ADDITION OF BROMELAIN INCREASES THE SENSORY QUALITY OF COARSELY CHOPPED DRY SAUSAGE.

MELENDO J.A., BELTRAN J.A., JAIME I., SANCHO R. and RONCALES P.

Dept. of Food Technology. Faculty of Veterinary Science. University of Zaragoza. ZARAGOZA. SPAIN.

S-VIA.47

SUMMARY

Commercial protease bromelain was added to a sausage mixture for coarsely chopped dry sausage 'chorizo' at different concentrations ranging from 6 to 600 U/100 g. Bromelain exerted a clear proteolytic effect, dependent of enzyme concentration, as revealed by non-protein nitrogen results and electrophoretic patterns. Proteolysis brought about a significant tenderising effect, evidenced by lower resistance to shear force. According to sensory analysis, tenderisation due to degradation of myofibrillar proteins was evaluated as excessive at high concentrations, while it resulted in higher quality sausages at low concentrations, noted particularly in texture perception. Difference with enzyme-free control was statistically significant, as demonstrated by both triangle and duo-trio discriminative tests.Bromelain was highly effective at refrigeration temperatures.

INTRODUCTION

Toughness is usually not included among the prevalent sensory characteristics of dry sausage. However, some of these meat products may result tough, chiefly to youths and the elderly. This is the case of most Spanish 'chorizos', whose meat is chopped very coarsely and ripening time is not as long as would be convenient for effective proteolytic tenderisation.

Changes affecting proteins throughout ripening of meat products have been shown to be due not only to microbial enzymes but also to endogenous proteases, chiefly cathepsins (Toldrá et al., 1991; Sárraga et al., 1993). Even though, proteolysis of dry sausage is a very slow process (Toldrá et al., 1991).

Proteases of different origins have been used for the tenderisation of meat (Etherington, 1991). Plant enzymes as papain and bromelain are the best known among them; besides these enzymes, other exogen proteases have been also proposed, namely cathepsins from spleen (Robbins and Cohen, 1976) or hialuronidases (Ouali, 1990).

Addition of enzymes to dry fermented sausages has been also considered, looking forward to obtain an enhanced final flavour. Lipases (Fernández et al., 1991) and aspartil-proteinases (Díaz et al, 1993) have been tried out for this purpose, with conflicting results.

The objective of this work is the assessment of a protease for obtaining a coarsely chopped dry sausage of a high quality for its sensory properties, specially referred to toughness, without the need of a long time of ripening.

The enzyme selected for this research was bromelain, E.C. 3.4.22.4., a plant endoprotease with optimum pH of 6 and temperature of 60C (Choi et al., 1992). The fact of being stable at 4°C (Beynon and Bond, 1989) was highly considered. Bromelain, besides its high activity upon myofibrillar proteins, possess a noticeable collagenolitic activity, similar to that of cathepsins B and L (Etherington, 1991). Its high specificity against myosin, as opposed to papain action (Kim and Taub, 1991), has been especially valued.

MATERIAL AND METHODS

"Chorizo" sausage was prepared as follows: 70% lean pork, 18% fat pork, 12% water and spices (powdered red pepper), 25 g/Kg sugars, 2 g/Kg common salt and nitrates, 1 g/Kg ascorbic acid, 2 g/Kg garlic.

Lean was minced using a 16 mm plate. Sausage mixture was divided in four batches and bromelain was added to three of them at either 6, 60 or 600 U/100 g. Batches were keeped at 0-4°C during 24 hours for improving emulsion stability and protein gelation. Samples were stuffed thereafter into 45 mm. diameter fibrous collagen casings and the sausages were held 48 hours at 20-24°C (80-85% relative humidity; RH) to allow fermentation. Afterwards, it was transferred to a drying room at 12-16°C (75-80% RH) and ripened for 36 days.

In addition to the sample taken from the non-stuffed mixture, individual "chorizo" samples were taken at several

times: after stuffing, 2, 4, 20 and 36 days. The sausages were ground in a meat grinder after removing the casing and the mixture was used for subsequent analysis.

Moisture was determined using the International Standard ISO 1442 (Spanish Ministry of Health and Social Security, 1985). pH was measured immediately after homogenization of a sample (3 g) in distiled water (30 ml) for 2 minutes. Total protein was analysed according to Kjeldahl method. International Standard ISO/ R 937 (Spanish Ministry of Health and Social Security, 1985). Non protein nitrogen was determined using the International Standard ISO/ R 937 (Spanish Ministry of Health and Social Security, 1985). Amine nitrogen was analysed at 570 nm. by a coloured compound formed by combination with ninhydrin and referred to a glycin standard curve. Ammonium nitrogen was determined using Johnson method (1941) with "chorizo" suspension obtained after precipitating the rest of the nitrogen fractions with tricloroacetic acid.

Sensory Analysis; sausages were evaluated at the end of the ripening process for sensory attributes by a jury of 20 semi-trained members using a 9 point intensity scale; significance between sample means was tested by ANOVA. A triangle and duo-trio tests were made according to the International Standard ISO CTC 34/SC 12 Regulation.

Electrophoretic study: myofibrillar proteins were obtained as described by Olson et al. (1976). Electrophoresis was performed in polyacrylamid gel with SDS according to Greaser et al. (1983).

Warner-Bratzler shear force was performed using an INSTRON model 4301. Samples were prepared from pieces of meat about 0,5 cm thick.

RESULTS AND DISCUSSION

As shown in Table 1, the evolution of pH and moisture content along the ripening of all sausages, independent of protease addition, was consistent with values reported previously for similar dry fermented sausages (Dierick et al, 1974; Palumbo et al., 1977; León Crespo et al., 1978; De Masi et al., 1991).

Addition of bromelain resulted in a dramatic enhancement of NNP formation (Figure 1); this effect was larger the higher was the concentration of added enzyme. Evolution of non protein nitrogen (NNP) appeared to be similar in all samples, showing a strong increase in the first days and a slow rise thereafter. It must be emphasised that the proteolityc activity proceeded chiefly at refrigeration temperatures, even before stuffing and fermentation. Díaz et al. (1993) found a similar evolution in other dry fermented sausage with addition of pronase E.

On the contrary, it may be observed that the formation of the different compounds which correspond to amine nitrogen (Figure 2), mainly aminoacids and small peptides, were produced in a lineal fashion, independent of the rapid action of bromelain. This behaviour is characteristic of endoproteases and of bromelain, since the large peptides firstly produced are only subsequently degraded to smaller peptides (Kim and Taub, 1991). Amine nitrogen levels were somehow related to the concentration of enzyme added.

Evolution of ammonium nitrogen (Figure 3) showed a similar shape, accordingly to the same considerations made above. Nevertheless, nitrogen levels were in this case absolutely independent of the concentration of enzyme.

The concentration of both forms of nitrogen found in our research were consistent with values reported previously by other authors (Dierick et al., 1974; Bello et al., 1974; Palumbo et al., 1977; Lois et al., 1987).

Sensory analysis of sausages was performed as described by Roncalés et al. (1991). In addition, a specific profile of textural parameters was also included. The sample with the higher bromelain concentration could not be evaluated because its softness was well over that expected in a dry sausage.

As shown in Figure 4, significant differences between the samples which contained 60 U of bromelain and the others were generally found, because its excessive lack of toughness and worst sensory ratings. The sausage containing 60 U differed greatly in overall toughness and toughness at first bite, leading to a lesser acceptability. Conversely, control and the 6 U batch showed minor differences in these texture parameters, and differed largely in total chewing and chewing force. Accordingly, most of the sensory ratings related to the sausage quality were higher in the 6 U bromelain sample than in the control. It is then demonstrated that bromelain exerted in the studied conditions a softening effect. This effect may be excessive if the bromelain concentration is too high, but there is a concentration of about 6 U which resulted in a possitive and desirable effect for 'chorizo' quality.

In order to validate the results obtained, we carried out triangle and duo-trio discriminative tests to differ between these two samples. Both tests showed that samples were different with a 90% of significance.

Consequently with these results, Table 2 is demonstrative of the differences found in the shear force using a Warner-Bratzler device among sausage samples differing in bromelain concentration added.

The electrophoretic study of the changes undergone by myofibrillar proteins throughout sausage ripening in the presence of varying concentrations of bromelain is shown in Figure 5. It can be first noticed that there were not many problems for myofibrillar proteins extraction, since electrophoretic density and mobility of most proteins was similar to those found in fresh meat. It is also very noticeable that the sample with 600 U had been hydrolysed

totally, since only peptides at the end of the run were found.

Myosin was degraded very intensely throughout control ripening, and totally in the presence of 60 U bromelain. Actin, on the contrary, was only slightly hydrolysed until a high amount of enzyme was added. Kim and Taub (1991) already reported that myosin was hydrolised by bromelain faster than actin, but this had never been reported in the ripening of a meat product.

Differences between control sausage and that with a lower concentration of bromelain were scarce. Only small differences referred to troponin T could be detected; its corresponding band disappeared and a peptide of lower molecular weight arose.

CONCLUSIONS

Bromelain exerted a significant tenderising effect on dry sausage. This effect resulted in an increase of desirable sensory characteristics when added at a concentration of 6 U/100 g. Bromelain was highly effective at refrigeration temperatures.

ACKNOWLEDGEMENT

The authors wish to thank the Diputación General de Aragón for the partial support of this research.

REFERENCES

Bello, J., Larralde, J., Saenz de Buruaga, I. (1974). "Estudio de las modificaciones proteicas que tienen lugar durante la curación de algunos de los derivados cárnicos". Anal. Brom., **26**: 195-210.

Beynon, R.J. & Bond, J.S. (1989). "Commercially available proteases". In "Proteolytic Enzymes" (Beynon, R.J. & Bond, J.S. eds.), IRL Press, Oxford, 232-240.

Choi, C., Son, G.M., Cho, Y.J., Chun, S.S., Lim, S.I. & Seok, Y.R. (1992). "Purification abd characteristics of bromelain from korean pineapple". J. Korean Agr. Chem. Soc. 35: 23-29.

De Masi, T., Wardlaw, F. Dick, R. & Acton, J. (1990). "Nonprotein nitrogen (NPN) and free amino acid contents of dry, fermented and nonfermented sausages." Meat Sci. **27:** 1-12.

Díaz, O., Fernández, M., García de Fernando, G., de la Hoz, L. & Ordóñez, J.A. (1993). "Effect of the addition of pronase E on the proteolysis in dry fermented sausages". Meat Sci. **34**: 205-216.

Dierick, N., Vandekerckhove, P. & Demeyer, D. (1974). "Changes in nonprotein nitrogen compounds during dry sausage ripening." J. Food Sci. **39:** 301-304.

Etherington, D.J. (1991). "Enzymes in the meat industry". In "Enzymes in food processing" (G.A. Tucker and L.F.J. Woods eds.), Van Nostrand-Reinhold, New York, 128-160.

Fernández, M., Díaz, O., Cambero, M.I., Hoz L. & Ordóñez, J.A. (1991). "Effect of the addition of pancreatic lipase on the lipolysis during the ripening of dry fermented sausages." Proc. 37th Int. Cong. of Meat Sci. and Technol. Kulmbach. 867.

Greaser, M.L., Yates, L. D., Krzywiki, K. & Roelde, D. L. (1983). "Electrophoretic methods for the separation and identification of muscle proteins:. Rec. Meat Conf. Proc. 36, 87-91.

Johnson, M.J. (1941). J.Biol.Chem., **137**: 575.

Kim, H.J. & Taub, L.A. (1991)."Specific degradation of myosin in meat by bromelain". Food Chem. **40**: 337-343.

León Crespo, F., Millán, R. & Serrano Moreno, A. (1978). "Cambios químicos durante la maduración del salchichón, 3: Modificaciones experimentadas por los compuestos nitrogenados solubles en agua." Arch. Zootecn. **27:** 105-116.

Lois, A.L., Gutiérrez, L.M., Zumalacárregui, J.M. & López, A. (1987). "Changes in several constituents during the ripening of chorizo - A Spanish dry sausage." Meat Sci. **19:** 169-177.

Ministerio de Sanidad y Consumo (1985). "Análisis de alimentos". Servicio de publicaciones del Ministerio de Sanidad y Consumo. Madrid.

Olson, D.G., Parrish, F.C.Jr. & Stromer, M.H. (1976). "Myofibril fragmentation and shear resistance of three muscles during postmortem storage". J. Food Sci. **41**:1036-1041.

Ouali, A. (1990). "Meat tenderisation: possible causes and mechanism. A review". J. Muscle Foods **1**: 129-165.

Palumbo, S.A., Smith, J.L. (1977). "Chemical and microbiological changes during sausage fermentation and ripening." ACS Symposium Series N 47: 279-294.

Robbins, F.M. & Cohen, S.H. (1976). "Effects of catheptic enzymes from spleen on the microstructure of

bovine muscle". J. Text. Stud. **7**:137-142.

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

Sárraga, C., Gil, M. & García-Regueiro, J.A. (1993). "Comparison of calpain and cathepsin (B, L y D) activities during dry-cured ham processing from heavy and light large white pigs". J. Sci. Food Agric. **62**, 71-75.

Toldrá, F., Motilva, M.J., Rico, E. & Flores, J. (1991). "Enzyme activities in the processing of dry-cured ham." Proc. 37th Int. Cong. of Meat Sci. Technol. Kulmbach. 954-956.

CAPTIONS TO TABLES AND FIGURES

TABLE 1.- Evolution of mean values for pH and dry matter (%) throughout ripening of all dry sausages.

TABLE 2.- Warner-Bratzler shear force of meat pieces from dry sausages containing varying concentrations of bromelain.

Figure 1.- Effect of the addition of bromelain on the concentration of non protein nitrogen throughout dry sausage ripening.

Figure 2.- Effect of the addition of bromelain on the concentration of amine nitrogen throughout dry sausage ripening.

Figure 3.- Effect of the addition of bromelain on the concentration of ammonium nitrogen throughout dry sausage ripening.

Figure 4.- Sensory evaluation of dry sausages containing varying concentrations of bromelain.

Figure 5.- SDS-PAGE using 15% acrylamide of myofibrillar proteins from dry sausages containing varying concentrations of bromelain. Control: 1) initial mixture, 2) ripened. Bromelain, ripened: 3) 6 U/ 100 g, 4) 60 U/100 g, 5) 600 U/100.