Research to lessen the amounts of curing agents in processed meat through use of rock salt and carbon monoxide

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Abstract. This study was carried out to examine the reddening of meat products due to the addition of natural yellow salt (YS) and carbon monoxide (CO). Following YS or NaCl addition at 2% to pork subsequent to nitrite (0~100 ppm) treatment, color development due to this addition was analyzed visually. Heme pigment content in the meat was also determined spectrophotometrically. YS was found to bring about greater reddening than NaCl, indicating residual nitrite and nitrate content to be significantly higher in meat containing YS, through the amount of either was quite small. The amount of nitrite required for a red color to develop was noted to vary significantly from one meat product to another. CO treatment of pork caused the formation of carboxy myoglobin (COMb) with consequent reddening of the meat. COMb was shown to be heat-stable and form stably at pH 5.0 to ~8.0 and to be extractable with water, but was barely extractable at all with acetone. Nitric oxide was found to have greater affinity toward myoglobin (Mb) than CO. Nitrosyl Mb was noted to be stable in all meat products examined. CO was seen to be capable of controlling the extent of lipid oxidation.

1. Introduction
The purchase of meat products is initially based on color prior to any assessment of parameters such as odor, taste or texture. Meat color is the primary determinant of any decision to purchase a meat product. Color developing agents such as nitrite and nitrate have been found quite useful but food safety considerations limit the extent of their usage.

In recent years, attention has come to be increasingly directed to food safety in response to consumer demands in this regard. Accordingly, techniques for effectively enhancing the red color of meat products through the least possible use of nitrite and nitrate, but with usage of naturally occurring ingredients, have thus become modern day focal points of emphasis [1,2]. As substitutes for curing agents, natural mineral substances are being avidly examined for potential application, both in Japan and foreign countries. The findings should prove valuable for enhancing and maintaining good food appearance as well as being in the best interests of human health.

However, unexpected reddening and discoloration problems have recently been pointed out and effective solutions must be found. The reddening effect of Himalayan rock salt on meat products was previously reported [3]. In uncured meat products, carboxy myoglobin (COMb) can be considered a major reason for unexpected meat reddening. Carbon monoxide (CO) binds strongly to myoglobin (Mb) to form COMb, which shows a stable bright red color. The addition of CO to food is prohibited by law in Japan, though in foreign countries, this compound is applied as a gas in food packing, and its use in meat products is being carefully examined [4]. Should CO be capable of lowering nitrite content in meat, its use should not meet with any consumer objections.
Natural salts, particularly those in Himalayan rock salts, should prove favorable candidates for reddening of meat products, since they contain nitrite along with nitrate in only small amounts, plus minerals. Yellow salt (YS) from Himalayan products was, therefore, examined in this study for its capacity to bring about a red coloration in meat products and the results were compared with those for ordinary cooking salts.

Additionally, the characteristics of CO, such as its strong binding to Mb, leading to an attractive red meat color, were also investigated in this study. A comparison was made of parameters such as heat stability, extractability and formation of COMb at different pHs. Mb derivatives were then investigated spectrophotometrically subsequent to CO flushing into nitrosylmyoglobin (NOMb). The antioxidative effects of CO on meat were also examined.

2. Materials and methods

2.1. Natural salts experiment
Meat taken from pig leg was depleted as much as possible of its fat and connective tissue and then minced. YS or NaCl was added at 2% to each meat sample, along with 0.1% sodium ascorbate and NaNO2 in the concentration range: 0, 10, 30, 50 or 100 ppm. After 4 days storage at 4°C under anaerobic conditions, the mince samples were cooked at 75°C for 30 min. After sample cooling, color was assessed visually and then with a spectral colorimeter.

The color forming ratio (CFR) [5], and heme pigment content were measured by acetone extraction. Residual nitrite and nitrate content were determined according to the method of Mirna and Schütz [6] and by copper-cadmium column reduction, respectively.

2.2. CO experiment
COMb was prepared in a model solution (0.1% Mb-0.2% Na2SO3O4, pH 5.5 with CO gas flushing) and cooked at 70°C for 20 min. The absorption spectra of COMb at pHs adjusted to 5.0-9.0 were monitored.

CO-treated meat and cured meat samples were prepared with pig thigh flushed with CO, to which 100ppm NaNO2 had been added, and the meats then kept at 2°C for 4 days. COMb and NOMb were extracted with water or 75% acetone and the absorption spectra of the solutions thus obtained were used to determine the extent of COMb formation. Lipid oxidation in each meat sample was also evaluated by 2-thiobarbituric acid (TBA) analysis [7].

Absorption spectra of NOMb solution (0.1% Mb-1% Na ascorbate-0.1% NaNO2, pH 5.5) previously flushed with CO was measured to determine the extent of COMb formation.
3. Results and Discussion
3.1. Effects of yellow rock salt and low levels of nitrite and nitrate on meat color
In mince with added NaCl and without nitrite, the CFR and a* (redness) were less than those measured in YS mince samples. In YS mince samples with and without nitrite, meat redness and CFR were found to exceed 70%, regardless of the low nitrite range, from 0 to 100 ppm (figure 1). Compared to NaCl mince samples, residual nitrite content was usually higher in mince with added YS (figure 2). The YS itself contained more nitrite than nitrate. YS was, thus, shown to effectively enhance meat reddening. Small amounts of nitrite, nitrate and minerals in the YS could possibly have contributed to this finding.

![Figure 1. Color forming ratio (CFR).](image1)

![Figure 2. Residual nitrite levels.](image2)

![Figure 3. Colour forming ratio as a function of heme pigment content.](image3)

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The CFR was noted to change with increase in heme pigment content in pork mince with added YS. When the content of heme was quite small, the CFR in the mince samples was larger than in mince with more heme (figure 3). This could possibly have been due to the limited capacity of heme nitrosation to bring about meat reddening.

3.3. Effects of CO gas on meat and NOMb
The meat sample extracts showed the specific absorption spectra of COMb. In a model solution, COMb was heat stable and stable throughout the given cooling times (figure 4), with pH maintained within a range of 5.0 to ~8.0. Meat pH is normally approximately 5.5, so this indicates the red color of meat should be maintained in the presence of CO.

The extraction of COMb was possible with water, though virtually impossible with acetone (figure 5), in contrast to NOMb (data not shown). Thus, the extent of COMb formation compared to total Mb could not be determined in the present study.

Even when CO was added to NOMb solution, the absorption spectrum of NOMb showed no change (data not shown), thus indicating the higher affinity of NO than CO toward Mb. The addition of NaNO₂ (100 ppm) to COMb resulted in a change in the absorption spectrum of the NOMb formed (data not shown).

![Figure 4. Absorption spectra of COMb solution after heating and cooling for up to 180 mins.](image1)

![Figure 5. Absorption spectra of CO-treated and cured meat after extraction with.](image2)

TBA values of raw meat increased significantly during storage, but TBA values for CO-treated pork decreased to nearly the same as those observed in the NO-group, compared to the control, from day 7 to day 21 of storage (figure 5). Therefore, CO was shown to be as capable as nitrite of controlling lipid oxidation in raw pork.

CO proved inhibitory to microorganism growth, as was also noted for nitrite (data not shown).
4. Conclusion
Himalayan rock salt was found to be effective for enhancing the red color of meat at very small nitrite content or even in the absence of nitrite. The CFR in cooked meat changed with heme pigment content in meat, owing to the low degree of nitrosation in the meat.

COMb was noted to form rapidly in meat when Mb and CO were combined, giving rise to a bright red color in the meat. It follows then, that CO could serve to significantly lessen the amount of nitrite added to meat products, while still supporting formation of an acceptable red color. CO could also function in a very similar fashion as nitrite in meat. CO itself could possibly be a factor for the unexpected reddening of meat as noted at food service facilities, but confirmation of this point will require additional research.

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References
[1] Mancini R A and Hunt M C 2005 Meat Sci. 1 100-121
[2] Sakata R 2010 Meat Sci. 86 243-248
[3] Kaneko M and Sakata R 2014 Proc. 60th Inter. Cong. Meat Sci. Technol., Punta del Uste, Uruguay, CD 068
[4] Suman S P, Hunt M C, Nair M N and Rentfrow G 2014 Meat Sci. 98 490-504
[5] Sakata R, Ohso M and Nagata Y 1981 Agric. Biol. Chem. 45 2077-2081
[6] Mirna A and Schütz G 1972 Fleischwirt. 52 337-1338
[7] Yamauchi K, Murata H, Ohashi T, Katayama H, Pearson A L, Okada T and Yamakura T 1991 Nippon Shokuhin Kogyo Gakkaishi 38 545-552
