CARBON MONOXIDE AS A COLORANT IN DRY FERMENTED SAUSAGES

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Introduction

Finding alternative colorants in dry cured meat products for nitrite ($NaNO_2$) and nitrate ($NaNO_3$), a precursor to nitrite, has been a long time challenge. Carbon monoxide (CO) is successfully used for improving the color of packaged fresh meat and heme-containing fish (Sørheim, 2005). A recent experiment showed that CO could substitute for nitrite in cooked beef and pork sausages, utilizing the high heat denaturation temperature of carboxymyoglobin (COMB) (Sørheim et al., 2004). The initial color of CO-treated cooked sausages was bright red, but the color stability was insufficient compared to nitrite sausages. CO also has the possibility of replacing nitrite in dry cured sausages, depending on the stability of COMB at the low pH in fermented products.

Objectives

To study the effect of substitution of nitrite with CO on the color of dry fermented beef sausages.

Methodology

The main experiment of dry fermented beef sausages consisted of 4 treatments: Raw materials pre-treated with 1 % CO/ 99 % N2 (CO-R), direct flushing of the batters with 1 % CO/ 99 % N2 (CO-D), addition of 120 ppm NaNO2 to the batters (N), and control with no CO or NaNO2 (C). All treatments, except the control, were produced with and without addition of 500 ppm ascorbic acid. The experiment was repeated, giving a total of 14 batches.

The basic recipe consisted of 91.5 % beef semimembranosus muscles, 4.5 % NaCl, 0.5 % glucose, and starter culture (BiocarnaTM Ferment CXK, Danisco, Copenhagen, Denmark) with 0.3 g per batch dissolved in 3.5 % water. Each batch weighed 1.5 kg.

Fresh meat was ground twice through a 4 mm plate. Meat for the CO-R treatment was placed in polyamide pouches, compressed to < 0.5 mm, packaged in 1 % CO / 99 % N2 with < 0.2 % residual O2, and stored at 3 °C for 5 days. Batters were prepared in a small Stephan UM5 chopper (A. Stephan u. Söhne, Hameln, Germany) with a lid and double bladed knives. The total chopping time was 2 min. For CO-R, pretreated meat with CO was used without additional supply of CO in the chopper. Batters for CO-D were flushed with 1 % CO/ 99 % N2 at 2 bars during the chopping. All batters were filled in fibrous casings of 43 mm x 50 cm (Viskase, Willowbrook, IL, USA). The fermenation started at 22°C and 95 % RH, were reduced to 18 °C and 90 % RH at 3 days, and was held at 15°C and 85 % RH for the remaining 9 days. The sausages were not smoked. pH and weight loss were recorded during the production.

CIE L*a*b* values (lightness, redness, yellowness) of the sausages were measured with a Minolta Chroma Meter CR-300 (Minolta Camera Co., Osaka, Japan) with 8 mm viewing port, 2° viewer angle and illuminant D65. Visual color evaluation was performed by three trained assessors using a 5 point scale of 1 = very red to 5 = extremely gray/brown. Instrumental and visual color was recorded 0, 15 and 60 min. after slicing of the sausages. Significant differences in color were evaluted by a multivariate method described by Langsrud (2002). Denaturation of myoglobin in CO-R and N sausages was analysed by the method of Krzywicki (1979). pH of batters and sausages was measured with an InLab 427 gel electrode (Mettler-Toledo, Urdorf, Switzerland).

Spectra of COMB and nitrosomyoglobin (NOMB) were obtained by using 0.2 % equine myoglobin (Sigma Chem. Co., St. Louis, MO, USA) dissolved in 0.5 M phosphate buffers at pH 5.6 and 4.7. The solutions were first converted to reduced deoxymyoglobin with solid sodium dithionite (Na2S2O4). CO (100 %) was lightly flushed through the solutions for 30 sec., and NaNO2 (100 ppm) was added. The solutions were scanned in the range 450 to 650 nm with 1 nm intervals using an Agilent 8453 UV-visible spectrophotometer (Agilent Technologies, Waldbronn, Germany). At pH 5.6, the solutions were scanned after 30 min., and at pH 4.7 after 30 min. and 20 hrs. Due to partial denaturation, the NOMB pH 4.7 solution at 30 min. and both pH 4.7 solutions at 20 hrs were filtered before scanning.

Results & Discussion

The color scores of the sausages immediately, 15 min. and 60 min. after slicing are shown in Fig. 1. Sausages of CO-R were more red than N initially, but the red color of CO-R sausages faded at 15 and 60 min. of air exposure. The CO-R batters were relatively firm and dense, minimizing CO loss via exchange with oxygen during chopping. CO-D was less efficient in preserving color of these sausages than CO-R. The finished CO-treated sausages were discolored at the rim, probably due to O2 uptake through the casings. Fig. 2 with a* redness values confirms the visual color scores. In addition, data of Fig. 2 reveals a beneficial color effect of the antioxidant ascorbic acid for the CO-R and N treatments ($p<0.05$). For all 4 treatments, L* lightness values decreased with display time, but b* yellowness values were not much affected (results not shown).

Initial pH of the batters was 5.6, and final pH of the fermented sausages was 4.7. Weight losses of the sausages ranged from 32 to 35 %. Denaturation of myoglobin was measured to be 61 – 65 % for CO-R and 84 – 90 % for N sausages. The high degree of

denaturation of the N sausages shows that nitrosoheme is a colorful hydrophobic pigment.

Fig. 3 demonstrates two characteristic and similar absorbance maxima at pH 5.6 for COMB (541 and 577 nm) and NOMB (547 and 578), in agreement with Nam & Ahn (2002). Low pH had no effect on the intensity of the COMB spectra, as they were virtually identical at pH 5.6 and 4.7. However, at pH 4.7 the NOMB spectra had lower absorbance at all wavelengths, compared to pH 5.6. The lower NOMB concentration at pH 4.7 was probably due to a more rapid conversion of nitrite ions and nitrous acid to nitric oxide (NO), which may have escaped as a gas before it could react with myoglobin. To minimize NO losses, Rust (1975) recommends nitrite and ascorbate curing brines to be held at alkaline or only slightly acidic conditions. Slinde (1987) found that during ripening of salami sausages the amount of extractable hydrophilic pigments decreased to zero. In our model experiment, the absorbance of COMB at low pH was only slightly lower at 20 hours, indicating that CO stabilizes myoglobin towards denaturation. It is likely that only the 6th position is occupied by CO and that the 5th position of the iron is still bound to the globin chain. The heme group as such may add one or two ligands of CO or NO. To what extent one or two molecules of CO and NO are bound to the denatured form needs to be studied further.

Conclusions

Dry fermented beef sausages with pH 4.7 were initially red after treatment of raw materials with a gas mixture with 1 % CO, and more red than sausages with 120 ppm nitrite added. Direct flushing of the batters with 1 % CO was less efficient for color formation than gas pretreatment. In accordance with previous findings of CO-treated cooked sausages, the color stability of both CO-R and CO-D samples upon air display was insufficient compared to nitrite sausages, and needs to be studied in more detail. Solutions of COMB and NOMB had absorbance maxima at similar positions at pH 5.6. COMB was more stable than NOMB at pH 4.7, indicating loss of NO at acidic conditions.

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Figures

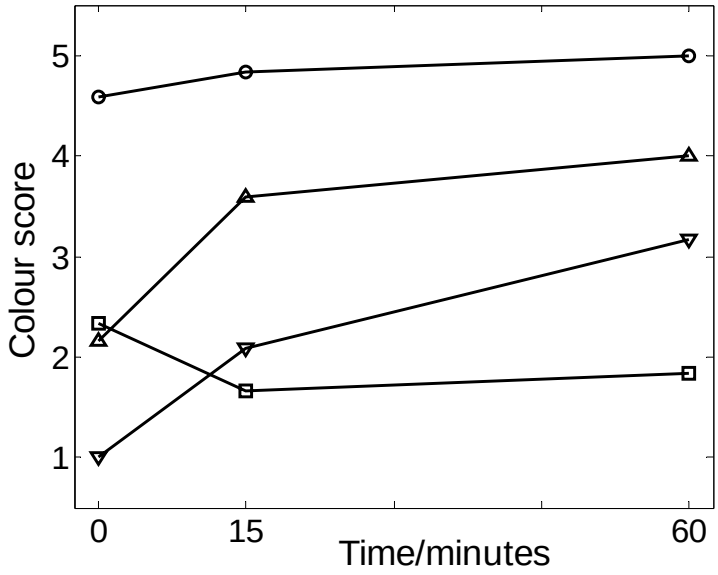

Fig. 1. Visual color score of sliced dry fermented sausages exposed to air for 60 minutes. Symbols: ∇ = CO pretreatment of raw materials (CO-R), Δ = CO flushing of batters (CO-D), □= nitrite (N), o = control with no CO/nitrite (C). Color scale: 1 = very red, 2 = some red, 3 = slightly red, 4 = some gray/brown, 5 = extremly gray/brown.

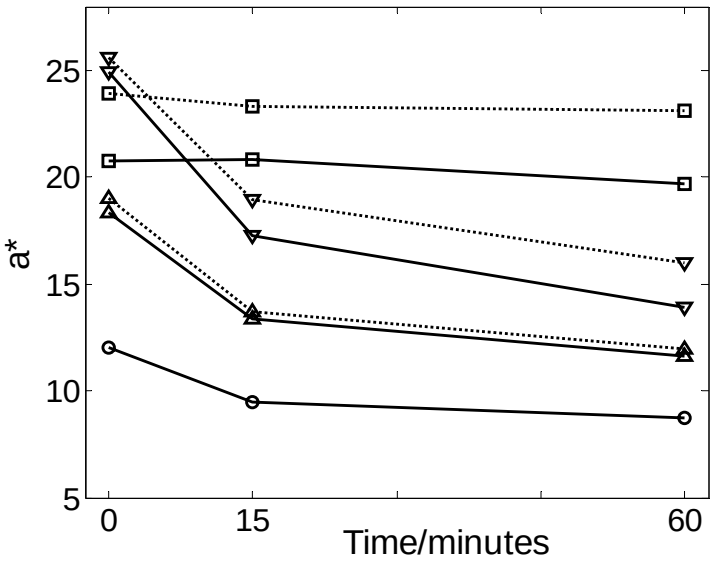

Fig. 2. CIE a* redness values of sliced dry fermented sausages exposed to air for 60 min. For symbols, see Fig. 1. No ascorbic acid = solid lines. With ascorbic acid = dotted lines.

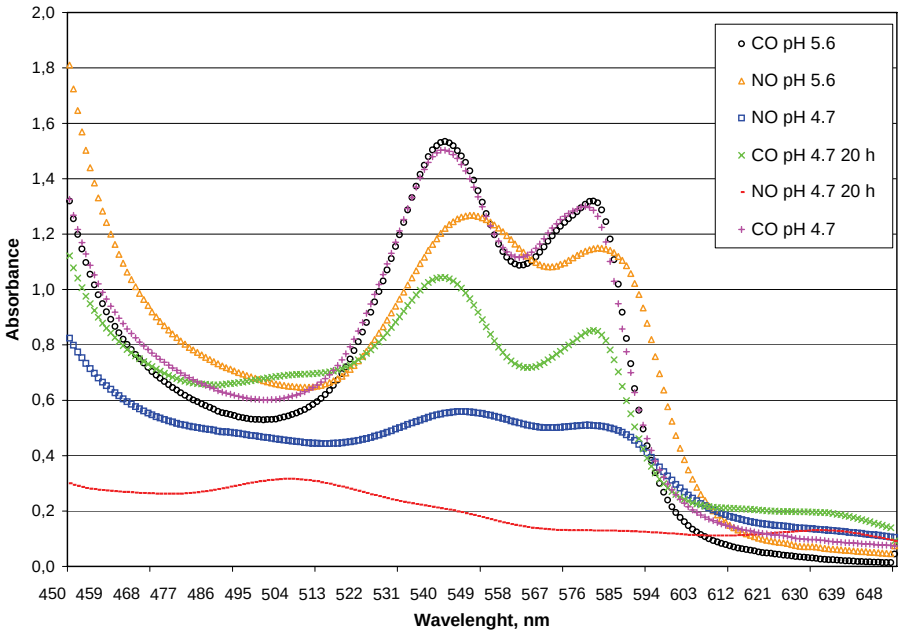

Fig. 3. Absorption spectra of carboxymyoglobin (CO) and nitrosomyoglobin (NO) in solutions with pH 5.6 and 4.7 at 30 minutes and 20 hours (20h).