Effect of High Pressure and 4-Hexylresorcinol on Enzymatic Activity and Darkening in Oysters

Miriam PÉREZ-MATEOS*, María Elvira LÓPEZ-CABALLERO, and Pilar MONTERO

Instituto del Frío (CSIC), Dpto. Ciencia y Tecnología de Carnes y Pescados

Ciudad Universitaria s/n, 28040 Madrid, Spain

Tel.: +34 91 54 2300

Fax +34 91 549 3627

*miriam@if.csic.es (author M.Pérez-Mateos to whom correspondence should be addressed)

[Escriba aquí]

ABSTRACT

Oysters treated with/without 4-hexylresorcinol were pressurized (400 MPa/7°C/10min) and stored chilled for 21 days either vacuum-packed or packaged aerobically. The results of visual evaluation of darkening and polyphenoloxidase (PPO) activity levels suggest that during storage non enzymatic browning occurred first, followed by blackening caused by PPO activity. PPO activity did not increase in the vacuum-packed oysters during storage, but pressurization was observed to activate the PPO. Autolytic activity in the oysters increased due to the high-pressure treatment, although higher autolysis levels were recorded in the unpressurized oysters at the end of storage. Inhibition of PPO activity by the 4-hexylresorcinol was observed chiefly in the vacuum-packed batches.

Key words: polyphenoloxidase, autolytic activity, darkening, oysters, high pressure

Short title: enzymatic activity in oyster

Introduction

Oysters are molluscs and are a very popular food in Europe. They are usually served and eaten while still alive, raw on the half shell. Since they are water-filtering organisms, they can accumulate pathogenic microorganisms, which sometimes cannot be removed by purification. This, in turn, gives rise to major health safety concerns, and for that reason a substantial proportion of the population does not eat raw oysters, even though they are a tasty delicacy. Another reason why consumers do not eat raw oysters is the unpleasant sensation of eating a living creature.

Recent studies on the effects of high pressure in the processing and storage of oysters have yielded promising results. Certain pressure-time-temperature conditions were able to preserve the raw appearance of oysters, which gained slightly in volume and were juicier (He and others 2000). The taste was virtually unchanged, although Hoover and others (1989) reported intensification of the flavor. Moreover, López-Caballero and others (2000a) observed that the appearance of the oyster meat was better when pressurization (400 MPa) was carried out under chilled conditions (7 °C), rather than at higher temperatures (20 and 37 °C), and also that one mechanical property, shear strength, was higher in pressure-treated oysters than in untreated oysters. In no case has pressurization resulted in lower product acceptability.

High pressure may also inhibit the availability of energy to microorganisms by affecting energy-producing enzymatic reactions, thereby reducing the viability of cells (Ashie and others 1996a). Reductions in all target microorganisms were achieved after pressurization (200-400 MPa/7 °C/10 min) of oysters and prawns (López-Caballero and others 2000a,b).

Calik and others (2001) reported that high pressures killed *Vibrio parahaemolyticus*. On the other hand, preliminary studies (López-Caballero and others 2000a) have shown that pressurizing killed the oysters as well, and in some cases packages have been pierced by sharp edges on the oyster shells, giving rise to pronounced darkening of the pressurized oysters, possibly due to enzymatic activity. However, to our knowledge, there have been no studies on PPO or autolytic activity levels in pressurized oysters.

Hydrostatic pressure has been shown to alter the activity of a wide range of enzymes in foods to varying degrees, depending on the pressure applied, the nature of the enzyme, and the duration of pressurization (Ohmori and others 1991; Ashie and Simpson 1996;; Weemaes and others 1998). Some workers (Asaka and Hayashi 1991; Eshtiaghi and Knorr 1996) have reported darkening after high-pressure treatment in fruits and vegetables due to activation of polyphenoloxidase (PPO); however, inactivation of PPO has also been reported at very high pressures, such as 900 MPa (Seyderhelm and others 1996).

Blackening is known to be caused principally by enzymatic oxidation of polyphenols present in the tissues and their subsequent polymerization by non enzymatic reactions. High levels of tyrosine, a phenol present in oysters, have been recorded (Jiang and others 1985). Oyster meat may turn dark during chilled storage. The brown color may be natural in old oysters; discoloration may also be caused by ruptured roe or kidney or by spoilage (Hardy and Smith 1970). Browning discoloration in oysters has been attributed to the Maillard reaction; and, hence, color may be leached out by holding oyster meat in iced water containing a small amount of an antioxidant acid, such as ascorbic acid. Discoloration in oysters is caused by the breakdown of glycogen, which can reach high levels in this species in the period running from the end of spawning to the following spawning season, or by oxidation of tyrosine, a

substance known to be high in oysters, in the presence of PPO and oxygen (Jiang and others 1985). However, acid levels may adversely affect flavor and texture, and high levels are not recommended. Jawahar and others (1994) achieved enhanced sensory acceptability of oysters by adding 0.2 % clove powder, and Jeong and others (1990) did the same using an deoxygenizer.

Low concentrations of selective tyrosinase inhibitors, such as resorcinol derivatives, have recently been used as an effective processing aid to inhibit browning in many food systems (McEvily and others 1991; Otwell and others 1992; Slattery and others 1995; Montero and others 2001). Monsalve-Gonzalez and others (1993) reported 4-hexylresorcinol in combination with ascorbic acid to be an effective anti-browning treatment for stored apple slices. Substituted resorcinols have several advantages over other conventionally used inhibitors, such as sulfites, in that they have greater specificity towards PPO, they do not bleach pigments to the extent that excess sulfites do, they are chemically more stable, and they are effective at very low concentrations (50 ppm) (Ashie and others 1996a).

Enzymes implicated in the deterioration of seafood texture have been shown to be susceptible to inactivation at high pressures (200 MPa/10 min) (Ashie and Simpson 1996). High pressures above 800 MPa/20 min inactivated squid (*Loligo bleekeri*) mantle proteases (Nagashima and others 1993). Also, acid and neutral/alkaline proteases were inactivated in meat at 500 and 400 MPa/10 min, respectively, while other tissue enzymes were inactivated at 300 MPa (Ohmori and others 1991), as were meat collagenases at 300 MPa/0-90 min (Dufour and others 1996). On the other hand, phosphatase and cathepsins have been reported to retain activity levels of 70-30 % in bovine liver and ruptured lysosomes after 506 MPa/10 min (Ohmori and others 1992), and high-pressure (100-300 MPa) activation of

proteolytic reactions has also been described in meat (Macfarlane 1985; Suzuki and others 1990; Kim and others 1992; Homma and others 1994).

Storage will, of course, be affected by the alterations produced by pressurization. The pressure conditions that inactivate enzymes or microorganisms may not be compatible with the conditions needed to preserve sensory properties. Therefore, the object of this study was to examine alterations in autolytic and polyphenoloxidase enzymatic activity during the storage of pressurized oysters. The effect of adding a potential blackening inhibitor, namely, 4-hexylresorcinol, to offset possible activation of PPO during storage was also studied.

Materials and Methods

Samples

Live cultured oysters (*Ostrea edulis*) were collected from an oyster farm located in the Ría de Cambados (Pontevedra, Spain) in April, after they had been purified. They were transported to the laboratory and maintained alive at about 5 °C for approximately 24 h before processing.

Sample preparation

All oysters were shucked and the flat shell valve removed; after that the oyster meats were immersed in cold water (5 °C) containing 3.5 % sea salt (Super Sal Lobos S.A., Santiago, Chile) for 10 min. For the inhibitor-containing batches 50 ppm of 4-hexylresorcinol (Sigma Chemical, St. Louis, MO, USA) was added to the salted water. After draining off the excess drip solution, samples were wrapped in polyethylene film. The control batch, with and without added inhibitor, was stored aerobically at 5 °C (unsealed bags). The remaining oysters were

vacuum-packed in bags with an oxygen permeability of 60 cm³ / 24 h / m² / atm at 23 °C (Cryovac BB-1; Grace, Barcelona, Spain). The vacuum-packed oysters, with and without inhibitor, were stored at 2 °C. Pressurized batches, with and without inhibitor, were prepared using a high-pressure pilot unit (ACB 665; Gec Alsthom, Nantes, France) operated at 400 MPa at 7 °C for 10 min (López-Caballero and others 2000a). Pressurized batches were then stored at 2 °C both vacuum-packed and aerobically packaged, the latter being intended to establish the effect of high-pressure treatment on the product when packages were pierced by sharp oyster shells. All batches were stored for 21 days at the indicated temperatures

In all, 8 batches of oysters were thus prepared: a control batch (C), a vacuum-packed batch (V), a pressurized vacuum-packed batch (PV), and a pressurized, non vacuum-packed batch (P). These same 4 batches also containing added inhibitor have been designated by adding “-I” to the batch designation.

Analyses

The following analyses were performed weekly for 21 days.

Proximate analysis of raw shucked oyster meat was performed according to AOAC procedures (AOAC 1984), except for the crude fat, which was determined using the method of Bligh and Dyer (1959).

Visual score. Color alterations during storage were evaluated by eight trained assessors using the following scale: 0 natural color, 2 slight darkening (or with approximately up to 20 % of oysters affected), 3 moderate darkening (or with approximately up to 50 % of oysters affected, being the threshold score above which the samples were deemed to be dark), 5 severe or complete darkening (or with approximately up to 80-100 % of oysters affected).

Polyphenoloxidase activity was determined based on work of Chen and others (1991): The mantle and gill of oysters, the parts where highest PPO activity levels have been recorded in previous experiments, were frozen in liquid nitrogen and ground to a fine powder by hand with a mortar and pestle. An amount of 3 g of the powder was added to 10 mL of 0.05 M sodium phosphate buffer, pH 7.2, containing 1 M NaCl and 0.2 % Brij 35 (Sigma Diagnostics, St. Louis, MO, USA). The extract was stirred at 4 °C under N₂ atmosphere for 3 h and the suspension then centrifuged (12000 x g, 30 min, 4 °C). Enzyme activity was measured continuously in a spectrophotometer (model UV-1601, Shimadzu, Kyoto, Japan) with a thermostatic controller (CPS-240) at 475 nm at 25 °C for 5 min. The substrate was DL-3,4-dihydroxyphenylalanine (DL-DOPA) (Sigma). Measurements were made on a mixture of 40 µL of enzyme extract and 280 µL of 20 mM DL-DOPA in water and 280 µL of 0.05 M sodium phosphate buffer, pH 7. Enzymatic activity was expressed as relative specific activity, being the percentage enzymatic activity per gram of protein expressed as $(A / A_{\text{máx}}) \cdot 100$, with A being the increase in optical density per min. Three replications of all determinations were performed in each of 2 separate experimental batches.

Autolytic activity. Autolytic activity was measured in the adductor muscle and body trunk. The chopped portions of oyster were mixed with 20 mM phosphate buffer, pH 6.5, at a ratio of 1:4 (w/v) and then homogenized in an Omni Mixer (Model 17106, Sorvall, Waterbury, CT, USA) blender at setting 4-5 in an ice water bath for 1 min. Autolytic activity was assayed according to a modified version of the method of Hurtado and others (1999) as follows. Immediately after preparation of the crude homogenate, 3 g aliquots were incubated in a thermostatic water bath (Unitronic, model S 320-100; J.P. Selecta, Barcelona, Spain) at 2 different temperatures, 0 °C and at 60 °C, for 1 h -minimum and maximum enzymativ activity,

respectively- (Hurtado and others, 1999). After incubation, the reaction was stopped by adding 6 mL of cold 10 % trichloroacetic acid (TCA). The mixture was held at 4 °C for 15 min to allow any unhydrolyzed proteins to precipitate, the precipitate then being removed by centrifugation at 6,100 x g at 4 °C for 15 min (model J2-MC, Beckman, Palo Alto, CA, USA). The soluble oligopeptide content in the supernatant was analyzed by the method of Lowry and others (1951) using tyrosine as standard. Activity (U) was expressed as nmoles of tyrosine released per g of muscle per h (nmol Tyr/g/h). The results given are the means of 3 replications of all determinations performed in each of 2 separate experimental batches.

Statistical analysis

Significance between pair mean values was evaluated using analysis of variance run on the mean measurement values, taking storage time as the covariance. Linear regression by treatment batch was also performed. The BMDP computer program (BMDP Statistical Software, Inc., Cork, Ireland) was used for statistical processing. The level of significance setting was $p \leq 0.05$.

Results and Discussion

Compositional analyses are expressed in percent: moisture: 84.70 ± 1.19 ; ash: 2.74 ± 0.55 ; crude protein: 9.50 ± 0.49 ; crude fat: 2.53 ± 0.56 . López-Caballero and others (2000a) found similar composition in the same specie of oyster.

The visual score was assessed as the attribute here designated as darkening, namely, the appearance of discoloration, rated on the basis of the 5-point scale described above. The visual scores are plotted in Figure 1. The refrigerated control batches, with (C-I) and without

(C) added inhibitor, did not exhibit deemed darkening (score 3-5) until day 21, beginning after two weeks of storage (Figure 1a); no differences with the addition of inhibitor. The vacuum-packed batches (V, V-I) followed a similar trend, but the visual score on day 21 was lower, and lower still in the inhibitor-containing batch. Linear regression performed on the visual scores for darkening yielded lines with slopes of 0.21 and 0.19 for batches C and C-I, respectively, and 0.16 and 0.11 for batches V and V-I, respectively. These results suggest that hexylresorcinols was more effective in vacuum batches. This may have been due to losses of liquid from the bags in the pressurized batches stored without vacuum in no sealed bags, allowing some of the inhibitor to drain away.

Darkening mainly took the form of browning, with blackening appearing only in some individuals. The higher degree of darkening in the control batches than in the vacuum-packed batches was probably due to the exclusion of oxygen during vacuum-packed storage, thus preventing discoloration. Jiang and others (1985) reported that discoloration resulting from oxidation caused by exposure of the oysters to air. The inhibitory effect of 4-hexylresorcinol was evident ($p \leq 0.05$) in batch V-I.

In general, darkening was more apparent in the pressurized batches (Fig1c,d) than the non pressurized batches (Fig.1a,b). The pressurized batches stored without vacuum packaging (batches P and P-I) exhibited more pronounced browning (Figure 1c) than the pressurized batch stored with vacuum packaging (Fig.1d). Significant differences ($p \leq 0.05$) between the batches that did and the batches that did not contain added inhibitor, where slopes of the regression lines were 0.16 and 0.18, respectively. Also, the pressurized batches stored with vacuum packaging (Fig.1d: PV and PV-I) showed higher scores by day 7, mainly in the batch containing no added inhibitor (batch PV). Nevertheless, by the end of storage (day 21) the

scores were no longer higher than those for batches V and V-I ($p > 0.05$). The slopes of the regression lines were 0.17 for batch PV and 0.11 for batch PV-I..

Also in pressurized samples, the addition of 4-hexylresorcinol was only effective in preventing darkening discoloration in the vacuum-packed batches. As mentioned above, this may have been due to losses of liquid from the bags in the pressurized batches stored aerobically, allowing some of the inhibitor to drain away. Jiang and others (1985) explained that the inhibitory effect of various antioxidants on blackening could be an outcome of their presence on the surface of the oysters as a coating but that, after some time in storage, drying of the surface took place, paving the way for discoloration to occur.

PPO activity in each of the batches was also measured during the storage period. In the unpressurized oyster batches, both the control batches and the vacuum-packed batches, the level of PPO activity remained rather steady throughout storage, and the regression lines for these batches were practically asymptotic with respect to the x-axis, indicating that there was no increase in relative PPO activity over the storage period (Figure 2). In contrast, a gradual increase was observed in the pressurized, vacuum-packed batches over time (the slope of the regression line being 175). This increase was still greater in the oyster batches that had been pressurized but not vacuum-packed (the slope of the regression line being 285). No significant differences due to the presence of 4-hexylresorcinol were found ($p > 0.05$).

The F-statistic for PPO activity (22.7) was higher than that for the visual score from the darkening evaluation (8.3), suggesting that PPO activity is a better differentiator for the study batches. Moreover the gradual darkening with storage time does not go exactly hand in hand

with PPO activity (Fig.1 and 2), this would appear to mean that darkening in the oysters was not ascribable to PPO activity alone, maybe there are some browning non enzymatic reaction. It should be borne in mind that molluscs contain very high levels of carbohydrate (glycogen) as compared to fish and crustaceans and that glycogen breakdown may give rise to browning.

The higher degree of darkening in the pressurized oysters may have been caused by pressure-induced tissue breakdown releasing polyphenoloxidase, which is then available to react in the presence of oxygen. Previously, López-Caballero and others (2000a) observed substantial darkening of pressurized oysters in vacuum-packages pierced by sharp oyster shells. The findings in the present study were consistent with those results and with the results for prawns reported by López-Caballero and others (2000b), who observed that high-pressure treatment aggravated blackening and spotting in the presence of oxygen.

Jiang and others (1985) studied blackening in frozen shucked oysters and pointed out that it could be brought about by lipid oxidation and probably also by the Maillard reaction as a result of the gradual reaction of free amino acids and glycogen breakdown products, and/or by oxidation of water-soluble components in the presence of PPO and oxygen. In this connection Hatano and others (1990) reported that the water-soluble fraction was responsible for the onset of unacceptable darkening and, according to Jawahar and others (1994), dark discoloration in oysters may be due to interactions between the hydrolyzed products of the lipid and protein components.

Figure 3 and 4, respectively represent autolytic activity in the different batches as measured at 60°C, the temperature at which most of the enzymes involved are most active (Hurtado

and others 1999), and at 0 °C, employed to simulate the conditions in chilled product. There was no autolytic activity in the control batches in the initial days of storage, because the oysters were alive. Increased activity was recorded over the course of storage, the increase being more apparent at 60 °C (Figure 3). After death biochemical alterations are triggered in fish muscles, which are catalyzed (including ATP breakdown and muscle proteolysis) by endogenous enzymes present in the muscle tissue as well as by enzymes leaking from the guts (Venugopal 1990; Ashie and others 1996b). Massive microorganism growth subsequently results in protein breakdown and accumulation of other substances (Liston 1982).

Autolysis was low in the vacuum-packed batches in the initial days of storage, probably because of the absence of the O₂ needed for enzyme-driven oxidation reactions as well as slower microorganism growth in the vacuum-packed oysters compared with the oysters stored aerobically (López-Caballero and others 2000a). Measured at 0 °C, only slight increases in autolytic activity were observed in the control batches (batches C and C-I) and in the vacuum-packed batch without inhibitor (batch V) until day 14 of storage. In contrast, when the extracts were incubated at 60 °C, increased autolytic activity was detected in batches C and V from day 7. The increased autolytic activity in these batches ($p \leq 0.05$) (Table 1, Figures 3a,b), attaining levels of up to 90 U (nmol Tyr/g/h) by the end of the storage period considered, can be attributed both to endogenous enzymes and to microbial enzymes. López-Caballero and others (2000a) reported an increase in the number of bacteria in oysters during storage under both aerobic and vacuum conditions.

Autolytic activity measurements followed a different pattern ($p \leq 0.05$) in the pressurized batches (Table 1). The pressure treatment (400 MPa/7 °C/10 min) increased the level of

proteolytic activity in the oysters (Figures 3 and 4), which seemed to be higher in the conditions of simulated perforation of the packages ($p \leq 0.05$). This result suggests that the exclusion of O_2 produced by vacuum-packaging and of microorganisms that might otherwise contaminate the pressurized batches, when the wrapping is breached, may affect enzymatic activity. Increased autolytic activity has been related to the destruction of the lysosomal membrane and leakage of the proteases involved into the cytosol that took place during pressurization at 100-200 MPa (Ohmori and others 1991). Assays of crude enzyme extracts obtained after pressure treatment of whole fish tissue seemed to indicate that the extent of inactivation of some of the proteases was even higher than in pressure-treated crude enzyme extract (Ashie and Simpson 1996). Our findings indicate that activation of proteolytic activity took place at 400 MPa/7 °C/10 min) (Figure 3 c,d and Figure 4 c,d).

Fish enzymes are generally more susceptible to high-pressure inactivation than are their mammalian counterparts (Ashie and Simpson 1996). Ashie and others (1996a) observed lower proteolytic activity in fish tissue with increasing pressure (up to 300 MPa) at constant pH. However, Ohmori and others (1991) found that pressures of 300 and 500 MPa denatured tissue proteins in pressurized beef extracts (25 °C/10 min) and that autolytic activity rose again at 500 MPa because protein denaturation may have outweighed protease inactivation.

At both temperatures employed in the autolytic activity assays, 0 °C and 60 °C, pressurization brought about an initial increase in autolytic activity in the pressurized batches; thereafter, activity levels levelled off until the end of storage at 0 °C and until day 14 at 60 °C. No reactivation of proteolytic activity was observed during storage (Figures 3 and 4). Furthermore, at 60°C a decrease in autolytic activity was recorded in the pressurized

aerobically stored oysters (Figure 3c). Previously, little or no reactivation of proteases from fish muscle tissue subjected to 100-300 MPa (25 °C/30 min) periodically assayed for activity during storage at 4 °C for 3 weeks was observed (Ashie and others 1996a), although the authors of that study attributed this finding to the effect of residual inhibitor in the extracts. Nevertheless, enzymes from bluefish and sheephead showed various levels of reactivation after 300 MPa/30 min when stored at 4-7 °C for up to 21 days (Ashie and Simpson 1996).

Low levels of enzymatic activity determined at 0 °C were recorded in batch V-I from the beginning of storage, but even so that batch still exhibited lower values than the counterpart batch (batch V) that did not contain inhibitor at the end of the storage period, indicative of a certain inhibitory effect of 4-hexylresorcinol on autolytic activity (Figure 4b). On the whole, the presence of inhibitor did not alter autolytic activity levels in the remaining batches. The differences that were recorded between the batches ($p \leq 0.05$) depended on whether or not the oysters underwent pressurization treatment (Table 1).

Summing up, higher levels of darkening were observed in the pressurized oysters, probably because of increased PPO activity. Increased autolytic activity was also recorded in the pressurized oysters. It would be interesting to study different immersion times for oysters in inhibitory solutions containing differing quantities of inhibitor to ascertain the effectiveness of hexylresorcinol in preventing blackening in oysters in combination with different conditions of treatment (pressure range, pH) capable of inactivating enzymatic activity in the oysters.

ACKNOWLEDGEMENTS

The authors thank Ms. Laura Barrios, Head of the Statistics and Operational Research

Service at the Centro Técnico de Informática (CSIC) in Madrid, for her advice. This study was funded by Spain's Comisión Interministerial de Ciencia y Tecnología (CICYT) [Interministerial Science and Technology Commission] under project no. AGL-2000-1497.

REFERENCES

- AOAC. 1984. Official Methods of Analysis, 12th edition. Washington, DC, USA: Association of Official Analytical Chemists
- Asaka M, Hayashi R 1991. Activation of polyphenoloxidase in pear fruits by high pressure treatment. *Agric Biol Chem* 55(9): 2439-2440.
- Ashie INA, Simpson BK. 1996. Application of high hydrostatic pressure to control enzyme related fresh seafood texture deterioration. *Food Res Int* 29(5-6): 569-575.
- Ashie INA, Simpson BK, Ramaswamy HS. 1996a. Control of endogenous enzyme activity in fish muscle by inhibitors and hydrostatic pressure using RSM. *J Food Sci* 61(2): 350-356.
- Ashie INA, Smith JP, Simpson BK. 1996b. Spoilage and shelf-life extension of fresh fish and shellfish. *Crit Rev Food Sci Nutr* 36(1&2): 87-121.
- Bligh E G, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. *Can J Biochem Phys* 37: 911-917.

385
386 Calik H, Morrissey MT, Reno PW, An H. 2001. Effects of high pressure processing on *Vibrio*
387 *parahaemolyticus* in pure culture and in *Pacific oysters*. Abstract #42-1. Presented at the IFT
388 Annual Meeting (New Orleans, LA) June 23-27.
389
390 Chen JS, Rolle RS, Marshall MR, Wei CI. 1991. Comparison of phenoloxidase activity from
391 Florida spiny lobster and western Australian lobster. J Food Sci 56(1)154-160.
392
393 Dufour E, Dalgalarondo M, Hervé G, Goutefongea R, Haertlé T. 1996. Proteolysis of type III
394 collagen by collagenase and cathepsin B under high hydrostatic pressure. Meat Sci 42(3):
395 261-269.
396
397 Eshtiaghi MN, Knorr D. 1996. High hydrostatic pressure thawing for the processing of fruit
398 preparations from frozen strawberries. Food Biotechnology 10(2): 143-148.
399
400 Hardy R, Smith JGM. 1970. Catching and processing scallops and queens. Torrey Research
401 Station. Note 46,1-11.
402
403 Hatano M, Iida H, Seki K, Takahashi K. 1990. Occurrence of unacceptable taste and coloring
404 of giant pacific oyster. Nippon Suisan Gakkaishi 56(9): 1481-1484 (in Japanese with an
405 English abstract).
406
407 He H, Adams RM, Morrissey MT. 2000. The use of high hydrostatic pressure to shuck
408 oysters and extend shelf-life. Abstract #51A-16. Presented at the IFT Annual Meeting (Dallas,
409 TX), June 10-13.

410

411 Homma N, Ikeuchi Y, Suzuki A. 1994. Effects of high pressure treatment on the proteolytic
 412 enzymes in meat. Meat Sci 38: 219-228.
 413

414 Hoover DG, Metrick C, Papineau AM, Farkas DF, Knorr D. 1989. Biological effect of high
 415 pressure of food microorganisms. Food Technol 43 (3): 99-107.
 416

417 Hurtado JL, Borderias J, Montero P, An HJ. 1999. Characterization of proteolytic activity in
 418 octopus (*Octopus vulgaris*) arm muscle. J Food Biochem 23(4): 469-483.
 419

420 Jawahar T, Balasundari S, Indra G, Jeyachandran P. 1994. Influence of antioxidants on the
 421 sensory quality and oxidative rancidity of frozen edible oyster. J Food Sci Technol 31(2): 168-
 422 170.
 423

424 Jeong BY, Ohshima T, Koizumi C, Kanou Y. 1990. Lipid deterioration and its inhibition of
 425 Japanese oyster *Crassostrea gigas* during frozen storage. Nippon Suisan Gakkaishhi 56:
 426 2083-2091.
 427

428 Jiang ST, Tsao CY, Lee TC. 1985. Prevention of blackening coloration and drip loss of frozen
 429 shucked oyster (*Crasostrea gigas*). In: Proceedings of Meetings of Commissions C2 & D3:
 430 International Institute of Refrigeration; Aberdeen (United Kingdom), p.349-354.
 431

432 Kim K, Ikeuchi Y, Suzuki A. 1992. Pressure-induced conversion of α -connectin to β -connectin.
 433 Meat Sci 32: 237-243.
 434

435 Liston J. 1982. Recent advances in the chemistry of iced fish spoilage. In: Martin RE, Flicks
 436 GJ, Herbard CE, Ward DE, editors. Chemistry and biochemistry of marine products. Westport,
 437 CT: AVI Publ. Co., p.27-36.
 438
 439 López-Caballero ME, Pérez-Mateos M, Montero P, Borderías JA. 2000a. Oyster preservation
 440 by high pressure treatment. J Food Protection 63(2): 196-201.
 441
 442 López-Caballero ME, Pérez-Mateos M, Borderías JA, Montero P. 2000b. Extension of shelf-
 443 life of prawns (*Penaeus japonicus*) by vacuum packaging and high-pressure treatment. J Food
 444 Protection 63(10): 1381-1388.
 445
 446 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin
 447 phenol reagent. J Biol Chem 193: 265.
 448
 449 McEvily AJ, Iyengar R, Otwell S. 1991. Sulfite alternative prevents shrimp melanosis. Food
 450 Technol 45(9): 80-86.
 451
 452 Macfarlane JJ. 1985. High pressure technology; meat quality. In: Lawler R, editor.
 453 Developments in meat science-3; London,UK: Elsevier Applied Science Pub., p.155-184.
 454
 455 Monsalve-Gonzalez A, Barbosa-Canovas GV, Cavalieri RP, McEvily AJ, Iyengar R. 1993.
 456 Control of browning during storage of apple slices preserved by combined methods. 4-
 457 hexylresorcinol as anti-browning agent. J Food Sci 58(4): 797-800, 826.
 458

459 Montero P, Ávalos A, Pérez-Mateos M. 2001. Characterization of polyphenoloxidase of
 460 prawns (*Penaeus japonicus*). Alternatives to inhibition: additives and high-pressure treatment.
 461 Food Chemistry (75)3: 317-324.
 462
 463 Nagashima Y, Ebina H, Tanaka M, Taguchi T. 1993. Effect of high hydrostatic pressure on the
 464 thermal gelation of squid mantle meat. Food Res Int 26: 119-123.
 465
 466 Ohmori T, Shigeshisa T, Taji S, Hayashi R. 1991. Effect of high pressure on the protease
 467 activities in meat. Agric Biol Chem 55(2): 357-361.
 468
 469 Ohmori T, Shigeshisa T, Taji S, Hayashi R. 1992. Biochemical effects of high hydrostatic
 470 pressure on the lysosome and proteases involved in it. Biosci Biotech Biochem 56(8): 1285-
 471 1288.
 472
 473 Otwell WS, Iyengar R, McEvily AJ. 1992. Inhibition of shrimp melanosis by 4-hexylresorcinol. J
 474 Aquatic Food Product Technol 1(1): 53-65.
 475
 476 Seyderhelm I, Boguslawski S, Michaelis G, Knorr D. 1996. Pressure induced inactivation of
 477 selected food enzymes. J Food Sci 61(2): 308-310.
 478
 479 Slattery SL, Williams DJ, Cusack A. 1995. A sulphite-free treatment inhibits blackspot
 480 formation in prawns. Food Australia 47(11): 509-514.
 481
 482 Suzuki A, Watanabe M, Iwamura K, Ikeuchi Y, Saito M. 1990. Effect of high pressure
 483 treatment on the ultrastructure and myofibrillar protein of beef skeletal muscle. Agric Biol
 484 Chem 54: 3085-3091.
 J.Food Sci. 2002;67(6):2107-2112

485

486 Venugopal V. 1990. Extracellular proteases of contaminant bacteria in fish spoilage: a review.
487 J Food Prot 53(4): 341-350.

488

489 Weemaes C, Ludikhuyze L, Van den Broeck I, Hendrickx M. 1998. High pressure inactivation
490 of polyphenoloxidases. J Food Sci 63(5): 873-877.

491

492

Figure 1. Visual scores for darkening in oysters during chilled storage: a) Control batch (C) and inhibitor containing (4-hexylresorcinol) control batch (C-I); b) vacuum-packed oysters, with (batch V-I) and without (batch V) inhibitor; c) pressurized oysters, packaged aerobically, with (batch P-I) and without (batch P) inhibitor; d) pressurized, vacuum-packed oysters, with (batch PV-I) and without (batch PV) inhibitor.

Figure 2. Polyphenoloxidase activity in oysters during chilled storage: a) Control batch (C) and inhibitor-containing (4-hexylresorcinol) control batch (C-I), b) vacuum-packed oysters, with (batch V-I) and without (batch V) inhibitor; c) pressurized oysters, packaged aerobically, with (batch P-I) and without (batch P) inhibitor; d) pressurized, vacuum-packed oysters, with (batch PV-I) and without (batch PV) inhibitor.

Figure 3. Proteolytic activity in oysters during chilled storage determined at 60 °C (a) Control batch (C) and inhibitor-containing (4-hexylresorcinol) control batch (C-I); b) vacuum-packed oysters, with (batch V-I) and without (batch V) inhibitor; c) pressurized oysters, packaged aerobically, with (batch P-I) and without (batch P) inhibitor; d) pressurized, vacuum-packed oysters, with (batch PV-I) and without (batch PV) inhibitor.

Figure 4. Proteolytic activity in oysters during chilled storage determined at 0 °C: a) Control batch (C) and inhibitor-containing (4-hexylresorcinol) control batch (C-I); b) vacuum-packed oysters, with (batch V-I) and without (batch V) inhibitor; c) pressurized oysters, packaged aerobically, with (batch P-I) and without (batch P) inhibitor; d) pressurized, vacuum-packed oysters, with (batch PV-I) and without (batch PV) inhibitor.

Table 1. Autolytic activity in oysters (at 60 °C and at 0 °C)

Temperature	Batch	Storage time (d)			
		0	7	14	21
60 °C	C	a/z	a/z	b/z	c/wz
	C-I	a/z	a/z	b/y	c/y
	V	a/z	a/z	b/xz	c/z
	V-I	a/z	a/z	b/y	c/x
	P	a/z	b/xy	c/wx	b/v
	P-I	a/z	b/x	c/wz	b/v
	PV	a/z	b/y	b/y	b/v
	PV-I	a/z	a/z	b/y	c/wx
0 °C	C	a/z	a/z	a/z	a/z
	C-I	a/z	a/z	a/z	b/wy
	V	a/z	a/z	a/z	b/xy
	V-I	a/z	ac/z	a/z	bc/vz
	P	a/z	b/y	c/x	d/tw
	P-I	a/z	bc/y	b/y	c/ty
	PV	a/z	a/z	a/z	b/uvy
	PV-I	a/z	a/z	b/y	b/uxz

Control batch (C) and inhibitor-containing (4-hexylresorcinol) control batch (C-I); vacuum-packed oysters, without (batch V) and with (batch V-I) inhibitor; pressurized oysters, packaged aerobically, without (batch P) and with (batch P-I) inhibitor; pressurized, vacuum-packed oysters, without (batch PV) and with (batch PV-I) inhibitor

Different letters (a, b, c, d) indicate significant differences during the storage period, and different letters (t, u, v, w, x, y, z) indicate significant differences among treatments ($p \leq 0.05$).

527

528

529

530

531

[Escriba aquí]

532

533

534

535

536

537

538

539

540

541