

**Extension of the Shelf Life of Prawns (*Penaeus japonicus*) by Vacuum Packaging and High-Pressure Treatment**

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**ABSTRACT**

The present study has investigated the application of high pressures (200 and 400 MPa) in chilled prawn tails, both conventionally stored (air) and vacuum packaged. Vacuum packaging and high-pressure treatment did extend the shelf life of the prawn samples, although it did affect muscle color very slightly, giving it a whiter appearance. The viable shelf life of 1 week for the air-stored samples was extended to 21 days in the vacuum-packed samples, 28 days in the samples treated at 200 MPa, and 35 days in the samples pressurized at 400 MPa. Vacuum packaging checked the onset of blackening, whereas high-pressure treatment aggravated the problem. From a microbiological point of view, batches conventionally stored reached about 6 log CFU/g or even higher at 14 days. Similar figures were reached in total number of bacteria in vacuum-packed samples and in pressurized at 200-MPa samples at 21 days. When samples were pressurized at 400 MPa, total numbers of bacteria were below 5.5 log CFU/g at 35 days of storage. Consequently, a combination of vacuum packaging and high-pressure treatment would appear to be beneficial in prolonging freshness and preventing spotting.

Spoilage in prawns is a consequence of the higher content in free amino acids in particular and higher quantities of nitrogenous materials in crustacean meats in general, making them quite susceptible to rapid attack by spoilage flora (24). Initial spoilage of crustacean meats is

accompanied by the production of large amounts of volatile base nitrogen compounds, some produced by the reduction in the trimethylamine oxide present in crustacean shellfish (24, 42).

Another aspect is the emergence of dark spots, i.e., melanosis or blackening, during the storage of prawns, starting on the cephalothorax and caudal tip of the tail and subsequently spreading to the rest of the cuticle, giving the product an unpleasant appearance culminating in rejection by consumers. Sulfite derivatives are the most commonly used legally permissible substances for combatting blackening, though they are relatively ineffective. For that reason, many fishermen use larger amounts of these substances than are permitted by present regulations (Directive 95/2/ EU Parliament and Council 20/2/1995) and may even use additives that are not lawful, such as boric acid. The appearance of spots on the surface that may later invade the muscle is brought about by the action of such enzymes as polyphenoloxidase that contributes to melanosis or browning in prawns by a process of oxidation (1, 8, 13, 15, 30). Such processing methods as vacuum packaging and high-pressure treatments may be used as auxiliary technologies in the refrigerated storage of products to completely or partially eliminate factors adversely affecting the storage of certain crustaceans. Ogawa et al. (31) reported that the absence of oxygen from air retarded blackening, even if trauma had occurred when the animals were alive. On the other hand, vacuum packaging may contribute to the growth of facultative anaerobes or strict anaerobes or both and thus to lower levels of food safety (22). Therefore, the applicability of high-pressure treatments is of considerable interest, because they inhibit microbial growth and alter enzymatic activity without changing the organoleptic attributes of the product (12, 23). A number of studies have demonstrated that high pressures can extend the shelf-life of such fish products as cod (*Gadus morhua*) (32), mackerel (*Scomber japonicus*) (17), cream of shrimp (18), bluefish (*Pomatomus saltatrix*) (5), salmon (*Salmon salar*) and herring (*Clupea harengus*) (29), and hake (*Merluccius capensis*) (23). However, how high pressure inactivates polyphenoloxidase in crustaceans is unknown. Experience with other substrates, chiefly vegetables, indicates that enzyme can be either inhibited or activated depending on the treatment conditions and the nature of the substrate itself (2, 10, 19, 21, 34–36).

Prawns are commonly sold whole in Spain; however, heading may be of considerable interest both technically and commercially, because the product then takes up less space and there is less chance of loss of vacuum due to puncture of the wrapping by the horns and antennae (7). A number of studies of headed prawns have been used (14, 27, 38, 40).

The object of the present study was to apply vacuum packaging and high pressure to extend the shelf life of headless prawns stored chilled and to study the action of those treatments on the development of spotting and spoilage.

## MATERIALS AND METHODS

**Sample preparation.** Live imperial tiger prawns (*Penaeus japonicus*) were obtained from Acuinova Andalucía, S.A., a company located in Ayamonte (Huelva, Spain) in the month of December. Mean size was  $13.0 \pm 0.9$  cm and mean weight  $21.5 \pm 4.5$  g. The prawns were anaesthetized by low-temperature shock, headed, and frozen and stored at  $-50^{\circ}\text{C}$  pending analysis. The prawns were defrosted overnight at  $4^{\circ}\text{C}$  and processed. They were first washed in cold water mixed with 3.5% sea salt at  $5^{\circ}\text{C}$  for 1 min. For the batches that contained added melanosis inhibitor, processing was the same as just described, but the cold water used for washing also contained 12,500 ppm of added sodium bisulfite (Sigma Chemical Co., St. Louis, Mo.). After washing, the tails were drained to remove excess solution and packed in vacuum bags with oxygen permeability of  $60 \text{ cm}^3/24 \text{ h/m}^2/\text{atm}$  at  $23^{\circ}\text{C}$  (Cryovac BB-1, Grace, Barcelona, Spain), six prawns to a bag.

Control samples were packaged in bags that were left open, the rest were vacuum sealed. The high-pressure samples then underwent further processing in ACB 665 high-pressure equipment (Gec Alsthom, Nantes, France) at 200 and 400 MPa at  $7^{\circ}\text{C}$  for 10 min. All batches were stored at  $1 \pm 1^{\circ}\text{C}$  in a forced-air cold store.

Melanosis score, chemical and microbiological analyses, shear strength, and water holding capacity were analyzed after 1 day of storage and then weekly. In addition melanosis score was determined initially (0 days) and after 3 days of storage.

All determinations were performed on raw prawns, except for shear strength that was performed on both raw and cooked prawns (to that end, several individuals from each of the treatments were cooked in boiling water for 1 min).

Prawn batch designations were as follows: C (control), V (vacuum packed), 200 (pressurized at 200 MPa, 10 min, 7°C), 400 (pressurized at 400 MPa, 10 min, 7°C). “-S” added to the batch designations indicates the use of added melanosis inhibitor (sodium bisulfite).

Proximate analyses. The proximate composition was measured according to the procedure recommended by the Association of Official Analytical Chemists (6) and in the case of fats according to the method of Bligh and Dyer (9).

Melanosis score. Black spotting during storage caused by melanosis was identified by visual inspection and evaluated as described by Otwell and Marshall (33) by a panel of eight trained assessors using a scale where 0 = absent; 2 = slight, noticeable on some prawns; 4 = slight, noticeable on most prawns; 6 = moderate, noticeable on most prawns; 8 = heavy, noticeable on most prawns; and 10 = heavy, totally unacceptable.

Chemical analyses. The pH of the prawn muscle was measured using a MeterLab model pHM 93 pHmeter (Radiometer Analytical, Denmark) after blending 10 g of muscle in 100 ml of distilled water.

Determination of the total volatile bases (TVB) was carried out according to the method of Antonocopoulos and Vyncke (4). Prawn muscle was homogenized with perchloric acid (6%) to precipitate out the muscle proteins. This was followed by centrifugation at 4000 × g at 5°C for 5 min and distillation of the supernatant (Tecator AB, Kjeltac System, model 1002, Höganäs, Sweden). The distillate was collected in boric acid (3%) and evaluated in hydrochloric acid (0.05 N). Results have been expressed as mg of nitrogen/100 g of sample.

Free formaldehyde was determined by the method of Castell and Smith (11). Nash's colorimetric reaction was performed using the aqueous extract obtained by washing the formaldehyde given off by 25 g of prawn muscle away in a vapor stream upon heating in the presence of phosphoric acid. The results have been expressed as μmoles of formaldehyde/g of prawn.

Microbiological analyses. At least 12 prawns per batch were taken for microbiological analysis as follows: 10 g of muscle (and 25 g to detect *Salmonella* spp. and *Shigella* spp.) were collected aseptically from different parts of individuals from the samples for each of the different treatments in a vertical laminar-flow cabinet (model AV 30/70, Telstar, Madrid, Spain) and placed in a sterile plastic bag (Sterilin, Stone, Staffordshire, UK) with 90 ml of buffered 0.1% peptone water (Adsa Micro, Barcelona, Spain). After 2 min in a stomacher blender (model Colwoth 400, Seward, London, UK), dilutions were prepared, also with buffered peptone water, for the following microorganism determinations: total number of bacteria and H<sub>2</sub>S-producer microorganisms on double-layered plates containing iron agar (Adsa Micro) after incubation at 20°C for 72 h; coliforms and suspected *Escherichia coli* on double-layered plates containing COLI ID medium (bioMe´rieux, Marcy l'Etoile, France) after incubation at 37°C for 48 h; *Salmonella* on spread plates containing SM ID medium (bioMe´rieux) and *Shigella* on spread plates containing SS medium (bioMe´rieux) after incubation at 37°C for 24 h [after enrichment in buffered peptone water (Adsa-Micro) 37°C/20 h followed by inoculation in Rappaport-Vassiliadis broth (Merck, Darmstadt, Germany), 43°C/24 h]; and finally sporulated and vegetative mesophilic clostridia on spread plates containing sulfite iron agar (Merck) after incubation in anaerobic jars (Oxoid, Basingstoke, UK) at 30°C for 48 h. Microbiological counts have been expressed as log CFU/g of sample, except for *Salmonella* and *Shigella* expressed as being absent or present in 25 g of sample. Analyses were performed in duplicate.

Shear strength. Muscle hardness was measured as the shear strength of raw and cooked samples at room temperature using an Instron model 4501 Texturometer (Instron Engineering Corp., Canton, Mass.). Prawn muscle was placed in a Kramer shear cell, and measurements were taken at a cross-head speed of 100 mm/ min using a 5-kN load cell. Readings were taken using the maximum force setting and have been expressed as the sample to weight ratio (N:g). The values given are the means of six replications.

Water holding capacity (WHC). It was determined by placing 1.5 g of muscle in a centrifugation tube with absorbent paper and centrifuging at 4,000 × g at room temperature for

15 min in a Sorvall model RT6000B centrifuge (E.I. du Pont de Nemours & Co., Wilmington, Del.). WHC has been expressed as the amount of water retained per 100 g of water present in the sample before centrifuging. At least three replications of all determinations were performed.

Statistical analyses. The significance between pairs of mean values was evaluated by means of analysis of variance run on the replicated measurement values taking storage time as the covariance. Linear regression by treatment group was also run. The BMDP statistical package (BMDP Statistical Software, Inc., Cork Technology Park, Cork, Ireland) was used.

## RESULTS AND DISCUSSION

According to the results of the proximate analysis, the percentage composition of the prawn muscle was: moisture  $78.13 \pm 0.62$ , ashes  $1.89 \pm 0.49$ , crude protein  $18.95 \pm 0.10$ , and crude fat  $1.15 \pm 0.16$ .

The appearance of the prawn batches with and without inhibitor was very similar after the different treatments, both vacuum packaging and pressurization (200 and 400 MPa). The only difference was that, while still looking raw, the muscle of prawns pressurized at 400 MPa was slightly more opaque and whitish in appearance.

Figure 1 plots the melanosis scores rated on the basis of the 10-point scale employed. The refrigerated control batch (Fig. 1a) exhibited slight spotting on the pleuron (the caudal portion of the tail) at the junction with the pleopods. The spotting increased with storage time until it had spread over the entire abdomen and the prawns were rejected as unacceptable on day 7. The sodium bisulfite-treated control batch did not display any appreciable differences in the melanosis rating scores with respect to the untreated batch, which calls into question the efficacy of the melanosis inhibitor used. In contrast, the appearance of the unpressurized vacuum-packed batches (Fig. 1b) remained good over the entire storage period, with no scores higher than 2. This can be attributed to the fact that the melanin-forming reaction requires sufficient amounts of oxygen that was not present in the vacuum-packed batches. The behavior of the batches pressurized at 200 and 400 MPa was quite similar to that of the batches that were vacuum packed only, except for earlier onset of spotting and a slight

increase in the number of spots (Fig. 1c and 1d). The use of sodium bisulfite in the pressurized batches turned the cooked prawns a brighter, stronger shade of red. Linear regression has been calculated from melanosis score; rating scores yielded a slope of 0.7 for the control batches, compared with slopes of less than 0.1 in all cases for the vacuum-packed and pressurized batches. Accordingly, the regression lines for the treated batches were practically asymptotic with respect to the abscissa (data not shown). In other words, there was virtually no increase in blackening over the storage period (Fig. 1).

The pH values for the prawn muscle were similar in all the different batches (Fig. 2). There was a sharp increase in the first week of storage, with values rising to higher than 8. Shamshad et al. (38) reported that *Penaeus merguensis* was not acceptable when the pH was greater than 7.6. Nevertheless, despite the pH values attained in the present experiment, the odor and overall appearance of the prawns remained acceptable. There were no significant differences between any of the batches either with added melanosis inhibitor or with high-pressure treatment ( $P \leq 0.05$ ) (Table 1).

The TVB content values, considered by Vanderzant et al. (41) to be chiefly ammonia, differed significantly according to treatment and storage time but not according to whether or not sodium bisulfite was added (Fig. 3) (Table 1). Initial concentration was between 9.26 and 18 mg N-TVB/100 g  $\pm$  0.40 of muscle, higher than the value reported by Shamshad et al. (38), who found initial levels of 4.5 mg N-TVB/100 g for banana shrimps (*P. merguensis*) from Pakistan stored at 0°C. From day 14 there was a pronounced increase in the TVB in the vacuum-packed batches, the increase being lowest in the prawns treated with sodium bisulfite ( $P \leq 0.05$ ). Working with banana shrimps with and without added sodium bisulfite (1.25% NaHSO<sub>3</sub>), Yamagata and Low (42) recorded values of 13.80 mg N-TVB/100 g during storage with added sodium bisulfite and 15.49 mg N-TVB/100 g during storage without added sodium bisulfite, suggesting that sodium bisulfite delayed spoilage. Despite presenting significant differences, the pressure-treated batches behaved similarly during the storage period (35 days), with values between 25 and 50 mg N-TVB/100 g.

Figure 4 depicts the trend for the free formaldehyde. The values were significantly higher in the control batch with added sodium bisulfite as compared to the control batch without added sodium bisulfite (Table 1). This was reported previously by Yamanaka et al. (43), who suggested a nonenzymatic reaction as a possible cause of conversion of the trimethylamine present into formaldehyde in the presence of sodium bisulfite. The formaldehyde content increased slightly in the batches that did not contain added melanosis inhibitor; conversely, in the batches that did contain added inhibitor, the increase was extremely pronounced between 0 and 20 days of storage. That increase may have been related to the presence of sodium bisulfite in the absence of air. Increased formaldehyde values when a vacuum was applied were reported earlier in cod by Lundstrom et al. (26), who attributed that finding to the reduction in the numbers of microorganisms under those conditions and consequently very low levels of conversion of trimethylamine oxide to trimethylamine, leading to an increase in the production of dimethylamine and formaldehyde. Despite the low trimethylamine oxide content in the prawns, in the present experiment there appeared to be a synergistic effect between vacuum packaging and the addition of melanosis inhibitor with respect to formaldehyde production. High pressure did not seem to have any clear influence on formaldehyde production. The decrease in free formaldehyde after 20 days of storage can be explained by a reduction in the free form through reaction with the proteins, as a result of the formation of covalent bonds and protein aggregation (39).

The total number of bacteria load in the prawns was initially quite low. This finding can be ascribed to the fact that the prawns were alive at the beginning of processing and were processed into the different batches immediately upon sacrifice to avoid subsequent contamination. Values in the control batches (C and C-S) were on the order of 6 (log CFU/g) after 14 days of storage. Shamshad et al. (38) reported similar values, with a microbiological shelf life of 3 days for shrimps stored at 0°C. In the vacuum-packed batches that level was attained after 21 days (Table 2). The high-pressure treatments reduced the total flora and following pressurization the counts were below the detection threshold. After a recovery phase, exponential growth occurred in the batch pressurized at 200 MPa with bisulfite (200-S) after 21

days, and growth commenced earlier and proceeded faster in the batch that did not contain the melanosis inhibitor (200) ( $P \leq 0.05$ ). No growth was observed in the batches pressurized at 400 MPa until day 14, and on day 28 levels were on the order of 7 log units in batch 400 compared to 3 log units in batch 400-S ( $P \leq 0.05$ ). These results differ from the findings for pike perch reported by Myllymäki et al. (29), who observed that the total flora was not affected at 500 MPa for 15 min, 15 to 20°C but was affected at 700 MPa for 15 min, 15 to 20°C. Gola et al. (18) also reported inactivation of microorganisms by high-pressure treatment. They found a shrimp cream pressurized at 700 MPa for 3 min to be stable for 3 to 4 months. On the other hand, according to Miyao et al. (28), most of the flora present in surimi (fish paste) was killed by treatment at between 300 and 400 MPa, with the gram-negative flora being more sensitive to pressure than the gram-positive flora. Greater ability of gram-positive flora to withstand pressure was likewise described by Fujii et al. (17) in minced mackerel (*S. japonicus*), though the gram-negative flora (*Pseudomonas*) was the predominant component at the time of spoilage. Those same workers reported an 80% decrease in the bacterial count following pressurization at 2,000 atm for 60 min and retardation of bacterial growth for 4 days under those treatment conditions.

H<sub>2</sub>S-producing microorganisms are not initially present in prawns, but they were detected during the first week of storage in the control and vacuum-packed batches (Table 2). H<sub>2</sub>S-producing microorganisms, of which *Shewanella putrefaciens* makes up the greater part (20), reached values of between 6 and 7 log CFU/g in batches V and V-S, respectively, after 28 days of storage. Highpressure treatment inhibited those microorganisms that did not appear until the end of the storage period. In the prawns pressurized at 200 MPa, counts reached a level of 6 log CFU/g after 28 days of storage, whereas counts in batch 200-S were below the detection threshold (<10 CFU/g), perhaps because of the added melanosis inhibitor.

No growth of H<sub>2</sub>S-producing microorganisms was detected in prawns pressurized at 400 MPa. The sensitivity of these microorganisms to high pressure was described previously by Myllymäki et al. (29) in fish and by LópezCaballero et al. (25) in oysters. Ammonia may be produced during the storage of iced shrimp by both microorganisms and tissue enzymes (16),

with the latter far exceeding bacterial enzyme production (44). This, together with the low concentration of trimethylamine in iced shrimps (42), could account for the levels of TVB attained (Fig. 3) despite the microbial counts recorded (Table 2) that were not very high, especially in the early stages of storage.

At the end of the experiment coliform levels were very low in the pressurized batches, except in batch 400, which would seem to indicate that addition of the melanosis inhibitor had no effect on those microorganisms ( $P \leq 0.05$ ). No presumptive *E. coli*, *Salmonella* spp., *Shigella* spp., or sulfite-reducing clostridia were detected in any of the batches during this experiment.

Shear strength changed very slightly over the storage period ( $P \leq 0.01$ ) in both the raw and cooked pressurized prawn muscle (Fig. 5). On the whole, values were lowest in the control batch. Furthermore, there were small but significant differences ( $P \leq 0.07$ ) in shear strength in the cooked prawns depending on whether or not sulfite was added. In the raw prawns, there were differences caused by the vacuum packaging and/or high-pressure treatments ( $P \leq 0.07$ ) (Table 1). Pressures of 200 and 400 MPa have been reported to soften the muscle in bluefish (*P. saltatrix*) by Ashie et al. (5) and in carp (*Cyprinus carpio*) by Yoshioka and Yamamoto (45). On the other hand, Angsupanich and Ledward (3) found that pressures of up to 400 MPa caused hardening in cod (*G. morhua*) muscle. No apparent softening was recorded in any of the batches in the present experiment. Ashie et al. (5) have postulated that enzymatic activity may first be inhibited in pressurized fish muscle and then recur during storage, causing softening. However, this was not observed in the present experiment, not even in the control batches. The addition of sodium bisulfite and the application of vacuum packaging led to the production of large amounts of formaldehyde (Fig. 4), as has already been discussed above. Those treatments are known to contribute to aggregation of the myofibrillar proteins, which in turn results in muscle hardness and dryness, but they did not bring about any increase in shear strength values.

WHC of the muscle was determined in order to try to ascertain whether processing brought about a reduction in the water content. WHC values fell off slightly ( $P \leq 0.01$ ) in all the batches over the storage period, with starting values of 78 to 82% and final values of 72 to 77% (data

not shown in tables or figures). Shaban et al. (37) recorded a 5% decrease in WHC after 4 days of storage in iced imperial tiger prawns (*P. japonicus*), the same species used in this experiment, though larger in size (35 g).

In summary, the control batches of the prawn tails considered here, stored at 3°C, exhibited a level of spotting sufficient to make the samples unfit for market after 3 days. However, vacuum packaging curbed the appearance of blackening for 35 days of storage at 3°C. Pressurization of the vacuum-packed samples at 200 and 400 MPa led to the appearance of some spots, though not sufficient to represent a problem from a commercial standpoint. The use of sodium bisulfite as a melanosis inhibitor and the method of application did not seem particularly effective at preventing blackening. On the basis of the microbiological indices, vacuum packaging extended the shelf life of prawns for up to 21 days, whereas pressurization at 200 MPa and 400 MPa in addition to vacuum packaging further extended the shelf life to 28 and 35 days, respectively. Ammonia odors caused by the nonmicrobial production of ammonia appeared in the unpressurized vacuum-packed batches after 14 to 21 days of storage, especially in the batches without added sodium bisulfite. Neither vacuum packaging nor high-pressure treatment affected the shear strength of the prawn muscle.

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