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Introduction

Nitrite and/or nitrate added in cured meat are involved in the formation of nitroso-compounds (nitrosamines, nitrosothiols and nitrosylheme) potentially mutagenic. Oxidation in meat also affects health by formation of mutagenic aldehydes.

An excessive consumption of cured meat is associated with an increased risk of colorectal cancer.

Therefore, the objective of this study was to evaluate the role of ascorbate regarding the reduction of nitrites in cured meat.

Materials and Methods

Cured meat was prepared with pork shoulder muscle, with different amounts of sodium nitrite (0 to 120 ppm) with or without ascorbate (300 ppm), and cooked at 68.5°C. A **recooking** at 180°C/7 min was also studied.

The **free iron** content was measured by ferrozine assay.

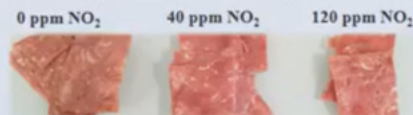
Lipid oxidation was evaluated by measurement of the thiobarbituric acid reactive substances (TBARS).

Nitrosylated heme iron was evaluated by extraction in acetone and visible spectroscopy.

To determine **nitrosothiols**, the S-NO bond was cleaved by HgCl₂ and the **nitrosamine** contents were determined by breaking N-NO bond with UV light. The nitrite released was measured by the Griess method.

Results

- 1) Addition of nitrite in cured meat decreased significantly **free iron** level.
- 2) For the uncured condition, **lipid oxidation** was significantly higher than in cured meat products and ascorbate did not show any significant protective effect.
- 3) Reducing nitrite from 120 to 40 ppm did not affect **nitrosylheme** content and color (see photo below).



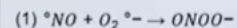
- 4) The level of **nitrosothiols** increased in cured meat from 40 ppm of added nitrite. This effect was especially pronounced in the absence of ascorbate.
- 5) **Nitrosamines** were not detected in this model with the exception of a low level at 40 ppm of nitrite.
- 6) With the **recooking**, mimicking a second cooking process in pizza for instance, production of nitrosamines was observed especially in absence of ascorbate.

Table 1. Effect of nitrite (NO₂) and ascorbate (AH) and of recooking on iron content, oxidation, nitrosation and nitrosylation in cured meat. (n=6; superscripts differ if *p*<0.01)

	0 ppm NO ₂ +AH	40 ppm NO ₂ +AH	80 ppm NO ₂ +AH	120 ppm NO ₂ +AH	120 ppm NO ₂	120 ppm NO ₂ +AH 180°C/7 min	120 ppm NO ₂ 180°C/7 min
Free iron (µM)	82.1 ^a +/- 1.7	28.4 ^b +/- 1.6	26.6 ^b +/- 0.9	34.9 ^c +/- 1.2	35.5 ^c +/- 0.8	-	-
TBARS (mg MDA/kg)	1.37 ^a +/- 0.03	0.69 ^b +/- 0.06	0.72 ^b +/- 0.15	0.51 ^b +/- 0.03	0.51 ^b +/- 0.04	-	-
Nitrosylated heme iron (µM)	1.2 ^a +/- 0.1	83.1 ^b +/- 7.5	98.5 ^b +/- 0.9	89.1 ^b +/- 6.1	82.2 ^b +/- 1.6	-	-
Nitrosothiols (µM)	7.7 ^a +/- 2.2	30.6 ^{ab} +/- 2.0	63.0 ^b +/- 9.1	35.2 ^{ab} +/- 14.1	207.4 ^c +/- 15.4	-	-
Nitrosamines (µM)	0	6.1 +/- 3.2	0	0	0	188.2 ^a +/- 34.4	439 ^b +/- 41.5

Discussion

1) Dismutation of superoxide radical (O₂^{•-}) gives hydrogen peroxide (H₂O₂), implicated in heme iron release. By preventing formation of superoxide radical (reaction 1), nitrite stabilizes heme iron.



2) In meat, free iron is implicated in oxidation *via* the formation of hydroxyl radical (HO[•]) (reaction 2). Thus, by decreasing H₂O₂ and oxygenated free radicals, nitrite acts as a powerful antioxidant.



3) A nitrite decrease could be considered without major impact on color up to 40 ppm.

4) Ascorbate prevents nitrosothiols formation and so limit the transfer of NO group from thiols to heme iron.

5) and 6) The increase of nitrosation observed during recooking can be limited by addition of ascorbate.

Conclusion

The protective effect of nitrite on lipid oxidation is counterbalanced by production of nitrosothiol and nitrosylheme. Ascorbate reduces nitrosamine formation due to recooking.

In future, we will examine the protective effect of some vegetable antioxidants against nitrosation and nitrosylation.