FORMATION OF ZINC PROTOPORPHYRIN IX DURING THE PRODUCTION PROCESS OF DRY FERMENTED SAUSAGES

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Abstract – The aim of this study was to follow the formation of zinc protoporphyrin IX (ZPP) during the production process of dry fermented sausages. Three batches were made, with the addition of nitrite salt, sodium chloride or sea salt, respectively. The formation of ZPP was largely inhibited by nitrite during production of dry fermented sausages. In nitrite-free dry fermented sausages ZPP was formed during production. In this study, the use of sea salt had a positive effect on the formation of ZPP, especially during the fermentation period.

Key Words – ZPP, Colour, Sodium nitrite, Sea salt

I. INTRODUCTION

One of the most important criteria of sensory quality in meat and meat products is colour [1]. The colour of meat products is determined by a combination of different factors including moisture and fat content, but more important is the concentration of myoglobin (Mb) [2], which comprises the majority of the total pigment content in meat and plays a major role in colour perception [3].

While fresh meat is mainly characterized by the presence of the red oxymyoglobin (OMbFe(II)), the meat will attain a dull brown colour (metmyoglobin, MMbFe(III)) during its aging [3]. Therefore, nitrite is added as a curing agent to meat products, in order to form nitrosomyoglobin (NOMbFe(II)) as the main contributor to the characteristic, relatively stable, red colour [4]. Although there are also other technological advantages, like antimicrobial and antioxidant activity, the use of nitrite is controversial. Because of its toxicity and as its involvement in the formation of *N*-nitrosamines [5], the interest remains to produce nitrite-free meat products.

Remarkably, traditional dry-cured Italian Parma ham, wherein only sea salt is added, obtains a stable red colour without any addition of nitrite or nitrate. Wakamatsu et al. [6] [7] assigned the bright red colour of Parma ham to the presence of zinc protoporphyrin IX (ZPP), wherein the ferrous ion in the heme molecule is replaced by zinc.

Three possible formation pathways have been suggested, (i) a slow non-enzymatic reaction; (ii) a bacterial enzymatic reaction, and (iii) an enzymatic reaction where an endogenous ferrochelatase interchanges the two metals Fe(II) and Zn(II) [8] [9].

ZPP is also present in other meat products such as the Spanish Iberian ham, lacking nitrite or nitrate as well. On the other hand, in dry cured ham, bacon and cooked ham with added nitrite, inhibition of ZPP was observed [2]. Using model systems, it was shown that nitrite inhibited the formation of not only ZPP but also of the not metallized form protoporphyrin IX [10]. Additionally, nitrite plays a unique role as an inhibitor of both the enzymatic and the non-enzymatic formation pathways [9].

The advantage of using sea salt is less clear until now. However, sea salt includes various components in addition to sodium chloride, which possibly influence the formation of ZPP in meat products [11].

Wakamatsu et al. [11] concluded that ZPP increased remarkably after 40 weeks of dry-cured ham processing, but there have been no reports on the changes in ZPP content in dry fermented sausages, which have in contrast with dry-cured hams a very short production time of three weeks.

In the present study the changes in ZPP content was followed in three batches of dry fermented sausages, containing nitrite salt, sodium chloride or sea salt, respectively.

II. MATERIALS AND METHODS

Sausage production and sampling

Three batches of dry fermented sausages were made using pork meat (69.6 %), pork back fat (26.8 %), salt (2.8 %), dextrose (0.70 %), sodium ascorbate (0.05 %), spices and a commercial starter culture containing *Lactobacillus sakei, Staphylococcus xylosus* and *Staphylococcus carnosus* (Texel SA306, Danisco, Belgium). Three different salts were investigated, the first batch was made with nitrite salt, containing sodium chloride with 0.6 % nitrite, the second batch was made with only sodium chloride and the third batch with sea salt (La Baleine iodé, gros, France). The meat batter was filled in casings of Ø 90 cm (Naturin, Rejo, Belgium), and after a rest period of 7 days at 4°C the sausages were fermented during three days (24°C/ 95RH %), smoked and subsequently dried (14°C/ 87RH %) until 20 % weight loss. Samples (transverse sections) are taken at day 0 (production day of the meat batter), 7 (before fermentation), 10 (after fermentation, before drying), 14, 21 and 28 (end of production). pH was measured at each sampling day.

Analyses

Dry matter The dry matter (DM) was analyzed according to ISO 1442-1973(E) [12].
Extraction of porphyrins Zinc protoporphyrin IX (ZPP) was isolated from the dry fermented sausages as described by Wakamatsu et al. [5] with minor modifications.
Briefly, minced sample (2.5 g) was homogenized with 10 volumes of 75% acetone (HPLC, Acros organics, Belgium) for 5 min, and the homogenate was filtered through a folded filter paper 313 (VWR International, Belgium).
Fluorescence analysis The fluorescence intensities (FI) for excitation at 420 nm and emission at 590 nm of the extracts were registered using a fluorescence spectrophotometer (Shimadzu, Japan). The index for fluorescence emission intensities was calculated from:

$$I_{fl} = FI_{sample\ (ex./em.\ 420/590\ nm)} \times DM^{-1} \quad (1)$$

Colour measurements. Hue angle values were calculated based on the recorded L*-, a*-, b*-values using a portable Chroma meter (Minolta CR 300, Japan):

$$Hue\ (°) = Bgtan\ \frac{b*}{a*} \quad (2)$$

Measurements were made with illuminant D65, 0° standard observer, and 8mm viewing area size.

III. RESULTS AND DISCUSSION

Dry fermented sausages were made using nitrite salt, sodium chloride or sea salt in order to evaluate the evolution of ZPP and its role on colour formation.

Evolution of pH during the production process

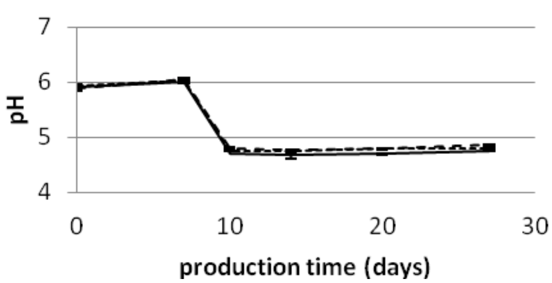

Figure 1. pH decline during the processing of three batches dry fermented sausages, using 2.8 % nitrite salt (containing 0.6% $NaNO_2$) (——), 2.8 % sodium chloride (– -) and 2.8 % La Baleine sea salt (---·). Data are expressed as means ± SD (n = 3).

Before fermenting, the sausages were stored at 4°C during 7 days, which gives the starter culture the time to adapt to the meat environment. As can be seen in figure 1, during this rest period no pH decline was observed, which is in accordance with the expectation that the starter culture would not grow at these conditions. From day 7, optimal conditions (24°C/ 95 % RH) for the fermentation were applied. As a consequence, the lactic acid bacteria were able to grow and a pH decline from circa 5.9 to 4.8 was observed. The pH is used as a control tool to check the efficiency of the fermentation process. Giving that all prepared batches show a similar pH evolution during the production process (Fig.1), it can be concluded that the fermentation was carried out properly.

ZPP formation during the production process

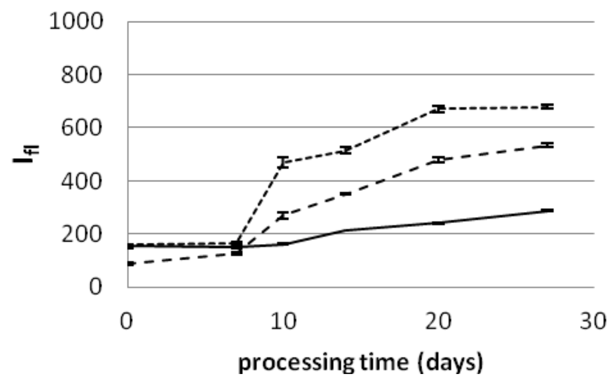

Figure 2. Changes in ZPP during the processing of three batches dry fermented sausages, using 2.8 % nitrite salt (containing 0.6% $NaNO_2$) (——) , 2.8 % sodium chloride (– -) and 2.8 % La Baleine sea salt (--·). Data are expressed as means ± SD (n = 3).

Figure 2 shows that during the cold storage in the first week, the index for fluorescence emission intensities (I_{fl}) remained constant, implicating no ZPP formation occurred in the meat product.

During fermentation (from day 7 until 10), no remarkable ZPP formation was observed when nitrite was added to the sausage preparation. Although the I_{fl} was increasing slowly during the drying process (from day 10 until the end of the processing), the increase is negligible compared to the nitrite-free dry fermented sausages. Based on these results, the inhibition of nitrite on the formation of ZPP, already stated in previous studies [2] [10], can be confirmed during the production of dry fermented sausages. When nitrite is omitted in the sausage formulation, the I_{fl} increase was initialized by the fermentation (from day 7) and pursued during the drying process (from day 10). The I_{fl} increase was more pronounced when sea salt was used. This suggests that 'impurities' in this specific sea salt used may cause an increased formation of ZPP, even within the short production period of three weeks. Moreover, the slope of the changes in I_{fl} during the fermentation was greater than during the drying process. Probably, temperature can play an important key role in this, although other variables should be taken into consideration.

This result is in contrast to the findings of other studies [11], where no positive effect of sea salt was found in dry cured hams. This may be explained by the use of another type of sea salt.

Colour evaluation during the production process

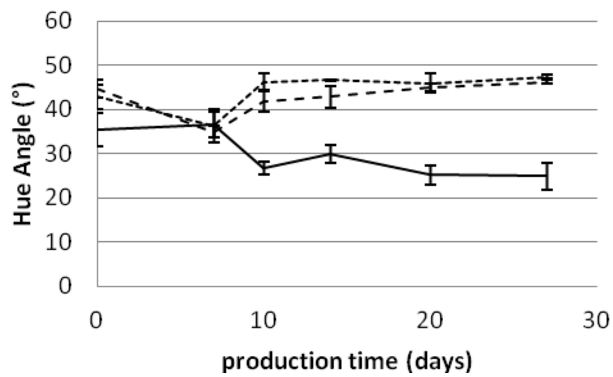

Figure 3. Changes in Hue Angle (°) during the processing of three batches dry fermented sausages, using 2.8 % nitrite salt (containing 0.6% $NaNO_2$) (——) , 2.8 % sodium chloride (– -) and 2.8 % La Baleine sea salt (--·). Data are expressed as means ± SD (n = 3).

Changes in Hue Angle (°) during the processing of the three batches were assessed (Fig. 3) to evaluate the colour formation in the dry fermented sausages. The Hue angle declined during the production of sausages with added nitrite. This implicates the development of the red meat colour by the formation of NOMbFe(II). In contrast, the Hue Angles of the nitrite-free dry fermented sausages are increasing during fermentation. Probably, due to the absence of NO molecules, MMbFe(III) was formed instead of NOMbFe(II). Moreover, the established ZPP formation in the nitrite-free dry fermented sausages was not sufficient to result in a smooth red coloured product. On the contrary, a dull brown colour was observed.

Although a difference in ZPP content was observed, no colour differences were measured between dry fermented sausages made with sodium chloride or sea salt.

IV. CONCLUSION

The formation of ZPP during the production of dry fermented sausages is largely inhibited by nitrite. In nitrite-free dry fermented sausages, however, the formation of ZPP is more pronounced. La Baleine sea salt had a positive effect on the formation of ZPP.

The formation of ZPP, even when sea salt was added, was not sufficient to achieve the expected red colour. More research has to be performed to elucidate the formation of ZPP and its role on meat colour in dry fermented sausages.

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Note: “The document reflects the author’s views. The interreg IVA 2 Seas Program Authorities are not liable for any use that may be made of the information contained therein.”

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