Effects of high pressure treatment on physicochemical quality of pre- and post-rigor palm ruff (*Seriolella violacea*) fillets

Running title: HHP effect on *Seriolella violacea* quality

Abstract

Fish and fish products are characterized for having a short shelf-life. Non-thermal processing techniques such as High Hydrostatic Pressure (HHP) have increasingly been employed to extend shelf-life of food products. The aim of this study was to evaluate changes on flesh physicochemical spoilage parameters (pH, Total Volatile Bases (TVB-N), Trimethylamine (TMA), Thiobarbituric Acid (TBA) and color) of palm ruff (*Seriolella violacea*) fillets in pre- and post-rigor conditions, subjected to two different HHP conditions: 450 MPa and 550 MPa, for 3 and 4 min each. Unpressurized and pressurized fillets were kept in chilled storage (4±1 °C) for 26 days to assess the effect of HHP on shelf-life. pH and TBA values increased after HHP treatment and with storage time for both unpressurized and pressurized samples. This is attributable to pressure induced lipid oxidation. Lightness (L*) values increased with pressure, where fish fillets became a cooked appearance. TMA and TVB-N values decreased after HHP treatment compared to the unpressurized samples, showing that HHP treatment is an efficient method to maintain the quality of palm ruff fillets. There was no clear difference between pre- and post-rigor in the parameters evaluated.

Keywords: Palm ruff, high pressure, rigor stage, trimethylamine, color.

Introduction

Quality and safety of food products are among the most important factors that influence consumer decisions (Considine et al., 2008). Currently, consumers demand for high quality foods and food products that are additive free, with long shelf-life and similar

characteristics to fresh products. With this aim, the food industry has investigated alternative food technology methods; some of these include pulsed electric fields, high hydrostatic pressure (HHP), ultrasound, microfiltration, intense pulses of light and irradiation (Tiwari et al., 2009). Among these methods, HHP has been a feasible alternative (economically and technologically) to the commonly used thermal processes (Patterson, 2005; Considine et al., 2008).

HHP, a non-thermal processing technique, is an effective method to increase food safety without causing major physicochemical changes in food (Mathias et al., 2010). This technique is recognized for increasing the shelf-life of food and food products (San Martín et al., 2002; Chouhan et al., 2015; Christensen et al., 2017; Kaur et al., 2016; Reyes et al., 2015) and for preserving their organoleptic properties (de Heiji et al., 2003; Nolwennig et al., 2010). The extent to which HHP improves food shelf-life and quality depends on processing variables, such as pressure and exposure time, in addition to the food composition and type of microorganisms involved. These effects are uniform and almost instantaneous and act independently of product geometry and equipment size (Torres-Arreola et al., 2007).

Palm ruff (*Seriolella violacea*) is a pelagic and native fish from Chile found along the Chilean coast. This fish presents significant comparative advantages over other native fish, which is evidenced by a fast growing and a high fertility rate (Oliva et al., 1996). Therefore, Palm ruff rises as an alternative to the national fishing industry, currently focused on salmon and mackerel farming, which in the last 10 years has suffered from huge economical losses due to fish diseases (SERNAPESCA, 2008) and scarcity.

Fresh fish have a short shelf-life due to the rapid post-mortem flesh deterioration caused by bacterial growth, autolytic enzymes activity (Dalgaard, 2000) protein degradation, lipid oxidation and ATP decomposition, all of which accelerate the loss of freshness and quality of fish (Ayala et al., 2010). These processes are delayed during chilled storage, however, significant losses of sensory and nutritional values still occur (Reyes et al., 2015). All muscle degradation processes occurring post-mortem start slowly during pre-rigor mortis and spike after post-rigor has resolved (Cherét et al., 2005). Thus, prolonging the pre-rigor state would retard flesh quality losses and increase shelf-life (Kristoffersen et al., 2007; Skjervold et al., 2001a; Tobiassen et al., 2006). Studies also indicate that extending the pre-

rigor stage improves the color and texture of fish (Skjervold et al., 2001b; Einen et al., 2002). The effect of HHP on pre- and post-rigor meat is not well studied and there is no information available on *S. violacea*. The aim of this study was to investigate the effect of HPP on physicochemical properties as a measure of quality on palm ruff muscle in pre- and post-rigor. For this purpose, samples were pressurized at 450 MPa or 550 MPa for 3 and 4 min each, and stored 26 days at 4±1 °C. Shelf life parameters such as pH, TVB-N, TMA, TBA and color were analyzed.

Materials and methods

Raw material and storage conditions

Palm ruff fish with an average weight of 700 g (70 fish samples) were obtained from a fish farm located at the Universidad Católica del Norte (Coquimbo, Chile). Live fish were transported to the laboratory on iced water. The fish were immediately filleted to 100 - 140 g filets. Fillets were individually placed in polyethylene bags, vacuum packed and separated in two batches, as pre- and post-rigor mortis samples. Pre-rigor samples were processed by HHP within 4 hours after being packaged. The post-rigor condition was achieved by cooling the packaged samples for 24 hours at 4±1 °C, then pressurized. Triplicate samples were kept in chilled storage (4±1 °C) for 26 days until further analysis. Samples were taken at 0, 4, 8, 14 and 26 days.

HHP treatments

Vacuum sealed fish fillets were placed in a cylindrical loading container at room temperature and pressurized at 450 and 550 MPa for 3 and 4 min (hereafter 450/3, 450/4, 550/3, 550/4) at a rate of 17 MPa/s and room temperature (20.0±2.0 °C) in a high-pressure unit (Avure Technol. Inc., Kent, WA, USA) using water as the pressure-transmitting medium. After pressurization, samples were kept under chilled storage at 4±1 °C. The experimental conditions were determined by a previous study to optimize the conditions of HHP that were used in another experiment with fish kept in chilled storage (Briones-Labarca et al., 2012).

Physicochemical analysis

pH measurements were made directly to the muscle using a pH meter (HANNA model Hl 99163, RI, USA). Color was determined from the fish fillets at a surface area were color appeared homogenous, with a colorimeter (HunterLab, model MiniScan XE Plus, Reston, VA, USA). *L* * (Lightness), *a** (+*a**: red, -*a**: green) and *b** (+*b*: yellow, -*b*: blue) values were recorded. TMA and TBA were measured from homogenized fillets with a dispersing instrument (ULTRA-TURRAX IKA T18 basic, Germany). TMA was measured according to the AOAC method N° 971.14 (AOAC, 1990) and expressed as mg TMA/100 g sample. TBA was determined by the method of Vyncke (1970) and results expressed in mg malonaldehyde/kg sample. TVB-N was measured from minced fillets according to the method of Botta et al. (1984). All measurements were performed in triplicate.

Statistical analysis

Statistical analyses were performed with the Statgraphics Plus 5 (Statistical Graphics Corp., Herndon, USA) applying a three-way analysis of variance (ANOVA) and Tukey multiple range test where significance was accepted at $P < 0.05$. Exponential evolution of freshness parameters with storage time was evaluated by exponential regression of the curves in Microsoft excel 2016.

Results and Discussion

Determination of pH

One of the first signs of fish decomposition is pH increase, presumably due to the production of basic nitrogen (Briones-Labarca et al., 2012). pH is also affected by changes in the concentration of hydrogen ions and free hydroxyls, resulting from variation in the redox balance in foods, microbial or enzymatic activity (Varlik et al., 2000). Thus, monitoring pH is one of the most used quality control methods in seafood (Varlik et al., 2000). The pH of pre- and post-rigor palm ruff was not significantly different ($P < 0.05$) (Table 4). At day 0, control samples were similar ($P > 0.05$) and averaged 6.12±0.05 (Table 1). HHP treatment increased the pH of the fish fillets independent of pressure intensity, holding time and pre- or post-rigor stage (average of all treatments at day 0, 6.45±0.10). Similar results were obtained in prawns (Bindu et al., 2012), salmon, cod and mackerel (Rode and Hovda, 2016).

HHP treatment induces protein unfolding and subsequent ionization of denatured proteins (Rode and Hovda, 2016). These changes in the tertiary and quaternary structures possibly expose alkaline amino acid radicals, such as imidazole of histidine, ionizing and alkalizing the medium (Ramírez-Suárez and Morrissey, 2006). During chilled storage, pH increased in control samples (untreated) from 6.15±0.04 at day 0 to 6.98±0.04 at day 26 (average pre- and post-rigor). All HHP treated samples showed lower pH values than the control, from 6.45±0.10 at day 0 to 6.73±0.05 at day 26 (average pre- and post-rigor for all treatments). These values did not exceed the acceptable pH limit (pH 6.8) set by Ludorff and Meyer (1973) whereas control samples surpassed it at day 26.

The increase in pH on fish muscle is likely due to accumulation of alkaline compounds such as ammonia, volatile bases and TMA, which are primarily derived from microbial action (Ludorff and Meyer, 1973). Although pH is a simple and fast monitoring system, our results suggest that it may not be such a reliable indicator of fish quality since the accepted limit was only reached after 26 days of chilled storage. At this time, the fillets were clearly in decomposition as measured by the other indicators (see below). Low pH increase was also reported for albacore tuna stored for 17 days (Ramirez-Suárez and Morrissey, 2006) whereas the pH in yellow croaker was found to decrease after 45 days of storage (Yang et al., 2015) and even fluctuate within 17 days' storage of red mullet fillets (Erkan et al. 2010a). Throughout the experiments, pre- and post-rigor samples showed no significant differences ($P > 0.05$). No significant difference ($P > 0.05$) was found in the pH values after applying 450/3 and 450/4. These pressures however, showed the lowest pH values among all tested pressures. Therefore, considering the shorter exposure time, an optimum condition of pH lower than the accepted limit would be 450/3.

Determination of TVB-N

Total volatile basic nitrogen (TVB-N) content is the most useful parameter in assessing the degree of fish deterioration (Marrackchi et al., 1990). TVB-N is produced post mortem during the breakdown of proteins induced by bacterial and enzymatic reactions (Liu and Wu, 2008; Botta et al., 1984). The TVB-N content from control and HHP-treated palm ruff fillets is shown in Table 2. The TVB-N content of pre- and post-rigor did not significantly differ ($P > 0.05$) after all HHP treatments and storage times (see Table 4). TVB-N values of

154 control samples at day 0 were lower in post-rigor (11.56 ± 0.28) than in pre-rigor
155 conditions (12.60 ± 1.28). In contrast, at day 0, TVB-N values from samples treated with
156 HHP were higher in post- than in pre-rigor.
157 Production of TVB-N decreased after HHP treatment (Table 2). TVB-N values of samples
158 treated at 450/3 and 450/4 did not significantly differ ($P > 0.05$), the same result was
159 obtained between 550/3 and 550/4. At day 0 the TVB-N content (average of pre- and post-
160 rigor) was 12.40±0.02 and 11.15±0.62 mg TVB-N/100 g for 450 MPa (3 and 4 min
161 respectively) and 11.11±0.70 and 10.53±0.07 mg TVB-N/100 g for 550 MPa (3 and 4 min
162 respectively). Similar pressure effects on TVB-N were reported in sardines (Gökodlu et al.,
163 1998), indian white prawn (Bindu et al., 2012), yellow croaker (Yang et al., 2015) and red
164 mullet (Erkan et al., 2010b).
165 TVB-N values increased during chilled storage, where control samples were in average *ca.*
166 3-fold higher at day 26 than at day 0, while HHP treated samples only increased in average
167 *ca.* 2-fold. During storage, TVB-N values from samples treated at 550 MPa remained lower
168 than of those treated at 450 MPa and control samples. However, at the end of the storage
169 time all pressurized samples reached a similar TVB-N value (24.49 ±0.91 and 23.37±1.15
170 mg TVB-N/100 g for 450 MPa 3/4 and 550MPa 3/4, respectively). As with pH, control
171 samples surpassed the rejection limit (30 mg TVB-N/100 g of sample, Büyükcan et al.,
172 2009) at 26 days of storage, whereas pressurized samples stayed within this limit
173 corroborating that the use of high pressures is an effective system to increase fish shelf-life.
174 The values of this parameter depend on the pressure at which the samples are subjected and
175 not on the exposure time. The treatment that obtained the lowest TVB-N value was 550/4.
176
177 **Evaluation of TMA**
178 Determination of trimethylamine (TMA) is a good complementary indicator (albeit not a
179 fast method) of fish deterioration and to assess quality and shelf-life of fish products
180 (Briones-Labarca et al., 2012). Trimethylamine oxide (TMAO) contained in fresh fish is
181 reduced during spoilage, releasing volatile base compounds. Two main possible pathways
182 for degradation of TMAO have been proposed, the first is an enzymatic reaction that
183 produces dimethylamine (DMA) and formaldehyde (FA) and is catalyzed by TMAOase
184 (Gigoakisa et al., 2003). The second pathway is a microbial decomposition reaction,

resulting in decreased redox potential, increased pH and electrical conductance, in which TMAO is degraded to TMA (Fu et al., 2008), which is responsible for the characteristic "fishy" odor during storage (Benjakul et al., 2004; Noseda et al. 2014).

Changes in TMA content of pre- and post-rigor palm ruff muscle are shown in Figure 1.

The effect of rigor stage (pre- and post-rigor) was significant ($P < 0.05$), the TMA values in pre- and post-rigor control samples were 0.154±0.019 and 0.146±0.003 mg TMA/100 g, respectively. In previous reports of fresh fish, the reported amount of TMA is very close to zero in albacore (Perez-Villareal and Pozo, 1990) and approximately 2 mg/100 g in cod (Dyer et al., 1945).

Application of high pressures affected TMA formation in pre- and post-rigor samples differently ($P <0.05$). Pre-rigor samples decreased TMA values after 450 and 550 MPa (3 and 4 min) reaching a similar value (average 0.135±0.003 mg TMA/100 g), while post-rigor fillets were not affected by HHP treatment ($P > 0.05$). After 26 days of chilled storage, TMA values from control samples increased 27.3-fold and 29.6-fold respect to day 0; this increase was steady until day 14, were it spiked 3-fold at day 26. Application of HHP delayed fish deterioration in all treatments, where TMA values increased from 5.9 to 8.8-fold at day 26 compared to day 0. Noteworthy, TMA values changed significantly ($P < 0.05$) with holding time and not with pressure intensity (Table 4).

Among all treatments, samples pressurized at 550 MPa (3 and 4 min) in post-rigor contained the lower TMA values after 26 days' storage, although in general post-rigor fillets showed lower TMA values than pre-rigor fillets. The delay in fish deterioration by high pressure treatment as measured by TMA values has been reported in squid (Jingyu et al., 2009), jack mackerel (Reyes et al., 2015), horse mackerel (Erkan et al., 2011), white prawn (Bindu et al., 2013), sea bream (Erkan et al., 2010b) and black tiger shrimp (Kaur et al., 2016). The reduction of TMA values in pressurized samples is attributed to inhibition of proteolytic activity by high pressures (Hernández-Andrés et al., 2005). Based on our results, the best conditions to reduce TMA formation in palm ruff is to treat the fillets in post-rigor with 550 MPa for 4 min.

Determination of TBA

The thiobarbituric acid (TBA) test evaluates rancidity in fish and is commonly used as an indicator of fish quality. Lipid oxidation was measured using the TBA test, which monitors levels of secondary oxidation products (Erkan et al., 2010a), specifically of malonaldehyde (MDA) content, one of the degradation products of lipid hydroperoxides formed during the oxidation process of polyunsaturated fatty acids by reaction with malonaldehyde TBA. TBA values are shown in Figure 2. No significant differences ($P > 0.05$) in TBA were found between pre- and post-rigor samples (see Table 4). Control pre- and post-rigor samples presented similar values and averaged 0.065±0.005 mg MDA/Kg at day 0. Increasing pressures produced a concomitant increment in TBA values, which averaged 0.112±0.015 and 0.227±0.032 mg MDA/Kg at 450 and 550 MPa, respectively. The maximum TBA observed was in (pre- and post-rigor) pressurized samples at 550 MPa, 3.5-fold higher than the content of control samples. This effect of high pressure on TBA may be related to pressure-induced denaturation of heme-proteins, which release free iron (Fe^{2+}) from heme groups, causing the oxidation of unsaturated fatty acids (Ohshima et al., 1993). The pressure dependent behavior of TBA was also reported in carp (Sequeira-Munoz et al., 2006), yellow croaker (Yang et al., 2015) and trout (Yağız et al., 2007). TBA of pre- and post-rigor control fillets increased exponentially during storage until day 14 ($R^2 = 0.9939$); this tendency was lost after 26 days' where TBA content reached an average of 0.822±0.009 mg MDA/Kg (approx. 13-fold higher than the initial values). During storage, TBA values remained higher in the pressurized samples compared to the controls, although these increased slowly until day 8, after which they spiked to reach at day 26, a 6-7-fold higher content than at day 0. Among treatment samples, pre- and post-rigor fillets pressurized at 550 MPa (3 and 4 min) reached the maximum TBA content after 26 days' storage (1.760±0.040 mg MDA/Kg average). In previous studies, the critical limit of mg MDA/kg was defined as 2.0 (Kaur et al., 2016) and 1.9 mg MDA/kg (Amanatidou et al., 2000). According to guidelines for MDA concentration in seafood, fish muscle with values above 0.72 mg MDA/kg will probably develop rancid flavors (Ke et al. 1976). TBA values exceeded this limit after 14 days' storage in all pressure treatments and control samples. However, there are several limits on TBA values, and we cannot conclud that palm fish reached the limit of oxidative rancidity.

TBA content has been shown to be dependent on fish species and to fluctuate in time, reaching a peak and decreasing after (Erkan et al., 2010a; Erkan et al., 2011). This behavior was not observed in palm ruff fillets but again, this could be attributed to the low initial TBA content. Considering the effects of pressure on lipid oxidation, HHP processing of fatty fish must be applied cautiously, which is why the use of 450 MPa is recommended.

Assessment of color

Color plays an important role in the acceptability of fish products. Changes in color are associated with degradation of blood pigments during spoilage (Pearson and Dutson, 2013). Thus, measuring color is considered as a routine procedure for the indirect measure of fish and meat freshness. Since palm ruff fillets pressurized at 550 MPa contained TBA values indicative of a high oxidative rancidity, color was measured only from samples pressurized at 450 MPa. Color values and statistical analysis of palm ruff muscle are shown in Table 3, and Table 4 respectively. At day 0, L^* control values were 44.76±1.03 in pre-rigor and 46.78±0.28 post-rigor samples. High pressure induced a lightening of the fish fillets, with average L^* values of 63.16±0.21 and 63.97±4.74 at 450 MPa 3 min and 4 min (pre- and post-rigor) respectively, similar results were obtained in turbot (Chevalier et al., 2001), red mullet (Erkan et al., 2010a) and sea bass (Teixeira et al., 2014). Lightness was higher in pre-rigor samples pressurized at 450 MPa 4 min (67.32±0.94) than in post rigor (60.61±1.21), although this difference was not maintained during storage time, where pre- and post-rigor sample values became comparable from day 8. After 26 days' lightness values did not differ from day 0. The increase in lightness could be seen with the naked eye, since the fillets lost "transparency" and presented a "cooked" appearance. Increased L^* values after HHP treatment have been reported for different seafood species such as salmon (Yagiz et al., 2009), turbot (Chevalier et al., 2001) and abalone (Briones-Labarca, 2012). The whitening effect is likely due to denaturation of myofibrillar and sarcoplasmic proteins (Ledward, 1998), specifically of globin. This occurs by the release or displacement of globin's heme group, or by oxidation of myoglobin to metamyoglobin (Carlez et al., 1995). The a^* parameter indicates color position between red/magenta (positive values) and green (negative values). Pre- and post-rigor control fillets showed positive values indicative of a red tint at day 0, which were reduced to negative values after pressure application in pre-

and post-rigor samples. After 26 days of storage, *a** values increased in all control and treated samples, although remained higher in unpressurized samples. As with lightness, a decrease in *a** values after HHP treatment may be due to globin denaturation (Bindu et al., 2013). The *b** parameter indicates color position between yellow (positive values) and blue (negative values). At day 0, *b** values from control samples were 10.10±0.06 and 11.74±0.12 in pre- and post-rigor respectively. During storage, *b** values fluctuated in all control and pressurized samples although at day 26, values were higher than at day 0. Unlike *L**, these differences in *a** and *b** parameters were not detectable with the naked eye. Negative *a** values after HHP treatment were also reported in turbot, although in this fish, *b** values were shown to increase with pressure intensity and holding times of 15 and 30 min (Chevalier et al., 2001). The results obtained indicate a slight decrease of *b** values, although we used shorter holding times (3 and 4 min). Nevertheless, *a** and *b** values have been shown to fluctuate with pressure intensity, holding times, temperature and fish species (Truong et al., 2015).

The increase in lightness in HHP treated fillets may be attributed to the degradation of pigments and/or protein coagulation. Protein coagulation would change sample surface properties, increasing light reflection and creating a cooked appearance. Given the importance that consumers give to meat color, optimization of HHP conditions must consider this parameter in order to produce fish fillets with a color accepted for consumers.

Conclusions

This research provided the impact of HHP treatment on pre-and post-rigor palm ruff (*S. violacea*) fillets, as a measure of fish quality. The biochemical indexes pH, TVB-N, TMA, TBA and the physical parameter color, showed significant differences after HHP treatment. However, there were no differences between pre- and post-rigor samples in any of the studied parameters. Based on these results and the difficulties associated to the processing of fish in pre-rigor, we suggest post-rigor processing as a viable alternative to maintain palm ruff quality after HHP processing. The choice of HHP conditions was based mainly on maintaining the quality of palm ruff fillets. As such, adverse effects such as changes in color and lipid oxidation should be considered before HHP processing. Based on this, we recommend a pressure of 450 MPa and a short holding time (3 min). In order to define the

best HHP conditions to be applied, complementary analyses of microbiological development ought to be carried out. We can conclude that HHP is able to maintain the quality of palm ruff (*S. violacea*). This research provides an initial characterization of the impact of HHP treatment on specific chemical and physical components that may influence palm ruff qualities for its future commercialization.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the FONDECYT N°1110782 project.

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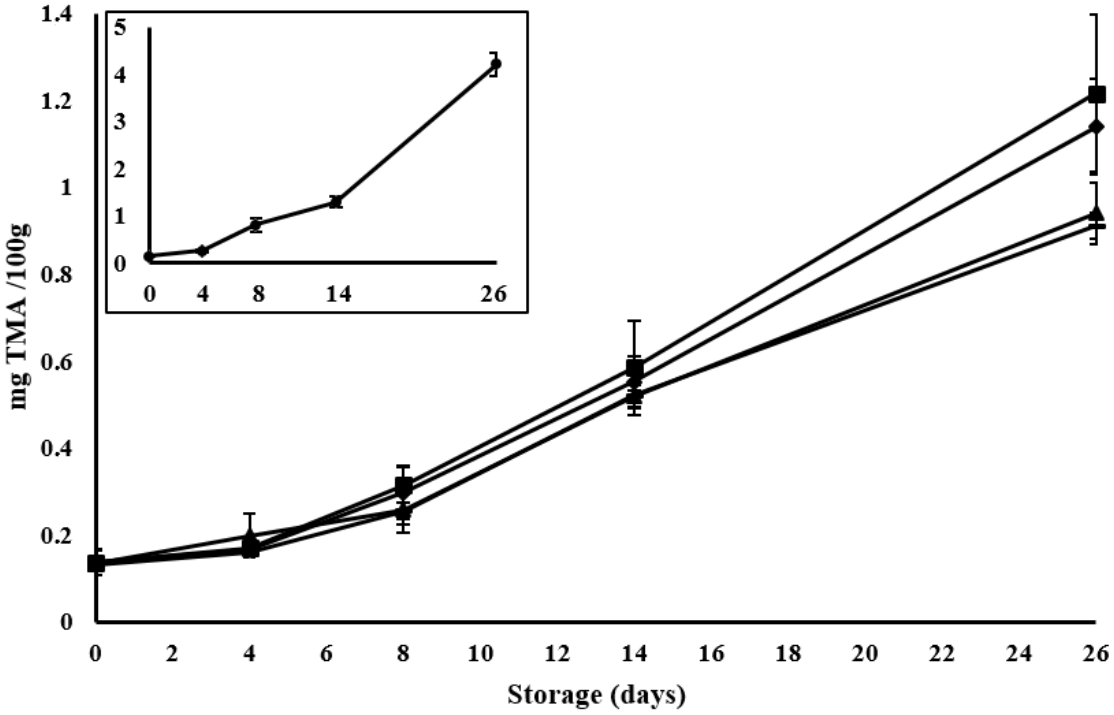
Figure 1. Changes in TMA-N in pre- (A) and post-rigor (B) control (inlet) and pressurized palm ruff samples during 26 days of storage. Symbols are means of three measurements ± SD. Control (●); 450 MPa/3 min (■); 450 MPa/4 min (♦); 550 MPa/3 min (▲); 550 MPa/4 min (▬).

A



B

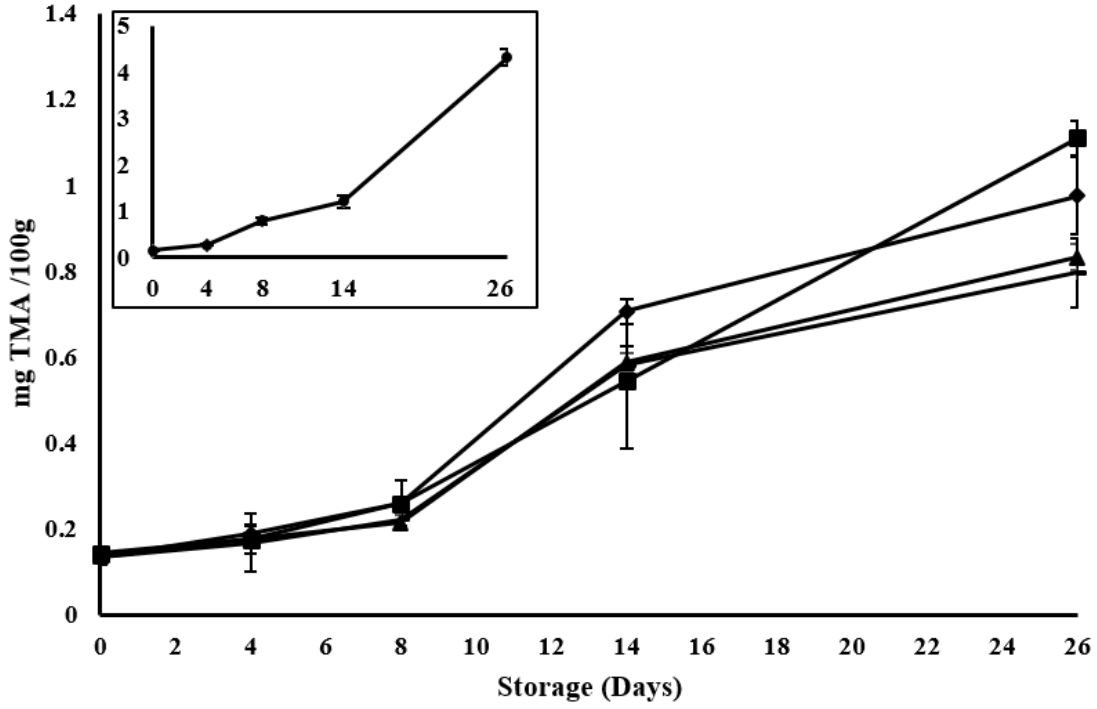
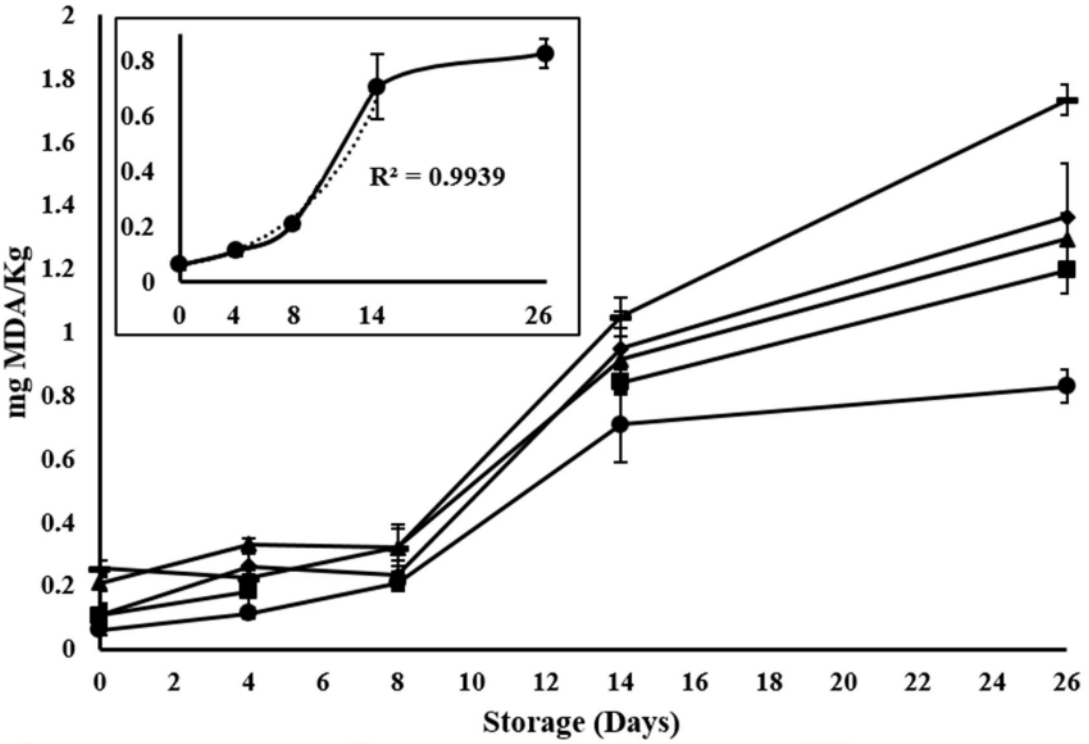


Figure 2. Changes in TBA in pre- (A) and post-rigor (B) control and pressurized palm ruff samples during 26 days of storage. Symbols are means of three measurements ± SD. Control (●); 450 MPa/3 min (■); 450 MPa/4 min (♦); 550 MPa/3 min (▲); 550 MPa/4 min (—). Inlet: Exponential fitting of control samples during 14 days of storage.

A



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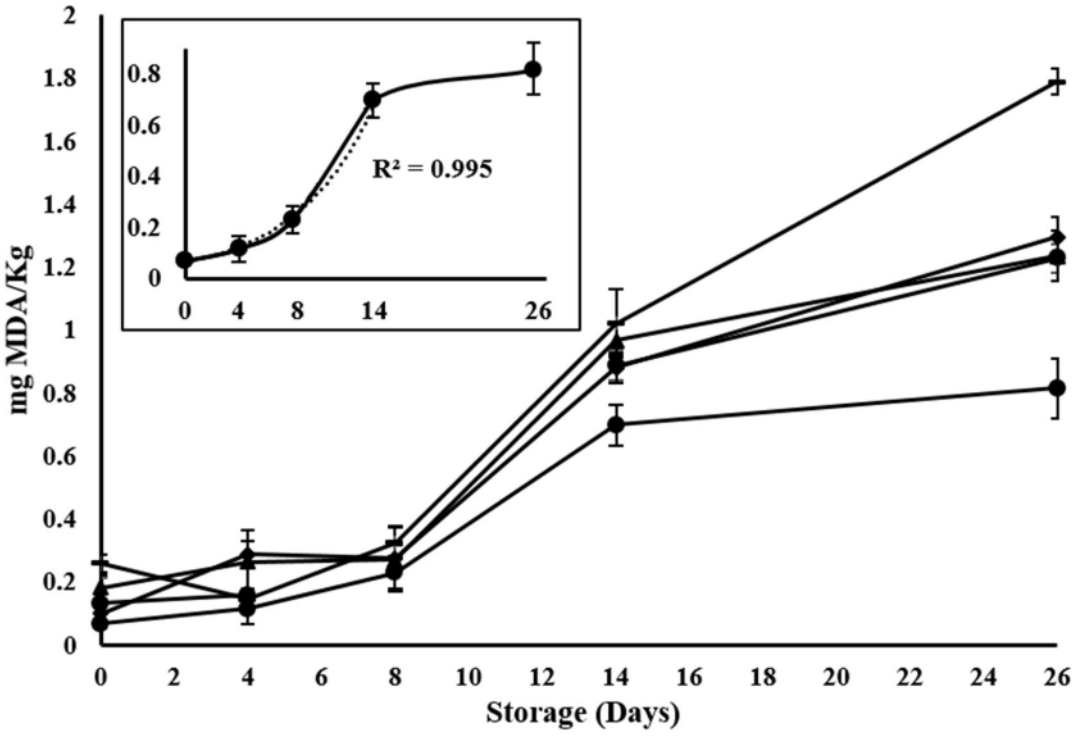

Table 1. pH values of control and HHP treated palm ruff fillets in pre- and post-rigor mortis.

Storage days	Control (untreated) (a)		High Pressure (MPa) / Time (minutes)							
			450/3 (b)		450/4 (c)		550/3 (d)		550/4 (e)	
	Pre rigor (a)	Post rigor (a)	Pre rigor (a)	Post rigor (a)	Pre rigor (a)	Post rigor (a)	Pre rigor (a)	Post rigor (a)	Pre rigor (a)	Post rigor (a)
0 (a)	6.12±0.05	6.17±0.10	6.48±0.05	6.29±0.10	6.45±0.02	6.30±0.01	6.50±0.01	6.49±0.01	6.52±0.02	6.54±0.01
4 (b)	6.36±0.02	6.30±0.04	6.48±0.08	6.26±0.08	6.32±0.02	6.37±0.01	6.54±0.02	6.54±0.03	6.54±0.02	6.56±0.02
8 (c)	6.38±0.01	6.35±0.05	6.51±0.04	6.53±0.03	6.55±0.01	6.40±0.04	6.58±0.01	6.58±0.01	6.60±0.01	6.60±0.01
14 (d)	6.72±0.02	6.69±0.02	6.73±0.03	6.71±0.01	6.51±0.04	6.76±0.01	6.75±0.01	6.76±0.01	6.75±0.02	6.78±0.01
26 (e)	6.95±0.02	7.01±0.02	6.72±0.02	6.74±0.02	6.68±0.01	6.65±0.01	6.75±0.01	6.77±0.01	6.76±0.01	6.79±0.03

All samples were done in triplicate. Different lowercase letters (a, b, c, d and e) denote statistically significant difference between groups ($P < 0.05$), corrected by Tukey test.

Table 2. Total volatile basic nitrogen (TVB-N) values expressed as mg TVB-N/100g of control and HHP treated palm ruff fillets in pre- and post-rigor mortis.

Storage days	Control (untreated) (a)		High Pressure (MPa) / Time (minutes)							
			450/3 (b)		450/4 (c)		550/3 (d)		550/4 (e)	
	Pre rigor (a)	Post rigor (a)	Pre rigor (a)	Post rigor (a)	Pre rigor (a)	Post rigor (a)	Pre rigor (a)	Post rigor (a)	Pre rigor (a)	Post rigor (a)
0 (a)	12.60±1.28	11.56±0.28	12.41±1.07	12.38±1.15	10.71±2.34	11.58±0.25	10.61±1.30	11.60±2.73	10.48±1.66	10.58±1.22
4 (b)	15.38±1.67	15.41±1.64	13.47±1.61	14.40±0.07	13.62±1.22	14.40±0.10	9.66±1.24	12.57±1.66	10.73±1.20	12.50±1.62
8 (c)	17.35±0.11	17.30±0.10	16.31±1.64	16.36±1.61	15.46±1.75	15.44±1.02	13.51±0.78	13.56±1.73	12.68±1.29	13.45±1.28
14 (d)	20.19±0.04	21.21±1.62	19.12±1.61	20.26±0.12	18.22±1.57	20.14±0.02	15.80±0.96	17.08±0.28	15.23±0.28	16.14±1.58
26 (e)	38.84±1.05	38.04±2.66	25.23±0.20	25.29±1.84	23.91±1.61	23.51±1.17	23.69±0.25	24.07±1.07	24.06±1.14	21.66±1.42

All samples were done in triplicate. All samples were done in triplicate. Different lowercase letters (a, b, c, d and e) denote statistically significant difference between groups ($P < 0.05$), corrected by Tukey test.

Table 3. *L**, *a** and *b** values of control and HHP treated palm ruff fillets in pre- and post-rigor mortis.

	Storage days	Control (untreated) (a)		High Pressure (MPa) / Time (minutes)			
				450/3 (b)		450/4 (c)	
		Pre rigor (a)	Post rigor (a)	Pre rigor (a)	Post rigor (a)	Pre rigor (a)	Post rigor (a)
L*	0 (a)	44.76±1.03	46.78±0.28	63.01±0.33	63.30±0.32	67.32±0.94	60.61±1.21
	4 (b)	44.89±0.20	47.06±1.02	65.26±0.24	60.91±1.24	64.86±0.26	62.04±0.65
	8 (c)	51.14±2.73	47.59±0.59	61.45±1.76	61.14±4.53	62.67±1.73	62.63±4.00
	14 (d)	45.90±0.23	42.04±0.40	60.41±2.32	60.05±0.55	59.87±4.14	62.84±2.57
	26 (e)	49.54±1.09	53.15±0.08	62.51±4.54	64.63±1.82	61.14±3.22	60.38±1.17
a*	0 (a)	1.43±0.36	1.15±0.11	-0.40±0.08	0.15±0.07	-1.38±0.14	-0.13±0.09
	4 (b)	3.14±0.39	0.73±0.05	-0.05±0.02	ND	0.71±0.09	1.97±0.27
	8 (c)	5.72±1.18	4.25±0.25	2.60±0.41	1.64±0.52	0.64±1.48	2.01±1.84
	14 (d)	4.10±0.17	5.51±0.39	1.41±0.96	-0.21±0.12	0.65±1.64	-0.10±0.35
	26 (e)	8.41±2.00	6.70±0.28	1.34±1.15	1.69±0.74	1.41±0.61	1.72±0.88
b*	0 (a)	10.10±0.06	11.74±0.12	10.00±0.06	14.30±0.27	9.66±0.55	10.39±0.02
	4 (b)	12.61±0.18	13.32±0.21	13.87±0.12	ND	12.78±0.23	17.31±0.53
	8 (c)	14.10±0.74	14.48±0.47	13.16±0.67	16.78±3.76	12.37±1.45	15.57±1.58
	14 (d)	14.48±0.53	14.36±0.50	12.62±0.89	13.46±0.90	12.87±0.86	12.53±0.38
	26 (e)	15.02±1.32	14.13±0.57	15.93±2.69	14.89±1.24	14.17±0.55	15.42±0.91

All samples were done in triplicate. ND: not determined. All samples were done in triplicate. Different lowercase letters (a, b, c, d and e) denote statistically significant difference between groups ($P < 0.05$), corrected by Tukey test.

Table 4. Three-way ANOVA of the effects of High Pressure/Time (HP/T), storage days (SD) and Rigor (R) on pH, TVB-N, TMA, TBA and color (L*a*b*) in palm ruff fillets, with significant effects shown by the p values < 0.05.

General effects	df	pH		TVB-N (mg TVB-N/100 g)		TMA (mg TMA/100 g)		TBA (mg MDA/Kg)		df	Color					
											L*		a*		b*	
		F	p-value	F	p-value	F	p-value	F	p-value		F	p-value	F	p-value	F	p-value
HP/T	4	103.31	0.0000	68.4	0.0000	11684.2	0.0000	2750.43	0.0000	2	725.19	0.0000	162.15	0.0000	9.32	0.0003
SD	4	718.58	0.0000	513.81	0.0000	26448.2	0.0000	49216.8	0.0000	4	8.29	0.0000	40.38	0.0000	33.19	0.0000
R	1	1.93	0.1676	3.26	0.074	33.93	0.0000	25.81	0.1327	1	2.8	0.0994	1.08	0.3039	40.74	0.0608
HP/T × SD	16	47.38	0.0000	11.36	0.0000	5038.05	0.0000	655.46	0.0000	8	7.52	0.0000	14.45	0.0000	3.58	0.0019
HP/T × R	4	10.25	0.0000	0.71	0.5883	3.25	0.0151	10.82	0.0000	2	5.64	0.0057	14.75	0.0000	5.69	0.0055
SD × R	4	14.6	0.0000	1.74	0.1477	28.76	0.0000	7.94	0.0000	4	0.74	0.5712	2.51	0.0511	8.56	0.0000
HP/T × SD × R	16	10.52	0.0000	0.38	0.9837	17.41	0.0000	19.46	0.0000	8	7.28	0.0000	3.14	0.005	2.17	0.0427
Error	100									60						