ELSEVIER

International Journal of Food Microbiology 42 (1998) 213–217

International Journal of Food Microbiology

Short communication

# Effect of nitrate and nitrite curing salts on microbial changes and sensory quality of non-fermented sausages

Y. Sanz, R. Vila, F. Toldrá*, J. Flores

*Instituto de Agroquímica y Tecnología de Alimentos (C.S.I.C.), Apt. 73, 46100 Burjassot, Valencia, Spain*

Received 6 August 1997; received in revised form 25 November 1997; accepted 28 April 1998

**Abstract**

The effects of nitrate and nitrite curing salts on microbial changes and sensory quality of non-fermented sausages of small diameter were investigated. During pre-ripening (day 5), levels of lactic acid bacteria and yeasts were slightly higher in nitrite-made sausages than in those made with nitrate. In contrast, nitrite discouraged the growth of psychrotrophs as occurs in fermented sausages. By the end of ripening (day 26), levels of microorganisms were similar in both batches of sausages except for psychrotrophs being higher in those made with nitrite. Nitrate-made sausages showed higher aroma and taste intensity. © 1998 Elsevier Science B.V.

*Keywords:* Non-fermented sausages; Curing salts; Sensory quality; Microbiology

## 1. Introduction

Traditional technology for dry sausage manufacture has been progressively replaced by rapid ripening methods which mainly rely on the use of controlled drying chambers and starter cultures to guarantee the safety and quality of the final product. The benefits of these new technologies are undoubted although the reduction of drying times and the use of starter inoculation has also resulted in poorer products as far as sensory quality is concerned (Árboles and Juliá, 1992). European consumers from the Mediterranean area reject intense acid flavours associated with rapid ripened sausages. So, in countries such as France, Italy and Spain, the production of fermented sausages is falling whilst the consumption of non-fermented small diameter ($<30$–$40$ mm) sausages is increasing (Flores and Bermell, 1996). The technology of this kind of sausage, based on the use of low ripening temperatures ($<10$–$12$°C), avoids an intense and rapid fermentation but reduces water activity ensuring the safety of the product. Either nitrate and/or nitrite curing salts are used for the manufacture of non-fermented sausages although nitrate is mainly utilised in Mediterranean countries (Flores, 1997). In slow-ripened processes, nitrate has been considered essential for the generation of flavour compounds (Durand, 1990). The positive effect of the use of nitrate on sensory quality has been related to the development of nitrite-sensitive microflora (Lücke,

*Corresponding author. Tel.: +34 96 390 0022; fax: +34 96 363 6301; e-mail: ftoldra@iata.csic.es


PII: S0168-1605(98)00072-5

1986) although such effect has not been confirmed in rapid ripened sausages when starters were inoculated (Katsaras et al., 1996a,b,c; Sanz et al., 1997a). On the other hand, the use of nitrite is of great importance for inhibiting the undesirable bacteria when combined with pH reductions as occurs in fermented sausages (Sanz et al., 1997a). Currently, the influence of curing salts on the microflora and organoleptic characteristics of non-fermented sausages still remains unclear.

The aim of this work was to study the effect of either nitrite or nitrate curing salts on microbial changes and sensory quality in small-diameter non-fermented sausages inoculated with a mould strain.

## 2. Materials and methods

### *2.1. Sausages manufacture and sampling*

The formulation of the two batches of sausages analysed in this work included lean pork and pork belly (1:1), 2.8% (w/w) NaCl, 3.0% (w/w) lactose, 1.0% (w/w) sodium caseinate, 0.5% (w/w) sodium ascorbate, 0.030% (w/w) $KNO_3$ (batch 1) or 0.015% (w/w) $NaNO_2$ (batch 2), 0.02% (w/w) spices.

The lean meat and fat were previously chilled at 4°C for two days, kept at −5°C overnight and then chopped through a 6-mm plate. The meat components were mixed with the remaining ingredients under vacuum (60 mmHg) and afterwards stuffed into 35–40-mm diameter regenerated collagen casings. The final weight of each sausage was 300 g, approximately. *Penicillium nalgiovensis* PNT 1 (Texel, Group Rhone-Poulenc, Madrid, Spain) was surface inoculated by dipping the sausages in a 0.04% (v/v) solution.

Both batches of sausages (nitrate and nitrite-made sausages) were kept at 10°C and 90% relative humidity for 2 days, and then at 15°C and the same relative humidity for 5 days to promote mould growth. Finally, sausages were dried at 12°C till weight losses were about 35%.

Four samples from each batch were taken at each of the different stages during the processing: raw minced meat (0 day), during pre-ripening (5th day), at the beginning of ripening (8th day), in the middle of ripening (13th day) and at the end of ripening (26th day).

### *2.2. Microbiological analyses*

Samples of 30 g were aseptically taken from the inner part of each sausage and homogenised for 3 min with Buffered Peptone Water (BPW) (Adsa Micro, Barcelona, Spain) at dilution 1:9 (w/v) in a Stomacher Lab-Blender 400 (London, UK). Serial decimal dilutions were made in BPW and then plated in duplicate for bacterial counts. Aerobic mesophilic bacteria were enumerated on Plate Count agar (Merck, Darmstadt, Germany) after 48-h incubation at 30°C. *Micrococcaceae* were enumerated on MSA agar (Merck, Darmstadt, Germany) and lactic acid bacteria on double-layer MRS agar (Merck, Darmstadt, Germany) after 72-h incubation at 30°C. *Enterobacteriaceae* counts were determined on VRBD agar (Merck, Darmstadt, Germany) after 24-h incubation at 37°C. Psychrotrophs were enumerated on KING FG agar (Adsa-Micro, Barcelona, Spain) after 5 days incubation at 15°C. Yeast numbers were determined on Rose Bengal agar (Adsa-Micro, Barcelona, Spain) after 3 days of incubation at 28°C.

### *2.3. Chemical analyses*

Moisture content was determined after dehydration at 100°C to constant weight. The pH was measured according to ISO (1974) using an Orion Research pH meter (Expandable Ion Analyzer EA920, Boston, USA). Nitrate and nitrite contents were determined according to ISO (1975a),ISO, 1975b) and expressed in mg/kg wet matter.

### *2.4. Sensory analyses*

A paired comparison test of the intensity and quality of colour, taste and aroma of both batches of sausages was performed at the end of the process. Samples were assessed by a panel of 27 non-trained panelists. The study consisted of four sessions (two sessions for each batch). In each session, two slices (2 mm thick) were presented to the panelists at 10 min intervals. The panelists were asked to indicate which sample had greater intensity of the characteristic being studied (Poste et al., 1991).

### *2.5. Data analyses*

Bacteriological and chemical data were analysed by one-way analysis of variance, and significant

differences among batches and different processing stages were determined at a confidence level of 95%. Sensory assessment data were subjected to a paired comparison test and differences between batches were established at a 95% confidence level.

## 3. Results and discussion

Microbial quality of raw minced meat used for sausage manufacture was evaluated. As shown in Fig. 1, low initial levels (around $10^3$ cfu/g) of lactic acid bacteria, *Micrococcaceae* and yeasts were detected as also found by other authors (Lücke, 1986; Samelis et al., 1994; Sanz et al., 1997b). Aerobic mesophilic counts and *Enterobacteriaceae* levels were also in the range usually reported for raw minced meat (Lücke, 1986; Domíngez et al., 1989; Sanz et al., 1997a).

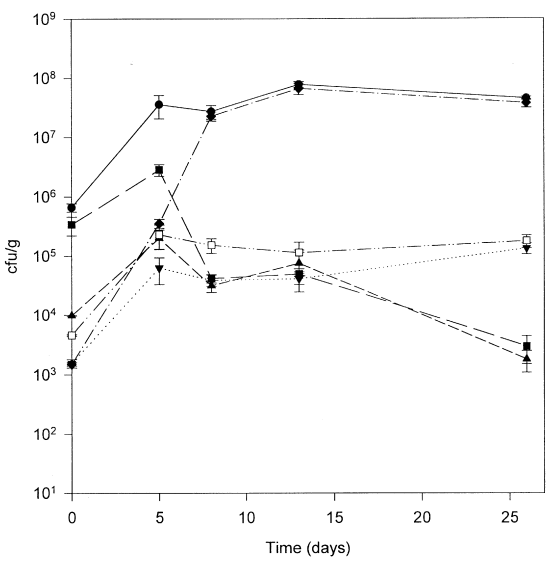


Fig. 1. Evolution of the microflora (● aerobic mesophilic bacteria, ♦ lactic acid bacteria, ▼ *Micrococcaceae*, △ *Enterobacteriaceae*, ■ psychrotrophs, □ yeast) during the processing of nitrate-made sausages (batch 1). Mean of four samples±standard error of mean (SEM, represented as vertical bars). Processing stages: day 0, raw minced meat; day 5, pre-ripening; day 8, beginning of drying; day 13, middle of drying; day 26, end of drying.

The evolution of the microflora during the processing of nitrate-made sausages (batch 1) is reflected in Fig. 1. The various microorganisms showed growth during the first 5 days. Lactic acid bacteria dominated the microflora since the beginning of ripening and their numbers were similar to aerobic mesophilic flora as occurs in fermented sausages (Samelis et al., 1994; Sanz et al., 1997a,b). *Micrococcaceae* and yeasts showed a second growth phase increasing ($P < 0.05$) at a late-ripening stage presumably as a consequence of the final rise in pH (Table 1), a characteristic of mould ripened sausages (Garriga et al., 1986; Roncalés et al., 1991).

The evolution of the microflora in nitrite-made sausages (batch 2) was similar (results not shown). However, levels of lactic acid bacteria and yeast were slightly higher ($P < 0.05$) than those of nitrate-made sausages (Fig. 1) by the 5th day of the process. So, nitrite apparently promoted the growth of these microbial groups at this stage when the reduction in pH took place (results not shown) even though lactic acid bacteria have been reported to be inhibited by nitrite in in vitro assays (Matsuoka et al., 1994). In contrast, the use of nitrite as curing agent discouraged the growth of psychrotrophs whose levels were significantly lower ($P < 0.05$) than in batch 1. The inhibitory effect of nitrite on psychrotrophs and *Enterobacteriaceae* growth has also been observed in fermented sausages when combined with reductions of pH (Lücke, 1986; Sanz et al., 1997a).

In the middle of ripening, slightly higher ($P < 0.05$) lactic acid bacteria and *Micrococcaceae* numbers were detected in nitrite-made sausages when compared with those made with nitrate. Stahnke (1995) also reported inhibition of *Micrococcaceae* growth by nitrate in sausages. However, such inhibitory effect was not reflected in MRS and MSA

Table 1
Sensory analysis of different batches of non-fermented sausages

| Sample | Aroma | | Colour | | Taste | |
|---|---|---|---|---|---|---|
| | Intensity | Quality | Intensity | Quality | Intensity | Quality |
| Batch 1 | 25[a] | 16[a] | 4[a] | 3[a] | 22[a] | 16[a] |
| Batch 2 | 2[b] | 10[a] | 19[a] | 19[a] | 5[b] | 10[a] |

[a,b] Different letters within a column indicates a significant difference ($P < 0.05$).

counts at the end of the process since no significant differences ($P < 0.05$) were detected between the two batches. As mentioned for nitrate-made sausages, *Micrococcaceae* and yeasts tend to significantly increase ($P < 0.05$) at the end of ripening. Indeed, the survival of *Micrococcaceae* mainly depends on the acidification (Johansson et al., 1994; Samelis et al., 1994) and parallel increases in yeast numbers and pH have been observed at advanced stages of ripening in fermented sausages (Samelis et al., 1994). *Enterobacteriaceae* numbers were low (about $10^3$ cfu/g) and similar for both batches at the end of ripening.

Differences in colour, taste and aroma between nitrate and nitrite-made sausages were evaluated by sensory analysis. The intensity of aroma and taste was significantly higher ($P < 0.05$) in nitrate-made sausages while differences in colour were not detected (see Table 1). Indeed, residual nitrate and nitrite levels were low in both batches (results not shown) indicating an effective nitrate and nitrite reduction although *Micrococcaceae* levels were not above $10^5$ cfu/g during the whole process. Likely, the inoculation of *Penicillium nalgiovensis* stabilised cured colour in both cases (Kröckel, 1995; Hammes and Knauf, 1994). The use of nitrate as curing agent has been associated with sausages of superior sensory scores. The improved organoleptic characteristics of nitrate-made sausages were related to the contribution of nitrite-sensitive microorganisms (*Micrococcaceae* and *Enterobacteriaceae*) to an increased aroma (Lücke, 1986) although differences in the occurrence of these bacteria were not detected in this study. On the contrary, differences in *Enterobacteriaceae* levels were detected in rapid fermented sausages but not in sensory quality (Sanz et al., 1997a).

In conclusion, the use of nitrate as curing agent in non-fermented sausages of small-diameter improves aroma and taste intensity, apparently without imposing higher hygienic risks, according to the microbiological results obtained.

## Acknowledgements

This work was supported by grant ALI 94-0723 from Comisión Interministerial de Ciencia y Tecnología (CICYT), Spain. The authors thank E. Costell and A. Feria for their help in sensory and microbial analyses, respectively, and P. Lorenzo for his technical assistance.

## References

Árboles, J., Juliá, E., 1992. Aroma in cured meat products. In: Smulders, F.J.M., Toldrá, F., Flores, J., Prieto, M. (Eds.), New Technologies for Meat and Meat Products. Audet, Nijmegen, pp. 109–123.

Domíngez, M.C., Gutiérrez, L.M., López, A., Seco, F., Zumalacarregui, J.M., 1989. Development of the principal groups of microorganisms during ripening of chorizo from León. Alimentaria 199, 11–15.

Durand, P., 1990. Interet technologique des nitrates et nitrites. Personal communication.

Flores, J., Bermell, S., 1996. Dry-cured sausages. Factors influencing souring and their consequences. Fleischwirtsch. 76, 163–165.

Flores, J., 1997. Mediterranean vs Northern European meat products. Processing technologies and main differences. Food Chem 59, 505–510.

Garriga, M.L., Selgas, M.D., Fernández, M., Ordoñez, J.A., 1986. Study of Proteolysis of a dry fermented pork sausage. Proc. Europ. Meet. Meat Res. Work., Copenhagen, 283–286.

Hammes, W.P., Knauf, H.J., 1994. Starter in processing of meat products. Meat Sci. 36, 155–168.

ISO, 1974. Measurement of pH. R 2917. Int. Standard Org.

ISO, 1975a. Determination of nitrate content. 3091. Int. Standard Org.

ISO, 1975b. Determination of nitrite content. 2918. Int. Standard Org.

Johansson, G., Berdagué, J-L., Larsson, M., Tran, N., Borch, E., 1994. Lipolysis, proteolysis and formation of volatile components during ripening of fermented sausages with *Pediococcus pentosaceus* and *Staphylococcus xylosus* as starter cultures. Meat Sci. 38, 203–218.

Katsaras, K., Lautenschlaeger, R., Boschkova, K., 1996. Physical–chemical processes in the production of pasterma. Fleischwirtsch. 76, 136–138.

Katsaras, K., Lautenschlaeger, R., Boschkova, K., 1996. Physical–chemical processes in the production of pasterma. Fleischwirtsch. 76, 141–142.

Katsaras, K., Lautenschlaeger, R., Boschkova, K., 1996. Physical–chemical processes in the production of pasterma. Fleischwirtsch. 76, 162.

Kröckel, L., 1995. Bacterial fermentation of meats. In: Fermented Meats. G. Campbell-platt and P.E. Cooks, eds. Champman and Hall, Glasgow, 69–102.

Lücke, F-K., 1986. Microbiological processes in the manufacture of dry sausage and raw ham. Fleischwirtsch. 66, 1505–1509.

Matsuoka, A., Furukawa, N., Takahashi, T., Yamanaka, Y., 1994. Utilization of lactic acid bacteria from kefir grains as starter cultures for fermented ground meat products. Animal Sci. Technol. 65, 732–737.

Poste, L.M., Mackie, D.A., Butler, G., Larmond, E. (1991). Laboratory methods for sensory analysis of food. Research Brand Agriculture Canada, Publication 1864/E, 19–29.

Roncalés, P., Aguilera, M., Beltrán, J.A., Jaime, I., Peiró, J.M., 1991. The effect of natural or artifical casing on the ripening and sensory quality of a mould-covered dry sausage. Int. J. Food Sci. Technol. 26, 83–89.

Samelis, J., Stavropoulos, S., Kakouri, A., Metaxopoulos, J., 1994. Quantification and characterization of the populations associated with naturally fermented Greek dry salami. Food Microbiol. 11, 447–460.

Sanz, Y., Vila, R., Toldrá, F., Nieto, P., Flores, J., 1997a. Effect of nitrate and nitrite curing salts on microbial changes and sensory quality of rapid ripened sausages. Int. J. Food Microbiol. 37, 225–229.

Sanz, Y., Flores, J., Toldrá, F., Feria, A., 1997b. Effect of preripening on microbial and chemical changes in dry fermented sausages. Food Microbiol. 14, 575–582.

Stahnke, L.-H., 1995. Dry sausages fermented with *Staphylococcus xylosus* at different temperatures and with different levels. 1. Chemical and bacteriological data. Meat Sci. 41, 179–191.