EFFECT OF THE ADDITION OF PAPAINE FROM Carica papaya ON THE DRY FERMENTED SAUSAGE PROTEOLYSIS DURING RIPENING

DIAZ O., FERNANDEZ M., GARCIA DE FERNANDO G.D., DE LA HOZ L. and ORDOÑEZ J.A.

Departamento de Nutrición y Bromatología III (Higiene y Tecnología de los Alimentos), Facultad de Veterinaria, Universidad Complutense, Madrid (Spain).

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SUMMARY

The effect of the addition of papaine on the proteolysis of dry fermented sausage ("salchichón") during ripening has been investigated. All batches, with or without (control) proteinase, showed a similar pattern in microbial and moisture changes, while the pH and water activity were different because of the papaine addition. As expected, nitrogen fractions (water soluble, non protein, 5% phosphotungstic acid soluble, 5% sulfosalicylic acid soluble and total volatile basic nitrogens) reached higher values in proteinase added sausages. Sensorially, the sausages manufactured with low papaine dose were similar to control batch while those with higher amount of proteinase were significantly different due to a remarkable softening.

Introduction

Dry fermented sausage manufacture involves a long period of time until the product reaches the desirable flavour. It is obvious that to shorten such period would be very interesting from an economical point of view. The same reasoning was made for cheese in the eighties and the most studied method for getting this aim was the enzyme (especially proteinases) addition. Proteolysis is one of the most important phenomena occurring in dry femented sausages during ripening. It yields peptides and free aminoacids that contribute to the sausage texture and flavour, together to others substances (aldehides, alcohols, amines, etc) generated in the nitrogen compounds degradation. Then, the use of proteolytic enzymes in dry sausage manufacture may be suitable for increasing the concentration of these compounds and, as a consequence, shortening the ripening time or, at least, to improve the sausage flavour. Some attempts have been recently made using proteases from Lactobacillus spp (NÆs et al., 1991, 1992), aspartyl proteinase from Aspergillus oryzae (Díaz et al., 1992) and pronase E from Streptomyces griseus (Díaz et al., 1993). In the presente work, papaine from Carica papaya has been assayed with the same aim.

Materials and methods

Dry fermented sausages were manufactured in an experimental plant of a local factory. The sausage composition was (% w/w): pork (56), beef (12), lard (25), dextrose (0.8), lactose (1.0), dextrin (1.8), salt (2.5), glutamate (0.25), nitrates (0.0085), nitrites (0.0065), black pepper (0.14) and ascorbate (0.046). Ingredients were mixed in a cutter, with particle size reduction to about 3 mm. Sausage mixture was divided in three batches (2 kg each). Papaine was added to two batches at concentrations of 800 and 4500 enzime units, named as 800P and 4500 P, respectively. One unit represented the amount of enzyme that produced an increase of 1 unit in the absorbance at 440 nm per hour, using azocasein (Sigma) as sustrate (0.8 % in Tris-HCl buffer 0.2M, pH 6.5). The third batch, without papaine, was the control.

Papaine (dissolved in about 200 ml of phosphate buffer 0.1M, pH 6.6) was mixed with sausage mixture and artificial casings (3 cm diameter) were then filled with the paste. The weight of the sausages was about 200 g. Sausages were ripened in a laboratory ripening cabinet (Kowell mod. CC-3-1) programmed to give the following conditions: the temperature was maintained at 22°C for 24 h and the relative humidity (RH) at 90 % for 12 h. After these periods, the temperature and the RH were gradually decreased up to 12°C and

75 %, respectively. These values were reached at the fourth day and maintained until the end of the experiment (26 days). Samples (1 sausage) of each batch were taken at various ripening times.

Total viable counts were determined on Plate Count Agar (Oxoid) and Micrococcaceae on Mannitol Salt Agar (Oxoid), both incubated at 32°C for 2 days and lactic acid bacteria on double-layer MRS agar (Oxoid) at pH 5.6, incubated for 4 days at 32°C.

The pH was assayed by inserting an electrode into the sausage sample, and dry matter by drying at 100°C to constant weight. Water activity (a_w) was measured in a dew point instrument (CX-1 from Decagon Devices Inc., Pullman, WA, USA).

For nitrogen fraction determinations, a portion of sausages (30 g) was homogenized with distilled water in a Polytron mod PTA 20TS to a final volume of 350 ml and centrifuged (6500g for 6 min) in a Sorvall RC5B centrifuge. The resulting pellet was re-extracted with an additional volume of water (100 ml) and recentrifuged under the same conditions. Supernatants were combined and the volume was recorded. This fraction was the water soluble nitrogen (WSN). Non protein (NPN), phosphotungstic acid soluble (PTN) and sulphosalicylic acid soluble (SSN) nitrogens were obtained mixing the same volumes (20 - 50 ml) of WSN with 25 % trichloroacetic, 10 % phosphotungstic or 10 % sulfosalicylic acid solutions, respectively. Mixtures were left at 4°C for 30 min (NPN and PTN) or 17 hours (SSN) and insoluble materials were removed by filtration through Whatman papaer No 2. Total nitrogen and WSN, NPN and PTN were determined by the Kjeldahl method. SSN was used for the free amino acid determination and measured with the ninhydrin reagent as described by Clark (1966). Other aliquot of WSN was used for total volatile basic nitrogen (TVBN) determination which was carried out by the Conway microdiffusion technique (Pearson, 1973).

At the end of the ripening, samples of the three batches were organoleptically judged by a panel composed by, at least, 18 members. Triangle test was made according to the I.S.O. (TC 34/SC 12 Regulation). Samples were also examined by panelists to judge colour, appearance, texture and flavour according to a hedonic scale from 1 (very bad) to 10 (very good). Overall quality factors were calculated considering the importance of each sensory characteristic for panelists.

Results and discussion

Microbial flora: Changes during ripening were similar in all batches. Therefore, no effects of the papaine were observed. The changes were similar to those reported by authors in other kinds of dry sausages (Lücke, 1984; Sanz et al., 1988; Selgas et al., 1988).

pH: This parameter showed a similar pattern in the 800P and control batches. Values decreased from 5.9 to 5.0 during fermentation and stabilized thereafter until the end of ripening. The pH of the 4500P batch was 0.2-0.3 units higher than those of the other batches, probably ought to the great amount of basic nitrogen compounds observed in the 4500P batch.

Water activity (a_w) and moisture: Changes in a_w and moisture followed the typical trend in these products, although 4500P batch a_w values were lower than those reached by the two other batches. This difference may be explained by the great proteolysis observed in these sausages, which increased the amount of low molecular weight compounds and, because of that, the osmotic activity.

Nitrogen fractions: Figure 1 shows the changes in WSN, NPN and PTN during ripening. A similar pattern was observed in all nitrogens fractions i.e. a slight increase of the values during the first days of ripening in control and 800P batches followed by stabilization until the end of the experience. 800P batch values were slightly higher than those of the control batch. However, the 4500P batch nitrogen levels increased strongly until 11th day and values were remarkably greater than those of the other batches. The levels and changes during ripening of NPN of control batch were similar to those reported by Dierick et al. (1974), DeMasi et al. (1990) and Astiasaran et al. (1990). The increase in NPN values observed in 800P and 4500P batches was no detected by NÆs et al. (1991) in dry fermented sausages manufactured with a proteinase from Lactobacillus paraceasei sp paracasei. SSN and TVBN (figure 2) showed a similar pattern in control and 800P batches. The levels of these nitrogen fractions were higher in 4500P batch, as it happened in other fractions, but the increase continued until 19th day of ripening.

As in the case of pronase E (Díaz et al., 1993) and aspartyl proteinase (Díaz et al., 1992), a deceleration of the protein breakdown was observed in further stages of ripening. It may be attributed to the

decrease of the pH during the fermentation phase, because papaine has its optimum activity at pH values of 6.0-7.5 (Belitz and Grosch, 1987). From this point of view, this enzyme is similar to pronase E and different to the aspartyl proteinase optimum. Other factor that may cause the proteolysis deceleration is the inhibition of the enzyme activity by the accumulation of resulting products from protein degradation. The influence of increasing the amount of enzyme on the proteolysis was higher with papaine than when pronase E (Díaz et al., 1993) and aspartyl proteinase (Díaz et al., 1992) were used.

Sensory properties: No differences were found between control and 800P batches. However, 4500P was significantly different ($p<0.01$) from the other two batches. The effect of papaine in some organoleptic characteristics of sausages are shown in table 1. The 800P batch reached close qualifications to control one. A remarkable softening was observed in 4500P batch, due to an excesive proteolysis which produced a great number of low molecular nitrogen compounds (figure 2)which could affect the sausage gel formation.

Conclusion

Papaine provokes an acceleration of the proteolytic phenomena, which, in turn, causes a shortening of the ripening process from the proteolytic point of view. However, a parallel improvement of the flavour is not observed, probably because a longer time is needed for the aminoacids conversion to volatile substances, such as aldehydes, cetoacids, etc..

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Figure 1. Effect of the addition of papaine (, control; , 800 units; , 4500 units) on the changes in (A) water soluble (WSN), (B) non protein (NPN) and (C) 5 % phosphotungstic acid soluble (PTN) nitrogens during the ripening of experimental dry fermented sausages.

Figure 2. Effect of the addition of papaine (, control; , 800 units; , 4500 units) on the changes in (A) 5 % sulfosalicylic acid soluble (SSN), and (B) total volatile basic (TVBN) nitrogens during the ripening of experimental dry fermented sausages.

Table 1. Effect of papaine (P) on selected sensorial characteristics of experimental dry fermented sausages after 26 days of ripening (0-10 scale)