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# Effect of fermentation temperature and nitrite nitrate on properties of dry fermented sausage

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**Abstract.** Sausages, produced with/without nitrite and nitrate, were fermented at 21°C (RH: 65-74%) or 8°C (RH: 75-85%). All sausages were dried until a final weight loss of 44% and analyzed for physicochemical and microbiological data. The removal of NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup> caused an increase in lipid oxidation, although the decrease in the fermentation temperature proved to be effective in controlling the lipid oxidation of ripened nitrite-free sausages. The effect of nitrite on the formation of red and bright colour was evident since the early stages of processing, while its removal resulted in the formation of a less red, less stable and lighter colour, in spite of fermentation temperature. The texture of ripened sausage did not show significant differences among groups. The elimination of NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup> had no effect on the typical microbiota (LAB and SNCP), while differences were observed for *B. thermosphacta*, enterococci and enterobacteria.

**Keywords.** Colour – Dry fermented sausage – Fermentation temperature – Microbiota – Nitrite – pH.

## **Effet de la température de fermentation et de l'élimination des nitrites et nitrates sur les paramètres du saucisson sec**

**Résumé.** Des saucissons produits avec et sans nitrates et nitrites, ont été fermentés à 21°C (RH 69-74%) et à 8°C (RH 75–85%). Les saucissons ont été maturés jusqu'à une perte de poids de 44%. Les analyses physico-chimiques et microbiologiques étaient effectuées au cours de la maturation. L'élimination de NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup> augmente l'oxydation des lipides mais la réduction des températures de fermentation contrôle l'oxydation des saucissons qui ont été produits sans nitrates. L'importance des nitrites dans la formation de la couleur rouge vif est évidente dès le début de leur production; leur élimination, indépendamment des températures de fermentation, détermine la formation d'une couleur rouge moins intense, moins stable et plus claire. Les paramètres de texture du salami mûré n'ont pas mis en évidence de différences significatives entre les groupes. L'élimination de NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup> n'affectait pas le microbiote typique (LAB et SNCP) tandis que des différences ont été observées à propos de *B. thermosphacta*, entérocoques et Enterobacteriaceae.

**Mots-clés.** Couleur – Saucisson sec – Températures de fermentation – Microbiote – Nitrate – pH.

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## **I – Introduction**

The use of nitrite in sausage production is important for its antibacterial, colour-forming, antioxidant and flavouring properties (Shahiti and Pegg, 1992). Despite these properties, there are problems with regard to toxicity of nitrite and nitroso-derivatives for human health (Cassens, 1997). The processing of the sausage is based on a drip and drying phases which reduce water activity; between these two phases there is a fermentation under controlled temperature and relative humidity, during which the growth of lactic acid bacteria, results in a pH decrease, thus the development of texture and colour and the control of spoilage bacteria and pathogens (Hugas and Monfort, 1997; Lucke, 1998). The aim of this research was to determine the effects of fermentation temperature and nitrite nitrate on the physicochemical and microbiological characteristics of dry fermented sausages.

## II – Materials and methods

### 1. Sausage formulation and processing

Three batches of dry fermented sausages, two without  $\text{NO}_2^-/\text{NO}_3^-$  (batches N-) and one with 80 ppm  $\text{NaNO}_2$  and 120 ppm  $\text{KNO}_3$  (batch N+) were produced from a common meat batter (approximately 75% lean pork meat and 25% pork fat). The pork lean and fat were ground through a 7 mm diameter mincing plate and mixed with salt (2.4%), sucrose (0.25%), sodium ascorbate (0.06%), white wine, whole black pepper, ground white pepper and garlic. A commercial starter culture containing *Lactobacillus curvatus*, *Staphylococcus carnosus* and *Kocuria varians* was added. Batters were stuffed into natural casing (80-85 mm diameter), and the final weight for each sausage was 1 kg. A mould starter, SK10 *Penicillium nalgiovensis*, was applied on the surface of sausages. For the batches N- two different processes (P) were applied: "P1" consisting of 3 d at  $21\pm 1^\circ\text{C}$  /65-74% relative humidity (RH) followed by a drying at  $17\pm 2^\circ\text{C}$ /70-80% RH and ripening at  $13\pm 1^\circ\text{C}$ /80-88 % RH; "P2" consisting of 13 d at  $8\pm 1^\circ\text{C}$ /75-85% RH. Afterwards the temperature was increased to  $16\pm 1^\circ\text{C}$  and 80-88% RH for 7 d and finally  $18\pm 1^\circ\text{C}$  and 80-88% RH until the end of the processing. The sausages produced with  $\text{NO}_2^-/\text{NO}_3^-$  (batch N+), were processed with P1 only. All sausages were processed until a final weight loss of 44% was reached. From each batch, samples of the sausage batter (100 g) were collected at days 0 (prior to stuffing) and three sausages were taken at 6, 13 and 29 d. At the end of the process 5 sausages for each batch were taken for analysis.

### 2. Analysis

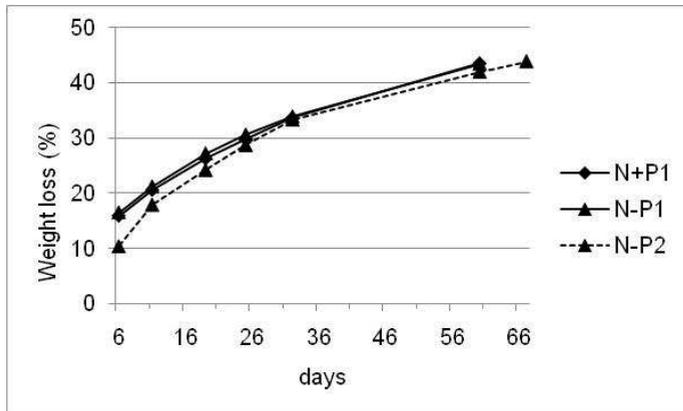
*Water activity* ( $a_w$ ) was measured at  $25^\circ\text{C}$  by means of Aqualab® equipment (Model Series 3TE, Decagon Devices Inc). *pH* was measured using a pH meter WTW (model 330) inserting the electrode (Hamilton) directly into the sausage. *Lipid oxidation* (TBARs) was determined using the 2-thiobarbituric acid method (TBA-test) described by Witte *et al.* (1970) using trichloroacetic acid 5% as solvent. *Textural Profile Analysis* (Bourne, 1978) was performed (Instron Texture Machine mod. 5565) using the central core of two slices of each sample. Each probe (15 mm height and 25 mm diameter) was compressed twice to 50% of original height. The following texture parameters were calculated: hardness (peak force during the 1<sup>st</sup> compression cycle), and cohesiveness (ratio of the positive force area during the 2<sup>nd</sup> compression to that during the 1<sup>st</sup> compression). *Colour* (Minolta CR-508d; illuminant D65), was measured by the CIE  $L^*a^*b^*$  system and the results were expressed as lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ). The sausages were cut into sections 2 cm thick and colour measurements were taken immediately after cutting, and after 24 hr of display in air at  $4^\circ\text{C}$ . Sausages were subjected to the following microbiological analyses: *Enterobacteriaceae* (ISO 7402), Gram-negative bacteria (G-) (violet red bile glucose agar  $30^\circ\text{C}/48$  hr), Lactic acid bacteria (LAB) (ISO 15214), Micrococci and staphylococci (Mannitol Salt Agar,  $30^\circ\text{C}/72$  hr) and enterococci (Slanetz Bartley agar  $37^\circ\text{C}/4$  hr and  $44^\circ\text{C}/44$  hr).

The chemical-physical and microbiological data were shown as mean values. One-way analysis of variance, (ANOVA, SPSS vr.11.5.0) was run to detect differences among batches at end of ripening (Bonferroni test).

## III – Results and discussion

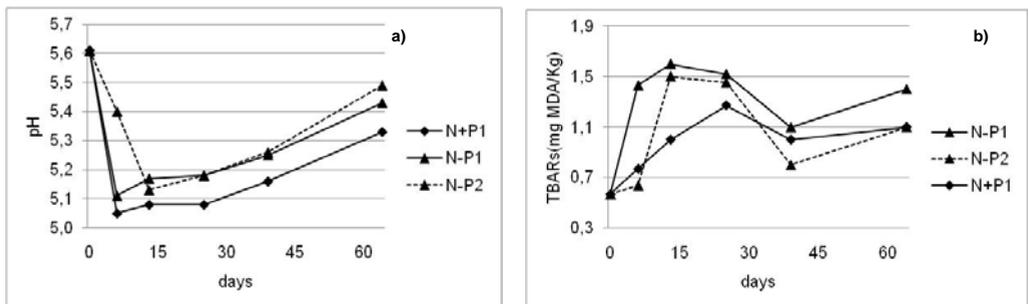
Figure 1 shows the weight losses of sausages throughout the ripening processes: a reduction approximately 44% (w/w) was reached in about 61 d for sausages processed with P1 and 67 d for sausages processed with P2, meaning that the slow ripening conditions of P2 were controlled to minimize differences in drying from a traditional process. During the processing  $a_w$  dropped from the initial value of 0.97 to the final average value of 0.86, without significant differences between the three batches.

All sausages showed at first a pH decrease and a final rise (Fig. 2a). The rate of pH decrease was affected by fermentation temperatures: the higher temperature in P1, favouring formation of organic acids, caused a faster pH decrease than in P2 (6 d vs 13 d). At the end of fermentation and at the end of ripening the pH values of batch N+ were lower ( $p < 0.005$ ) than in batches N-.



**Fig. 1.** Mean values of weight loss in the different batches during the ripening of sausages (N-P1: no  $\text{NO}_2/\text{NO}_3^-$  added sausage, process P1; N-P2: no  $\text{NO}_2/\text{NO}_3^-$  added sausage, process P2; N+P2:  $\text{NO}_2/\text{NO}_3^-$  added sausage, process P2).

For all batches TBARs values showed an increase followed by a reduction (Fig. 2b). The lipid oxidation was more rapidly increased in N-P1 than in N-P2 or in N+P1 batches but, at 13 d the highest TBARs values were observed in both N- batches. At end of ripening process, ANOVA revealed that oxidation of N-P1 sausages was higher ( $p = 0.017$ ) than N+P1 and N-P2.



**Fig. 2.** Mean values of pH (a) and TBARs (b) in the different batches during ripening.

The effect of nitrite on the formation of a red and bright colour was evident since the early stages of processing (data not show), and its removal resulted, at end of ripening, in the formation of a less red, more yellow and lighter colour (Fig. 3). The storage (24 hr at  $4^\circ\text{C}$ ) of sliced sausages determined an increase of differences in color index among batches N+ and N- despite processing condition.

pH and moisture are major factors affecting texture properties and the increase of hardness (maximum force required to compress the sample) and cohesiveness (strength of the internal bonds making up the body of the sample) observed during ripening. The observed differences of hardness and cohesiveness (Fig. 4) among the batches during the first phases of ripening

could be explained by the differences in the rate of pH decrease and in the weight loss. At end of ripening process, the texture parameters of sausages did not show significant differences among batches.

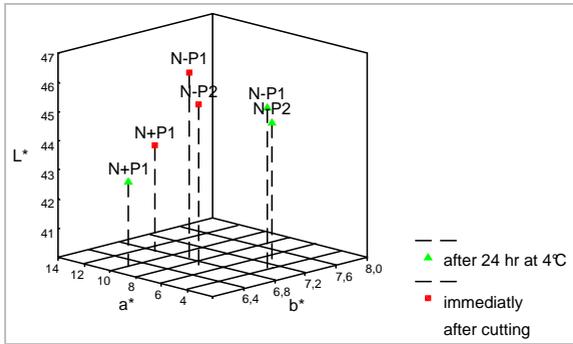


Fig. 3. Colour parameters, after cutting and after 24 hr at 4°C, of the ripened sausage.

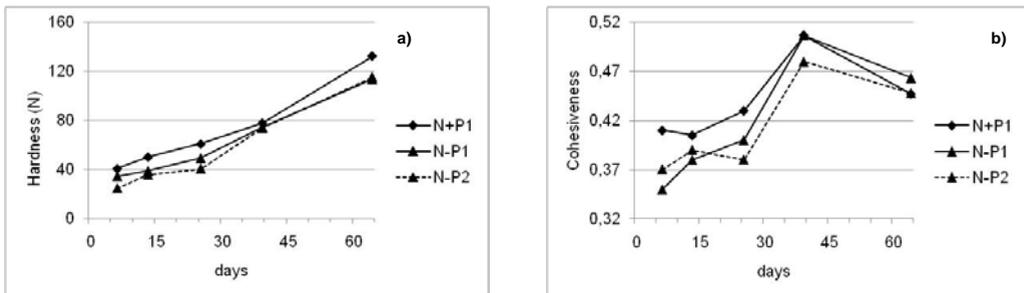


Fig. 4. Mean values of hardness and cohesiveness in the different batches.

Figure 5 shows the fate of typical and spoilage microorganisms during the processing of different batches of sausages. In P1 the elimination of  $\text{NO}_2^-/\text{NO}_3^-$  had no effect on the typical microbiota: LAB increased during the first 10 d, reaching values in the order of  $10^9$  cfu/g, staphylococci not coagulase positive (SNCP), introduced with the starter at level of  $10^6$  to  $10^7$  cfu/g, remained the same for the entire process. Differences were observed for the behaviour of G-, *B. thermosphacta* and enterococci. Trends of G-, in the batch N+P1 revealed, in the first 10 d, a drastic and progressive reduction to reach at the end of process values less than 10 cfu/g; *B. thermosphacta* showed a similar pattern. In batch N-P1 a change was observed in the load of G- that after 30 d, were still around  $10^3$  cfu/g and only at the end of maturing showed values <10 cfu/g. The behaviour of *B. thermosphacta* during the first 30 d is almost similar to that of the batch N-P1 but, at the end of processing, a great variability ( $10$ - $10^4$  cfu/g) among the values, was detected in the different samples. In the N-P2 batch the use of temperature as low as 8°C during the fermentation allowed more control over the evolution of the spoilage bacteria. G-bacteria decreased steadily reaching, after maturing, values <10cfu/g; enterococci remained at the same level until the end of processing. *B. thermosphacta*, after an initial reduction of about 2 log, showed a sharp increase and, at the end, the level was higher than initial one.

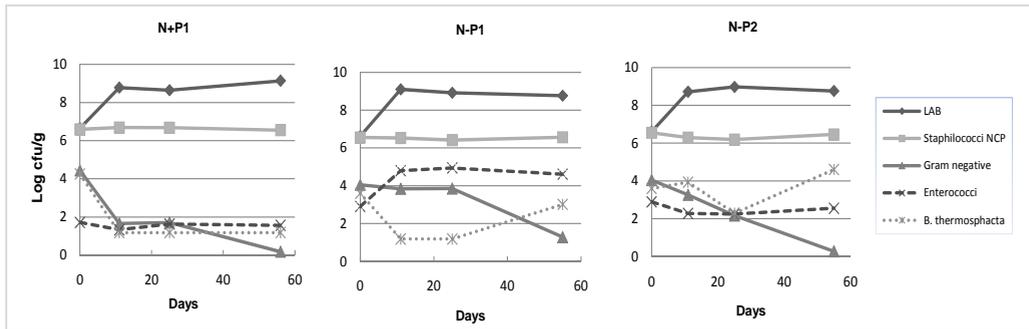


Fig. 5. Performance of the main microbial groups during ripening of sausage.

## IV – Conclusions

Under the conditions of the present study, the production of dry cured sausages without nitrate and nitrite resulted in a not very stable colour and in an uncontrolled lipid oxidation specially at higher temperature. No effect on the typical microbiota was detected whereas spoilage bacteria growth wasn't controlled. The slower rate of acidification at low temperature (8°C) of fermentation did not significantly affect the weight loss texture parameters and it allowed more control of the evolution of Gram negative and enterococci.

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