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Dry Fermented Sausages Elaborated with *Lactobacillus plantarum-Staphylococcus carnosus.* Part II: Effect of Partial Replacement of NaCl with KCl on the Proteolytic and Insolubilization Processes.

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ABSTRACT

The effect of partial replacement of NaCl with KCl (3% NaCl by 1·5% NaCl + 1% KCl) on the proteolytic and insolubilization processes that affect the nitrogen fractions of dry fermented sausages produced with L. plantarum-S. carnosus *as starter culture was analysed. The percentage of solubility was significantly lower in the modified product, which is consistent with its significantly higher percentage of insoluble fraction observed from the beginning of the process. The myofibrillar fraction was more affected by the proposed modification than the sarcoplasmic fraction: the loss of solubility for the modified and control products were 33·6 and 27·6% for myofibrillar fraction and 9·9 and 9·3% for the sarcoplasmic fraction, respectively. Also a higher intensity of the proteolytic process was observed. The modified product was found to be slightly less hard but had a better salted taste.* © *1997 Elsevier Science Ltd*

INTRODUCTION

The most important constituents of sausages, from both the nutritional and technological point of view, are proteins. They are the principal functional and structural components of processed meats. Their functional properties include swelling, solubility, viscosity, water and fat binding, gelation and possibly emulsification (Acton *et al.*, 1983; Hermansson, *et al.*, 1986).

Protein structure and conformation are affected by proteolysis and insolubilization (Bello *et al.*, 1974*a*,*b*; Lois *et al.*, 1987; Astiasarán *et al.*, 1989; Astiasarán *et al.*, 1990*a*; García de Fernando and Fox, 1991). Several studies have indicated that the solubility of

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both the myofibrillar and sarcoplasmic proteins in fermented sausages decreases during the ripening due to the increase in salt concentration and the decrease in pH (Wardlaw, *et al.*, 1974; Cid *et al.*, 1992). Denaturation or, more accurately, insolubilisation in salt solution, gives rise to increases in the insoluble nitrogen fraction during ripening (Bello *et al.*, 1974*b*; Leon Crespo *et al.*, 1985; Lois *et al.*, 1987; Astiasarán *et al.*, 1990*a*,*b*). Also, proteolytic processes can be affected (Astiasarán *et al.*, 1990*a*).

Changes in protein solubility and degradation would affect the functional properties of proteins giving rise to textural defects.

Many authors have estimated that 33 to 77% of the sodium which is consumed comes from processed foods (Fregly, 1983; Mattes and Donnelly, 1991). Changing manufacturing practice was considered the most important factor in reducing sodium intake (Adams *et al.*, 1995) together with the health benefits.

With the recent development of low-salt and low-fat meat products the need to understand, modify and control the functionality of meat proteins will become even more important (Smith, 1988). Astiasarán *et al.* (1993) found that the parameters related to protein integrity were notably affected by the time of addition of nitrite/nitrate salts and sodium chloride. A reduction of NaCl could probably affect the protein functionality, modifying its solubility.

In Part I of the present work, the effect of a partial replacement of NaCl with KCl on stability and the nitrosation process of dry fermented sausages made with *Lactobacillus plantarum-Staphylococcus carnosus* as starter culture was analysed (Ibañez *et al.*, 1996). The purpose of the present study was to investigate the effects of a similar modification on the proteolytic and insolubilization processes that affect the nitrogen fractions of dry fermented sausages. Furthermore, the repercussion on some sensorial attributes was analysed.

MATERIAL AND METHODS

Samples

An experiment consisting in the manufacture of a traditional dry fermented sausage with 3% NaCl (control products) and a modified product, with 1·5% NaCl + 1% KCl, was carried out. The description of the formulations and technological process employed was given in the Part I of the work (Ibañez *et al.*, 1996). The study was repeated four times providing four different batches of both types of fermented sausages. The physicochemical analysis were carried out on one sausage of each batch at the next phases: 0 (mixed product), 1, 3 (fermented product), 10, 17 and 24 (ripened product) days. Sensorial and microbiological analysis was carried out only on the finished products (24 days of drying).

Analytical methods

Microbiological parameters

Hygienic quality was determined according to the following methods: *Salmonella* ISO 3565 (ISO, 1983), *Escherichia coli* (Pascual, 1992), sulfite reducing Clostridia (Pascual, 1992) and *Staphylococcus aureus* enterotoxigenic (UNE, 1985).

Nitrogen fractions

The following nitrogen fractions were determined: sarcoplasmic protein N (SN), myofibrillar protein N (MN), non-protein (NPN), soluble N in 0·1N NaOH (IN) and total N (TN), according to the methods described by Astiasarán *et al.* (1990*a*). This method

involved the extraction of the samples with buffers of different ionic strength for the sarcoplasmic (0·08 and pH = 7·4) and myofibrillar (0·1 and pH = 7·4) fractions, with 10% trichloroacetic acid for the non-protein nitrogen and with NaOH for the insoluble protein. The solubility of sarcoplasmic and myofibrillar nitrogen fractions was estimated as a percentage of total nitrogen.

Sensory evaluation

The panel was composed of 10 judges previously trained and selected by triangle tests. Panel members independently evaluated each sample for appearance (visual appraisal of the surface based on the lean-fat binding), hardness (force required to bite completely through sample between molar teeth), saltiness (amount of saltiness perceived during mastication) and overall acceptability. All of them were evaluated on a 1–9 hedonic scale (scores were grouped in the following intervals: 1–4: deficient in appearance, extremely soft, no salt, very bad acceptability; 4·1–6: normal in appearance, hardness, saltiness and overall acceptability; 6·1–9: very good appearance, extremely hard and salty, very good overall acceptability).

Data analysis

The evolution of the chemical parameters through all the ripening was analysed with an ANOVA with a confidence level of 95%. Differences between control and modified products were evaluated with a Student's *t*-test, showing the level of significance in the tables.

RESULTS AND DISCUSSION

The change in the formulation gave rise to a Na^+/K^+ ratio decreased from 4·35 in the control to 0·86 in the modified product; and a significantly higher water activity and acidification were found (Ibañez *et al.*, 1996).

Table 1 shows the results of the microbiologial analysis. No differences were detected in the hygienic quality in both types of products, in spite of the higher water activity observed in the modified products (0·914 in the modified products and 0·844 in the control).

Acidification is one of the reasons for protein insolubilization. In the previous paper, a significantly ($p < 0·001$) greater acidification was observed in the modified products throughout all the ripening. Indeed, the insoluble fraction was significantly higher in the modified products during nearly all the ripening (Table 2). This fraction showed an increase of 26·4% in the modified product and 18·2% in the control. The loss of protein solubility is not uniform and depends, among other factors, on the technology applied (Astiasarán *et al.*, 1993). Tables 3 and 4 show the evolution of sarcoplasmic and myofibrillar nitrogen fraction solubility, respectively, during ripening. In both sarcoplasmic and myofibrillar fractions, the percentage of solubility was significantly lower in the modified product, which is consistent with its higher percentage of insoluble fraction. The loss of

TABLE 1
:Results of Microbiological Analysis in the Finished Products

	Control	*Modified*
Salmonella(in 25g)	absence	absence
Staphylococcus aureus Enterotoxigenic (c.f.u.g^{-1})	< 100	< 100
Sulphite reducing Clostridia (c.f.u.g^{-1})	< 100	< 100
Escherichia coli (c.f.u.g^{-1})	< 100	< 100

TABLE 2
Insoluble Nitrogen Fraction (mg IN mg^{-1} NT × 100)

Days	*Control*	*Modified*	*LS*
0	9·4 (0·9[a]; 9·57)	12·9 (1·0[a]; 7·75)	**
1	9·1 (0·9[a]; 9·89)	14·7 (0·7[a]; 4·76)	***
3	21·0 (1·2[b]; 5·71)	39·1 (1·1[b]; 2·81)	***
10	32·5 (1·5[c]; 4·61)	37·7 (1·4[b]; 3·71)	**
17	36·2 (1·0[d]; 2·76)	38·1 (1·5[b]; 3·94)	NS
24	27·6 (1·2[e]; 4·35)	39·3 (1·0[e]; 2·54)	***

Mean values and standard deviations of eight values. ANOVA: for each type of product values with the same letter are not significantly different ($p > 0·05$).
Student's *t*-test: LS (level of significance), NS (not significant, $p > 0·05$), ** ($p < 0·01$), *** ($p < 0·001$).

myofibrillar solubility was higher in modified products (33·6%) than in the control (27·6%). However, the loss of solubility affecting the sarcoplasmic fraction was very similar in both types of product: 9·9% in modified product and 9·3% in the control. These results suggest that the myofibrillar fraction is more affected by the proposed modification than the sarcoplasmic fraction.

Besides insolubilization, proteins are attacked by proteolytic enzymes during ripening. Modified products showed a significantly higher NPN fraction from the beginning of the process than the control (Table 5). This positive effect on proteolytic activity could be explained by the contribution of three factors. First, by the greater acidification, which could favour proteolytic activity (Astiasarán *et al.*, 1990*a*); Burgos (1981) also observed in sausages higher proteolytic activities when the pH decreased. Secondly, salt reduction

TABLE 3
Sarcoplasmic Nitrogen Fraction (mg SM mg^{-1} NT × 100)

Days	*Control*	*Modified*	*LS*
0	16·9 (0·9[a]; 5·32)	14·5 (0·7[a]; 4·83)	**
1	15·4 (0·6[a]; 3·90)	13·7 (0·8[a]; 5·84)	*
3	20·2 (1·0[b]; 4·95)	12·9 (2·1[a]; 16·28)	***
10	21·5 (0·6[b]; 2·79)	10·0 (0·8[b]; 8·00)	***
17	8·7 (0·8[c]; 9·19)	8·5 (0·4[b]; 4·70)	NS
24	7·6 (0·3[c]; 3·95)	4·6 (0·6[c]; 13·04)	***

Mean values and standard deviations of eight values. ANOVA: for each type of product values with the same letter are not significantly different. ($p > 0·05$).
Student's *t*-test: LS (level of significance), NS (not significant, $p > 0·05$), ** ($p < 0·01$), *** ($p < 0·001$).

TABLE 4
Myofibrillar Nitrogen Fraction (mg MN mg^{-1} NT × 100)

Days	*Control*	*Modified*	*LS*
0	36·9	38·2	*
	(0·7[a]; 1·90)	(0·7[a]; 1·83)	
1	30·0	21·2	***
	(1·1[b]; 3·63)	(0·9[b]; 4·24)	
3	23·3	12·4	***
	(1·1[c]; 4·72)	(0·6[c]; 4·84)	
10	22·4	11·9	***
	(0·7[c]; 3·12)	(0·5[c]; 4·20)	
17	15·2	11·2	***
	(0·3[d]; 1·97)	(0·2[c]; 1·78)	
24	9·3	4·6	***
	(0·6[e]; 6·45)	(0·5[d]; 10·90)	

Mean values and standard deviations of eight values. ANOVA: for each type of product values with the same letter are not significantly differents ($p > 0.05$).
Student's *t*-test: LS (level of significance), NS (not significant, $p > 0.05$), ** ($p < 0.01$), *** ($p < 0.001$)

TABLE 5
Non Protein Nitrogen Fraction (mg NPN mg^{-1} NT ×100)

Days	*Control*	*Modified*	*LS*
0	11·8	16·9	***
	(0·7[a]; 5·93)	(0·7[a]; 4·14)	
1	12·5	17·3	***
	(0·7[a]; 5·60)	(0·8[ab]; 4·62)	
3	5·3	17·7	***
	(0·7[b]; 13·21)	(0·8[abc]; 4·52)	
10	8·3	18·0	***
	(0·5[c]; 6·02)	(0·8[abc]; 4·44)	
17	17·3	19·2	**
	(0·6[d]; 3·47)	(0·8[bc]; 4·17)	
24	15·0	19·1	***
	(0·5[e]; 3·33)	(1·1[c]; 5·76)	

Mean values and standard deviations of eight values. ANOVA: for each type of product values with the same letter are not significantly differents ($p > 0.05$).
Student's *t*-test: LS (level of significance), NS (not significant, $p > 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

could favour the activity of cathepsins. Toldrá (1992) pointed out that the activity of cathepsins could be affected by different salt levels, cathepsin H and D activities being substantially inhibited by salt. Finally, *Micrococcaceae* counts (datas showed in Ibañez *et al.*, 1996) were higher in the modified products during the first weeks and the marked proteolytic and lipolytic acivities of these micro-organisms have been widely recognised (Nordal and Slide, 1980, Lücke, 1987, Sanz *et al.*, 1988).

The observed modifications to protein solubility and degradation could have been expected to have some effect on the sensorial properties of the final products. Table 6 shows the results of the sensorial analysis. The binding capacity of the proteins was sensorially analysed through the evaluation of the slices' appearance. No significant differences were found in the mean scores between modified and control products, indicating

TABLE 6
Sensorial Analysis of the Finished Products

	Control	*Modified*	*LS*
Appearance	7·75 (0·26; 3·35)	7·82 (0·28; 3·58)	NS
Hardness	5·64 (0·63; 11·17)	4·69 (0·46; 9·91)	**
Saltiness	6·40 (0·56; 8·75)	4·59 (0·33; 7·19)	***
Overall acceptability	6·66 (0·51; 7·66)	6·17 (0·88; 14·26)	NS

Mean values of 80 evaluations with the standard deviations.
Student's *t*-test: LS level of significance. NS (not significant), ** ($p < 0{\cdot}01$), *** ($p < 0{\cdot}001$).

that no negative effects of this functional property took place. Hardness was considered by the panellists as normal in both types of products, although a significant lower score was observed in the modified products. Saltiness was evaluated in the range of normal for modified products and in the range of excessive for control products. The best scores for this characteristic of the modified products is in line with the new tendencies in the tastes of health-conscious consumers. In other studies, results indicate that a salt-substitute containing equal concentrations of NaCl and KCl can replace higher concentrations of NaCl without affecting consumer acceptance (Ainsworth *et al.*, 1993; Adams *et al.*, 1994). Adams *et al.* (1995) suggested that the concentration of sodium alone does not determine the perception of saltiness in a complex food system.

In summary, the proposed modification gave rise to significant changes in the nitrogenous fractions with some repercussions in the functional properties of proteins expressed by a slight reduction in the hardness evaluation of the product. Nevertheless, a better evaluation of the salted taste was found which makes the development of products with a reduction in common salt more interesting.

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