EFFECT OF TIME-TEMPERATURE ABUSE ON MICROBIOLOGICAL AND PHYSIOCHEMICAL PROPERTIES OF BARRAMUNDI *(LATES CALCARIFER*, BLOCH*)* FILLETS

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ABSTRACT

The effect of time-temperature abuse (TTA) on quality and shelf life of barramundi (*Lates calcarifer*) fillets using microbiological and physiochemical tools was investigated. Fillets were subjected to 3 different pre-blast freezing (PBF) temperatures viz. 5°C, 0°C and -20°C for 0h, 1h, 1 day, 2 days, 4 days, 8 days and 16 days, after which fillets were exposed to -80°C for 8 hours and then stored at -20°C for 20 days. Color and rheological parameter values changed as PBF time period progressed at each temperature tested. There was minimal change to the microbiological and physiochemical properties of fillets stored at -20°C from 0h to 16 days. TVC, TVBN, pH, protein, color and rheological parameters of fillets that underwent PBF temperature period at 0°C and 5°C for 16 days deteriorated significantly compared to those treated at -20°C. The maximum PBF shelf life of barramundi fillets at 0°C and 5°C was 8 days.

PRACTICAL APPLICATIONS

After slaughter, the fish are likely to be exposed to inconsistent storage conditions (temperature abuse) for a limited period during transportation and subsequent storage. This temperature abuse may accelerate quality and shelf life changes in fillets. The purpose of the present study was to investigate the effects of different PBF temperature periods and temperatures (time-temperature index).

INTRODUCTION

Following slaughter, fish are likely to be exposed to inconsistent temperatures during transportation and subsequent storage. This temperature abuse may accelerate quality and shelf life changes in fillets. Therefore, it is important that adequate chill/storage procedures are in place to ensure that perishable foods not only achieve their required shelf lives but are safe for consumption by the end user (Jol *et al.* 2006). Exposure to higher temperatures and/or fluctuations of storage temperature produces cumulative adverse effects on the quality of stored foods, which is the primary cause of damage to food marketed through retail channels (Blond and Le Meste 2004).

Temperature control of stored fish is essential, not only to maintain quality but also to minimize changes in microbiological and physiochemical properties. The optimum range for successfully handling and displaying refrigerated foods is -1 to 2°C, certainly never higher than 5°C (Almonacid-Merino and Torres 1993). However, many of the retail display cases cycle up to 7 to 10°C (Young 1987). Domestic refrigerator temperatures are often higher than the recommended temperature of 5°C (Notermans *et al.* 1997; Nauta *et al.* 2003). The usual method to preserve the quality of fresh fish is storage in ice fish in ice. However, during iced storage of raw fish the quality of the fish muscle will deteriorate (Hultmann and Rustad 2007). Poor postharvest handling practices may enhance the rate of deterioration (Ashie *et al.* 1996). Freezing and frozen storage of fish can also lead to structural and physiochemical changes that alter the properties of the fish muscle causing quality deterioration to different degrees (Burgaard and Jørgensen 2010). In addition, the longer the storage period the softer the texture of the fish will be (Jiang *et al.* 2008). The impact of time-temperature abuse differs between species of fish. There is currently no information available on the quality and shelf life changes in barramundi (*Lates calcarifer*) fillets caused by exposure to different temperatures prior to freezing (pre-blast freezing temperatures).

The expansion of barramundi markets is presently limited because of quality loss during the freezing process (Zakhariya *et al.* 2014). Barramundi has a reputation as a high quality commercial species, with premium eating qualities (Australian Barramundi Farmers Association 2008). Barramundi is an important and valuable product in the Australian fish processing

industry, with an estimated aquaculture farm gate value of AU$45 million per annum (Australian Barramundi Farmers Association 2014). However, the fish may occasionally be subjected to inadequate storage conditions (temperature abuse) for a limited period during distribution from slaughter to consumer. The aim of the present experiment was to investigate the effects of different pre-blast freezing temperature periods and temperatures (time-temperature index) on the quality and shelf life of barramundi fillets.

MATERIALS AND METHODS

Sample Preparation

Aquacultured barramundi reared in marine water and harvested from Marine Farms Pty Ltd, Exmouth, Western Australia, Australia (latitude 21° 54' S; longitude 114° 10' E) were used for the study. The fish were kept at a temperature of 0-5°C throughout harvest and shipment. The average whole weight of barramundi used was 3.35 kg. Upon arrival, each whole barramundi was washed under running tap water (18- 20°C), and filleted prior to packing. The fillets were then cut into slices of approximately 2 cm thick. Fillet portions of approximately 200 g were then packed into separate sealed polythene bags.

Experimental Procedure

Fillets were divided into four batches, with four replicates of each: the control (fresh) batch of barramundi fillets (BF) were analysed immediately after being received and were not subjected to freezing, the second batch underwent pre-blast freezing treatment at 5°C for 0 h, 1 h, 1 day, 2 days, 4 days, 8 days, and 16 days, the third batch underwent pre-blast freezing treatment at 0°C for the same time intervals, and the fourth batch underwent pre-blast freezing treatment at -20°C for the same time intervals before blast freezing. All barramundi fillets were then individually frozen on a polystyrene dish in an air blast freezer with 5 m/s air velocity at –80°C for 8 h at the Department of Agriculture and Environment, Curtin University, Perth, Western Australia. All the frozen fillets were subsequently stored in a freezer at –20°C at CARL for 20 days. At the end of each treatment samples were thawed under running tap water (18- 20°C). Microbiological and physiochemical analyses of barramundi fillets were then carried out. The fillets were sub sampled in the laboratory under hygienic conditions and macerated in an acid washed glass

blender before being analysed for their quality and shelf life. Quality and shelf life of barramundi fillets were evaluated using the microbiological and physiochemical analyses described below:

Microbiological Analysis

TVC were determined using standard plate counts according to the method described by Association of Official Analytical Chemists (1995). The surface of the 0.5 g flesh sample and the weighing dish were swabbed with 70% ethanol. 4.5 mL of 0.85% NaCl and the flesh sample were then added to a sterile test tube and homogenised with a sterile glass rod. 0.1 mL of selected dilution was then inoculated onto a plate count nutrient agar plate (Plate Count Agar, PCA). The number of colony forming units (c.f.u.) was counted after 48 ± 3 h incubation at 25°C.

Proximate analysis

The muscle was homogenised and the moisture content of 5 g of homogenate was determined by drying the sample at 105°C until a constant weight was obtained (Association of Official Analytical Chemists 1990). Ash was determined by using the basic Association of Official Analytical Chemists (1990) method, involving heating the samples in a furnace at 550°C for 8–12 h. Total protein nitrogen content was measured by the standard method as described in Association of Official Analytical Chemists (1990) with a Kjeltec Auto 1030 Analyser (Tecator, *Höganäs,* Sweden) and the final protein content is expressed on a dry matter basis.

pH

The pH of barramundi fillets was determined using a TPS WP-80 pH meter. 5 g of barramundi meat was ground with 45 mL of distilled water in a test tube with a glass rod and pH was then measured.

TVBN

The total volatile base nitrogen (TVBN) was determined by the macro Kjeldahl method (Pearson 1981). The analysis was based on titration with 0.1 M sodium hydroxide, of a distillate of fish

muscle triturate (10 g) in water (300 mL) and magnesium oxide (2 g). The results were expressed in mg 100 g^{-1} of muscle.

Texture

Sample preparation

Fig. 1 indicates the section of the barramundi fillets analysed for rheological parameters. The middle (belly) of each fillet was collected and cut into 3 cm x 2 cm x 1.5 cm pieces with a sharp knife. Four fillets per treatment were subjected to hardness, cohesiveness, springiness, gumminess, chewiness, and stiffness testing. Four determinations of each texture variable were made on each fillet. Prior to analysis, samples were allowed to thaw to equilibrate at room temperature (18-20°C, 2 h).

Texture profile analysis (TPA)

Texture profile analysis was conducted using a texture analyser (TA Plus; AMETEK Lloyd Instruments Ltd., Fareham, UK). The machine interfaced to a personal computer with Nexygen™ Software (Version 4.6; AMETEK Lloyd Instruments Ltd.) with a load cell of 500 N. Measurements were taken with a Magness-Taylor probe (4 mm in diameter) and the crosshead operated at a constant speed of 2 mm s^{-1} to 7.5 mm depth. A trigger force of 1 N was used to puncture the fillets for all determinations. The test conditions were two consecutive cycles of 30% compression with 5 s between cycles. Each sample was placed on top of the square-base table and the gap size between the sample and the probe was at least 2 mm. The following rheological parameters of the barramundi fillets were determined (with units in brackets): fillet hardness (firmness) (Newtons (N), springiness (cm), gumminess (kilogram force (kgf)), chewiness (kilogram force millimetre (kgf.mm)) and stiffness (kg force per millimetre (kg f mm^{-1})). No specific expressed units were used for measurements of cohesiveness.

Colour measurement

Colour measurements were performed on samples according to Schubring (1999) using a colorimeter Minolta Spectrophotometer CM-508i. The colour reading includes lightness (L*), redness (a*) and yellowness (b*).

Statistical analysis

Statistical analyses were performed using SPSS software version 19.0. All results data were expressed as means ± S.E. (Standard Error) of four replicate samples. Analysis of variance (ANOVA) followed by Tukey post hoc analysis was used to determine significant differences between treatments at $\alpha < 0.05$ levels. All data were tested for homogeneity of variance by Levene's test.

RESULTS

The proximate composition of fresh barramundi fillets was 72.38±0.93% w.b. (wet basis) moisture, 1.02±0.04 % ash and 62.54±0.47 % d.b. (dry basis) protein. Fillet moisture content increased over time and was significantly higher ($P<0.05$) after 16 days of PBF temperature period than in fresh fillets for each of the PBF temperatures (Table 1). The increase in mean % moisture content of fillets subjected to 16 days of PBF temperature period at 5°C, 0°C and -20°C was 5.24% w.b., 3.86% w.b. and 3.17% w.b., respectively. One day of PBF temperature period resulted in a significant ($P<0.05$) increase in fillet ash content at each tested temperature except at 5°C. Ash content then decreased significantly ($P<0.05$) after 2 days PBF temperature period at 5°C and 0°C, and after 4 days PBF temperature period at -20°C (Table 1).

The protein content of fillets decreased as pre-blast freezing temperature period increased at 5°C and 0°C, with protein content significantly lower ($P<0.05$) after 16 days at 0°C and after 4 days and longer at 5°C compared to fresh fillets. Conversely, the protein content increased over time at -20°C, with protein content significantly higher ($P<0.05$) after 16 days treatment than in fresh fillets and after 4 days than at the other temperatures (Table 1). pH increased significantly ($P<0.05$) over the pre-blast freezing temperature period (0-16 days) from 6.34±0.00 to 6.78±0.00 at 5°C and to 6.68±0.00 at 0°C. However pH increased to a much lesser degree over 16 days at -20°C, from 6.34±0.00 to 6.49±0.01 (Table 2). Fillets that underwent PBF temperature period at 5°C for 16 days had significantly higher ($P<0.05$) pH than at 0°C and -20°C.

The TVBN of barramundi fillets rose from 6.25±0.02 to 54.14±0.18 mg 100 g^{-1}, and 49.19±0.05 mg 100 g^{-1} after 16 days when subjected to PBF temperature period at 5°C and 0°C, respectively, but only to 11.63±0.23 mg 100 g^{-1} at -20°C. TVBN levels increased significantly ($P<0.05$)

compared to fresh fillets when fillets were exposed to PBF temperature period for one hour and longer at all temperatures (Table 3). TVC on fresh fillets was 2.44±0.03 log CFU g^{-1}. 16 days PBF temperature period at 5°C, 0°C, and -20°C resulted in TVC values increasing significantly ($P<0.05$) to 8.58±0.20, 9.96±0.12 and 4.18±0.06, log CFU g^{-1} respectively. TVC increased significantly ($P<0.05$) between 0 days and 4 days, and between 4 days and 16 days PBF temperature period at 5°C and 0°C. However PBF temperature treatment at -20°C had relatively minimal impact as TVC was significantly lower ($P<0.05$) with treatment at -20°C than at 0 and 5°C for 8 days and longer (Table 4).

The mean L* value of the fresh fillets was 50.19±0.00, the mean a* value was -2.43±0.16 and the mean b* value was 0.28±0.00. L*, a* and b* increased significantly ($P<0.05$) when subjected to PBF temperature period at 0°C and 5°C from 0h to 16 days (Table 5). Fillets that underwent PBF temperature period at 5°C and 0°C for 16 days had significantly higher ($P<0.05$) L*value (lighter) than at -20°C. Fillets that underwent PBF temperature period at 5°C, had higher a* values (more greenish) and b* value (more yellowish) than fillets that underwent PBF temperature period at 0°C and -20°C after 16 days.

Each rheological parameter decreased significantly ($P<0.05$) after 16 days of PBF temperature period at 5°C, 0°C and -20°C, compared to fresh fillets. The most significant ($P<0.05$) decrease in rheological parameters (hardness, cohesiveness, springiness, gumminess, chewiness and stiffness) occurred between fresh fillets and fillets exposed to between 1 hour and 1 day PBF temperature period at all temperatures. With the exception of hardness, which decreased to a greater degree at 5°C and 0°C than at -20°C, each PBF temperature treatment had a similar effect on rheological parameters (Table 6).

DISCUSSION

Temperature control is a critical parameter to retard quality deterioration of perishable foodstuffs, such as fresh fish, during storage and transport from processing to consumers (Margeirsson *et al.* 2012). Zakhariya *et al*. (2014) demonstrated that it is important to prevent temperature variations or abuse during freezing and transport to avoid the detrimental effect of freezing and thawing so as to extend the quality and shelf-life of barramundi fillets. Temperature

196 abuse may shorten the freshness period and storage life of fish products (Margeirsson *et al.*
197 2012). Thus, fillets in the present study exposed to PBF temperature period at 5°C deteriorated
198 more rapidly than did fillets exposed to PBF temperature period at 0°C and -20°C. This may
199 have involved post-mortem myofibrillar degradation of the fish muscle, which is a major
200 problem for the fisheries industry (Jasra *et al.* 2001).

201 Studies on carp (*Labeo rohita*) (Gandotra *et al.* 2012), crab (*Scylla serrata*) (Zamir *et al.* 1998),
202 Arctic char (*Salvelinus alpinus*) (Bao *et al.* 2007) and snakehead (*Puntius* spp.) (Siddique *et al.*
203 2011) have shown that flesh moisture content increases with freezing time. Zamir *et al.* (1998)
204 attributed this increase to the loss of water holding capacity of the tissue. Fish with higher flesh
205 moisture content have a higher proportion of loosely bound water (Odoli 2009). There was a
206 gradual increase in moisture content in the present study. This increase in moisture content as
207 spoilage progressed could be due to activities of proteolytic enzymes (Fazal and Ramesh 2013).
208 However, ash content only increased to one day, then decreased over time during PBF
209 temperature period at all temperatures in the present study. Studies conducted by Okeyo *et al.*
210 (2009) on Nile perch (*Lates niloticus*) and Emire *et al.* (2009) on tilapia *(Oreochromis niloticus)*
211 reported a decrease in total ash content during its frozen storage. Drip loss during the thawing
212 process might be the reason for the decrease in the ash and protein contents in the present study
213 (Beklevik *et al.* 2005).

214 The decrease in the crude protein content of barramundi fillets in the present study from 0 to 16
215 days of PBF temperature period at 0°C and 5°C can be attributed to the leaching of the soluble
216 components, especially water-soluble protein and urea, from the fillets (Ashok Kumar *et al.*
217 2000; Singh and Balange 2005). Benjakul and Bauer (2001) reported that the proteins in fish
218 flesh are soluble proteins, which are localised in the cell and released when the cells are
219 damaged. This muscle drip loss can lower acceptability due to the loss of tasteful constituents,
220 e.g. some amino acids or nucleotides (Benjakul and Bauer 2001). Maria Macedo Viegas *et al.*
221 (2013) stated that increase in drip loss in frozen cod fillets is the result of muscle protein
222 denaturation and disruption of membranes, cytoskeleton, and extracellular matrix leading to loss
223 of intracellular compounds along with proteins. In contrast fillets exposed to PBF temperature
224 period at -20°C had higher protein content after 16 days in the present study. This increase in
225 protein content has also been observed during the frozen storage of fish cutlets, fish burgers and

fish sticks (Raju *et al*. 1999; Vanitha *et al*. 2013), and fish fingers from perches (Lakshminatha *et al*. 1992) and this could be due to the release of oxidative enzymes and pro-oxidants from various ruptured cellular organelles (Xia *et al.* 2009).

Post mortem pH of fish flesh varies from 6.0 to 7.1 (Simeonidou *et al.* 1998; Ozogul *et al.* 2005). This was confirmed for barramundi fillets in the present study (pH: 6.34 – 6.78). Abbas *et al*. (2009) stated that pH can act as an indicator of fish freshness as pH is low at the early stages of storage when the nutritional state is still good and then increases after storage for a certain period of time. Fillet pH increased significantly ($P<0.05$) with increasing storage time and temperature in the present study (0-16 days), indicating that alkaline compounds were accumulated through autolytic activities or microbial metabolism (Pons-Sanchez-Cascado *et al.* 2006). The pH is an important determinant of microbial growth and seafood with a high pH has a high spoilage potential and a short shelf life (Newton and Gell 1981).

The level of TVBN in freshly caught fish is generally between 5 and 20 mg N 100 g^{-1} muscle (Ozogul *et al.* 2005). The TVBN value of PBF (0 h) barramundi fillets in the present study was 6.26±0.11 mg 100 g^{-1}. A level of 30-35 mg 100 g^{-1} is considered the upper limit, above which fish products are considered unfit for human consumption (Ludorf and Meyer 1973, Oehlenschlager 1992). This is as a result of microorganisms influencing changes in some volatile nitrogen bases, causing fillet deterioration (Odoli 2009). In the present study, TVBN increased at each PBF treatment temperature, but to a greater extent at 5°C and 0°C. TVBN increased above the safe limit for human consumption (30-35 mg 100 g^{-1}) between 4 and 8 days PBF temperature period at 5°C, between 8 and 16 days PBF temperature period at 0°C and remained below this limit for 16 days at -20°C. This confirms that temperature abuse may shorten the freshness period and storage life of barramundi fillets particularly at 0°C and above. 10^4-10^6 TVC/cm^2 or g^{-1} is considered an acceptable range of TVC in the Australian meat industry (Meat Standards Committee 2002). Therefore, the TVC of the barramundi fillets in the present study was unacceptable after 8 days PBF temperature period at 0°C and 5°C, 6.38±0.12 and 8.17±0.33, respectively but remained acceptable (less than 10^7 cfu g^{-1}) at -20°C, even after 16 days. The growth in microbial load, as represented by TVC, accelerated with increasing temperature in the present study, demonstrating that enzymatic and microbiological processes are greatly influenced

by temperature (Huss 1995). This demonstrates the significant effect that time-temperature abuse has on barramundi fillet deterioration.

Colour changes in cod (*Gadus morhua*) include loss of surface glossiness, muscle opacity, or chalky appearance and are thought to be due to irreversible changes in the muscle proteins (Shenouda 1980). Dias *et al.* (1994) stated that colour changes in black scabbard fish (*Aphanopus carbo*) and silver scabbard fish (*Lepidopus caudatus*) can occur during frozen storage due to lipid oxidation and pigment degradation processes (Dias *et al.* 1994). During 12 d of refrigerated storage, the yellow discoloured catfish (*Ictalurus punctatus*) fillets became darker and more yellow (Li *et al.* 2013). Similarly, fillets were more yellowish after 16 d at 5°C than at 0 and -20°C in the present study. Fillets were also lighter and more greenish after 16 d at 5°C than at 0 and -20°C. The present study confirms that although fillet colour changes are slow at freezer temperatures, the rate of change is still temperature dependent and the colder the storage temperature, the slower the colour change (Spooncer *et al.* n.d.).Haard (1992) suggested that texture of fish flesh was influenced by many factors including postmortem pH decline, proteolysis, fat content, composition and its distribution in the fish muscle (Liu *et al.* 2010). Hardness decreased significantly ($P<0.05$) as a result of 16 days of PBF temperature period treatment at all temperatures in the present study. Schubring (2002) stated that the increasing softness during refrigerated storage is a result of proteolysis caused by endogenous and microbial enzymes. These enzymes caused increased proteolysis and resultant lower hardness at 0 and 5°C than at 20°C in the present study. The decrease in firmness as well as in elasticity may be due partly to the muscle softening as a result of proteolytic activity. Texture softening is mainly influenced by the autolysis and denaturation of muscle protein during chilled and frozen storage (Tsuchiya *et al.* 1992; Benjakul *et al.* 1997). The decrease in rheological parameters in the present study demonstrates that time-temperature abuse or just freezing at -20°C results in significant changes in barramundi fillet texture over time. The decrease in fillet cohesiveness, springiness, gumminess, chewiness and stiffness values (Table 6) after PBF treatments at 0°C, 5°C and, -20°C for 16 days in the present study could be due to the corresponding softening of fillets.

CONCLUSION

In conclusion, based upon microbiological analysis of barramundi fillets, the maximum PBF temperature shelf life was 8 days for fillets at 0˚C and 5˚C. In contrast, fillets subjected to PBF temperature period at -20°C have a shelf life of more than 16 days PBF temperature period. PBF temperature period at all temperatures deteriorated the L*, a*, b* values, and rheological parameters. TVC, TVBN, pH, protein, colour and rheological parameters deteriorated significantly after 16 days PBF temperature period at 0°C and 5°C. PBF treatment at -20°C from 0h to 16 days had only a minor effect on the microbiological and physiochemical properties. This observation, combined with the subsequent 20 day storage period, demonstrates that barramundi fillets stored at -20°C remain acceptable in terms of TVC and pH, TVBN, protein, and colour for at least 36 days. The largest detrimental changes to fillets in the present study occurred through PBF temperature period at 5°C, followed by 0°C. This demonstrates the inadequacy of storage at these higher temperatures for maintaining the quality and shelf life of barramundi fillets.

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TABLE 1.

CHANGES IN THE MOISTURE CONTENT % W.B., ASH CONTENT % AND PROTEIN CONTENT % OF BARRAMUNDI (*LATES CALCARIFER*) FILLETS BEFORE FREEZING (BF) AND PRE-BLAST FREEZING TREATMENTS AT 0°C, 5°C AND, -20°C FOR 0H, 1H, 1 DAY, 2 DAYS, 4 DAYS, 8 DAYS AND 16 DAYS.

Moisture content %

Treatment	0h	1h	1 day	2 days	4days	8days	16days
5°C	$72.38\pm0.93^{a}_{A}$	$72.52\pm0.39^{a}_{A}$	$73.57\pm0.11^{a}_{A}$	$73.92\pm0.12^{a}_{A}$	$74.03\pm0.90^{a}_{A}$	$75.34\pm0.09^{ab}_{B}$	$77.62\pm0.12^{b}_{A}$
0°C	$72.38\pm0.93^{a}_{A}$	$71.78\pm0.31^{a}_{A}$	$72.53\pm0.30^{a}_{A}$	$72.27\pm0.05^{a}_{A}$	$73.79\pm0.39^{ab}_{A}$	$74.00\pm0.31^{ab}_{A}$	$76.24\pm0.03^{b}_{B}$
-20°C	$72.38\pm0.93^{ab}_{A}$	$71.79\pm0.25^{a}_{A}$	$72.45\pm0.96^{ab}_{A}$	$73.08\pm0.74^{abc}_{A}$	$74.69\pm1.09^{abc}_{A}$	$75.37\pm0.40^{bc}_{A}$	$75.55\pm0.16^{c}_{C}$

Ash content %

Treatment	0h	1h	1day	2days	4days	8days	16days
5 °C	$1.02\pm0.04^{abc}_{A}$	$1.18\pm0.08^{bc}_{A}$	$1.24\pm0.07^{c}_{A}$	$0.96\pm0.01^{ab}_{B}$	$0.92\pm0.01^{a}_{B}$	$0.91\pm0.02^{a}_{B}$	$0.90\pm0.00^{a}_{B}$
0 °C	$1.02\pm0.04^{a}_{A}$	$1.11\pm0.05^{ab}_{A}$	$1.26\pm0.07^{b}_{A}$	$1.02\pm0.00^{a}_{B}$	$1.10\pm0.03^{ab}_{A}$	$1.00\pm0.02^{a}_{A}$	$0.97\pm0.02^{a}_{A}$
-20 °C	$1.02\pm0.04^{ac}_{A}$	$1.10\pm0.04^{ac}_{A}$	$1.33\pm0.03^{b}_{A}$	$1.18\pm0.06^{bc}_{A}$	$1.09\pm0.04^{ac}_{A}$	$0.96\pm0.02^{ac}_{AB}$	$0.92\pm0.01^{a}_{AB}$

Protein content %

Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	$62.54\pm0.47^{a}_{A}$	$61.15\pm0.29^{a}_{A}$	$60.26\pm0.36^{ab}_{B}$	$60.63\pm0.11^{a}_{AB}$	$58.05\pm0.37^{bc}_{B}$	$57.33\pm0.22^{c}_{C}$	$55.99\pm0.16^{c}_{C}$
0°C	$62.54\pm0.47^{a}_{A}$	$62.23\pm0.63^{a}_{A}$	$62.03\pm0.50^{a}_{AB}$	$61.08\pm0.69^{ab}_{B}$	$61.28\pm0.20^{ab}_{C}$	$61.27\pm0.27^{ab}_{B}$	$59.26\pm0.36^{b}_{B}$
-20°C	$62.54\pm0.47^{ab}_{A}$	$61.47\pm0.72^{a}_{A}$	$62.53\pm0.71^{ab}_{A}$	$63.04\pm0.89^{abc}_{A}$	$64.25\pm0.53^{bc}_{A}$	$64.98\pm0.31^{bc}_{A}$	$65.08\pm0.02^{c}_{A}$

All values are the means ± SE of four replicates, n=4

Values followed by different superscript letters in the same row are significantly different at $\alpha=0.05$

Values followed by different subscript capital letters in the same column are significantly different at $\alpha=0.05$

TABLE 2.

CHANGES IN THE PH OF BARRAMUNDI (*LATES CALCARIFER*) FILLETS BEFORE FREEZING (BF) AND PRE-BLAST FREEZING TREATMENTS AT 0°C, 5°C AND, -20°C FOR 0H, 1H, 1 DAY, 2 DAYS, 4 DAYS, 8 DAYS AND 16 DAYS.

Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	$6.34\pm0.00^{a}{}_{A}$	$6.34\pm0.01^{a}{}_{A}$	$6.49\pm0.00^{cde}{}_{B}$	$6.51\pm0.00^{def}{}_{A}$	$6.56\pm0.01^{efg}{}_{B}$	$6.63\pm0.00^{gh}{}_{B}$	$6.78\pm0.00^{i}{}_{C}$
0°C	$6.34\pm0.00^{a}{}_{A}$	$6.39\pm0.02^{ab}{}_{A}$	$6.42\pm0.01^{abcd}{}_{A}$	$6.46\pm0.01^{bcd}{}_{A}$	$6.55\pm0.00^{efg}{}_{B}$	$6.58\pm0.00^{fg}{}_{B}$	$6.68\pm0.00^{h}{}_{B}$
-20°C	$6.34\pm0.00^{a}{}_{A}$	$6.38\pm0.02^{ab}{}_{A}$	$6.40\pm0.00^{abc}{}_{A}$	$6.44\pm0.02^{bcd}{}_{A}$	$6.41\pm0.04^{abc}{}_{A}$	$6.44\pm0.01^{bcd}{}_{A}$	$6.49\pm0.01^{cde}{}_{A}$

All values are the means ± SE of four replicates, n=4

Values followed by different superscript letters in the same row are significantly different at $\alpha=0.05$

Values followed by different subscript capital letters in the same column are significantly different at $\alpha=0.05$

TABLE 3.

CHANGES IN THE TVBN OF BARRAMUNDI (*LATES CALCARIFER*) FILLETS BEFORE FREEZING (BF) AND PRE-BLAST FREEZING TREATMENTS AT 0°C, 5°C AND, -20°C FOR 0H, 1H, 1 DAY, 2 DAYS, 4 DAYS, 8 DAYS AND 16 DAYS.

Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	$6.25\pm0.02^{a}{}_{A}$	$10.45\pm0.02^{cd}{}_{B}$	$12.01\pm0.05^{f}{}_{B}$	$17.55\pm0.11^{g}{}_{C}$	$29.28\pm0.22^{i}{}_{C}$	$41.46\pm0.22^{j}{}_{C}$	$54.14\pm0.18^{l}{}_{C}$
0°C	$6.25\pm0.02^{a}{}_{A}$	$9.61\pm0.23^{bc}{}_{A}$	$12.38\pm0.19^{f}{}_{B}$	$12.51\pm0.25^{f}{}_{B}$	$17.37\pm0.07^{g}{}_{B}$	$19.17\pm0.05^{h}{}_{B}$	$49.19\pm0.05^{k}{}_{B}$
-20°C	$6.25\pm0.02^{a}{}_{A}$	$9.44\pm0.22^{b}{}_{A}$	$10.14\pm0.01^{bcd}{}_{A}$	$10.24\pm0.06^{bcd}{}_{A}$	$10.56\pm0.30^{d}{}_{A}$	$10.77\pm0.27^{de}{}_{A}$	$11.63\pm0.23^{ef}{}_{A}$

All values are the means ± SE of four replicates, n=4

Values followed by different superscript letters in the same row are significantly different at $\alpha=0.05$

Values followed by different subscript capital letters in the same column are significantly different at $\alpha=0.05$

TABLE 4.

CHANGES IN THE TVC OF BARRAMUNDI (*LATES CALCARIFER*) FILLETS BEFORE FREEZING (BF) AND PRE-BLAST FREEZING TREATMENTS AT 0°C, 5°C AND, -20°C FOR 0H, 1H, 1 DAY, 2 DAYS, 4 DAYS, 8 DAYS AND 16 DAYS.

Treatment	**0h**	**1h**	**1day**	**2days**	**4days**	**8days**	**16days**
5°C	$2.44\pm0.03^{a}{}_{A}$	$2.62\pm0.15^{ab}{}_{A}$	$3.33\pm0.16^{bc}{}_{A}$	$3.42\pm0.22^{cd}{}_{A}$	$3.76\pm0.08^{cd}{}_{A}$	$6.38\pm0.12^{f}{}_{B}$	$8.58\pm0.20^{g}{}_{B}$
0°C	$2.44\pm0.03^{a}{}_{A}$	$2.61\pm0.18^{ab}{}_{A}$	$3.29\pm0.14^{bc}{}_{A}$	$3.49\pm0.23^{cd}{}_{A}$	$5.11\pm0.12^{e}{}_{B}$	$8.17\pm0.33^{g}{}_{C}$	$9.96\pm0.12^{h}{}_{C}$
-20°C	$2.44\pm0.03^{a}{}_{A}$	$2.51\pm0.06^{a}{}_{A}$	$3.52\pm0.07^{cd}{}_{A}$	$3.73\pm0.09^{cd}{}_{A}$	$3.80\pm0.02^{cd}{}_{A}$	$3.86\pm0.05^{cd}{}_{A}$	$4.18\pm0.06^{d}{}_{A}$

All values are the means ± SE of four replicates, n=4

Values followed by different superscript letters in the same row are significantly different at $\alpha=0.05$

Values followed by different subscript capital letters in the same column are significantly different at $\alpha=0.05$

TABLE 5:
CHANGES IN THE L*, A* AND B* VALUES OF BARRAMUNDI (*LATES CALCARIFER*) FILLETS BEFORE FREEZING (BF) AND PRE-BLAST FREEZING TREATMENTS AT 0°C, 5°C AND, -20°C FOR 0H, 1H, 1 DAY, 2 DAYS, 4 DAYS, 8 DAYS AND 16 DAYS.

L*

Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	$50.19\pm0.00^{a}_{A}$	$51.32\pm0.04^{b}_{A}$	$52.38\pm0.09^{c}_{A}$	$52.78\pm0.05^{c}_{A}$	$54.81\pm0.11^{e}_{A}$	$56.48\pm0.18^{f}_{A}$	$58.89\pm0.01^{g}_{A}$
0°C	$50.19\pm0.00^{a}_{A}$	$50.68\pm0.10^{a}_{B}$	$50.31\pm0.08^{a}_{C}$	$51.49\pm0.13^{b}_{B}$	$52.44\pm0.08^{c}_{B}$	$53.51\pm0.12^{d}_{B}$	$56.64\pm0.14^{f}_{B}$
-20°C	$50.19\pm0.00^{a}_{A}$	$50.17\pm0.03^{a}_{C}$	$51.56\pm0.11^{b}_{B}$	$51.54\pm0.19^{b}_{B}$	$52.77\pm0.08^{c}_{B}$	$53.65\pm0.17^{d}_{B}$	$54.62\pm0.07^{e}_{C}$

a*

Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	$-2.43\pm0.16^{a}_{A}$	$-2.23\pm0.02^{b}_{AB}$	$-2.47\pm0.05^{a}_{B}$	$-1.74\pm0.04^{c}_{A}$	$-1.50\pm0.03^{d}_{A}$	$-1.42\pm0.20^{d}_{A}$	$-0.54\pm0.01^{e}_{A}$
0°C	$-2.43\pm0.16^{a}_{A}$	$-2.37\pm0.06^{a}_{B}$	$-2.49\pm0.10^{a}_{B}$	$-1.24\pm0.01^{e}_{A}$	$-1.20\pm0.02^{e}_{A}$	$-1.37\pm0.16^{e}_{A}$	$-1.47\pm0.19^{e}_{B}$
-20°C	$-2.43\pm0.16^{a}_{A}$	$-2.17\pm0.02^{b}_{A}$	$-2.11\pm0.00^{b}_{A}$	$-2.34\pm0.21^{a}_{B}$	$-2.40\pm0.16^{a}_{B}$	$-2.13\pm0.01^{b}_{B}$	$-2.06\pm0.01^{c}_{C}$

b*

Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	$0.28\pm0.00^{a}_{A}$	$0.32\pm0.00^{a}_{A}$	$0.57\pm0.06^{ab}_{A}$	$1.17\pm0.02^{c}_{AB}$	$2.51\pm0.03^{d}_{A}$	$3.48\pm0.16^{ef}_{A}$	$5.52\pm0.20^{g}_{A}$
0°C	$0.28\pm0.00^{a}_{A}$	$0.32\pm0.02^{a}_{A}$	$0.52\pm0.09^{a}_{A}$	$1.00\pm0.05^{bc}_{B}$	$2.22\pm0.14^{d}_{A}$	$3.23\pm0.04^{e}_{A}$	$3.92\pm0.03^{f}_{B}$
-20°C	$0.28\pm0.00^{a}_{A}$	$0.33\pm0.00^{a}_{A}$	$0.43\pm0.08^{a}_{A}$	$1.30\pm0.11^{c}_{A}$	$2.25\pm0.10^{d}_{A}$	$2.37\pm0.07^{d}_{B}$	$3.52\pm0.13^{ef}_{B}$

All values are the means ± SE of four replicates, n=4
Values followed by different superscript letters in the same row are significantly different at $\alpha=0.05$
Values followed by different subscript capital letters in the same column are significantly different at $\alpha=0.05$

TABLE 6.

CHANGES IN THE HARDNESS (N), COHESIVENESS, SPRINGINESS (CM), GUMMINESS (KGF), CHEWINESS (KGF.MM), AND STIFFNESS (KGF/MM) OF BARRAMUNDI (*LATES CALCARIFER*) FILLETS BEFORE FREEZING (BF) AND PRE-BLAST FREEZING TREATMENTS AT 0°C, 5°C AND, -20°C FOR 0H, 1H, 1 DAY, 2 DAYS, 4 DAYS, 8 DAYS AND 16 DAYS.

Hardness (N)

Treatment	**0h**	**1h**	**1day**	**2days**	**4days**	**8days**	**16days**
5°C	$1.98\pm0.04^{a}_{A}$	$1.76\pm0.01^{b}_{A}$	$1.68\pm0.02^{bc}_{A}$	$1.66\pm0.00^{bc}_{B}$	$1.59\pm0.02^{ce}_{B}$	$1.46\pm0.01^{d}_{C}$	$1.46\pm0.02^{de}_{B}$
0°C	$1.98\pm0.04^{a}_{A}$	$1.72\pm0.02^{b}_{A}$	$1.71\pm0.00^{b}_{A}$	$1.71\pm0.01^{b}_{B}$	$1.64\pm0.02^{b}_{B}$	$1.60\pm0.02^{bc}_{B}$	$1.48\pm0.00^{c}_{B}$
-20°C	$1.98\pm0.04^{a}_{A}$	$1.79\pm0.02^{b}_{A}$	$1.75\pm0.01^{b}_{A}$	$1.84\pm0.0^{b}_{A}$	$1.84\pm0.01^{b}_{A}$	$1.84\pm0.01^{b}_{A}$	$1.76\pm0.01^{b}_{A}$

Cohesiveness

Treatment	**0h**	**1h**	**1day**	**2days**	**4days**	**8days**	**16days**
5°C	$0.08\pm0.00^{a}_{A}$	$0.07\pm0.00^{a}_{A}$	$0.06\pm0.00^{a}_{A}$	$0.01\pm0.00^{b}_{B}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$
0°C	$0.08\pm0.00^{a}_{A}$	$0.07\pm0.00^{a}_{A}$	$0.05\pm0.00^{ab}_{A}$	$0.03\pm0.00^{bc}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$
-20°C	$0.08\pm0.00^{a}_{A}$	$0.04\pm0.01^{b}_{A}$	$0.01\pm0.00^{bc}_{A}$	$0.00\pm0.00^{c}_{B}$	$0.00\pm0.00^{c}_{A}$	$0.00\pm0.00^{c}_{A}$	$0.00\pm0.00^{c}_{A}$

Springiness (cm)

Treatment	**0h**	**1h**	**1day**	**2days**	**4days**	**8days**	**16days**
5°C	$0.08\pm0.00^{a}_{A}$	$0.03\pm0.00^{b}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$
0°C	$0.08\pm0.00^{a}_{A}$	$0.04\pm0.00^{b}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$
-20°C	$0.08\pm0.00^{a}_{A}$	$0.02\pm0.00^{b}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$	$0.01\pm0.00^{c}_{A}$

Gumminess (kgf)

Treatment	**0h**	**1h**	**1day**	**2days**	**4days**	**8days**	**16days**
5°C	$0.10\pm0.00^{a}_{A}$	$0.05\pm0.02^{ab}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$
0°C	$0.10\pm0.00^{a}_{A}$	$0.07\pm0.02^{a}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$
-20°C	$0.10\pm0.00^{a}_{A}$	$0.07\pm0.02^{a}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$	$0.00\pm0.00^{b}_{A}$

Chewiness (kgf.mm)

Treatment	**0h**	**1h**	**1day**	**2days**	**4days**	**8days**	**16days**
5°C	$0.04\pm0.00^{a}_{A}$	$0.02\pm.00^{ab}_{A}$	$0.02\pm0.00^{ab}_{A}$	$0.01\pm0.00^{ab}_{B}$	$0.01\pm0.00^{b}_{B}$	$0.01\pm0.00^{b}_{A}$	$0.01\pm0.00^{b}_{A}$
0°C	$0.04\pm0.00^{ab}_{A}$	$0.03\pm.00^{ab}_{A}$	$0.05\pm0.00^{a}_{A}$	$0.05\pm0.00^{a}_{A}$	$0.03\pm0.00^{ab}_{A}$	$0.01\pm0.00^{b}_{A}$	$0.01\pm0.00^{b}_{A}$
-20°C	$0.04\pm0.00^{a}_{A}$	$0.03\pm0.01^{ab}_{A}$	$0.02\pm0.00^{ab}_{A}$	$0.02\pm0.00^{ab}_{B}$	$0.01\pm0.00^{b}_{B}$	$0.01\pm0.00^{b}_{A}$	$0.01\pm0.00^{b}_{A}$

Stiffness (kgf/mm)

Treatment	**0h**	**1h**	**1day**	**2days**	**4days**	**8days**	**16days**
5°C	$0.25\pm0.01^{a}_{A}$	$0.06\pm0.00^{b}_{B}$	$0.06\pm0.00^{b}_{B}$	$0.04\pm0.00^{bc}_{A}$	$0.01\pm0.00^{c}_{B}$	$0.02\pm0.00^{bc}_{A}$	$0.01\pm0.00^{bc}_{A}$
0°C	$0.25\pm0.01^{a}_{A}$	$0.07\pm0.00^{b}_{AB}$	$0.07\pm0.00^{bc}_{AB}$	$0.05\pm0.01^{bcd}_{A}$	$0.03\pm0.00^{cd}_{AB}$	$0.02\pm0.00^{d}_{A}$	$0.02\pm0.00^{d}_{A}$
-20°C	$0.25\pm0.01^{a}_{A}$	$0.10\pm0.00^{b}_{A}$	$0.09\pm0.00^{b}_{A}$	$0.06\pm0.01^{bc}_{A}$	$0.04\pm0.00^{c}_{A}$	$0.03\pm0.00^{c}_{A}$	$0.03\pm0.00^{c}_{A}$

All values are the means ± SE of four replicates, n=4

Values followed by different superscript letters in the same row are significantly different at α=0.05

Values followed by different subscript capital letters in the same column are significantly different at α=0.05