosamine production in processed meat products. This in turn has led to problems of decrease in colour formation, discoloration and yellowing. The red cell fraction from slaughtered animal blood presently has few uses and this possible applications in foodstuffs are being sought. Animal blood Hb is used as a natural colorant in meat products, but its preparation is difficult.

Hb is a heme pigment in meat and, with myoglobin, is directly related to colour development. We prepared Hb from cattle blood and examined the possibility of its use for accelerating cured meat coloration (Sakata et al, 1990; 1992). Using cattle blood Hb powder, the best conditions for producing NOHb from Hb in regard to $NaNO_2$-NaAsA concentration, pH, time and temperature were determined. The nitrosation of Hb in a Hb reaction mixture proceeded rapidly and the colour forming ratio (CFR) was high and stable. Colour formation increased in sausage following the addition of the Hb reaction mixture. NOHb quantitatively contributed to meat product colour that was assessed based on nitrosoheme pigments (NOHP).

The present study was conducted:
(1) to separate and prepare Hb from cattle blood to determine optimum conditions for nitrosation and storage of NOHb; and
(2) to establish a method for using Hb as a natural colorant.

MATERIALS AND METHODS

Preparation of Hb solution and estimation of Hb purity

Blood from Holstein cows (average 5 years old) just after slaughter was made anti-coagulating. The blood was centrifuged to obtain the red cell fraction which was washed with physiological saline (0.9% NaCl) solution. This fraction was dialysed against water for hemolysis and then centrifuged. The Hb sediment was used as the Hb solution (Hb conc. 26.0%), whose method of preparation is shown in Figure 1. The Hb solution was diluted to 1/100 with water and the visible absorption spectral pattern was recorded. The solution was subjected to Toyopearl HW-50F gel chromatography (Toyo Soda Co. Ltd.). Absorbance at 525nm (isobestic point for heme pigment derivatives, Bowen, 1949; Krzywicki, 1982) and 280nm of the eluent was measured. Hb purity was determined from the elution profiles.

Preparation of the Hb reaction mixture

The mixture was prepared as previously reported (Sakata *et al.*, 1990; 1992). pH of the Hb solution was adjusted to 4.5 with conc. lactic acid (85.0-92.0%) and $NaNO_2$, AsA and NaCl powders were added at 25mM, 25mM and 2%

respectively. A mixture containing 40% glucose was also prepared. The mixtures were incubated at 2°C and 20°C respectively and NOHb (estimated by CFR) was measured periodically.

Determination of CFR and residual nitrite

CFR and nitrite in the Hb reaction mixture and sausage sample were determined. CFR was expressed as % NOHP to total heme pigments (TOHP). TOHP (Hb in the reaction mixture) and NOHP (NOHb in the reaction mixture) were extracted with 75% acetone-0.7% HCl and 75% acetone respectively. The contents of both pigments were determined based on absorbency at each maximum absorption wavelength (TOHP: 383nm; NOHP: 395nm). CFR was calculated from the values for absorbency. Residual nitrite content was measured using the acetone extract of NOHP following the method of Mirna and Schütz (1972) with a slight modification.

Aerobic bacterial count

This parameter was determined using standard plate agar medium (37°C). Hb solution immediately following its preparation and the Hb reaction mixture stored for three days were used.

Preparation of sausage containing the Hb reaction mixture

Non-meat ingredient solution containing proteins was added to porcine loin meat at the same weight and cured with 2% NaCl, 0.01% $NaNO_2$, 0.1% NaAsA and 0.3% polyphosphates. The Hb reaction mixture was added to above system at 0.5 or 1.0% by weight, and each sample was mixed, stuffed and cooked (75°C, 60 minutes) to obtain sausage.

RESULTS AND DISCUSSION

Purity of Hb prepared from cattle blood

As evident from the spectrum of the Hb solution in Figure 2, bovine blood Hb was a typical oxygenated derivative and the Hb solution was bright red. Based on the gel chromatogram in Figure 2, the Hb solution contains very small amounts of proteins as impurities (calculated area ratio of first large elution peak at 280nm: 98.1%). The maximum peak of commercial bovine Hb (Sigma H-2500) eluted under the same conditions showed the same elution position as the Hb solution. The present Hb from cattle blood was thus highly pure and suitable for subsequent experiments.

Effects of added glucose on CFR and residual nitrite content of the Hb reaction mixture

Figure 3 shows the effects of added glucose on the nitrosation of Hb in the reaction mixture. More than 80% of total Hb was rapidly nitrosated following the condition of 25mM $NaNO_2$-25mM AsA and adjustment of pH to 4.5 and the system was stored at 20°C and 2°C. On the third day of storage, very little residual nitrite could be detected in the Hb reaction mixture (Table 1). Glucose added to the reaction mixture caused CFR to increase by more than 90% after reaction for one day. Glucose may possibly promote nitrite reduction by ascorbate as well as colour formation (Nagata and Ando, 1971). It lowers water activity and delay putrescence by bacteria. Accordingly, commercial Hb solution prepared from cattle blood is made to have more than 40% glucose (ex. Harimex Hemo Colour). In this study, glucose content was 40%. The NOHb formed in the Hb reaction mixture remained essentially unchanged for 20 days at 2°C (Figure 4).

Bacterial count of the Hb reaction mixture

Table 2 shows bacteria count data for the Hb reaction mixture. No aerobic bacteria could be detected in the solution after three days, as was also observed for samples without glucose. This was considered due not to glucose but inhibitory effect of added nitrite on bacterial growth (Pearson and Tauber, 1984).

Effects of Hb reaction mixture on experimental sausage

The extent of colour formation was determined in the sausage containing pork, commercial soy protein isolates, blood plasma, egg white and Hb reaction mixture (Table 3). The more of these non-meat proteins are used in processing meat products, the less is the desired colour of the products. Colour formation is due only to heme pigments of meat, mainly myoglobin. Thus, in the present study, many non-meat proteins were added to meat as shown in Table 3 so as to determine the roles of the Hb colorant. The content of NOHP in the sausage increased when 0.5% or 1.0% of the Hb reaction mixture were added to the system. Based on this content, increase in NOHP was 106.5% and 206.5% respectively that of the control. NOHb in the Hb reaction mixture thus quantitatively contributes to the colour formation of meat products. The reasons why the addition of Hb reaction was limited to within 1% are as follows:
(1) Heme pigment finally attains this content in consideration of commercial meat products; and
(2) Colour cannot be visually assessed following addition of the Hb reaction mixture at more than 1%.

The Hunter value of sausage surface was calculated (Table 4). Redness increased with the addition of Hb reaction mixture (Hunter-a value). Virtually no decrease in this parameter could be detected at two weeks of storage at 2°C (in the dark room). NOHb in added Hb reaction mixture thus remans stable during heat exposure, and pigment may possibly impart and augment cooked cured meat colour to the end products. In the discoloration test with fluorescent lighting (15W, irradiated from the height of 30cm), the sample containing the Hb reaction mixture showed a Hunter-a value higher than that of the control. Significant decrease in this value was not seen. The red colour could be virtually observed to be well maintained.

TBA value of the experimental sausage was determined by the distillation method (Yamauchi and Ando, 1973) after

Table 1. Residual NO^-_2 (ppm) in Hb reaction mixture stored for three days.

+ Glucose		- Glucose	
2°C	20°C	2°C	20°C
8.5	ND	ND	ND

ND = not detected

Table 2. Bacterial count of the Hb reaction mixture with 40% glucose.

Sample	Aerobic plate count
Hb solution just after preparation	8.6×10^3 cfu/g
Hb reaction mixture after three days*	Not detected

* stored at 2°C or 20°C

Table 3. Effects of adding the Hb reaction mixture to experimental sausage (I).

Sample[1]	Hb reaction mixt[2] added	NOHP -Hb(A)	NOHP +Hb(B)	Increase rate of NOHP (B-A)/A x100	CFR %
Sausage	0.5	0.046	0.095	106.5	80.4
	1.0	0.046	0.141	206.5	87.3

[1] Non-meat ingredient solution containing 5% soy protein isolates, 3% blood plasma and 3% egg white was added to ground porcine loin meat at the same weight and cured with 2%NaCl, 0.01%$NaNO_2$, 0.1%NaAsA and 0.3% polyphosphates. Hb reaction mixture was added in curing. The cured meat was stuffed into polyvinylidene chloride casing (50mm) and cooked at 75°C for 60 minutes.

[z] Hb reaction mixture containing 40% glucose was stored fro seven days at 2°C after being prepared (CFR=91.0%).

Table 4. Effects of adding the Hb reaction to experimental sausage (II).

Hb reaction mixture added (%)	Storage period at 2°C (weeks)	Hunter value L	a	b	Hunter value (after lighting)* L	a	b
----	0	62.7	11.3	10.3	63.5	9.4	10.4
	2	62.2	11.8	10.4	63.5	9.0	11.3
0.5	0	53.8	16.1	8.5	55.6	13.0	9.0
	2	55.1	15.6	8.6	54.5	13.0	9.2
1.0	0	47.7	17.6	7.8	50.1	15.4	7.8
	2	50.6	17.1	7.9	49.4	15.7	9.2

* Fresh and stored samples were irradiated under florescent lighting for two hours at room temperature.

Table 5. TBA values of experimental sausage containing the Hb reaction mixture.

Hb reaction mixture added (%)	TBA value (MAmg/kg)*
----	0.045
0.5	0.034
1.0	0.062

* Sausage was analyzed after two weeks of storage at 2°C.