PRESSURE/HEAT TREATMENTS EFFECT ON PROTEASE ACTIVITY OF BEEF EXTRACT

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Background

Pressure-assisted gelation depends on levels and sequence of pressure/temperature combinations. Heating under high pressure conditions limits the gelling of meat systems (Jiménez Colmenero et al., 1998). This behaviour has been associated with increased protease activity in meat protein due to these high pressure processing (HPP) conditions. A number of authors have conducted studies to determine the effects of high pressure (applied at cold or ambient temperatures) on proteolytic enzymes in meat systems, reporting both quantitative and qualitative enzymatic variations (Kurth, 1986; Ohmori et al., 1991; Homma et al., 1994; Jung et al., 2000). In this connection it has been reported that pressure-induced increase in endogenous proteolytic activity was due to the release of enzymes from lysosomes (Ohmori et al., 1991; Homma et al., 1994; Jung et al., 2000). Homma et al. (1994) investigating the pressure effect (100-500 MPa/2 °C) on the enzymes themselves, measured enzymatic activity in pressurized crude extract and found that enzymatic activity decreased as pressure increased, while Jung et al. (2000) reported enzymatic activation (purified enzyme) at moderate pressure levels (up to 400MPa/10 °C). Kurth (1986) reported that pressure/heat treatments (150 MPa/30-80 °C) appeared to protect cathepsin B1 against heat inactivation, maximum activity occurring at 60 °C and 150 MPa. To help understand the possible effect of endogenous proteases on pressure/heat assisted gelation, more information is needed about the effect of pressure/temperature treatments on the enzymatic activity in muscle. In this connection it would be useful to evaluate enzymatic activity in assay conditions rather than assessing their effect on the basis of post-treatment residual enzymatic activity in the meat system.

Objectives

Research has been conducted on the effect of pressure (400 MPa)/heat (36, 55, 70 °C) combinations on enzymatic activity of crude beef extract. Enzymatic activity takes place in the pressure vessel (vials containing enzymes and substrate) during HPP. Since the amount of enzyme is constant, the observed differences in enzymatic activity can be attributed to the effect of pressure/heat treatment on the enzymes themselves.

Material and Methods

The study was conducted on post-rigor beef muscle (topside). The assay of protease activity was based on Anson's (1938) procedure as described by Garcia (2001).

Preparation of beef extract: After removal of fat and connective tissue, 50 g of muscle was suspended in 100 ml of buffer phosphate 20 mM, pH 7.0, 0.9 % NaCl by homogenizing for 60 s. This suspension was centrifuged 10 000 g for 20 min at 4 °C.

Incubation sample: The incubation mixture was prepared by combining 13 ml de beef extract with 52 ml of denatured haemoglobin previously dissolved in buffer (boric acid 0.025M, orthophosphoric acid 0.025 M and acetic acid 0.025M, pH 4.0). All the reaction mixture was put into vials (2.2 ml), which were completely filled to exclude air. The reaction mixture temperature was less than 10 °C in all cases.

Pressure and thermal treatments: Samples were pressurized in an ACB model AGIP Nº 665 high-pressure pilot unit (GEC, Alsthom, Nantes, France). The reaction mixtures (4 vials per treatment) were pressurized at 400 MPa and heated (13 min) using water at 36, 55, and 70 °C as the heating and pressurizing medium. Pressure was increased at a rate of 2.25 MPa/s and released in 10s. Non-pressurized (NP) control samples (atmospheric pressure) were made under the same conditions as the pressurized (P) samples. The pressure was released immediately after treatment. Both samples (pressurized and control) were rapidly opened and 0.7 ml of stopping reagent (50% w/v trichloroacetic acid, TCA) was added to the incubation mixture. Samples were then kept at room temperature for 6 h, centrifuged (10 000 g, 20 min) and filtered through Whatman 4 filter.

Absorbance was measured at 280 nm with a Perkin-Elmer spectrophotometer. Protease activity was estimated by the difference between sample absorbance and blank absorbance (where TCA was added at the beginning). One unit of activity was expressed as a 0.001-unit increase of absorbance. The experiment was performed twice.

Results and discussion

Enzymatic activity increased with temperature in controls (atmospheric pressure) and pressurized samples (400 MPa), but to different extents (Fig. 1); non pressurized samples exhibited greater enzymatic activity than the corresponding pressurized samples at 36 and 55 °C, but activity was similar at 70 °C.

The following considerations may help to understand the consequences of the treatment used. The amount of enzyme in beef extract is constant, and therefore the observed differences in protease activity during treatment are attributable to the enzymes themselves. Secondly, while pressure is transmitted in a uniform (isostatic) and quasi-instantaneous manner throughout the sample, heat transmission by conduction is a relatively time-consuming process, so that a temperature gradient occurs within the sample. The small sample size helped minimize this gradient, but it was still different at each of the three experimental temperatures because the thermal gradient between the initial (< 10 °C) and the final temperature (36, 55, 70 °C) of the sample was different in each case. The amount of the TCA soluble fraction is therefore determined by the enzymatic activity taking place at constant pressure and variable temperature.

The fact that enzymatic activity increased with temperature and was highest at 70 °C (Fig. 1), at which temperature only some residual activity remained due to thermal denaturation of the enzyme, would appear to be a consequence of the methodology used. Unlike when muscle is thermally treated prior to the measurement of residual enzymatic activity, in this experiment the concentration of peptides detected (absorbance) was the product of the enzymatic activity occurring from the start of heating through to the final temperature (36, 55, 70 °C) and the end of treatment; in other words, this is a cumulative process. Thus, even if maximum activity levels cannot be established, we can determine that there is considerable activity at more than 55 °C.

The effect of high pressure on protease activity seems to be dependent on temperature (Fig. 1). Whereas there was no formation of TCA-soluble peptides at 36 °C, enzymatic activity appeared to be enhanced at between 55 and 70 °C, since there were no differences between the

two samples (control and pressurized) treated at 70 °C. The results of this experiment point to the existence of considerable enzymatic activity during the application of 400 MPa/heat combinations.

Pertinent literature

- Anson, M. L. (1938). The estimation of pepsin, trypsin, papain and cathepsin with haemoglobin. *J. Gen. Physiol.*, 22, 79-89.
- Garcia, R. (2001). Efecto de las enzimas endógenas sobre las propiedades bioquímicas y estructurales de carne de bovino durante la maduración. Maestria en Biotecnología. Unidad Iztapalapa. Universidad Autonoma Metropolitana. México.
- Homma, N., Ikeuchi, Y. & Suzuki, A. (1994). Effects of high pressure treatment on the proteolytic enzymes in meat. *Meat Sci.*, 38, 219-228.
- Jiménez-Colmenero, F., Cofrades, S., Carballo, J. Fernández, P., & Fernández-Martín, F. (1998). Heating of chicken and pork meat batters under pressure conditions: Protein interactions. *J. Agric. Food Chem.*, 46, 4706-4711.
- Jung, S., Lamballerie-Anton, M., Taylor, R.G., & Ghoul, M. (2000). High-pressure effects on lysosome integrity and lysosomal enzyme activity in bovine muscle. *J. Agric.Food Chem.*, 48, 2467-2471
- Kurth, L. B. (1986). Effect of pressure-heat treatments on cathepsin B1 activity. *J. Food Sci.*, 51, 663-664, 667.
- Ohmori, T., Shigehisa, T., Taji, S & Hayashi, R. (1991). Effect of high pressure on the protease activities of meat. *Agric. Biol. Chem.*, 55, 357-361.

Acknowledgements

This research was supported by the projects ALI99-1105, AGL2000-1497 and Programa de Cooperación Científica con Iberoamérica (Mexico).

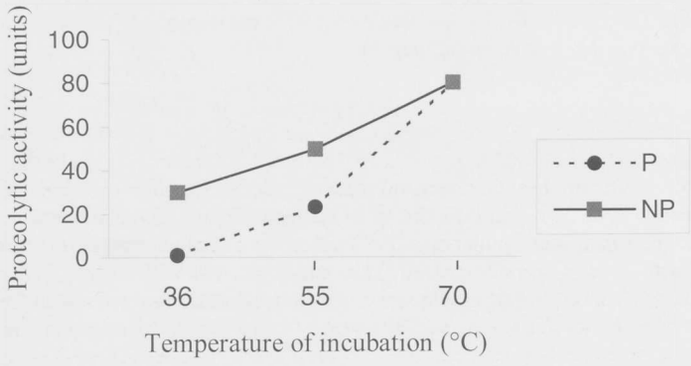

Figure 1. Effect of pressure/heat combination on proteolitic activity of beef extract. NP = non-pressurized and heated at 36, 55 and 70 °C; P = pressurized (400MPa) and heated at 36, 55 and 70 °C.