EFFECT OF THE FUNGAL EXTRACELLULAR PROTEASE EPg222 IN TEXTURE OF CONTROLLED RIPENED PORK

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Background

Protein denaturation takes place during the first stages of dry-cured meat processing (Córdoba et al., 1994; García et al., 1997). This phenomenon lead to an increase of hardness and chewiness of the ripened products (Monin et al. 1997). In addition, proteolysis yielding non protein nitrogen compounds related to flavour development could be limited, since denatured proteins may be not suceptible to atack by endogenous and microbial proteases (Córdoba et al., 1994). An increase of proteolysis in the first stages of processing before proteins denaturation could lead to a higher accumulation of non protein nitrogen compounds and a more desirable texture. Meat protein hydrolysis is mainly catalysed by endogenous enzymes, such as cathepsins and trypsin-like peptidases (Toldrá et al., 1993; Toldrá et al., 1997). However, NaCl and other curing agents (Rico et al., 1991) produce a strong inhibitory effect on these enzymes. To stimulate protein hydrolysis in the first stages of dry-curing meat products, exogenous proteases active at the usual NaCl concentration (3-5%) of these products could be used. In addition, the effect of exogenous proteases could be restricted by their ability to penetrate to deep tissues, especially in whole meat pieces. The use of exogenous proteases able to reach deep tissues may be of great interest to improve the sensorial characteristics of dry-cured meat products. Several proteases have been assayed to accelerate ripening of dry fermented sausages (Hagen et al., 1996; Díaz et al., 1997; Zapelena et al., 1999). However, very litle work has been done with proteases in whole ripened pieces. An extracellular protease from *Penicillium chrysogenum* (Pg222) showing high proteolytic activity against myofibrillar proteins and collagen up to 3M NaCl (Benito et al. 2002) could be adequate for these dry-cured meat products.

Objectives

The aim of this work has been to investigate the proteolytic activity of the protease EPg222 on whole meat pieces of pork loins in the first stages of dry-cured meat processing and to know its effect on texture.

Material and Methods

Sterile pork loins were obtained as described Martín et al. (2002), cut into pieces of c.a. 200 g and put into sterile bags. Each piece was added of 5% (wt/vol) sterile NaCl and kept 18 h at 4ºC. Then 30 ml of a sterile solution containing 4 mg/ml chloranphenicol, 1 mg/ml cicloheximide and 0.012 mg/ml of the enzyme EPg222 were added. Samples were ripened at 20ºC for 32 days in sterile conditions. Untreated controls were incubated at the same conditions. Samples were taken at 3, 5, 10, 17, 24 and 32 days of ripening by triplicate. The samples were analysed once a layer down to 10 mm from surface had been removed. Myofibrillar proteins were extracted with 1.1 M potassium iodide + 0.1 M sodium phosphate, pH 7.4 buffer (Córdoba et al.1994). Protein concentrations were determined according to Bradford (1976). Myofibrillar proteins were characterised by 7.5% SDS-PAGE (Laemmly, 1970). Non-protein nitrogen (NPN) was determined by Nessler method using 4 g of sample after protein precipitation with 0.6 M perchloric acid, as described by De Ketelaere et al. (1974). For microscopical observation slices were cut from deep tissues, fixed in 10% formalin, and processed using conventional histological techniques. Sections of 3 mm thick were stained with haematoxylineosin for muscle fibre observation (Córdoba et al. 1994). Texture evaluation of samples was performed as texture profile analysis (TPA) (Bourne, 1982) at room temperature, using TA-XT2 texture analyser with XT-RA dimension software (Stable Micro Systems, Godalming, UK). Cubic samples of 1 x 1 x 1 cm were compressed twice to 60% of their original height with a compression platen of 40 mm inch. diameter. Force-time curves were recorded at crosshead of 0.6 mm/s.

Results and discussion

Total amount of myofibrillar protein decreased in both treated and untreated samples during incubation time (Figure 1). At 17, 24 and 32 days of ripening this protein fraction showed lower values ($P<0.01$) than control. However, SDS-PAGE analysis of this protein fraction revealed higher proteolysis in treated than control pork at 10 days of ripening (Figure 2). These results confirm in controlled ripened pork the ability to hydrolyse the main myofibrillar proteins shown by EPg222 in vitro (Benito et al., 2002). The level of NPN was higher ($P<0.01$) in treated than in control samples after 17 days of ripening (Figure 3).

Microscopical observation reveals loss of muscle fibres structure in treated sampled as compared with control, where muscular fibres remain almost unaltered after 32 days of incubation (Figure 4). This effect could be due to the proteolytic effect of the enzyme on myofibrillar proteins.

Results of texture profile analysis are shown in figures 5 and 6. The addition of EPg222 lowered ($P<0.05$) hardness and chewiness after 10 days of ripening. These differences are mainly due to the higher hydrolysis of myofibrillar, detected only by SDS-PAGE analysis (Figure 2). The fact that the maximum difference in the texture was found at 10 days of ripening, when little changes in myofibrillar proteins are found, reveals that structural proteins other than myofibrillar proteins such as collagen have to be affected by the enzyme.

In conclusion the enzyme EPg222 is able to increase protein hydrolysis in controlled ripened pork with 5% NaCl at the first stages of processing, increasing nitrogen soluble compounds and decreasing hardness and chewiness. Given that these effects can influence sensorial characteristics of the ripened products, this enzyme could be of interest to stimulate protein hydrolysis in the first stages of the dry-curing meat processing.

Pertinent literature

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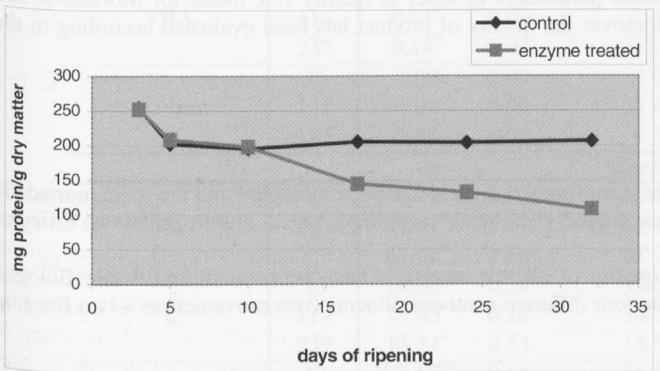

Figure 1. Myofibrillar proteins concentration during ripening of EPg222 treated and control pork.

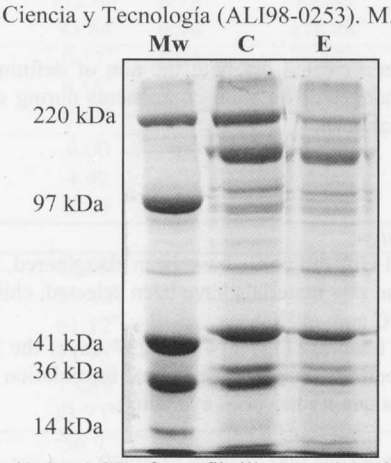

Figure 2. SDS-PAGE of myofibrillar proteins after 10 days of ripening of EPg222 treated (E) and control (C) pork. Mw, molecular weight marker.

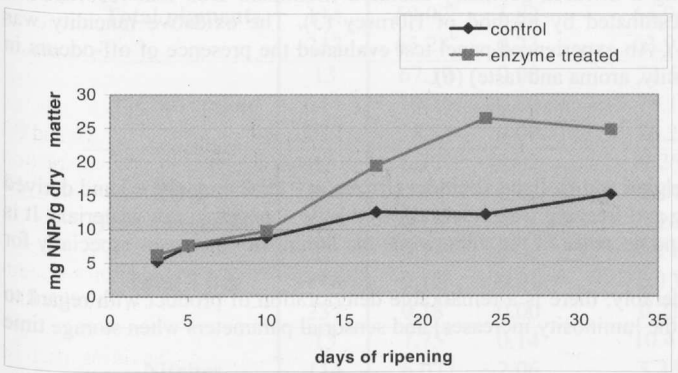

Figure 3. Evolution of non-protein nitrogen after different days of ripening in tretated (EPg222) and control ripened pork.

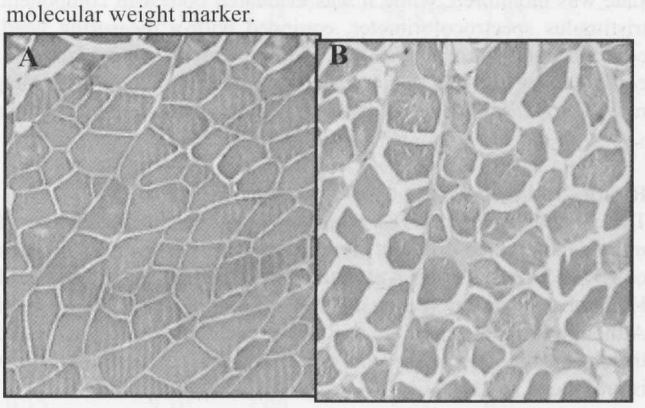

Figure 4. Microscopical observation (10x) of mucle tissue from untreated control (A) and EPg222 treated (B) pork at 32 days of ripening.

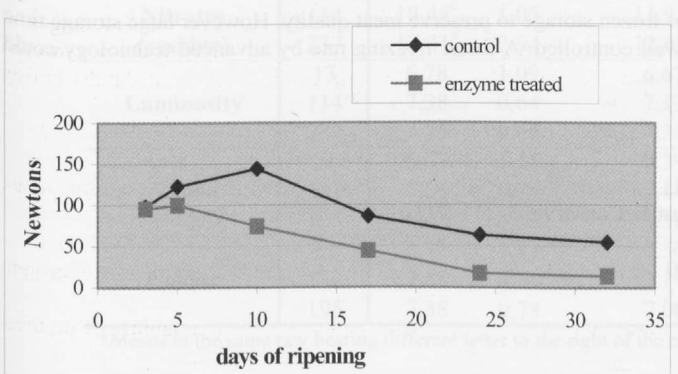

Figure 5. Evolution of hardness throughout the ripening in control and enzyme treated pork.

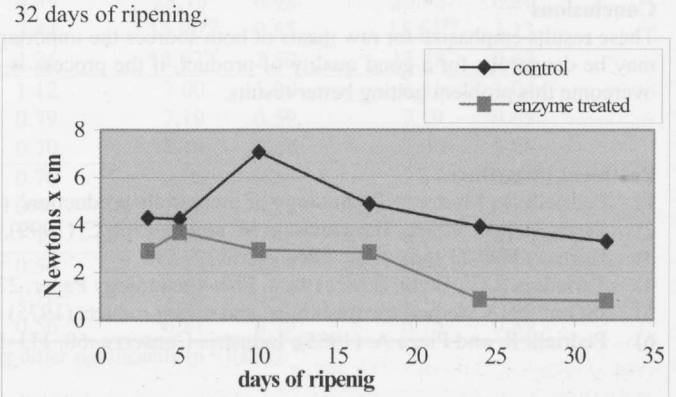

Figure 6. Evolution of chewiness throughout the ripening in control and enzyme treated pork.