

Effect of High Hydrostatic Pressure Treatment on the Qualities of Cultured Large Yellow Croaker (*Pseudosciaena crocea*) during Cold Storage

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This study was carried out to evaluate the effect of high hydrostatic pressure on quality changes of cultured yellow croaker (*Pseudosciaena crocea*) muscle during storage time at 4 °C. High hydrostatic pressure (HHP) treatments were applied at 100, 200, 300, 400, and 500 MPa for 10 and 15 min. Quality changes include pH, water activity, total volatile basic nitrogen (TVB-N), thiobarbituric acid content (TBA) and trimethylamine (TMA). Total bacterial count of *P. crocea* muscle's samples were determined immediately after treatment and throughout subsequent storage at 4 °C as well. Results have shown *P. crocea* muscle's pH value, TBA value content and TMA content increased significantly with the increase of treated pressure, while Aw and TVB-N content decreased as the pressure increased, and the growth of total bacterial count significantly inhibited with increasing pressure. The results showed that the pH value and Aw of *P. crocea* muscle treated with HPP increased and then decreased during storage; the TVB-N and TMA contents both increased (no more than 35 mg/100 g and 5 mg/100 g, respectively) and were effectively controlled on day 45 after the treatment at 500 MPa for 15 min; and the total colony count of *P. crocea* was 5.7×10^4 CFU/mL on day 45 after the treatment at 500 MPa for 15 min. Together these results indicate that the HHP treatment at 500 MPa for 15 min is optimal for the preservation of cultured *P. crocea*.

Key words: *Pseudosciaena crocea*; high hydrostatic pressure; quality.

The aquatic products of particular economic fish species are of high significance to economic development. The large yellow croaker (*Pseudosciaena crocea*) is becoming one of the most important marine fish species and is widely favored by domestic and overseas consumers for its tender meat, delicious muscle, high protein content, and low steroid levels as well as its beneficial effects of treating anemia and nourishing the body (Lu, 2011). In the late 1990s, cultivation

technologies for *P. crocea* improved gradually and farming of *P. crocea* rapidly increased. The resulting annual output of *P. crocea* soon exceeded 50,000 tons in China, which indicated that cultured *P. crocea* was one of the most valuable aquatic products for export. However, due to the perishable mature seafood, the development of satisfactory methods for shelf life extension that ensures quality maintenance and continuous supply of refrigerated quality products with minimum losses, has occupied the attention of food technologists. With the aid of processing technology, these large yellow croakers can be stored and transported with minimal quality degradation, and processed to value-added products that will draw attention from consumers worldwide. Moreover the use of safe preservation

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technologies is of practical significance to seafood security as well as long-distance transport and quality stabilization during their processing and storage (Yang, 2004).

High hydrostatic pressure (HHP) treatment is a pure physical processing technology that has been applied to food preservation. Its advantages include preserving freshness and quality, extending storage life, retaining original nutrients and flavor, and improving food quality; thus, it is considered a promising method of seafood preservation, Liu, 2008 & Wei, 2009. High pressure processing is a promising seafood preservation method. HHP treatment is increasingly employed for the commercial processing of sea cucumbers, eng, 2009, oysters (Murchie *et al.*, 2005), fish (Erkan, Üretener, & Alpas, 2010) and surimi (Chung *et al.*). The HHP technology can also be used for disinfection, enzyme inactivation, improving texture, and sterilization. This new technology reportedly provides long shelf-life and minimum quality loss since it does not have many undesirable changes, which are associated with thermal processing because it retains a fresh taste, and has shown enhanced flavour with no indication of oxidation. However, structure, texture colour and biochemical can be negatively affected (V. Briones-Labarca, 2012).

Nevertheless, the effects of HHP treatments on quality changes of *P. crocea* muscle have not been reported. The aim of this study was to investigate the effects of high hydrostatic pressure on quality changes (pH, water activity (Aw), total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA), trimethylamine (TMA), as well as total bacterial count) of cultured large yellow croaker (*Pseudosciaena crocea*) during cold storage time.

MATERIALS AND METHODS

Sample preparation and HHP treatment

Large yellow croaker (length: 20 ± 1 cm) were purchased from an aquatic products market in Ningbo City, Zhejiang Province, China. They were delivered alive in plastic bags acclimated with aerated seawater to the lab. The fish were killed by knocking head. Their heads and tails were removed. The remaining part was eviscerated and the muscle fillets (100 ± 10 g) were transferred into polyethylene

flexible pouches. Samples were kept under a refrigerated room (4°C) no more than one hour before further analysis. The samples were prepared in duplicate for water content, protein and fat content, and in triplicate for other parameters.

The fillets were individually vacuum packed and hermetically sealed in high density polyethylene bags. The prepared muscle samples were loaded into cylindrical loading container and HHP-treated at 100, 200, 300, 400, and 500 MPa for different time at room temperature (25°C) in a 5L high pressure device (Tianjin Huataisen Biological Engineering Technology Co., Ltd., Tianjin, China). The HHP treatment was conducted at room temperature (20°C) with water as the pressure-transmitting medium at 10 MPa/s.

Determination of pH

The prepared and ground muscle samples (10 g) were transferred into a volumetric flask followed by the addition of distilled water to a constant volume of 100 mL. The suspensions were mixed thoroughly and then allowed to stand for 30 min. Thereafter, the suspensions were filtered on a filtration machine and the filtrate was used for pH measurements using a calibrated pH meter (Mettler Toledo Fe20, Zurich, Switzerland). Measurements were carried out in triplicate.

Determination of Water activity

The water activity values of homogenised were determined by the AW-Sprint Novasina water activity instrument (TH-500, Novasina Co., Ltd., Switzerland). The samples at a constant temperature ($15\text{--}25^{\circ}\text{C}$) were weighed into sample boxes and flattened. A gauge outfit equipped with a sensor was then placed on the sample boxes and gently tightened. The sample boxes were kept at a constant temperature for 2 h, during which the changes in the sensor's pointer were continuously observed. Once the pointer had stabilized, the value it indicated was recorded as the Aw value of the sample at this temperature (Gan, 2012). Measurements were carried out in triplicate.

Determination of Total volatile basic nitrogen (TVB-N)

Muscle tissue was thawed at room temperature by lotic water for use. It weighs about 10.0 g and place it in a conical flask. Add 90 mL of water, shake it from time to time, and filter it after 30 min of dipping to be prepared for use.

Prepare 40 % NaOH solution and 4 % boric acid solution. Add mixed indicator into the boric acid solution (add 10 ml of indicator into 4 % 1000 ml of boric acid solution). So set them respectively at the specified position of the automatic Kjeldahl apparatus (automatic Kjeldahl analyzer UDK 152, Italy VELP company, Brianza, Italy).

Correctly imbibe 5.0 ml of the above filtrate in the digestive tract and then set it in the distillation reaction chamber of UDK 152 automatic Kjeldahl analyzer. Place the calibrated standard hydrochloric acid solution in the absorbing place. Then operate the page of the analyzer and set the standard value. After pressing the “Start” button, the analyzer automatically starts to distill and titrate. At last, the value of the volume of hydrochloric acid consumed was recorded, and the reagent blank control group was made (Huang, 1993). Measurements were carried out in triplicate. All used reagents were of analytical grade. The solvents and reagents were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China with analytical grade.

The result can be expressed by the mg number containing N in the sample per 100g.

$$\text{TVB-N, mg/100g} = [(V_1 - V_2) \times C \times 14 / (m \times 5 / 50)] \times 100$$

Where, for the volume of hydrochloric acid standard titrant consumed by V1-sample, the unit is mL; For the volume of hydrochloric acid standard titrant consumed by V2-sample blank, the unit is mL. For the concentration of C-hydrochloric acid standard titration solution, the unit is mol/L. m is the volume of the sample.

Determination of Thiobarbituric acid (TBA)

The TBA value was measured as follows (Huang, 1993): homogenized *P. crocea* muscle samples (10 g) were weighed into colorimetric tubes with stoppers, followed by the addition of 50 mL of 7.5% trichloroacetic acid (containing 0.1% ethylenediaminetetraacetic acid [EDTA]). The suspensions were oscillated for 30 min and then filtered with double-layer filter paper. An aliquot (5 mL) of 0.02 M TBA solution was taken and incubated in a boiling water bath for 40 min, then cooled for 5-min centrifugation. The supernatant was mixed thoroughly with 5 mL of chloroform and then allowed to stand for 30 min. The resulting supernatant was subjected to colorimetric assays at 532 nm and 600 nm, respectively. The absorbance of the supernatant was recorded and then used for

calculation with the following formula (TBA solution: 0.288 g of TBA was heated and dissolved in 90% water to a final volume of 100 mL; 7.5% TBA: 7.5 g of TBA and 0.1 g of EDTA were dissolved in water to a final volume of 100 mL):

$$\text{TBA (mg/100 g)} = (A_{532} - A_{600}) / 155 \times 1/10 \times 72.6 \times 100.$$

All reagents were of analytical grade. The solvents and reagents were all purchased from Sinopharm Chemical Reagent Co., Ltd.

Determination of Total bacterial count

Total colony counting was conducted according to Huang (Huang, 1993). The processed *P. crocea* muscle samples (10 g) were aseptically weighed into disinfectant conical flasks containing 100 mL of disinfectant saline solution. The suspensions were oscillated to form 1:10 uniform solutions. Aliquot (1 mL) of the 1:10 solutions were transferred using a 1-mL disinfectant pipettor into test tubes containing 9 mL of disinfectant saline along the tube wall at 1 cm above the surface of the diluent. The resulting solutions were mixed to form 1:100 dilutions. The 1:10 dilution procedure was followed with a new 1-mL disinfectant pipettor each time. The resulting solutions were mixed thoroughly before further dilution. The solution series was diluted to 10^{-3} or an appropriate dilution for colony counting. All reagents were of analytical grade. The solvents and reagents were all purchased from Sinopharm Chemical Reagent Co., Ltd.

Determination of Trimethylamine (TMA)

Gradient volumes of TMA standard solution (0, 1, 2, 3, 4, and 5 mL) were dispensed into 125-mL separatory funnels and filled with water to a volume of 5 mL. Next, 1 mL of magnesium carbonate formaldehyde solution, 10 mL of toluene, and 3 mL of saturated potassium carbonate solution were added to each funnel. The resulting mixtures were oscillated vigorously for 1 min and then allowed to stand for 5 min. The toluene layer of each mixture was separately transferred into a capped tube containing 0.5 g of anhydrous sodium sulfate for dehydration. A 5-mL aliquot of the resulting solution was taken and mixed thoroughly with 5 mL of picric acid solution in toluene, followed by chromometric assay at 410 nm. Sample absorbance was measured with 1-cm cuvettes and a reagent blank was used as a reference solution. *P. crocea* muscle samples (5 g) were weighed into

250-mL conical flasks, followed by the addition of 40 mL of distilled water. The suspensions were oscillated for 15 min and then filtered into separate 100-mL volumetric flasks. The filtrates were diluted with distilled water to the mark of the maximum volume and mixed thoroughly. Aliquot (5 mL) of the diluted solution were transferred into 125-mL separatory funnels, with 5 mL of water used as a blank control. The following procedure was the same as that used to prepare the performance curve, and a reagent blank was used as a reference solution. The corresponding TMA content of the test samples was derived from absorbance measurements using the performance curve (Xu, 2005). All used reagents were of analytical grade. The solvents and reagents were purchased from Sinopharm Chemical Reagent Co., Ltd.

Statistical analysis

The effect of high hydrostatic pressure on each quality parameters were estimated using SPSS19.0 (SPSS Corp., Norman, California, USA). The results were analyzed by analysis of variance (ANOVA) and Duncan's multiple range tests with a significance level of $\pm = 0.05$ and a confidence interval of 95% ($P \leq 0.05$). One-way ANOVA was conducted for analyzing the factor of storage time within a pressure and within a pressure-holding time. For all of the samples, two or three different groups ($n = 2$ or 3) were considered and analyzed separately to perform a statistical analysis. The results were displayed as: mean \pm SEM

RESULTS AND DISCUSSION

Effects of different HHP treatments on quality indicators of cultured *P. crocea* muscle

The fresh *P. crocea* muscle had a slightly acidic pH (6.72). The pressure level had a significant effect on pH ($P < 0.05$; Table 1). After the HHP treatment, the *P. crocea* muscle pH value increased slightly, indicating that the increase in the samples' water content might have resulted from a dilution effect of the HHP treatment. Alternatively, the HHP treatment might have changed the protein conformation and, thus, caused certain protein denaturation; the resulting unfolding of the protein structure led to the exposure of a number of essential amino acids.

The Aw does not represent the absolute moisture of the food but is a measure of the level

Table 1. Effects of same pressure and different holding pressure times on cultured yellow croaker characteristics

Pressure (MPa/min)	pH	Aw	TVB-N (mg/100 g)	TMA (mg/100 g)	TBA (mg/100 g)	CFU/mL
CK	6.72 \pm 0.10 ^{ab}	0.901 \pm 0.001 ^c	7.24 \pm 0.01 ^f	0.508 \pm 0.002 ^a	0.508 \pm 0.002 ^a	2.1 \times 10 ⁵ \pm 1.4 \times 10 ⁴
400/5	6.71 \pm 0.02 ^a	0.875 \pm 0.003 ^b	6.84 \pm 0.16 ^b	2.11 \pm 0.03 ^a	7.520 \pm 0.002 ^a	3.1 \times 10 ³ \pm 1.1 \times 10 ^{2a}
400/10	6.77 \pm 0.01 ^b	0.870 \pm 0.003 ^b	6.79 \pm 0.01 ^b	2.46 \pm 0.01 ^b	8.760 \pm 0.007 ^b	2 \times 10 ³ \pm 2.8 \times 10 ^{2a}
400/15	6.81 \pm 0.02 ^c	0.865 \pm 0.001 ^b	6.51 \pm 0.23 ^b	3.01 \pm 0.04 ^c	9.699 \pm 0.119 ^c	9.3 \times 10 ² \pm 14 ^b
400/20	6.83 \pm 0.01 ^c	0.774 \pm 0.021 ^a	5.61 \pm 0.32 ^a	3.32 \pm 0.02 ^d	11.227 \pm 0.325 ^d	4.1 \times 10 ² \pm 12 ^b

a, b, c, d, e, f, and g denote differences among various pressures ($P < 0.05$).

TVB-N, total volatile basic nitrogen; TMA, trimethylamine; TBA, thiobarbituric acid

and experience of microorganisms to endure drying. The results of the present study showed that the *P. crocea* muscle Aw under HHP treatment showed a decreasing trend with increasing pressure (Table 1). The pressure level had significant effects on the fish muscle Aw ($P < 0.05$), while the Aw of the control group was 0.901. The significant influence of Aw on the preservation effect of HHP treatment is that a low Aw can enhance the sterilization and inactivation effects of HHP (Guan, 1996). The lower Aw of a food, the stronger its ability to retain moisture and the less available water provided for the propagation of microorganisms (Hao, 2012).

Factors that affect the freshness of aquatic products include physical, chemical, and biological elements. These factors are mutual constraints, of which microbially induced deterioration is the most popular and active element (Zhang, 2007). In a study by Gram et al. (Gram, 1996), the deterioration of aquatic products was mainly reflected by the growth and metabolism of certain microorganisms and the resulting production of amines, sulfides, alcohols, aldehydes, ketones, and organic acids with undesirable smells and odors, which are not consumer friendly. The results of the present study showed that the total bacterial counts *P. crocea* muscle samples under HHP treatment decreased with the increasing of treated pressure (Table 1). And the pressure level significantly affected the total bacterial counts *P. crocea* muscle samples ($P < 0.05$). At an HHP < 300 MPa, increasing the treatment pressure significantly reduced the total bacterial counts the muscle samples. Further increasing the pressure was associated with slowed growth of the number of colonies. That is, only increasing the pressure had a slight sterilizing effect, whereas at the HHP of 500 MPa, the total bacterial counts *P. crocea* muscle sample was 3.4×10^2 CFU/mL.

The TBA value can be used to measure the level of lipid oxidation. The experimental results showed that the TBA value of *P. crocea* muscle samples under HHP treatment slightly increased with increasing pressure (Table 1). The TBA value of the *P. crocea* control group was 0.508 mg/100 g on day 0 and reached 9.797 mg/100 g after the 500 MPa treatment. This result demonstrated that HHP treatment had a strong effect on the TBA value of *P. crocea* muscle. The pressure level had

significant effects on the TBA value of the *P. crocea* muscle samples ($P < 0.05$). HHP treatment can improve myoglobin and deoxymyoglobin, thereby promoting lipid oxidation within the muscle. The TVB-N level is an important indicator for determining the freshness of protein-rich foods. In the deterioration process of aquatic products, bacterial growth and reproduction and enzymatic reactions induce the breakdown of proteins and the subsequent production of volatile basic N-containing substances such as amines and ammonia (Liu, 2008). The experimental results of this study showed that the TVB-N level of *P. crocea* muscle samples in the control group was 7.24 mg/100 g on day 0, which declined to 6.59 mg/100 g after the treatment of 500 MPa for 15 min. There was a decreasing trend in the TVB-N level of *P. crocea* muscle samples under HHP treatment with increasing pressure. Pressure level had significant effects on the TVB-N levels of the *P. crocea* muscle samples ($P < 0.05$).

The TMA is a reduction product of TMA N-oxide in aquatic animals (especially marine fish species) by facultative anaerobes, whose content gradually increases due to fish freshness deterioration (V. Briones-Labarca, 2012). The TMA content of the *P. crocea* muscle samples showed no significant changes between the 100 and 200 MPa treatments but significantly increased to 3.07 mg/100 g under the 500 MPa treatment for 15 min. The level of pressure significantly affected the TMA content of *P. crocea* muscle samples ($P < 0.05$).

Together, the above experimental data and the data analysis results indicate that 400 MPa preservation treatment of cultured *P. crocea* is more suitable than other pressure conditions. Thus, the *P. crocea* muscle samples were treated at 400 MPa for 5, 10, 15, and 20 min, followed by determination of the changes in terms of quality indicators. The determination of pH value is one of the most frequently used methods for controlling the physical quality of seafood products.

The results showed that pH values of the *P. crocea* muscle samples slightly increased with increasing pressure-holding time (Table 2). The pressure-holding time had a significant effect on the pH value of *P. crocea* muscle samples ($P < 0.05$). Under the same pressure condition, different pressure-holding times can change the balance of

oxidation-reduction reactions in the sample and result in the variations of free radical and hydroxyl group concentrations, thereby changing the pH value.

In the present study, the Aw of *P. crocea* muscle samples showed a decreasing trend with increasing pressure-holding time. The holding time had no significant effects on the Aw of *P. crocea* muscle samples ($P > 0.05$). Although a decrease in the Aw may help microorganisms resist the destructive effect of pressure, the low Aw can suppress the repair capacity of sublethal microbial cells. Different pressure-holding times resulted in significant differences in the TVB-N content of the samples ($P < 0.05$). With increasing pressure-holding time, the protease activity continued to decrease, thereby inhibiting the protein decomposition.

The TMA content of *P. crocea* muscle samples increased with increasing pressure-holding time, and the pressure-holding time showed significant effects on the TMA content of the samples ($P < 0.05$). The TBA value indicates the tissue accumulation of aldehydes and is a measure of the level of lipid oxidation. The pressure-holding time had significant effects on the TBA value of *P. crocea* muscle samples ($P < 0.05$), as longer pressure-holding times would promote lipid oxidation in fish fat. Additionally, the pressure-holding time had a significant effect on the total colony counts of *P. crocea* muscle samples ($P < 0.05$).

The longer the pressure-holding time, the stronger the sterilizing effect. HHP treatment mainly inhibited bacterial growth by degenerating the necessary enzymes. However, when the residual bacterial rate reached a certain value, simply increasing the HHP treatment time had no significant sterilizing effect but further improved the sterilizing effect in combination with other treatments.

pH of cultured *P. crocea* muscle during storage after different HHP treatments

The detection of pH is one of the most frequently used physical quality control methods for seafood products, which is affected by the changes in the concentrations of free hydrogen and hydroxyl ions because of the shifts in the oxidation-reduction balance of the food by the activity of microorganisms or enzymes (Var11k, C.,

Table 1. Effects of different pressures and the same holding pressure time on cultured yellow croaker characteristics

Pressure (MPa/min)	pH	Aw	TVB-N (mg/100 g)	TMA (mg/100 g)	TBA (mg/100 g)	CFU/mL
CK	6.72 ± 0.10 ^{ab}	0.901 ± 0.001 ^c	7.24 ± 0.01 ^f	0.508 ± 0.002 ^a	0.508 ± 0.002 ^a	2.1 × 10 ⁵ ± 1.4 × 10 ⁴
100/10	6.70 ± 0.02 ^a	0.874 ± 0.020 ^{ab}	7.09 ± 0.01 ^{ef}	1.79 ± 0.11 ^a	3.598 ± 0.011 ^b	1.4 × 10 ⁵ ± 2.8 × 10 ^{4b}
200/10	6.73 ± 0.27 ^{ab}	0.877 ± 0.001 ^b	6.98 ± 0.02 ^{de}	1.95 ± 0.07 ^a	4.129 ± 0.012 ^c	2.8 × 10 ⁴ ± 3.5 × 10 ^{3a}
300/10	6.78 ± 0.25 ^{abc}	0.872 ± 0.003 ^{ab}	6.86 ± 0.05 ^{cd}	2.33 ± 0.01 ^b	6.696 ± 0.004 ^d	2 × 10 ³ ± 2.8 × 10 ^{2a}
400/10	6.70 ± 0.11 ^{abc}	0.869 ± 0.001 ^{ab}	6.80 ± 0.01 ^{bc}	2.46 ± 0.01 ^b	8.760 ± 0.007 ^e	2 × 10 ³ ± 2.8 × 10 ^{2a}
500/10	6.81 ± 0.01 ^c	0.863 ± 0.001 ^{ab}	6.65 ± 0.17 ^{ab}	2.90 ± 0.01 ^c	9.797 ± 0.005 ^f	4.1 × 10 ² ± 14 ^a

a, b, c, d, e, f, and g denote differences among various pressures ($P < 0.05$).

TVB-N, total volatile basic nitrogen; TMA, trimethylamine; TBA, thiobarbituric acid

2000). During storage, the pH value of untreated *P. crocea* muscle samples in the control group decreased from 6.72 on day 0 to 6.41 on day 45 (Table 3). Differently, the pH value of HHP-processed *P. crocea* muscle samples showed a decreasing trend within 12 d of storage, with increases to different extents observed in the late

storage period. There were significant differences in the pH value of *P. crocea* muscle samples between different HHP treatments ($P < 0.05$). The pH of cod, turkey and beef also increases on HHP treatment; this has been proposed to be linked to protein denaturation (Angsupanich & Ledward, 1998).

Table 3. Effects of high pressure treatment on pH changes of cultured large yellow croaker samples during 4°C preservation

Preservation days	High pressure				
	0.1 MPa (control)	100 MPa/10 min	300 MPa/10 min	500 MPa/10 min	500 MPa/15 min
3	6.66 ± 0.06 ^{cA}	6.65 ± 0.04 ^{bA}	6.75 ± 0.03 ^{cdB}	6.76 ± 0.03 ^{bcB}	6.78 ± 0.01 ^{bcB}
6	6.64 ± 0.02 ^{bA}	6.63 ± 0.08 ^{abB}	6.69 ± 0.01 ^{bB}	6.76 ± 0.01 ^{bcC}	6.76 ± 0.03 ^{bcC}
9	6.63 ± 0.56 ^{bA}	6.56 ± 0.06 ^{aAB}	6.60 ± 0.02 ^{aBC}	6.69 ± 0.01 ^{aBC}	6.71 ± 0.01 ^{aC}
12	6.42 ± 0.52 ^{bA}	6.66 ± 0.03 ^{bB}	6.75 ± 0.01 ^{bcC}	6.70 ± 0.01 ^{aBC}	6.70 ± 0.01 ^{aC}
15	6.50 ± 0.01 ^{bA}	6.75 ± 0.02 ^{cB}	6.74 ± 0.02 ^{cdB}	6.74 ± 0.04 ^{bB}	6.78 ± 0.01 ^{bcB}
30	6.48 ± 0.01 ^{abA}	6.76 ± 0.04 ^{cB}	6.78 ± 0.03 ^{dB}	6.79 ± 0.02 ^{cB}	6.80 ± 0.02 ^{cdB}
45	6.41 ± 0.06 ^{aA}	6.80 ± 0.02 ^{cB}	6.83 ± 0.05 ^{fB}	6.80 ± 0.03 ^{cB}	6.79 ± 0.03 ^{dB}

a, b, c, d, e, and f denote significant difference between the column samples and the control group ($P < 0.05$); A, B, and C denote significant differences between the line samples and the control group ($P < 0.05$).

In the early storage stage, lactic acid was produced through glycolysis, and acid substances such as phosphoric acid were produced from the decomposition of ATP and phosphocreatine. In the late storage stage, the volatile basic N-containing substances were produced as a result of the decomposition of N-containing substances such as amino acids, which increased the pH value of the fish muscle. The active compensation for a progressive acidosis stops when death occurs and the pH rapidly falls: therefore decreasing pH values is a clear indicator of death (Aaraas et al., 2004).

Aw of cultured *P. crocea* muscle during storage after different HHP treatments

During storage, the Aw of the *P. crocea* muscle samples first increased and then decreased in both the control and experimental groups (Table 4). In the storage period, there were significant differences in the Aw of samples processed under different HHP treatments ($P < 0.05$), with higher values found in the experimental group than in the control group. The decreased Aw may help the microorganisms resist the damaging effects of pressure, but a low Aw can also inhibit their ability

Table 4. Effect of high pressure treatment on water activity changes of cultured large yellow croaker samples during 4°C preservation

Preservation days	High pressure				
	0.1 MPa (control)	100 MPa/10 min	300 MPa/10 min	500 MPa/10 min	500 MPa/15 min
3	0.902 ± 0.001 ^{dE}	0.878 ± 0.001 ^{aD}	0.881 ± 0.003 ^{cC}	0.867 ± 0.002 ^{aB}	0.864 ± 0.001 ^{aA}
6	0.905 ± 0.001 ^{deD}	0.883 ± 0.001 ^{bD}	0.885 ± 0.001 ^{cdC}	0.871 ± 0.001 ^{aB}	0.869 ± 0.001 ^{bA}
9	0.906 ± 0.001 ^{eE}	0.891 ± 0.001 ^{cD}	0.888 ± 0.003 ^{deC}	0.875 ± 0.001 ^{aB}	0.872 ± 0.002 ^{cA}
12	0.907 ± 0.001 ^{eD}	0.901 ± 0.001 ^{gC}	0.891 ± 0.002 ^{eB}	0.879 ± 0.005 ^{aA}	0.879 ± 0.002 ^{eA}
15	0.898 ± 0.001 ^{cC}	0.898 ± 0.002 ^{dC}	0.886 ± 0.001 ^{dB}	0.876 ± 0.001 ^{aA}	0.875 ± 0.001 ^{dA}
30	0.878 ± 0.001 ^{bc}	0.889 ± 0.002 ^{cD}	0.874 ± 0.004 ^{bb}	0.871 ± 0.001 ^{aAB}	0.870 ± 0.001 ^{bcA}
45	0.873 ± 0.003 ^{aA}	0.879 ± 0.003 ^{aA}	0.868 ± 0.002 ^{aA}	0.877 ± 0.002 ^{aA}	0.865 ± 0.002 ^{aA}

a, b, c, d, e, and f denote significant differences between the column samples and the control group ($P < 0.05$); A, B, C, D, and E denote significant differences between the line samples and the control group ($P < 0.05$).

to repair semi-lethal cells. The experimental results demonstrate that the HHP treatment decreases the *P. crocea* muscle Aw within an acceptable range.

TVB-N of cultured *P. crocea* muscle during storage after different HHP treatments

Changes in total volatile basic nitrogen (TVB-N) analysis for all samples are given in mg/100 g samples (Table 5). The TVB-N content of the *P. crocea* muscle samples gradually increased with progressing deterioration, demonstrating a positive correlation with the level of deterioration. This TVB-N analysis is often used as an index in assessing the shelf-life and storage quality of

seafood products (Botta et al., 1984). Higher pressure was more favorable for suppressing deterioration than untreated sample. Nevertheless, the TVB-N values in the different seafood samples analysed, based on the results of the studies of the freshness, the suitability limits of TVB-N values were fixed range of appropriate TVB-N content as follows: TVB-N < 25 mg N/100 g, excellent; 25 mg N/100 g < TVB-N < 30 mg N/100 g, good; 30 mg N/100 g < TVB-N < 35 mg N/100 g, salable; and TVB-N > 35 mg N/100 g, deteriorated (Büyükcan, Bozoglu, & Alpas, 2009).

Table 5. Effect of no treatment versus high pressure treatment on TVB-N changes of breeding large yellow croaker samples during 4°C preservation (mg/100 g)

Preservation days	High pressure				
	0.1 MPa (control)	100 MPa/10 min	300 MPa/10 min	500 MPa/10 min	500 MPa/15 min
3	10.130 ± 0.017 ^{aC}	9.877 ± 0.011 ^{aB}	8.127 ± 0.012 ^{aA}	12.110 ± 0.104 ^{aE}	10.953 ± 0.021 ^{aD}
6	17.993 ± 0.566 ^{bCD}	18.553 ± 0.574 ^{bD}	17.677 ± 0.015 ^{bC}	15.407 ± 0.618 ^{bB}	12.797 ± 0.127 ^{bA}
9	27.163 ± 0.006 ^{cD}	20.873 ± 0.012 ^{cC}	16.233 ± 0.011 ^{cA}	20.537 ± 0.586 ^{cBC}	20.130 ± 0.641 ^{cB}
12	35.937 ± 0.006 ^{dE}	32.730 ± 0.010 ^{dD}	24.847 ± 0.012 ^{dB}	25.683 ± 0.006 ^{dC}	23.883 ± 0.011 ^{dA}
15	45.837 ± 0.508 ^{eD}	41.713 ± 1.154 ^{eC}	31.503 ± 1.155 ^{eB}	30.237 ± 0.453 ^{eB}	27.557 ± 0.384 ^{eA}
30	87.817 ± 0.110 ^{fD}	69.967 ± 0.473 ^{fC}	56.840 ± 0.035 ^{fB}	30.387 ± 0.514 ^{eA}	30.577 ± 0.185 ^{fA}
45	225.313 ± 0.046 ^{gE}	150.543 ± 0.769 ^{gD}	91.017 ± 0.263 ^{gC}	35.503 ± 1.132 ^{fB}	33.137 ± 0.962 ^{gA}

a, b, c, d, e, f, and g denote the significant difference between the column samples and the control group ($P < 0.05$); A, B, C, D, and E denote the significant difference between the line samples and the control group ($P < 0.05$). TVB-N, total volatile basic nitrogen

The TVB-N content of the *P. crocea* muscle samples in both the control and experimental groups increased with storage time, but the changes were significantly different among the pressure treatments. Compared with the control group, the experimental group of the *P. crocea* muscle samples had significantly lower TVB-N content. The greater the pressure is applied, the slower the increase is in the TVB-N content. After 9 days of storage, the TVB-N content of the *P. crocea* muscle samples in the control group exceeded the raw food standards, whereas that of the HHP-processed group remained <25 mg/100 g ± 0.00. During the storage period, significant changes in the TVB-N content of *P. crocea* muscle samples were detected among the different HHP treatments ($P < 0.05$). Based on the results obtained from this study, TVB-N value could be useful in assessing the level of *P. crocea* muscle samples deterioration more than in evaluating the changes

occurring during the first stages of storage. The formation of TVB-N significantly ($p < 0.05$) increased with the storage time with values of 225.313 mg/100 g ± 0.046 at day 45 in control samples when compared with HHP-treated samples, which attained much lower values of 150.543 mg /100 g ± 0.769, 91.017 mg /100 g ± 0.263, 35.503 mg/100 g ± 1.132 and 33.137 mg/100 g ± 0.962 for 100, 300, 500 MPa at 10 min and 500 MPa at 15 min, respectively, at day 45 (Table 5). 500 MPa HHP-treated samples were classified as salable (30 mg N/100 g < TVB-N < 35 mg N/100 g). Sikorski, Kolakowska, and Burt (1990) reported an acceptability level of TVB-N of 17 mg N/100 g in oysters. Lopez-Caballero et al. (2000) observed the HHP treatment at 400 MPa, 7 °C for 5 min increased the TVB-N values of non purified (13.3 mg N/100 g) and purified (11.2 mg N/100 g) oysters up to 25–30 mg N/100 g after 6 weeks of storage. Because the slow increase in TVB-N was reported to exist

because of general acidification of the high glycogen content that is converted to lactic acid.

TMA of cultured *P. crocea* muscle during storage after different HHP treatments

TMA content is often used as a biochemical index to assess keeping quality and shelf-life of fresh fish and seafood. Fresh fish inherently contain TMA N-oxide, the content of which changes with aquatic product type, environment, season, size, and age (Hunkar & Esra, 2009). The quantity of TMA found in fish is used as an index of spoilage.

In the storage period, the TMA content of the *P. crocea* muscle samples after different HHP treatments varied widely ($P < 0.05$, Table 6). With prolonged storage period (number of days), the TMA contents of *P. crocea* muscle samples in both the control and experimental groups increased

gradually, suggesting that the freshness of the fish muscle had decreased. The TMA content of the samples in the control group was 1.75 mg/100 g on day 0 and increased to 33.88 mg/100 g on day 45. After the treatment of 500 MPa for 10 and 15 min, the TMA contents of samples were 6.222 and 4.957 mg/100 g, respectively, only on day 45 of storage. About the untreated samples stored at 5°C, the trimethyl content reached the upper limit of 10 mg/100 g on day 21. According to the experimental data (Table 6), the TMA content of the *P. crocea* muscle samples prepared at 500 MPa did not exceed the abovementioned upper limit even after 45 d of storage. Together these results indicate that the HHP treatment helps suppress the reduction of TMA N-oxide levels and, thus, reduces TMA production.

Table 6. Effect of no treatment versus high pressure treatment on the TMA changes of breeding large yellow croaker samples during 4°C preservation (mg/100 g)

Preservation days	High pressure				
	0.1 MPa (control)	100 MPa/10 min	300 MPa/10 min	500 MPa/10 min	500 MPa/15 min
3	1.723 ± 0.067 ^{aA}	1.906 ± 0.121 ^{aB}	2.560 ± 0.017 ^{aC}	1.850 ± 0.069 ^{aAB}	2.873 ± 0.065 ^{abD}
6	1.467 ± 0.011 ^{aA}	2.080 ± 0.121 ^{aB}	2.009 ± 0.137 ^{aC}	2.167 ± 0.063 ^{abB}	3.011 ± 0.010 ^{abcD}
9	1.617 ± 0.012 ^{aA}	2.313 ± 0.144 ^{abB}	2.730 ± 0.156 ^{aC}	2.333 ± 0.006 ^{abB}	2.833 ± 0.127 ^{aC}
12	3.740 ± 0.451 ^{bc}	3.057 ± 0.029 ^{bcB}	3.113 ± 0.040 ^{abB}	2.427 ± 0.092 ^{ba}	3.102 ± 0.199 ^{cdB}
15	12.880 ± 0.156 ^{cd}	11.977 ± 0.820 ^{cc}	6.160 ± 0.061 ^{bb}	2.923 ± 0.051 ^{ca}	3.063 ± 0.064 ^{bcA}
30	20.190 ± 0.086 ^{dd}	19.163 ± 0.681 ^{dc}	16.067 ± 0.150 ^{cb}	3.407 ± 0.275 ^{da}	3.283 ± 0.109 ^{da}
45	33.857 ± 0.150 ^{ee}	27.923 ± 0.629 ^{ed}	20.893 ± 0.755 ^{dc}	6.222 ± 0.468 ^{eb}	4.957 ± 0.064 ^{ea}

a, b, c, d, e, and f denote significant differences between the column samples and the control group ($P < 0.05$); A, B, C, D, and E denote significant differences between the line samples and the control group ($P < 0.05$); TMA, trimethylamine.

TBA of cultured *P. crocea* muscle during storage after different HHP treatments

Table 7 gives the TBA content of untreated and treated *P. crocea* muscle samples. During storage, the TBA values of *P. crocea* muscle samples prepared under different HHP treatments showed significant variations ($P < 0.05$). The experimental data showed that the control and 100 MPa-processed *P. crocea* groups had increasing TBA values, while the HHP-processed *P. crocea* muscle samples had decreasing TBA values with storage duration (Table 7). The higher the pressure was applied, the greater the decrease was in the TBA values of the HHP-processed samples except those of the control and 100 MPa-processed

groups. The formation of TBA increased significantly ($p < 0.05$) with storage time from 1.434mg/100 g±0.023 at day 0 to 33.765mg/100 g±0.225 at the end of day 45 in the fresh *P. crocea* sample (control), when compared with those treated samples, which attained much lower values of 27.811±0.002, 16.586±0.011, 6.413±0.021 and 4.330±0.061mg/100 g at day 45, respectively (Table 7). Wada(1992) stated that lipid oxidation decomposition after HHP treatment involves the release of metal ions under the synergistic effect of denaturalized proteins in muscle, which causes the changes in muscle products in terms of color, smell, tissue structure, and even nutritional compositions.

Table 7. Effect of no treatment versus high pressure treatment on TBA changes of breeding large yellow croaker samples during 4°C preservation (mg/100 g)

Preservation days	High pressure				
	0.1 MPa (control)	100 MPa/10 min	300 MPa/10 min	500 MPa/10 min	500 MPa/15 min
3	1.434 ± 0.023 ^{abA}	1.993 ± 0.007 ^{eb}	2.004 ± 0.012 ^{gC}	2.968 ± 0.061 ^{gD}	3.077 ± 0.187 ^{gE}
6	1.249 ± 0.082 ^{aA}	3.874 ± 0.003 ^{dB}	6.541 ± 0.094 ^{fc}	4.915 ± 0.087 ^{fd}	5.811 ± 0.266 ^{fE}
9	1.303 ± 0.012 ^{aA}	3.497 ± 0.021 ^{cb}	5.942 ± 0.854 ^{cC}	3.301 ± 0.009 ^{cd}	4.594 ± 0.174 ^{cd}
12	9.707 ± 0.008 ^{baA}	6.198 ± 0.006 ^{bb}	5.845 ± 0.005 ^{dc}	5.404 ± 0.271 ^{dd}	4.411 ± 0.051 ^{dd}
15	13.299 ± 0.430 ^{caA}	9.008 ± 0.007 ^{aA}	4.984 ± 0.079 ^{eb}	5.067 ± 0.067 ^{eb}	4.105 ± 0.108 ^{eb}
30	21.427 ± 0.075 ^{de}	19.511 ± 0.003 ^{bd}	10.137 ± 0.035 ^{baA}	6.475 ± 0.032 ^{bc}	3.395 ± 0.033 ^{bb}
45	33.756 ± 0.225 ^{ed}	27.811 ± 0.002 ^{gC}	16.586 ± 0.011 ^{aB}	6.413 ± 0.021 ^{aAB}	4.330 ± 0.061 ^{aA}

a,b,c,d,e,f & g denote significant differences between the column samples and the control group ($P < 0.05$); A,B,C,D & E denote significant differences between the line samples and the control group ($P < 0.05$). TBA, thiobarbituric acid.

Total bacterial count of cultured *P. crocea* muscle during storage after different HHP treatments

The changes in total bacterial count in *P. crocea* muscle samples prepared under different pressure conditions were examined after 3, 6, 9, 12, 15, 30, and 45 days of storage (Table 8). The results showed that the total bacterial counts *P. crocea* muscle samples of the control group were uncountable from day 9, while those of the samples

processed at 500 MPa had $<10^6$ CFU/mL on day 45 of storage. In the storage period, there were significant variations in the total bacterial counts *P. crocea* muscle samples prepared under different HHP conditions ($P < 0.05$). Together these experimental results demonstrate that HHP treatment both has a strong instant sterilizing effect and effectively extends the shelf life of *P. crocea*.

Table 8. Effect of no treatment versus high pressure treatment on TVC changes of breeding large yellow croaker samples during 4°C preservation (CFU/mL)

Preservation days	High pressure				
	0.1 MPa (control)	100 MPa/10 min	300 MPa/10 min	500 MPa/10 min	500 MPa/15 min
3	$1.7 \times 10^6 \pm 1.4 \times 10^{5aC}$	$1.2 \times 10^6 \pm 2.8 \times 10^{5aB}$	$1.6 \times 10^4 \pm 5.6 \times 10^{3aA}$	$10^3 \pm 2.8 \times 10^{2aA}$	$7.3 \times 10^2 \pm 98^{aA}$
6	$8.6 \times 10^6 \pm 4.2 \times 10^{5B}$	$5.7 \times 10^6 \pm 4.2 \times 10^{5B}$	$3.8 \times 10^5 \pm 2.8 \times 10^{4cA}$	$5.3 \times 10^3 \pm 9.9 \times 10^{3aA}$	$3.1 \times 10^3 \pm 1.4 \times 10^{2abA}$
9	"	"	$5.1 \times 10^6 \pm 4.2 \times 10^{5b}$	$10^4 \pm 2.8 \times 10^{2ab}$	$6.8 \times 10^3 \pm 2.8 \times 10^{2ab}$
12	"	"	"	$2 \times 10^4 \pm 2.8 \times 10^{3bc}$	$10^4 \pm 2.8 \times 10^{2bc}$
15	"	"	"	$2.8 \times 10^4 \pm 2.8 \times 10^{3c}$	$1.7 \times 10^4 \pm 1.4 \times 10^{3c}$
30	"	"	"	$8 \times 10^4 \pm 2.8 \times 10^{3d}$	$3.8 \times 10^4 \pm 5.6 \times 10^{3d}$
45	"	"	"	$1.1 \times 10^5 \pm 1.4 \times 10^{4e}$	$5.7 \times 10^4 \pm 7 \times 10^{3e}$

a, b, c, d, and e denote the significant differences between the column samples and the control group ($P < 0.05$); A, B, and C denote the significant difference between the line samples and the control group ($P < 0.05$); TVC, total viable counts.

CONCLUSIONS

The HHP technique can effectively kill the majority of harmful microorganisms in aquatic products for consumer safety and inhibit the activities of a number of endogenous enzymes. It

can completely preserve the color, flavor, taste, and nutrients of aquatic products and effectively extend their storage life and qualities. HHP treatment significantly changed pH, Aw, TVB-N, TMA, TBA and total bacterial count of *P. crocea* muscle compared with untreated *P. crocea* muscle.

This research provides an initial specific characterization of the impact of HHP treatment on specific chemical and physical components that may influence *P. crocea* qualities.

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