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Limited proteolysis of myofibrillar proteins by bromelain decreases toughness of coarse dry sausage

J. A. Melendo, J. A. Beltrán, I. Jaime, R. Sancho & P. Roncalés*

Department of Food Technology, Faculty of Veterinary Science, University of Zaragoza, c. Miguel Servet 177, 50013, Zaragoza, Spain

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Commercial protease bromelain was added to a coarsely chopped dry sausage, 'chorizo', at different concentrations ranging from 6 to 600 U per 100 g. Bromelain exerted a clear proteolytic effect on myosin and other myofibrillar proteins, depending on enzyme concentration, as revealed by non-protein nitrogen data and electrophoretic patterns. Proteolysis brought about a significant ($P < 0.1$) tenderising effect on sausages, according to sensory analysis. Tenderisation was evaluated as being excessive and undesirable at high concentrations, while it showed no significant differences in parameters related to sausage quality when used at low concentrations. The difference with enzyme-free control was statistically significant ($P < 0.1$), as demonstrated by both triangle and duo–trio discriminative tests. Bromelain exerted its activity mainly within an initial 48 h period at refrigeration temperatures. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Toughness is usually not included among the prevalent sensory characteristics of dry sausage. However, some of these meat products may appear tough, chiefly to the young and the elderly. This is the case for most 'chorizo' sausages, in which the meat is chopped very coarsely and the ripening time is not sufficient for effective proteolytic tenderisation.

Changes affecting proteins during the ripening of meat products have been shown to be due not only to microbial enzymes but also to endogenous proteases, mainly cathepsins (Toldrá & Etherington, 1988; Sárraga *et al.*, 1993); even so, proteolysis of dry sausage is a very slow process (Toldrá *et al.*, 1993).

Addition of enzymes to dry fermented sausages to enhance flavour has been already considered. Lipases (Fernández *et al.*, 1991) and aspartyl-proteinases (Díaz *et al.*, 1993) have been tried, but with conflicting results.

The objective of this work was the assessment of a protease for obtaining a coarsely chopped dry sausage with high quality sensory properties, especially texture, without the need for a long ripening time. The enzyme selected for this research was bromelain, EC 3.4.22.4, a plant endoprotease with a pH optimum of 6 and temperature optimum of 60°C (Choi *et al.*, 1992). Its stability at 4°C (Beynon & Bond, 1989) was a major consideration. In addition to its high activity on myofibrillar proteins, bromelain possesses a noticeable collagenolytic activity, similar to that of cathepsins B and L (Etherington, 1991). Its high specificity for myosin, compared with the action of papain (Kim & Taub, 1991), has been especially considered.

*To whom correspondence should be addressed.

MATERIALS AND METHODS

'Chorizo' sausage was prepared as follows: 70% lean pork shoulder, 18% pork back fat, 12% water and spices (powdered red pepper, 20 g kg^{-1}), 25 g kg^{-1} sugar, 20 g kg^{-1} common salt, 0.1 g kg^{-1} potassium nitrate, 1 g kg^{-1} ascorbic acid and 2 g kg^{-1} garlic.

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

In addition to the triplicate samples taken from the non-stuffed mixture, three individual 'chorizo' sausages from each batch were taken at the following times: 2, 4, 20 and 36 days (i.e. after stuffing, after fermentation,

half-dried and dried, respectively). The sausages were ground separately in a meat grinder after removing the casing, and duplicates of each mixture were used for subsequent analysis. Results were expressed as the mean values of data obtained from every three sausages.

Moisture was determined using the International Standard ISO 1442 (MSC, 1985). Sausage pH was measured with a combined electrode immediately after homogenisation of a sample (3 g) in distilled water (30 ml) for 2 min. Total protein was analyzed according to the International Standard ISO/R 937 (MSC, 1985). Non-protein nitrogen (NPN) was determined using the International Standard ISO/R 937 (MSC, 1985) after precipitation of proteins with 0.6 M trichloroacetic acid. Amino nitrogen was analysed by measuring absorbance at 570 nm of the coloured compound formed by combination of free α-amino groups with ninhydrin, and referred to a glycine standard curve. Ammonia nitrogen was determined using the method of Johnson (1941) with a 'chorizo' suspension obtained after precipitating the rest of the nitrogen fractions with 0.6 M trichloroacetic acid.

Sausages were evaluated at the end of the ripening process for sensory attributes by a jury of 15 trained members using a 9-point intensity scale, according to Roncalés *et al.* (1991). A specific texture profile, as shown in Fig. 3, was especially designed for this research. Significance (at the 90% level) between sample means was tested by ANOVA. Triangle and duo–trio tests were carried out by a panel of 30 untrained members, according to the British Standard Institution (BS-5929-3/1984 and BS-5929-4/1985, respectively).

For the electrophoretic study, myofibrillar proteins were obtained as described by Olson *et al.* (1976). Electrophoresis was performed in 15% polyacrylamide gel slabs with sodium dodecyl sulphate, according to Greaser *et al.* (1983).

RESULTS AND DISCUSSION

Changes in pH and moisture content during the ripening of sausages (not shown) were consistent with results reported previously for other dry fermented sausages (Dierick *et al.*, 1974; Palumbo & Smith, 1977; Lois *et al.*, 1987; De Masi *et al.*, 1990).

Addition of bromelain resulted in a dramatic enhancement of NPN formation (Fig. 1), related to the concentration of added enzyme. Evolution of NPN showed a strong increase in the first days and a slow rise thereafter. It must be emphasised that the proteolytic activity proceeded chiefly at refrigeration temperatures, before stuffing and prior to the main fermentation. Díaz *et al.* (1993) found a similar evolution in other dry fermented sausages with addition of pronase E.

Conversely, it can be seen in Fig. 2 that the formation of amino nitrogen was linear, independent of the rapid action of bromelain. This behaviour is characteristic of endoproteases, and therefore of bromelain, since the large peptides produced by the action of these enzymes are subsequently degraded to smaller peptides and free amino acids (Kim & Taub, 1991). Amounts of amino nitrogen formed were also related to the concentration of enzyme added, although differences among batches were not as large as for NPN formation. Evolution of ammonia nitrogen (not shown) showed a similar pattern, according to the same considerations noted above. Nevertheless, nitrogen levels in this case were almost independent of the concentration of enzyme added.

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

As shown in Fig. 3, significant differences ($P < 0.1$) between the samples that contained 60 U of bromelain and the others were generally found. These sausages differed largely ($P < 0.1$) in most of the texture profile parameters. The control and the 6 U batch showed smaller differences in these textural parameters, but differed significantly ($P < 0.1$) in toughness at first bite, total chewing, chewing force and overall toughness. All this gave rise to a greatly reduced overall toughness in the 60 U sausage, while reduction was only slight in the 6 U batch, when compared to control sausages.

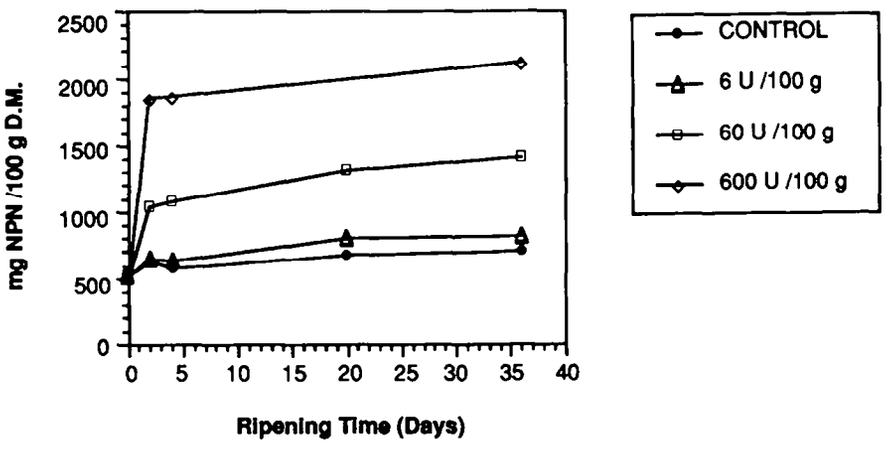

Fig. 1. Effect of the addition of bromelain on the concentration of non-protein nitrogen (NPN) during dry sausage ripening.

The 6 U bromelain sample and the control did not differ for most of the sensory ratings related to sausage quality, while sensory ratings were significantly lower ($P < 0.1$) for the sausages containing 60 U of bromelain. Flavour was found to be more intense in these latter sausages, while its quality was evaluated as significantly ($P < 0.1$) lower. Results concerning the batch that contained the highest amount of enzyme essentially agreed with those obtained previously by Díaz *et al.* (1993). Therefore, the softening effect of bromelain under the conditions studied appeared to be excessive if its concentration is too high, but the lower concentration of about 6 U resulted in a presumably desirable effect for 'chorizo' sausage quality (i.e. more tender with the same flavour).

In order to check if differences could be appreciated by consumers, we carried out triangle and duo–trio tests to discriminate between control and 6 U bromelain samples.

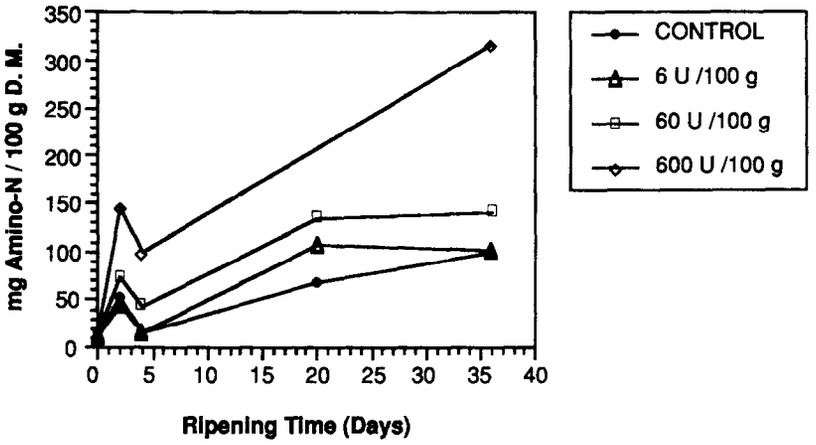

Fig. 2. Effect of the addition of bromelain on the concentration of amino nitrogen during dry sausage ripening.

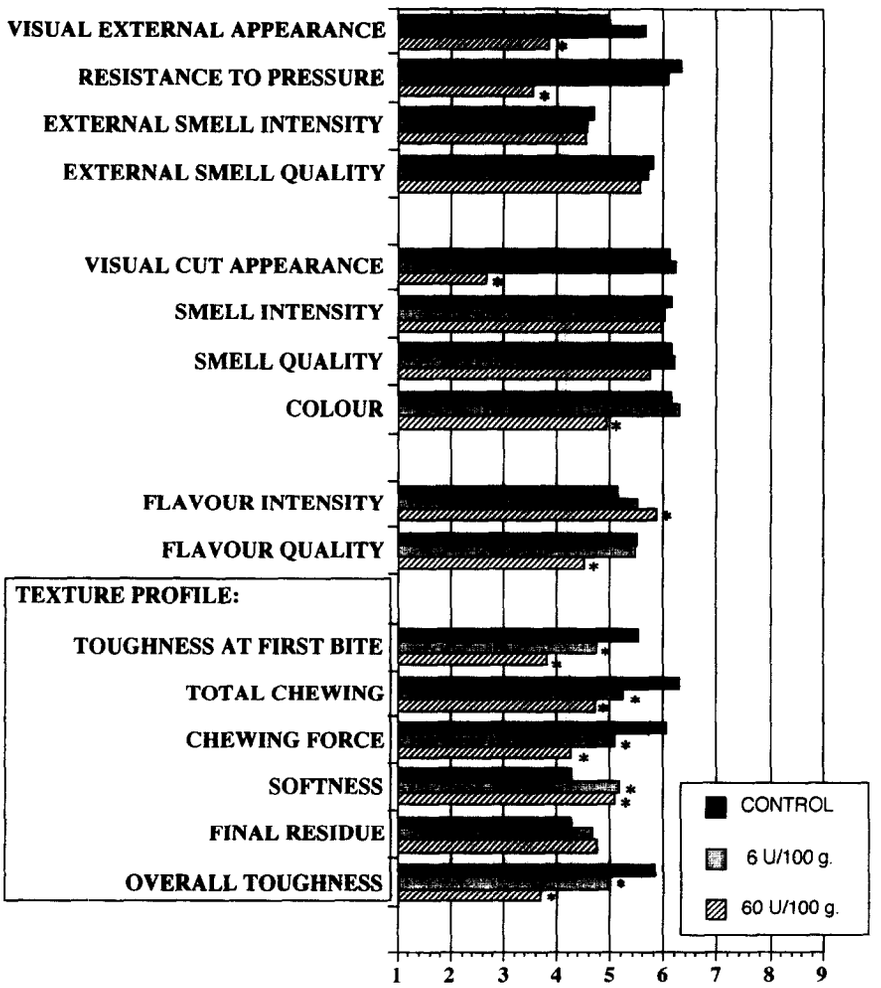

Fig. 3. Sensory evaluation of dry sausages containing varying concentrations of bromelain: 9 denotes maximum intensity or quality; 1 denotes minimum intensity or quality. *Mean values differ significantly at the 90% level from the control.

Both tests (results not shown) demonstrated that samples were different with a 90% level of significance.

The electrophoretic study of the changes undergone by myofibrillar proteins during sausage ripening in the presence of varying concentrations of bromelain is shown in Fig. 4. There appeared to be no interference with myofibrillar protein extraction, since relative electrophoretic density and mobility of most proteins were similar to those found in fresh meat. It is also very noticeable that myofibrillar proteins of the sample with 600 U had been hydrolysed totally, since only small peptides located at the end of the run were present.

Myosin was degraded very intensely throughout the ripening of control sausages, and totally in the presence of 60 U bromelain. Actin, on the other hand, was only slightly hydrolysed unless a high amount of enzyme was added. Kim & Taub (1991) have already reported that bromelain hydroysed myosin faster than actin, but this has never been reported for the ripening of a meat product.

Differences between the control sausage and that with the lower concentration of bromelain were few. However, small differences related to the myosin heavy chain and troponin T could be detected, although the latter is hardly noticeable in the gel photograph shown. Their corresponding bands decreased in intensity and peptides of lower molecular weight were formed. Degradation of myosin could therefore be related to the tenderising effect of bromelain.

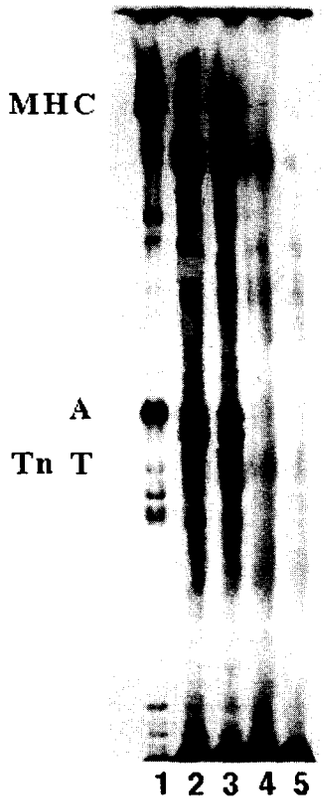

Fig. 4. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (using 15% acrylamide) of myofibrillar proteins from dry sausages containing varying concentrations of bromelain. Control: (1) initial mixture, (2) ripened. Bromelain added, ripened: (3) 6 U per 100 g, (4) 60 U per 100 g, (5) 600 U per 100 g.

CONCLUSIONS

Bromelain exerted a significant ($P<0.1$) tenderising effect on dry sausage. When the enzyme was added at a concentration of 6 U per 100 g, the other sensory parameters of 'chorizo' were not affected. Bromelain exerted its activity mainly within an initial 48 h period at refrigeration temperatures.

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