Response surface methodology multivariate analysis of properties of high pressure - induced fish mince gel

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Abstract

Factorial analyses were performed to determine the effect of high pressure treatment on characteristics of blue whiting mince of good gel - forming quality. Pressure, time and temperature independently influenced gel characteristics. Temperature had the most influence of the three. High - pressure gels were generally distinguished by lower adhesiveness, higher water holding capacity and lower yellowness than heat induced gels. The combination of pressure and temperature produced more elastic gels, whereas gels made under high pressure at chilling temperatures were much harder, more deformable and more cohesive. There was no correlation between elasticity and deformability.

INTRODUCTION

There have been numerous studies on thermal gelation of muscle, and for some years now researchers have been examining the possibility of using high pressure technology as an alternative to thermal gelation to obtain products with new textures and high - quality added value (1, 2). However, our current data cannot readily be related with precision to other reports on high pressure because mechanical properties depend not only on treatment conditions – pressure - time - temperature (2, 3, 4) – but are also affected by such factors as physicochemical conditions of the medium (salt level, pH, etc) (2, 5) and other factors such as species or gel - forming ability of muscle proteins. In this same sense, Pérez - Mateos and Montero (6) reported that the effect of high pressure on the gelation of washed sardine mince depended on mince quality; they found that pressure improved the mechanical properties of washed sardine minces that exhibited poor gel - forming ability in response to heat treatment, but it did not exert any sizeable influence on minces of high gel - forming ability. Previous experiments on high

pressure gelling of fish mince (6, 7) showed that temperature had a pronounced effect on all properties and that the pattern was very different depending on whether low or high temperature were used, the reason being that the effect of temperature was so great that it annulled or predominated over the effects of pressure. All experiments with blue whiting muscle have shown that this has poor gel - forming ability and starch at least is needed to improve that ability. In principle, it will behave differently, even although it is from the same specie, if muscle demonstrates good gel - forming ability without gelling aids

The chief aim of this work was to ascertain the effect of the variables pressure, time and a narrow temperature range (0 - 37 °C) on characteristics of myofibrillar proteins of blue whiting gels using a good gel - forming ability mince without the influence of texturing additives.

MATERIALS AND METHODS

Blue whiting (*Micromesistius poutassou* Risso) used in this study was caught off the Cantabrian coast in November. Average size was: 23.4 ± 1.2 cm and average weight: 77.8 ± 12.3 g. The proximate composition (8) was (%): crude protein 12.34 ± 0.32, moisture 82.18 ± 0.16, crude fat 0.47 ± 0.04 and ash 0.63 ± 0.01 (analyses do not show added cryoprotectant). Preparation of mince, gel - making were carried out as described previously with crushed ice to give the required final gel moisture of 80 % (8). Folding test, puncture test (breaking deformation, breaking force, work of penetration), Texture Profile Analysis (hardness, adhesiveness, cohesiveness) and stress-relaxation test (elasticity), colour (L*, a* b*) and water holding capacity were determinated as Montero *et al*. (9).

Statistical analysis. *Response surface methodology* was used to study the simultaneous effect of three independent variables (pressure: 200, 245, 310, 375, 420 MPa; time: 10, 14, 20, 26, 30 min; temperature: 0, 8, 20, 33, 38) according to a central composite rotatable design, using a statistical program (Statgraphic, STSC Inc., Rockville MD). Assessment of error was derived from replication of one treatment as suggested in the design (10). Each parameter was represented by a second - order polynomial expression which predicted the effect of the combination of independent variables with a multiple correlation coefficient (r):

$$Y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} b_{ii} x^2_i + \sum_{i<j}^{k} b_{ij} x_i x_j$$

where the estimated response is *Y*; equation parameter estimates (β_0 for constant, β_i for linear terms, β_{ii} for quadratic terms, β_{ij} for interaction terms); levels of factors are x_i, x_j and the number of factors is *k*. The significance of the equation parameters for each response variable was assessed by F test in order to check the goodness of the model as a whole.

Multivariate analysis. This was performed using the BMP computer programme (BMDP Statistical Software, Inc., Cork Technology Park, Cork, Ireland) for factor analysis (BMDP 4M) of all means of values for blue whiting muscle gels made by high - pressure treatment according to the response surface design (RSM).

One - way analysis of variance was carried out in characteristics of gels made to determine differences due to gelling treatments: atmospheric pressure and high pressure. The difference of means between pairs was resolved by means of confidence intervals using Bonferroni test (BMDP 7D). Level of significance was set for $p \leq 0.05$.

RESULTS AND DISCUSSION

To know the effect of pressure and temperature in the high pressure treatment, all properties of gels including samples obtained by RSM in a range of pressure (200 - 420 MPa), time (10 - 30 min) and temperature (0 - 38 °C) were collected. For all the pressure - time - temperature conditions assayed, gels were obtained which scored maximum in the folding test without added gelling aids. Pérez - Mateos and Montero (6), working within similar ranges of pressure - time - temperature on muscle of sardine (*Sardina pilchardus*), a fatty specie, likewise obtained gels with maximum folding test scores in all cases. However, at temperatures over 40 ºC high - pressure treatment did not prevent the onset of *modori* or irreversible gel breakdown, probably due to the action of *proteasas*, so that gel - forming ability, and hence folding test scores, were reduced (6, 7, 8).

In breaking deformation (Table 1), temperature negatively influenced the linear and quadratic terms ($p \leq 0.01$) throughout the experimental range (0 - 38 °C), whereas pressure affected the quadratic term ($p \leq 0.01$) of the function (300 - 420 MPa). Time did not shown significant influence in the range assayed. Thus, gels tended to present higher breaking deformation when they were made at pressures below 300 MPa and low temperatures.

These results are in reasonable agreement with other authors (11), who reported the highest breaking deformation values in similar conditions (200 - 300 MPa, 0 °C, 10 min) in Alaska pollack (*Theragra chalcogramma*) *surimi* gels. As regards the influence of temperature, Pérez - Mateos and Montero (6) also reported a significant linear and quadratic effect on breaking deformation in sardine muscle gels of high quality. Other studies (7) suggest that pressurizing times longer than 10 min increases breaking deformation in blue whiting (*Micromesistius poutassou*) muscle gels with added starch.

According to the coefficients estimated for breaking force (Table 1), all the parameters considered (pressure, time and temperature) negatively influenced the linear term ($p \leq 0.01$). According to some authors (7, 9) this may be consistent with the fact that pressure treatment produces a lower proportion of covalent bonds and a greater presence of weaker bonds, especially hydrophobic interactions (12), specifically in the myosin heavy chain (2, 11, 13, 14).

In experiments on blue whiting (*Micromesistius poutassou*) mince of poor gel - forming ability it was found that time and temperature were the variables that influenced breaking force (7), while in sardine (*Sardina pilchardus*) minces temperature alone induced gelling under high pressure (6).

The work of penetration (Table 1) also negatively influenced the linear term of breaking force versus pressure ($p \leq 0.01$), time ($p \leq 0.05$) and temperature ($p \leq 0.01$). Ko *et al*. (1) found that work of penetration increased with pressure (100 MPa - 500 MPa) applied in gels made from minced muscle of sardine and Alaska pollack. Maximum values occur at different pressures depending on the species, for example: *Sardinops melanostictus, Theragra chalcogramma, Nemipterus tambuloides, Pollachius virens, Micromesistius poutassou* (1, 7, 8, 15, 16).

Hardness (Table 2) decreased linearly with increased pressure ($p \leq 0.01$) and temperature ($p \leq 0.01$), so that the hardest gels were obtained at relatively low pressures (200 MPa) and chilling temperatures (< 10 °C). Time exhibited two opposite effects: around 10-20 min, negative in the linear term ($p \leq 0.01$) and longer than 20 min, positive in the quadratic term ($p \leq 0.05$).

Pérez - Mateos *et al*. (7) also obtained harder gels from blue whiting (*Micromesistius poutassou*) muscle under pressure at not very high temperatures (less than 40 °C). Carlez *et al*. (15) found that gels were harder at higher pressures (300 MPa, 5 - 10 °C, 15 min) working with *Nemipterus tambuloides surimi* gels. Okamoto *et al*. (17), on the other hand, found that the hardness of carp actomyosin gels increased with the pressure level applied. In this sense, Angsupanich and Ledward (18) recently reported on fillet cods that hardness of gels induced at 400 or 600 MPa increased significantly when compared with fresh samples or samples treated at lower or higher pressure (200 MPa and 800 MPa respectively).

These differences may be due to the differences in high - pressure gelling conditions. According to Fernández - Martín *et al*. (19), when pressure is applied at non - denaturing temperature, this causes the actin to aggregate, thus increasing hardness; whereas when pressure is applied at denaturing temperature, this protects the myosin from denaturation, so that gels are softer.

Adhesiveness (Table 2) tended to decrease with increased pressure ($p \leq 0.01$ linear), processing temperature ($p \leq 0.01$ linear) and pressurizing time ($p \leq 0.05$ quadratic), so that gels tended to be most adhesive with treatments at around 200 MPa, < 10 °C, 10 min. Pérez - Mateos *et al*. (7) also found that blue whiting (*Micromesistius poutassou*) muscle gels were more adhesive with low pressure and temperature (200 MPa, ≤ 15 °C) or intermediate pressure and temperature (300 MPa, 40 °C). This agrees with studies carried out by Okamoto et al. (17), who reported that adhesiveness of carp actomyosin gels decreased with the pressure due to the different gelation mechanism between pressure and heating.

The evolution of cohesiveness values (Table 2) was similar to that of adhesiveness. The

most cohesive gels were obtained with relatively low pressure (200 MPa), short time (10 min) and chilling temperature. Pérez - Mateos *et al*. (7) produced cohesive blue whiting (*Micromesistius poutassou*) muscle gels at low pressure and temperature, as both pressure and temperature negatively affected cohesiveness.

In experiments on cod fillets, Angsupanich and Ledward (18) found that the effect of pressure on cohesiveness decreased from 0 to 200 MPa and elasticity increased over this range; they also reported a sharp decrease in adhesiveness. According to these authors, loss of myosin structure leads to decreased adhesiveness, gumminess and cohesiveness; in contrast, unfolding of actin at 400 and 600 MPa leads to increased in gumminess, hardness and adhesiveness.

Pérez - Mateos and Montero (6) found that heating during pressurization of washed muscle of sardine (*Sardina pilchardus*) caused softening of gels and reduced adhesiveness; if these conditions were maintained for a longer time, the gels were also less cohesive.

As regards elasticity (Table 2), pressure - induced gels tended to be more elastic with more severe conditions: high pressure applied for long processing times at heating temperatures ($p \leq 0.01$ linear). These results indicate that treatment conditions favourable to gel elasticity are the opposite of conditions favourable to breaking deformation. According to Angsupanich and Ledward (18) the formation of the hydrogen bonds must contribute to increase in springiness over 0 to 300 MPa.

Pérez - Mateos *et al*. (7) also found that blue whiting (*Micromesistius poutassou*) muscle gels compressed to 50 % were more elastic when pressure - induced than when heat - induced, especially at pressures over 300 MPa in gels with 1 % NaCl. On the other hand,

Pérez - Mateos and Montero (6) found no significant differences in elasticity values of pressure induced (*Sardina pilchardus*) gels.

Although differences in elasticity vary according to species and gel - forming ability, there are other factors that influence compression - determined properties, such as twin - cycle compression or tension relaxation by compression at constant deformation, and above al the degree of compression (20).

Water holding capacity (WHC) was not influenced by pressure - time - temperature in the given conditions, although this may have been because it was quite high (around 90 %) in all cases anyway (Table 3). According to some authors, high pressure treatment helps reduce water loss probably due to disaggregation and unfolding of proteins (21).

On the other hand, Okazaki (3) reported reduced water holding capacity of soluble sarcoplasmic proteins from sardine muscle under 20 min pressurizing at 500 MPa or more. In blue whiting (*Micromesistius poutassou*) muscle gels containing starch, Pérez - Mateos *et al*. (7) found that water holding values were highest with long treatments (200 MPa, 25 min) carried out cold. Pérez - Mateos and Montero (6) found a tendency for sardine (*Sardina pilchardus*) muscle gels to attain maximum estimated water holding capacity values at the same pressure (200 MPa) and temperatures around 40 °C for shorter times (10 min).

Temperature ($p \leq 0.01$) and pressure ($p \leq 0.01$) clearly affected lightness in the linear part of the function (Table 3), and values were highest with high pressure (400 MPa) and moderate heating temperature (40 °C). Other authors have also found higher lightness values with increased pressure (22, 23, 24) and with increased temperature (6, 7).

Redness (*a**) and yellowness (*b**) both tended to increase with the process temperature ($p \leq 0.01$ linear), probably because the temperature caused more intense changes than the pressure owing to the difference in their effects on protein denaturation.

In general, the pressure treatment that produced the highest values of the target properties was about 200 MPa applied at chilling temperature (< 10 °C) for short times. Gels made with higher pressure, temperature and time exhibited higher elasticity and lightness (375 MPa, 20 min, 37 °C).

On the basis of this experimental design, a multivariate analysis was carried out to relate processing conditions (pressure - time - temperature) and fish gel characteristics (mechanical properties, WHC and colour), so that we could determine which were influenced by the different parameters of the high pressure process.

The results in the correlation matrix (Table 4) showed a positive relationship between the pressure applied and the lightness and elasticity of the gel. On the other hand, pressure correlated negatively with all the other mechanical properties considered. Temperature correlated strongly with most gel characteristics (breaking force, work of penetration, hardness, adhesiveness, cohesiveness, elasticity, redness and yellowness). Time, on the other hand, correlated less strongly with variables considered.

In addition, the dependent variables correlated strongly with one another. Breaking deformation and breaking force correlated positively, and hence the work of penetration correlated positively with both. At the same time the work of penetration correlated positively with hardness, adhesiveness and cohesiveness in such a way that the greater the gel forming ability as reflected by high work of penetration values, the more strongly was the network connected (hardness) and the stronger were the interaction with the

exterior (high adhesiveness) and the inter - molecular interaction (cohesiveness). On the other hand, there was a negative correlation between elasticity and breaking deformation, which could indicate that the gel was highly deformable but did not recover well after compression. In spite of the fact that there is correlation, they are measurements obtained by different techniques – breaking penetration and compression without breaking. Montero *et al*. (25) reported that the penetrometer test measured the compactness of density of the gel and constituted an index of firmess, whereas the compression test measured the degree of binding.

Factor analysis was used to study the interrelationships between the variables using the exploratory data. In this method variation in the data set was summarized by a minimun number of factors or principal components. Variables with high loadings on the same factor tend to be highly correlated with each other. Only the directions of main variance in the data matrix are given attention; the more subtle sources of variance may pass unnoticed.

The sorted rotated factor loadings patterns (Table 5) extracted four factors which accounted for 95.2 % of data variance. Factor 1 is the majority factor explaining total variance, of which it accounts for 69.1 %; part of this is temperature, which influences mainly adhesiveness, hardness, breaking force and redness. Factor 2 explains 13.4 %; it is the majority factor accounting for the effect of pressure on lightness (L*), although it also affects other variables such as: adhesiveness, hardness, breaking force, elasticity, cohesiveness and water holding capacity. The third factor only explains 9.7 %; it is a temperature - related term that has a negative loading on breaking deformation and work of penetration and a positive loading on other variables like yellowness (b*). The pressurizing time is part of the fourth factor, which accounts for 7.8 % of total variance, reflecting its positive influence on water holding capacity. The fact that temperature was

part of two factors (1 and 3), although chiefly the first, in which neither pressure nor time were parts indicates that there is no interaction among these variables and that their effects are independent. This suggests that pressure and temperature induce different gelling mechanisms. But despite that, some characteristics like breaking force, elasticity, work of penetration, cohesiveness and water holding capacity are altered by all three variables if not always to the same extent.

The conditions for gelling of muscle would be about 200 MPa for short times (10 min) at chilling temperature (< 10 °C) (gel L); this does not apply to elasticity and lightness, which are favoured by conditions in the region of 375 MPa, 20 min, 37 °C (gel H). Thus our results are in reasonable agreement with previous reports (7, 8) on work with the same species but of poor gel forming ability and using added starch.

Figure 1 shows the characteristics of gels induced by these gelling treatments. Gel L (200 MPa, < 10 °C, 10 min) presented the highest values for the properties measured in the penetration test (breaking deformation, breaking force and work of penetration); it was also very hard, cohesive and less elastic than the other two gels (T and H). The heat - induced gel (T) presented very high values of adhesiveness ($p \leq 0.05$), higher than the pressure - induced gels, but lower water holding capacity ($p \leq 0.05$). As to colour, the thermal gel (T) was lighter (L*) than the pressure gels (L and H) but it was also yellower (b*). This may be because temperature induced more drastic changes (denaturation, pigment oxidation) than pressure, which tends to alter less the sensory characteristics of the product.

According to Pérez - Mateos *et al*. (8) working on blue whiting gels with 5 % starch, high pressure induced gels were more elastic than heat induced gels. The gels in lot L were characterized by high values of breaking force, breaking deformation and cohesiveness,

whereas the gels in lot T had the highest values of hardness and water holding capacity. The authors attributed these differences to the fact that the heat induced gel exhibited more aggregation than the pressure induced gels and that there was a higher proportion of hydrophobic interactions in the lot H gels.

CONCLUSIONS

Pressure, time and temperature independently influenced the characteristics of blue whiting muscle gels according to the response surface design plotted in the experimental conditions. Temperature was the factor that most influenced the process (about 80 % of explained variance). Increased pressure, time and temperature favoured elasticity and water holding capacity in the given conditions but reduced all the other mechanical properties considered. As to colour, temperature intensified the colouring of gels (red and yellow), while pressure tended to increase the lightness more. Hence, the conditions for muscle gelation were identified as around 200 MPa for short times (10 min) at chilling temperature (< 10 °C) (gel L); and to favour the majority of the gel characteristics but not elasticity and lightness, it could be better around 375 MPa, 20 min, 37 °C (gel H). Although pressure only participated as a variable in factor 2 (13.4 % of total variance) and therefore correlated strongly with the characteristics expressed by that factor, pressurization as a method for gelling at a given temperature is useful for obtaining diversified gel properties.

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