USE OF *Penicillium olsonii* EXTRACTS TO POTENTIATE THE DRY FERMENTED SAUSAGE FLAVOUR

BRUNA, J., FERNÁNDEZ, M., HIERRO, E., de la HOZ, L and ORDÓÑEZ, J.A.
Departamento de Higiene y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad Complutense, 28040 Madrid (Spain).

INTRODUCTION

Several attempts have been made to accelerate the ripening of dry fermented sausages by using either lipases (Zalacaín et al., 1995, 1996 and 1997a,b; Fernández et al., 1995a,b) or proteinases (Naes et al., 1995; Blom et al., 1996; Zapelena et al., 1997; Melendo et al., 1996). Pronase E from *Streptomyces griseus*, aspartyl proteinase from *Aspergillus oryzae* and papain have been used by our group (Díaz et al., 1993, 1996 and 1997) also with this aim. It was concluded (Díaz et al., 1997) that:" to shorten the ripening of dry sausages, the addition of proteinases and lipases may be useful to provide substrates, which must be transformed in aromatic compounds. Therefore, it is also necessary, besides the addition of proteinases and lipases, to create conditions or to add either an efficient starter or other kind of enzymes, so that the above mentioned volatiles may be formed in a shorter time than the usual from free amino acids and fatty acids generated by the enzymes".

The present work is an attempt to potentiate the dry fermented sausage flavour by adding both a protease and a mould extract.

METHODS

***Penicillium* extract preparation**: *Penicillium olsonii* was growth in Czapeck Dox liquid medium (Oxoid) for 15 days. Then, it was centrifuged (1000 g, 15 min) and the supernatant was washed until an absorbance value lower than 0.05. A mixture of 4 grams of mycellium, 10 ml of glass sand and 14.5 ml of phosphate buffer 0.2 M, pH 5.5 was disrupted in a cell homogeneizer (Braun MSK) during 2 min. The final mixture was filtered throught Whatman 4 and the mycellium was subjected again to the same treatment. The filtered extracts were collected and assayed for nitrogen content using the method of Lowry (1951).

Sausage preparation: The ingredients were prepared to give the final composition (%): pork 55; beef 13.4; fat 25; dextrine 1.8; glucose 0.8; lactose 1.0; phosphates 0.3; NaCl 2.5; $NaNO_2$ 0.0065; $NaNO_3$ 0.0085; ground pepper 0.14; sodium ascorbate 0.046. After grinding and before mixing the ingredients were inoculated with a starter composed by a mixture (100:1) of *Lactobacillus plantarum* 4045 and *Staphylococcus carnosus*. Four batches of sausages were manufactured. Batch control (C) composed only by the above mixture, batch PE added with 600 units of Pronase E per kg of sausage, batch PE+P added with 600 units of Pronase E and 37.5 ml of the mould extract (2.7 mg N/ml) per kg of sausage and batch P added only with the same mould extract amount. About 4 kg of every sausage batch were prepared and ripened in a ripening cabinet (Kowell Mod. CC3AFY) programmed 48 h at 22°C and relative humidity (RH) 90%; thereafter at 12°C and RH 85%.

Analytical procedures: Free amino acids and amines were analysed by HPLC as previously described by Díaz et al., (1993) and Ordóñez et al. (1991), respectively. Volatile compounds were determined by GC-MS as Hierro et al. (1995).

Texture profile analysis were carried out in a Texture analyser (Stable Micro Systems) equipped with a cylinder probe P/25 (hardness, cohesiveness, adhesiveness, gumminess, chewiness and springiness) or a reversible probe (cutting force and cutting work).

Sensory analysis: At the end of ripening, samples of the four batches were assessed by a panel composed of at least 18 members. A triangle test was made according to the International Standards Organization (I.S.O.) (TC 34/SC 12 Regulation). Samples were also examined by panellists to judge the color, appearance, texture and flavor according to a hedonic scale from 1 (very bad) to 10 (very good). Overall quality was calculated considering the importance of each sensory characteristic for panellists.

Statistical analysis: Results were statistically treated by applying the ANOVA, using a Statview program running in an Apple Macintosh PowerPC Computer. Results are from three different sausage manufactures.

RESULT AND DISCUSSION

Figure 1 shows the changes in the total free amino acids during ripening of sausages. Two different trends were observed. The first, with values reaching 4000 mg/100 g D.M. after 5 days of ripening corresponded to the batches added with Pronase E and the second, with values almost three times lower corresponded to the batch control or only added with the *Penicillium* extract. The higher free amino acid liberation is obviously due to the Pronase E activity (Díaz et al., 1993). The two batches of sausages added with the *Penicillium* extract showed a slightly lower total free amino acid content than that of the corresponding non added batch. This effect is clearer observed in the batch PE+P *vs* the PE. This fact seems to indicate an amino acid degradative activity from the *Penicillium* extract. The amines triptamine, phenylethylamine, putrescine, histamine, cadaverine, tyramine, spermidine and spermine were detected. As previously described (Díaz et al., 1997), the addition of Pronase E produced a higher accumulation of amines (data not shown), excepting spermine and spermidine. The addition of the *Penicillium* extract only produced an increase of the putrescine content when was combined with Pronase E. However, the amine values observed in this batch were lower than those described as dangerous (Renner, 1987; Halasz et al., 1994). All batches had the same volatile compound pattern although PE+P showed the higher concentration followed by PE (data not shown).

Table 1 shows the rheological parameters of the ripened dry fermented sausages. Batches added with Pronase E were softer than control, indicating a greater proteolytic activity. This fact was also corroborated with a lower gumminess, chewiness, cutting force and cutting work. In general, the addition of the *Penicillium* extract does not modify the reological parameters.

Triangle test sensory analysis (data not shown) showed that every bathch was significantly different from each other ($p<0.05$).

Table 2 shows the results of the descriptive sensory analysis. As in the case of amino acid content and rheological parameters, the main effect may be attributed to the Pronase E addition. However, the addition of the *Penicillium* extract produced slight increases on the scores of odour, texture, flavour and, obviously, global quality.

These results confirm the positive action of pronase E and they seem to indicate that the addition of *Penicillium* extracts could contribute to a better sensory atributes of dry fermented sausages. The results are promising and, therefore, a higher dosis of mould extract has to be proved.

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Table 1. Rheological parameters of dry fermented sausages after 26 days of ripening.

	Batch				
	C*	PE*	PE+P*	P*	*p*
Hardness (N)	269.2± 9.5 [a]	167.3± 12.8 [b]	166.8± 6.8 [b]	273.2± 4.3 [a]	0.0001
Adhesiveness (N)	-0.85± 0.3	-1.28± 0.5	-1.04± 0.3	-0.75± 0.1	n.s.
Springiness	0.686± 0.1	0.646± 0.1	0.587± 0.04	0.660± 0.03	n.s.
Cohesiveness	0.379± 0.009 [a]	0.346± 0.02 [a,b]	0.320± 0.02 [b]	0.385± 0.007 [a]	0.0002
Gumminess (N)	102.1± 5.2 [a]	57.8± 5.3 [b]	53.4± 5.4 [b]	105.1± 3 [a]	0.0001
Chewiness (N)	70.3± 11 [a]	37.3± 5 [b]	31.3± 3.3 [b]	69.4± 3.7 [a]	0.0001
Cutting force (N)	156.4± 3.1 [a]	110.2± 12.2 [b]	102.1± 2.5 [b]	158.51± 2.8 [a]	0.0001
Cutting work (J)	1485.1± 16.9 [a]	929.1± 3.1 [b]	813.8± 3.1 [c]	1397.7± 24.9 [d]	0.0001

* C: control batch; PE: control batch added with 600 u. of Pronase E per Kg; PE+P: batch PE added with *Penicillium olsonii* extract; P: control batch added with *Penicillium olsonii* extract.
a, b, c, d: values in a row with different letters are significantly different ($p<0.05$)

Table 2. Descriptive sensory analysis of dry fermented sausages after 26 days of ripening.

	Batch				
	C*	PE*	PE+P*	P*	*p*
Colour	7.7± 0.8	7.7± 1.2	7.8± 1.5	7.6± 0.8	n.s.
Odour	6.6± 0.7 [a]	7.3± 0.8 [a,b]	8± 1.2 [b]	7.7± 1.5 [a,b]	0.0001
Texture	6.6± 0.8 [a]	8.2± 1.3 [b]	8.4± 0.8 [b]	7.1± 1.8 [a,b]	0.0002
Flavour	6.8± 1 [a]	7.5± 0.9 [a,b]	8.5± 1 [b]	7.1±1.4 [a]	0.0003
Global quality	6.8± 0.7 [a]	7.7± 0.5 [b,c]	8.4± 0.5 [b]	7.2± 1 [a,c]	0.0001

*, a, b, c, d: similar to those of Table1.
**: Global quality= (Colour x 0.1)+ (Texture x 0,25)+ (Odour x 0,15) + (Flavour x 0,5)

Figure 1. Total free amino acid content (g/100 g D.M.) of dry fermented sausages