Effect of high pressure on the proteolytic activity and
gel-forming ability of minced sardine muscle

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INTRODUCTION
Endogenous, heat-stable proteases can hinder heat-induced gelation by disintegrating the myofibrillar proteins. Washing during surimi production can be an effective way of eliminating sarcoplasmic proteins and thereby minimizing the effect of the enzymes involved in gel degradation. High-pressure technology is increasingly being used as an alternative means of food preservation, not only by helping to destroy bacteria but also by inactivating enzymes. Accordingly, high-pressure technology could also be used to improve the gelling ability of muscle. High-pressure treatment of Pacific whiting surimi circumvented the protease problem and formed strong gels (1). Moreover, pressurizing considerably improved the rheological properties of mince with low gel-forming ability under heat treatment (2).

The present study examined the effects of high-pressure treatment and washing on proteolytic activity in sardine muscle as well as their influence on the muscle’s gelling ability, and in parallel SDS-PAGE was used to gain insight into structural changes in the protein molecules.

MATERIALS AND METHODS
Sample preparation and treatment: Headed and gutted sardines were purchased at a local market. The skin was removed and mince prepared in a deboning machine. To study the effect of high pressure and washing on the proteolytic activity of the minced muscle, the mince was washed in water (1:3 w/v) at 0-3°C. Washed (W) and unwashed (UW) minces were vacuum-packed and batches were pressurized (P: 300 MPa, 7°C, 20 min) or left unpressurized (UP: atmospheric pressure, 7°C).

The effect of high pressure on the proteolytic activity of homogenates made from the minced muscle was also studied. Unwashed mince was homogenized in a proportion of 1:3 (w/v) in cold 0.2 M phosphate buffer pH 6 (muscle pH) or McIlvaine buffer (0.2 sodium phosphate and 0.1 M sodium citrate) pH 3 (optimal pH). Subsequently, the
Homogenates were pressurized (P: 300 MPa, 7°C, 20 min) or left unpressurized (UP: atmospheric pressure, 7°C).

**Determination of proteolytic activity:** Proteolytic activity (μmol Tyr/g/h) was determined in triplicate at 55°C (optimal temperature) at pH 3 and pH 6 for 1 h (3).

**Gel preparation:** Gels prepared containing 2 % salt and 81 % water were cooked in two steps, 37°C for 30 min and 90°C for 50 min. The folding test (4) was applied to the gels, and the dynamic viscoelastic properties of the batters were also analysed (5).

**Electrophoretic analysis:** SDS-PAGE of the salt-soluble proteins (0.6 M NaCl, 20-mM phosphate, pH 7) from the batters was carried out (6) using 10 % polyacrylamide gels.

**Statistical analysis:** Analysis of variance was performed using the SPSS package (p≤0.05).

**RESULTS AND DISCUSSION**

Differences in proteolytic activity according to the pH were observed in the minced muscle. Table 1 shows higher proteolytic activity at the optimal pH (pH 3) than at the muscle pH (pH 6) in all the batches tested. At pH 3, the UW mince had lower proteolytic activity values in P, probably due to enzyme inactivation by the pressure treatment. In the W mince there was no difference between the pressurized and unpressurized batches, probably due to removal of the sarcoplasmic proteins and therefore, proteolytic enzymes. This would also explain the lower values for the W mince generally. The behaviour was different at pH 6, pressurization did not induce any changes in UW mince activity. However, in the W mince, higher proteolysis values were recorded for the pressurized batch, suggesting increased activity of the enzymes involved, which were not removed by washing, or greater accessibility of the substrate to the enzymes due to structural changes brought about by pressurization.

When high-pressure treatment was applied to the muscle homogenates (data not shown), proteolysis increased sharply, probably because of disruption of the lysosomes (7) and greater accessibility of the proteins as a result of structural changes caused by pressure. However, activity levels at pH 3 were not higher than at pH 6, probably due to greater sensitivity of the enzymes in the homogenate to
pressure. In this sense, Lakshmanan et al. (8) showed that pressurization substantially reduced the activity levels of enzyme extracts. The UW-UP gels scored “3” on the 5-point folding test scale. This value was indicative of poor gel-forming ability. The rest of the batches scored even lower “1”, suggesting that both pressurization and washing hindered the gelation process. These results contrasted with those obtained for the modulus of elasticity (G’) of the batters. G’ values peaked at 50-55°C. G’ for the W-UP gels reached higher values (239 KPa) than for the UW-UP gels (190 KPa). The results were similar with pressurization, with higher G’ values for the W-P gels (196 KPa) than for the UW-P gels (112 KPa). Higher G’ values for the washed samples may indicate a higher degree of heat-induced protein aggregation but not necessarily better gelling capacity. Protein aggregation may also have been heightened by removal of the proteases by washing. In any case, better values were obtained for the UP gels than for the P gels, which suggests that protein denaturation induced by high pressure could hinder subsequent aggregation during thermal processing.

The electrophoresis profile (Figure 1) reveals the higher myofibrillar protein content in the UW-UP batter, which also formed the best gel. The bands for the main myofibrillar proteins, i.e., myosin heavy chain (MHC) and actin (Ac), nearly disappeared in the P batters, denoting a high degree of myofibrillar protein denaturation, in turn yielding a low-quality mince for purposes of gelling. High pressure often induces dissociation, unfolding, denaturation, and aggregation of proteins (9, 10). Furthermore, the MHC and Ac bands were slightly weaker after washing. Although it is widely accepted that sodium chloride is necessary to solubilize myofibrillar proteins, numerous studies have indicated that myofibrillar proteins are soluble in water and become more soluble with prolonged washing (11).

CONCLUSIONS
Pressure treatment decreased the proteolytic activity in sardine mince. However, it also impaired the gel-forming ability of the minces, most likely because of its denaturing/aggregating effect on myofibrillar proteins. Thus, pressurized sardine mince does not seem to be an appropriate raw material for making gels.

Acknowledgements: This research was supported by the Spain’s “Comisión Interministerial de Ciencia y Tecnología” mainly under project ALI AGL2000-1497.
The authors thank the “Comunidad de Madrid” (CAM) for Ms. Hernández’s predoctoral fellowship.

REFERENCES

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<th>Unwashed mince (UW)</th>
<th>Washed mince (W)</th>
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<tbody>
<tr>
<td>pH 6</td>
<td>1.24 ± 0.08</td>
<td>0.56 ± 0.04</td>
</tr>
<tr>
<td>pH 3</td>
<td>2.92 ± 0.12</td>
<td>1.70 ± 0.12</td>
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Table 1. Proteolytic activity (µmol Tyr/g/h) in sardine minces.

Figure 1. SDS-PAGE migration pattern for salt-soluble proteins from sardine mince batters.