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

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11

12 **ABSTRACT**

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

23 **PRACTICAL APPLICATIONS**

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

29

## 30 INTRODUCTION

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

39 Temperature control of stored fish is essential, not only to maintain quality but also to minimize  
40 changes in microbiological and physiochemical properties. The optimum range for successfully  
41 handling and displaying refrigerated foods is -1 to 2°C, certainly never higher than 5°C  
42 (Almonacid-Merino and Torres 1993). However, many of the retail display cases cycle up to 7 to  
43 10°C (Young 1987). Domestic refrigerator temperatures are often higher than the recommended  
44 temperature of 5°C (Notermans *et al.* 1997; Nauta *et al.* 2003). The usual method to preserve the  
45 quality of fresh fish is storage in ice fish in ice. However, during iced storage of raw fish the  
46 quality of the fish muscle will deteriorate (Hultmann and Rustad 2007). Poor postharvest  
47 handling practices may enhance the rate of deterioration (Ashie *et al.* 1996). Freezing and frozen  
48 storage of fish can also lead to structural and physiochemical changes that alter the properties of  
49 the fish muscle causing quality deterioration to different degrees (Burggaard and Jørgensen 2010).  
50 In addition, the longer the storage period the softer the texture of the fish will be (Jiang *et al.*  
51 2008). The impact of time-temperature abuse differs between species of fish. There is currently  
52 no information available on the quality and shelf life changes in barramundi (*Lates calcarifer*)  
53 fillets caused by exposure to different temperatures prior to freezing (pre-blast freezing  
54 temperatures).

55 The expansion of barramundi markets is presently limited because of quality loss during the  
56 freezing process (Zakhariya *et al.* 2014). Barramundi has a reputation as a high quality  
57 commercial species, with premium eating qualities (Australian Barramundi Farmers Association  
58 2008). Barramundi is an important and valuable product in the Australian fish processing

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

## 65 **MATERIALS AND METHODS**

### 66 *Sample Preparation*

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

### 74 **Experimental Procedure**

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

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

### 89 **Microbiological Analysis**

90 TVC were determined using standard plate counts according to the method described by  
91 Association of Official Analytical Chemists (1995). The surface of the 0.5 g flesh sample and the  
92 weighing dish were swabbed with 70% ethanol. 4.5 mL of 0.85% NaCl and the flesh sample  
93 were then added to a sterile test tube and homogenised with a sterile glass rod. 0.1 mL of  
94 selected dilution was then inoculated onto a plate count nutrient agar plate (Plate Count Agar,  
95 PCA). The number of colony forming units (c.f.u.) was counted after  $48 \pm 3$  h incubation at  
96 25°C.

### 97 **Proximate analysis**

98 The muscle was homogenised and the moisture content of 5 g of homogenate was determined by  
99 drying the sample at 105°C until a constant weight was obtained (Association of Official  
100 Analytical Chemists 1990). Ash was determined by using the basic Association of Official  
101 Analytical Chemists (1990) method, involving heating the samples in a furnace at 550°C for 8–  
102 12 h. Total protein nitrogen content was measured by the standard method as described in  
103 Association of Official Analytical Chemists (1990) with a Kjeltex Auto 1030 Analyser (Tecator,  
104 *Höganäs*, Sweden) and the final protein content is expressed on a dry matter basis.

### 105 **pH**

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

### 109 **TVBN**

110 The total volatile base nitrogen (TVBN) was determined by the macro Kjeldahl method (Pearson  
111 1981). The analysis was based on titration with 0.1 M sodium hydroxide, of a distillate of fish

112 muscle triturate (10 g) in water (300 mL) and magnesium oxide (2 g). The results were expressed  
113 in mg 100 g<sup>-1</sup> of muscle.

## 114 **Texture**

### 115 *Sample preparation*

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

### 122 **Texture profile analysis (TPA)**

123 Texture profile analysis was conducted using a texture analyser (TA Plus; AMETEK Lloyd  
124 Instruments Ltd., Fareham, UK). The machine interfaced to a personal computer with  
125 Nexygen™ Software (Version 4.6; AMETEK Lloyd Instruments Ltd.) with a load cell of 500 N.  
126 Measurements were taken with a Magness-Taylor probe (4 mm in diameter) and the crosshead  
127 operated at a constant speed of 2 mm s<sup>-1</sup> to 7.5 mm depth. A trigger force of 1 N was used to  
128 puncture the fillets for all determinations. The test conditions were two consecutive cycles of  
129 30% compression with 5 s between cycles. Each sample was placed on top of the square-base  
130 table and the gap size between the sample and the probe was at least 2 mm. The following  
131 rheological parameters of the barramundi fillets were determined (with units in brackets): fillet  
132 hardness (firmness) (Newtons (N), springiness (cm), gumminess (kilogram force (kgf)),  
133 chewiness (kilogram force millimetre (kgf.mm)) and stiffness (kg force per millimetre (kg f mm<sup>-1</sup>)).  
134 No specific expressed units were used for measurements of cohesiveness.

### 135 **Colour measurement**

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

139 **Statistical analysis**

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

145 **RESULTS**

146 The proximate composition of fresh barramundi fillets was  $72.38 \pm 0.93\%$  w.b. (wet basis)  
147 moisture,  $1.02 \pm 0.04\%$  ash and  $62.54 \pm 0.47\%$  d.b. (dry basis) protein. Fillet moisture content  
148 increased over time and was significantly higher ( $P < 0.05$ ) after 16 days of PBF temperature  
149 period than in fresh fillets for each of the PBF temperatures (Table 1). The increase in mean %  
150 moisture content of fillets subjected to 16 days of PBF temperature period at  $5^{\circ}\text{C}$ ,  $0^{\circ}\text{C}$  and  $-20^{\circ}\text{C}$   
151 was  $5.24\%$  w.b.,  $3.86\%$  w.b. and  $3.17\%$  w.b., respectively. One day of PBF temperature period  
152 resulted in a significant ( $P < 0.05$ ) increase in fillet ash content at each tested temperature except  
153 at  $5^{\circ}\text{C}$ . Ash content then decreased significantly ( $P < 0.05$ ) after 2 days PBF temperature period at  
154  $5^{\circ}\text{C}$  and  $0^{\circ}\text{C}$ , and after 4 days PBF temperature period at  $-20^{\circ}\text{C}$  (Table 1).

155 The protein content of fillets decreased as pre-blast freezing temperature period increased at  $5^{\circ}\text{C}$   
156 and  $0^{\circ}\text{C}$ , with protein content significantly lower ( $P < 0.05$ ) after 16 days at  $0^{\circ}\text{C}$  and after 4 days  
157 and longer at  $5^{\circ}\text{C}$  compared to fresh fillets. Conversely, the protein content increased over time  
158 at  $-20^{\circ}\text{C}$ , with protein content significantly higher ( $P < 0.05$ ) after 16 days treatment than in fresh  
159 fillets and after 4 days than at the other temperatures (Table 1). pH increased significantly  
160 ( $P < 0.05$ ) over the pre-blast freezing temperature period (0-16 days) from  $6.34 \pm 0.00$  to  $6.78 \pm 0.00$   
161 at  $5^{\circ}\text{C}$  and to  $6.68 \pm 0.00$  at  $0^{\circ}\text{C}$ . However pH increased to a much lesser degree over 16 days at -  
162  $20^{\circ}\text{C}$ , from  $6.34 \pm 0.00$  to  $6.49 \pm 0.01$  (Table 2). Fillets that underwent PBF temperature period at  
163  $5^{\circ}\text{C}$  for 16 days had significantly higher ( $P < 0.05$ ) pH than at  $0^{\circ}\text{C}$  and  $-20^{\circ}\text{C}$ .

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

167 compared to fresh fillets when fillets were exposed to PBF temperature period for one hour and  
168 longer at all temperatures (Table 3). TVC on fresh fillets was  $2.44 \pm 0.03$  log CFU g<sup>-1</sup>. 16 days  
169 PBF temperature period at 5°C, 0°C, and -20°C resulted in TVC values increasing significantly  
170 ( $P < 0.05$ ) to  $8.58 \pm 0.20$ ,  $9.96 \pm 0.12$  and  $4.18 \pm 0.06$ , log CFU g<sup>-1</sup> respectively. TVC increased  
171 significantly ( $P < 0.05$ ) between 0 days and 4 days, and between 4 days and 16 days PBF  
172 temperature period at 5°C and 0°C. However PBF temperature treatment at -20°C had relatively  
173 minimal impact as TVC was significantly lower ( $P < 0.05$ ) with treatment at -20°C than at 0 and  
174 5°C for 8 days and longer (Table 4).

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

182

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

## 190 **DISCUSSION**

191 Temperature control is a critical parameter to retard quality deterioration of perishable  
192 foodstuffs, such as fresh fish, during storage and transport from processing to consumers  
193 (Margeirsson *et al.* 2012). Zakhariya *et al.* (2014) demonstrated that it is important to prevent  
194 temperature variations or abuse during freezing and transport to avoid the detrimental effect of  
195 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

226 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  
227 various ruptured cellular organelles (Xia *et al.* 2009).

229 Post mortem pH of fish flesh varies from 6.0 to 7.1 (Simeonidou *et al.* 1998; Ozogul *et al.* 2005).  
230 This was confirmed for barramundi fillets in the present study (pH: 6.34 – 6.78). Abbas *et al.*  
231 (2009) stated that pH can act as an indicator of fish freshness as pH is low at the early stages of  
232 storage when the nutritional state is still good and then increases after storage for a certain period  
233 of time. Fillet pH increased significantly ( $P < 0.05$ ) with increasing storage time and temperature  
234 in the present study (0-16 days), indicating that alkaline compounds were accumulated through  
235 autolytic activities or microbial metabolism (Pons-Sanchez-Cascado *et al.* 2006). The pH is an  
236 important determinant of microbial growth and seafood with a high pH has a high spoilage  
237 potential and a short shelf life (Newton and Gell 1981).

238 The level of TVBN in freshly caught fish is generally between 5 and 20 mg N 100 g<sup>-1</sup> muscle  
239 (Ozogul *et al.* 2005). The TVBN value of PBF (0 h) barramundi fillets in the present study was  
240  $6.26 \pm 0.11$  mg 100 g<sup>-1</sup>. A level of 30-35 mg 100 g<sup>-1</sup> is considered the upper limit, above which  
241 fish products are considered unfit for human consumption (Ludorf and Meyer 1973,  
242 Oehlenschlager 1992). This is as a result of microorganisms influencing changes in some volatile  
243 nitrogen bases, causing fillet deterioration (Odoli 2009). In the present study, TVBN increased at  
244 each PBF treatment temperature, but to a greater extent at 5°C and 0°C. TVBN increased above  
245 the safe limit for human consumption (30-35 mg 100 g<sup>-1</sup>) between 4 and 8 days PBF temperature  
246 period at 5°C, between 8 and 16 days PBF temperature period at 0°C and remained below this  
247 limit for 16 days at -20°C. This confirms that temperature abuse may shorten the freshness  
248 period and storage life of barramundi fillets particularly at 0°C and above.  $10^4$ - $10^6$  TVC/cm<sup>2</sup> or g<sup>-1</sup>  
249 <sup>1</sup> is considered an acceptable range of TVC in the Australian meat industry (Meat Standards  
250 Committee 2002). Therefore, the TVC of the barramundi fillets in the present study was  
251 unacceptable after 8 days PBF temperature period at 0°C and 5°C,  $6.38 \pm 0.12$  and  $8.17 \pm 0.33$ ,  
252 respectively but remained acceptable (less than  $10^7$  cfu g<sup>-1</sup>) at -20°C, even after 16 days. The  
253 growth in microbial load, as represented by TVC, accelerated with increasing temperature in the  
254 present study, demonstrating that enzymatic and microbiological processes are greatly influenced

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

257 Colour changes in cod (*Gadus morhua*) include loss of surface glossiness, muscle opacity, or  
258 chalky appearance and are thought to be due to irreversible changes in the muscle proteins  
259 (Shenouda 1980). Dias *et al.* (1994) stated that colour changes in black scabbard fish  
260 (*Aphanopus carbo*) and silver scabbard fish (*Lepidopus caudatus*) can occur during frozen  
261 storage due to lipid oxidation and pigment degradation processes (Dias *et al.* 1994). During 12 d  
262 of refrigerated storage, the yellow discoloured catfish (*Ictalurus punctatus*) fillets became darker  
263 and more yellow (Li *et al.* 2013). Similarly, fillets were more yellowish after 16 d at 5°C than at  
264 0 and -20°C in the present study. Fillets were also lighter and more greenish after 16 d at 5°C  
265 than at 0 and -20°C. The present study confirms that although fillet colour changes are slow at  
266 freezer temperatures, the rate of change is still temperature dependent and the colder the storage  
267 temperature, the slower the colour change (Spooner *et al.* n.d.). Haard (1992) suggested that  
268 texture of fish flesh was influenced by many factors including postmortem pH decline,  
269 proteolysis, fat content, composition and its distribution in the fish muscle (Liu *et al.* 2010).  
270 Hardness decreased significantly ( $P < 0.05$ ) as a result of 16 days of PBF temperature period  
271 treatment at all temperatures in the present study. Schubring (2002) stated that the increasing  
272 softness during refrigerated storage is a result of proteolysis caused by endogenous and microbial  
273 enzymes. These enzymes caused increased proteolysis and resultant lower hardness at 0 and 5°C  
274 than at 20°C in the present study. The decrease in firmness as well as in elasticity may be due  
275 partly to the muscle softening as a result of proteolytic activity. Texture softening is mainly  
276 influenced by the autolysis and denaturation of muscle protein during chilled and frozen storage  
277 (Tsuchiya *et al.* 1992; Benjakul *et al.* 1997). The decrease in rheological parameters in the  
278 present study demonstrates that time-temperature abuse or just freezing at -20°C results in  
279 significant changes in barramundi fillet texture over time. The decrease in fillet cohesiveness,  
280 springiness, gumminess, chewiness and stiffness values (Table 6) after PBF treatments at 0°C,  
281 5°C and, -20°C for 16 days in the present study could be due to the corresponding softening of  
282 fillets.

283

284 **CONCLUSION**

285 In conclusion, based upon microbiological analysis of barramundi fillets, the maximum PBF  
286 temperature shelf life was 8 days for fillets at 0°C and 5°C. In contrast, fillets subjected to PBF  
287 temperature period at -20°C have a shelf life of more than 16 days PBF temperature period. PBF  
288 temperature period at all temperatures deteriorated the L\*, a\*, b\* values, and rheological  
289 parameters. TVC, TVBN, pH, protein, colour and rheological parameters deteriorated  
290 significantly after 16 days PBF temperature period at 0°C and 5°C. PBF treatment at -20°C from  
291 0h to 16 days had only a minor effect on the microbiological and physiochemical properties. This  
292 observation, combined with the subsequent 20 day storage period, demonstrates that barramundi  
293 fillets stored at -20°C remain acceptable in terms of TVC and pH, TVBN, protein, and colour for  
294 at least 36 days. The largest detrimental changes to fillets in the present study occurred through  
295 PBF temperature period at 5°C, followed by 0°C. This demonstrates the inadequacy of storage at  
296 these higher temperatures for maintaining the quality and shelf life of barramundi fillets.

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310 **References**

- 311 ABBAS, K., SALEH, A., MOHAMED, A. and LASEKAN, O. 2009. The relationship between  
312 water activity and fish spoilage during cold storage: a review. *Journal of food, agriculture*  
313 *& environment*. 7.
- 314 ALMONACID-MERINO, S.F. and TORRES, J.A. 1993. Mathematical models to evaluate  
315 temperature abuse effects during distribution of refrigerated solid foods. *Journal of Food*  
316 *Engineering*. 20(3), 223-245.
- 317 ASHIE, I.N.A., SMITH, J.P., SIMPSON, B.K. and HAARD, N.F. 1996. Spoilage and shelf-life  
318 extension of fresh fish and shellfish. *Critical Reviews in Food Science and Nutrition*.  
319 36(1-2), 87-121.
- 320 ASHOK KUMAR, K., RAVISHANKAR, C.N., BADONIA, R. and SOLANKI, K.K. 2000.  
321 Quality changes in whale shark (*Rhinodon typus* Smith) meat during storage in ice. *Fish*.  
322 *Technol*. 37(1), 15–18.
- 323 ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC). 1990. *Official Methods*  
324 *of Analysis*, Association of Official Analytical Chemists. Washington, DC.
- 325 ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC). 1995. *Official Methods*  
326 *of Analysis*, Association of Official Analytical Chemists. Washington, DC.
- 327 AUSTRALIAN BARRAMUNDI FARMERS ASSOCIATION. 2008. Barramundi. Retrieved  
328 March 10th 2013.
- 329 BAO, H.N.D., ARASON, S. and ÞÓRARINSDÓTTIR, K.A. 2007. Effects of Dry Ice and  
330 Superchilling on Quality and Shelf Life of Arctic Charr (*Salvelinus alpinus*) Fillets.  
331 *International Journal of Food Engineering*. 3(3).
- 332 BEKLEVIK, G., POLAT, A. and OZOGUL, F. 2005. Nutritional value of Sea Bass  
333 (*Dicentrarchus labrax*) fillets during frozen (-18°C) storage. *Turkish Journal of*  
334 *Veterinary and Animal Science*. 29, 891-895.
- 335 BENJAKUL, S. and BAUER, F. 2001. Biochemical and physicochemical changes in catfish  
336 (*Silurus glanis* Linne) muscle as influenced by different freeze–thaw cycles. *Food*  
337 *Chemistry*. 72(2), 207-217.
- 338 BENJAKUL, S., SEYMOUR, T.A., MORRISSEY, M.T. and AN, H. 1997.  
339 Physicochemical changes in Pacific whiting muscle proteins during iced storage. *J. Food*  
340 *Sci*. 62, 729-733.
- 341 BLOND, G. and LE MESTE, M. 2004. Principles of frozen storage. In *Food science and*  
342 *technology*, Marcel Dekker, Inc., New York, NY.
- 343 BURGAARD, M.G. and JØRGENSEN, B.M. 2011. Effect of Frozen Storage Temperature on  
344 Quality-Related Changes in Rainbow Trout (*Oncorhynchus mykiss*). *Journal of Aquatic*  
345 *Food Product Technology*. 20(1), 53-63.
- 346 DIAS, J., NUNES, M.L. and MENDES, R. 1994. Effect of frozen storage on the chemical and  
347 physical properties of black and silver scabbardfish. *Journal of the Science of Food and*  
348 *Agriculture*. 66(3), 327-335.
- 349 EMIRE, A.S. and GEBREMARIAM, M.M. 2009. Influence of frozen period on the proximate  
350 composition and microbiological quality of Nile tilapia fish (*Oreochromis niloticus*)  
351 *Journal of Food Processing and Preservation*. 34, 743-757.
- 352 FAZAL, A.A. and RAMESH, M. 2013. Effect of storage temperatures on the quality of pink  
353 perch surimi. *Innovare Journal of Food Science*. 1(1).

354 GANDOTRA, R., KOUL, M., GUPTA, S. and SHARMA, S. 2012. Change In Proximate  
355 Composition And Microbial Count By Low Temperature preservation In Fish Muscle Of  
356 *Labeo Rohita* (Ham-Buch). IOSR J. Pharm. Biol. Sci. (IOSRJPBS) 2, 13–17.

357 HAARD, N.F. 1992. Control of chemical composition and food quality attributes of cultured  
358 fish. Food Research International. 25(4), 289-307.

359 HULTMANN, L. and RUSTAD, T. 2007. Effects of temperature abuse on textural properties  
360 and proteolytic activities during post mortem iced storage of farmed Atlantic cod (*Gadus*  
361 *morhua*). Food Chemistry. 104(4), 1687-1697.

362 HUSS, H.H. 1995. Quality and quality changes in fresh fish. *FAO fisheries technical paper*.  
363 (348).

364 JASRA, S.K., JASRA, P.K. and TALESARA, C.L. 2001. Myofibrillar protein degradation of  
365 carp (*Labeo rohita* (Hamilton)) muscle after post-mortem unfrozen and frozen storage.  
366 Journal of the Science of Food and Agriculture. 81(5), 519-524.

367 JIANG, M., WANG, Y., VAN SANTEN, E. and CHAPPELL, J.A. 2008. Evaluation of textural  
368 properties of channel catfish (*Ictalurus punctatus* Rafinesque) fillet with the natural  
369 contour method. LWT-Food Science and Technology. 41(9), 1548-1554.

370 JOL, S., KASSIANENKO, A., WSZOL, K. and OGGEL, J. 2006. Issues in time and temperature  
371 abuse of refrigerated foods. Food Safety. 11(6), 30-32.

372 LAKSHMINATHA, R., SETTY, T.M.R. and DORA, K.C. 1992. Studies on the storage  
373 behaviour of fish fingers from croakers and perches. Journal of Fishery Technology. 29,  
374 35-39.

375 LI, Y. and BELL, L.N. 2013. Color and Carotenoid Content Changes of Yellow Discolored  
376 Channel Catfish, *Ictalurus Punctatus*, Fillets During Refrigerated Storage. Journal of the  
377 World Aquaculture Society. 44, 148-53.

378 LIU, S., FAN, W., ZHONG, S., MA, C., LI, P., ZHOU, K., PENG, Z. and ZHU, M. 2010.  
379 Quality evaluation of tray-packed tilapia fillets stored at 0C based on sensory,  
380 microbiological, biochemical and physical attributes. African Journal of Biotechnology.  
381 9(5), 692-701.

382 LUDORF, M. and MEYER, W. 1973. *Fische und fischerzeugnisse*, Paul Parey Verlag,  
383 Hamburg-Berlin.

384 MARIA MACEDO VIEGAS, E., REGINA BARBIERI DE CARVALHO, M., ROBERTO  
385 CAMPAGNOLI DE OLIVEIRA FILHO, P., GABERZ KIRSCHNIK, P., SHINDY  
386 AIURA, F. and CRISTINA VARGAS, S. 2012. Changes During Chilled Storage of  
387 Whole Tilapia and Short-Term Frozen Storage of Tilapia Fillets. Journal of Aquatic Food  
388 Product Technology. 22(2), 192-200.

389 MARGEIRSSON, B., LAUZON, H.L., PÁLSSON, H., POPOV, V., GOSPAVIC, R.,  
390 JÓNSSON, M.P. and ARASON, S. 2012. Temperature fluctuations and quality  
391 deterioration of chilled cod (*Gadus morhua*) fillets packaged in different boxes stored on  
392 pallets under dynamic temperature conditions. International Journal of Refrigeration.  
393 35(1), 187-201.

394 MEAT STANDARDS COMMITTEE. 2002. Microbiological testing for process monitoring in  
395 the meat industry. October 30, 2002.

396 NAUTA, M.J., LITMAN, S., BARKER, G.C. and CARLIN, F. 2003. A retail and consumer  
397 phase model for exposure assessment of *Bacillus cereus*. International Journal of Food  
398 Microbiology. 83(2), 205-218.

399 NEWTON, K.G. and GELL, C.O. 1981. The microbiology of DFD fresh meats: A review. *Meat*  
400 *Science*. 5(3), 223-232.

401 NOTERMANS, S., DUFRENNE, J., TEUNIS, P., BEUMER, R., TE GIFFEL, M. and  
402 PEETERS WEEM, P. 1997. A risk assessment study of *Bacillus cereus* present in  
403 pasteurized milk. *Food Microbiology*. 14(2), 143-151.

404 ODOLI, C.O. 2009. Optimal storage conditions for fresh farmed tilapia (*Oreochromis niloticus*)  
405 fillets (Doctoral dissertation, University of Iceland).

406 OELENSCHLÄGER, J. 1992. Evaluation of some well established and some underrated indices  
407 for the determination of freshness and/or spoilage of ice stored wet fish. In Huss, H.H.  
408 (ed.) *Quality Assurance in the Fish Industry*. Elsevier Science Publishers.

409 OKEYO, G., LOKURUKA, M. and MATOFARI, J. 2009. Nutritional composition and shelflife  
410 of the lake victoria Nile perch (*Lates niloticus*) stored in ice. *African Journal of Food,*  
411 *Agriculture, Nutrition and Development*. 9(3).

412 ÖZOGUL, Y., ÖZYURT, G., ÖZOGUL, F., KULEY, E. and POLAT, A. 2005. Freshness  
413 assessment of European eel (*Anguilla anguilla*) by sensory, chemical and microbiological  
414 methods. *Food Chemistry*. 92(4), 745-751.

415 PEARSON, D. 1981. *Chemical Analysis of Food*, 8th Ed., Churchill Livingstone, Edinburgh.

416 PONS-SÁNCHEZ-CASCADO, S., VECIANA-NOGUÉS, M.T., BOVER-CID, S., MARINÉ-  
417 FONT, A. and VIDAL-CAROU, M.C. 2006. Use of volatile and non-volatile amines to  
418 evaluate the freshness of anchovies stored in ice. *Journal of the Science of Food and*  
419 *Agriculture*. 86(5), 699-705.

420 RAJU, C.V., DHANANJAYA, S. and REDDY, G.V.S. 1999. Preparation and cold storage  
421 studies on ready-to-fry sticks from pink perch (*Nemipterus japonicus*). *Journal of Aquatic*  
422 *Biology*. 14, 111-114.

423 SCHUBRING, R. 1999. DSC studies on deep frozen fishery products. *Thermochimica Acta*.  
424 337(1-2), 89-95.

425 SCHUBRING, R. 2002. Texture measurement on gutted cod during storage in ice using a hand-  
426 held instrument. *Informationen für die Fischwirtschaft aus der Fischereiforschung*. 49(1),  
427 25-27.

428 SHENOUDA, S.Y.K. 1980. Theories of protein denaturation during frozen storage of fish flesh.  
429 *Adv. Food Res.* 26, 275-311.

430 SIDDIQUE, M.N., HASAN, M.J., REZA, M.Z., ISLAM, M.R., BODURUZAMAN, M.,  
431 FORHADUR, M. and REZA, S. 2011. Effect of freezing time on nutritional value of  
432 Jatpunti (*Puntius sophore*), Sarpunti (*P. sarana*) and Thaisarpunti (*P. gonionotus*).  
433 *Bangladesh Res. Publ. J.* 5, 387-392.

434 SIMEONIDOU, S., GOVARIS, A. and VARELTZIS, K. 1997. Quality assessment of seven  
435 Mediterranean fish species during storage on ice. *Food Research International*. 30(7),  
436 479-484.

437 SINGH, R.K. and BALANGE, A.K. 2005. Characteristics of pink perch (*Nemipterus japonicus*)  
438 surimi at frozen temperature. *Journal of food processing and preservation*. 29(1), 75-83.

439 SPOONCER, W.F., SMITH, D.R. and POWELL, V.H. n.d. The effect of Chilling & Freezing on  
440 Meat Quality, CSIRO Meat Research Laboratory.

441 TSUCHIYA, H., KITA, S. and SEKI, N. 1992. Postmortem changes in  $\alpha$ -actinin and connectin  
442 in carp and rainbow trout muscles. *Nippon Suisan Gakkaishi*. 58, 793-798.

443 VANITHA, M., DHANAPAL, K., SRAVANI, K. and REDDY, G.V.S. 2013. Quality evaluation  
444 of value added mince based products from catla (*Catla catla*) during frozen storage.

445 XIA, X., KONG, B., LIU, Q. and LIU, J. 2009. Physicochemical change and protein oxidation in  
446 porcine longissimus dorsi as influenced by different freeze-thaw cycles. Meat Sci. 83,  
447 239-245.

448 Young, L.L. 1987. Next supermarket revolution - fresh prepared food. The National Provisioner.  
449 21 Nov., p. 28.

450 Zamir, R., Qasim, R. and Ullah, A. 1998. Changes in physical and chemical constituents of crab  
451 meat during storage at refrigerator temperature ( $7\pm 2^{\circ}\text{C}$ ). Pakistan Journal of  
452 Pharmaceutical Sciences. 11(1), 27-33.

453 Zakhariya S.Y., Fotedar R. and Prangnell D. 2014. Effects of Refreezing on Microbiological and  
454 Physicochemical Properties of Barramundi (*Lates calcarifer*, Bloch) Fillets. Journal of  
455 Food Processing and Preservation, In Press.

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475 TABLE 1.  
 476 CHANGES IN THE MOISTURE CONTENT % W.B., ASH CONTENT % AND PROTEIN  
 477 CONTENT % OF BARRAMUNDI (*LATES CALCARIFER*) FILLETS BEFORE FREEZING  
 478 (BF) AND PRE-BLAST FREEZING TREATMENTS AT 0°C, 5°C AND, -20°C FOR 0H, 1H,  
 479 1 DAY, 2 DAYS, 4 DAYS, 8 DAYS AND 16 DAYS.

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Moisture content %							
Treatment	0h	1h	1 day	2 days	4days	8days	16days
5°C	72.38±0.93 <sup>a</sup> <sub>A</sub>	72.52±0.39 <sup>a</sup> <sub>A</sub>	73.57±0.11 <sup>a</sup> <sub>A</sub>	73.92±0.12 <sup>a</sup> <sub>A</sub>	74.03±0.90 <sup>a</sup> <sub>A</sub>	75.34±0.09 <sup>ab</sup> <sub>B</sub>	77.62±0.12 <sup>b</sup> <sub>A</sub>
0°C	72.38±0.93 <sup>a</sup> <sub>A</sub>	71.78±0.31 <sup>a</sup> <sub>A</sub>	72.53±0.30 <sup>a</sup> <sub>A</sub>	72.27±0.05 <sup>a</sup> <sub>A</sub>	73.79±0.39 <sup>ab</sup> <sub>A</sub>	74.00±0.31 <sup>ab</sup> <sub>A</sub>	76.24±0.03 <sup>b</sup> <sub>B</sub>
-20°C	72.38±0.93 <sup>ab</sup> <sub>A</sub>	71.79±0.25 <sup>a</sup> <sub>A</sub>	72.45±0.96 <sup>ab</sup> <sub>A</sub>	73.08±0.74 <sup>abc</sup> <sub>A</sub>	74.69±1.09 <sup>abc</sup> <sub>A</sub>	75.37±0.40 <sup>bc</sup> <sub>A</sub>	75.55±0.16 <sup>c</sup> <sub>C</sub>
Ash content %							
Treatment	0h	1h	1day	2days	4days	8days	16days
5 °C	1.02±0.04 <sup>abc</sup> <sub>A</sub>	1.18±0.08 <sup>bc</sup> <sub>A</sub>	1.24±0.07 <sup>c</sup> <sub>A</sub>	0.96±0.01 <sup>ab</sup> <sub>B</sub>	0.92±0.01 <sup>a</sup> <sub>B</sub>	0.91±0.02 <sup>a</sup> <sub>B</sub>	0.90±0.00 <sup>a</sup> <sub>B</sub>
0 °C	1.02±0.04 <sup>a</sup> <sub>A</sub>	1.11±0.05 <sup>ab</sup> <sub>A</sub>	1.26±0.07 <sup>b</sup> <sub>A</sub>	1.02±0.00 <sup>a</sup> <sub>B</sub>	1.10±0.03 <sup>ab</sup> <sub>A</sub>	1.00±0.02 <sup>a</sup> <sub>A</sub>	0.97±0.02 <sup>a</sup> <sub>A</sub>
-20 °C	1.02±0.04 <sup>ac</sup> <sub>A</sub>	1.10±0.04 <sup>ac</sup> <sub>A</sub>	1.33±0.03 <sup>b</sup> <sub>A</sub>	1.18±0.06 <sup>bc</sup> <sub>A</sub>	1.09±0.04 <sup>ac</sup> <sub>A</sub>	0.96±0.02 <sup>ac</sup> <sub>AB</sub>	0.92±0.01 <sup>a</sup> <sub>AB</sub>
Protein content %							
Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	62.54±0.47 <sup>a</sup> <sub>A</sub>	61.15±0.29 <sup>a</sup> <sub>A</sub>	60.26±0.36 <sup>ab</sup> <sub>B</sub>	60.63±0.11 <sup>a</sup> <sub>AB</sub>	58.05±0.37 <sup>bc</sup> <sub>B</sub>	57.33±0.22 <sup>c</sup> <sub>C</sub>	55.99±0.16 <sup>c</sup> <sub>C</sub>
0°C	62.54±0.47 <sup>a</sup> <sub>A</sub>	62.23±0.63 <sup>a</sup> <sub>A</sub>	62.03±0.50 <sup>a</sup> <sub>AB</sub>	61.08±0.69 <sup>ab</sup> <sub>B</sub>	61.28±0.20 <sup>ab</sup> <sub>C</sub>	61.27±0.27 <sup>ab</sup> <sub>B</sub>	59.26±0.36 <sup>b</sup> <sub>B</sub>
-20°C	62.54±0.47 <sup>ab</sup> <sub>A</sub>	61.47±0.72 <sup>a</sup> <sub>A</sub>	62.53±0.71 <sup>ab</sup> <sub>A</sub>	63.04±0.89 <sup>abc</sup> <sub>A</sub>	64.25±0.53 <sup>bc</sup> <sub>A</sub>	64.98±0.31 <sup>bc</sup> <sub>A</sub>	65.08±0.02 <sup>c</sup> <sub>A</sub>

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

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

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Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	6.34±0.00 <sup>a</sup> <sub>A</sub>	6.34±0.01 <sup>a</sup> <sub>A</sub>	6.49±0.00 <sup>cde</sup> <sub>B</sub>	6.51±0.00 <sup>def</sup> <sub>A</sub>	6.56±0.01 <sup>efg</sup> <sub>B</sub>	6.63±0.00 <sup>gh</sup> <sub>B</sub>	6.78±0.00 <sup>i</sup> <sub>C</sub>
0°C	6.34±0.00 <sup>a</sup> <sub>A</sub>	6.39±0.02 <sup>ab</sup> <sub>A</sub>	6.42±0.01 <sup>abcd</sup> <sub>A</sub>	6.46±0.01 <sup>bcd</sup> <sub>A</sub>	6.55±0.00 <sup>efg</sup> <sub>B</sub>	6.58±0.00 <sup>fg</sup> <sub>B</sub>	6.68±0.00 <sup>h</sup> <sub>B</sub>
-20°C	6.34±0.00 <sup>a</sup> <sub>A</sub>	6.38±0.02 <sup>ab</sup> <sub>A</sub>	6.40±0.00 <sup>abc</sup> <sub>A</sub>	6.44±0.02 <sup>bcd</sup> <sub>A</sub>	6.41±0.04 <sup>abc</sup> <sub>A</sub>	6.44±0.01 <sup>bcd</sup> <sub>A</sub>	6.49±0.01 <sup>cde</sup> <sub>A</sub>

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

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530 TABLE 3.  
 531 CHANGES IN THE TVBN OF BARRAMUNDI (*LATES CALCARIFER*) FILLETS BEFORE  
 532 FREEZING (BF) AND PRE-BLAST FREEZING TREATMENTS AT 0°C, 5°C AND, -20°C  
 533 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±0.02 <sup>a</sup> <sub>A</sub>	10.45±0.02 <sup>cd</sup> <sub>B</sub>	12.01±0.05 <sup>f</sup> <sub>B</sub>	17.55±0.11 <sup>g</sup> <sub>C</sub>	29.28±0.22 <sup>i</sup> <sub>C</sub>	41.46±0.22 <sup>j</sup> <sub>C</sub>	54.14±0.18 <sup>l</sup> <sub>C</sub>
0°C	6.25±0.02 <sup>a</sup> <sub>A</sub>	9.61±0.23 <sup>bc</sup> <sub>A</sub>	12.38±0.19 <sup>f</sup> <sub>B</sub>	12.51±0.25 <sup>f</sup> <sub>B</sub>	17.37±0.07 <sup>g</sup> <sub>B</sub>	19.17±0.05 <sup>h</sup> <sub>B</sub>	49.19±0.05 <sup>k</sup> <sub>B</sub>
-20°C	6.25±0.02 <sup>a</sup> <sub>A</sub>	9.44±0.22 <sup>b</sup> <sub>A</sub>	10.14±0.01 <sup>bcd</sup> <sub>A</sub>	10.24±0.06 <sup>bcd</sup> <sub>A</sub>	10.56±0.30 <sup>d</sup> <sub>A</sub>	10.77±0.27 <sup>de</sup> <sub>A</sub>	11.63±0.23 <sup>ef</sup> <sub>A</sub>

537 All values are the means ± SE of four replicates, n=4  
 538 Values followed by different superscript letters in the same row are significantly different at α=0.05  
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573 TABLE 4.  
 574 CHANGES IN THE TVC OF BARRAMUNDI (*LATES CALCARIFER*) FILLETS BEFORE  
 575 FREEZING (BF) AND PRE-BLAST FREEZING TREATMENTS AT 0°C, 5°C AND, -20°C  
 576 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±0.03 <sup>a</sup> <sub>A</sub>	2.62±0.15 <sup>ab</sup> <sub>A</sub>	3.33±0.16 <sup>bc</sup> <sub>A</sub>	3.42±0.22 <sup>cd</sup> <sub>A</sub>	3.76±0.08 <sup>cd</sup> <sub>A</sub>	6.38±0.12 <sup>f</sup> <sub>B</sub>	8.58±0.20 <sup>g</sup> <sub>B</sub>
0°C	2.44±0.03 <sup>a</sup> <sub>A</sub>	2.61±0.18 <sup>ab</sup> <sub>A</sub>	3.29±0.14 <sup>bc</sup> <sub>A</sub>	3.49±0.23 <sup>cd</sup> <sub>A</sub>	5.11±0.12 <sup>e</sup> <sub>B</sub>	8.17±0.33 <sup>g</sup> <sub>C</sub>	9.96±0.12 <sup>h</sup> <sub>C</sub>
-20°C	2.44±0.03 <sup>a</sup> <sub>A</sub>	2.51±0.06 <sup>a</sup> <sub>A</sub>	3.52±0.07 <sup>cd</sup> <sub>A</sub>	3.73±0.09 <sup>cd</sup> <sub>A</sub>	3.80±0.02 <sup>cd</sup> <sub>A</sub>	3.86±0.05 <sup>cd</sup> <sub>A</sub>	4.18±0.06 <sup>d</sup> <sub>A</sub>

580 All values are the means ± SE of four replicates, n=4  
 581 Values followed by different superscript letters in the same row are significantly different at α=0.05  
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616 TABLE 5:  
 617 CHANGES IN THE L\*, A\* AND B\* VALUES OF BARRAMUNDI (*LATES CALCARIFER*)  
 618 FILLETS BEFORE FREEZING (BF) AND PRE-BLAST FREEZING TREATMENTS AT 0°C,  
 619 5°C AND, -20°C FOR 0H, 1H, 1 DAY, 2 DAYS, 4 DAYS, 8 DAYS AND 16 DAYS.  
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L\*

Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	50.19±0.00 <sup>a</sup> <sub>A</sub>	51.32±0.04 <sup>b</sup> <sub>A</sub>	52.38±0.09 <sup>c</sup> <sub>A</sub>	52.78±0.05 <sup>c</sup> <sub>A</sub>	54.81±0.11 <sup>e</sup> <sub>A</sub>	56.48±0.18 <sup>f</sup> <sub>A</sub>	58.89±0.01 <sup>g</sup> <sub>A</sub>
0°C	50.19±0.00 <sup>a</sup> <sub>A</sub>	50.68±0.10 <sup>a</sup> <sub>B</sub>	50.31±0.08 <sup>a</sup> <sub>C</sub>	51.49±0.13 <sup>b</sup> <sub>B</sub>	52.44±0.08 <sup>c</sup> <sub>B</sub>	53.51±0.12 <sup>d</sup> <sub>B</sub>	56.64±0.14 <sup>f</sup> <sub>B</sub>
-20°C	50.19±0.00 <sup>a</sup> <sub>A</sub>	50.17±0.03 <sup>a</sup> <sub>C</sub>	51.56±0.11 <sup>b</sup> <sub>B</sub>	51.54±0.19 <sup>b</sup> <sub>B</sub>	52.77±0.08 <sup>c</sup> <sub>B</sub>	53.65±0.17 <sup>d</sup> <sub>B</sub>	54.62±0.07 <sup>e</sup> <sub>C</sub>

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 622 a\*  
 623

Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	-2.43±0.16 <sup>a</sup> <sub>A</sub>	-2.23±0.02 <sup>b</sup> <sub>AB</sub>	-2.47±0.05 <sup>a</sup> <sub>B</sub>	-1.74±0.04 <sup>c</sup> <sub>A</sub>	-1.50±0.03 <sup>d</sup> <sub>A</sub>	-1.42±0.20 <sup>d</sup> <sub>A</sub>	-0.54±0.01 <sup>e</sup> <sub>A</sub>
0°C	-2.43±0.16 <sup>a</sup> <sub>A</sub>	-2.37±0.06 <sup>a</sup> <sub>B</sub>	-2.49±0.10 <sup>a</sup> <sub>B</sub>	-1.24±0.01 <sup>e</sup> <sub>A</sub>	-1.20±0.02 <sup>e</sup> <sub>A</sub>	-1.37±0.16 <sup>e</sup> <sub>A</sub>	-1.47±0.19 <sup>f</sup> <sub>B</sub>
-20°C	-2.43±0.16 <sup>a</sup> <sub>A</sub>	-2.17±0.02 <sup>b</sup> <sub>A</sub>	-2.11±0.00 <sup>b</sup> <sub>A</sub>	-2.34±0.21 <sup>a</sup> <sub>B</sub>	-2.40±0.16 <sup>a</sup> <sub>B</sub>	-2.13±0.01 <sup>b</sup> <sub>B</sub>	-2.06±0.01 <sup>c</sup> <sub>C</sub>

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 625  
 626 b\*  
 627

Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	0.28±0.00 <sup>a</sup> <sub>A</sub>	0.32±0.00 <sup>a</sup> <sub>A</sub>	0.57±0.06 <sup>ab</sup> <sub>A</sub>	1.17±0.02 <sup>c</sup> <sub>AB</sub>	2.51±0.03 <sup>d</sup> <sub>A</sub>	3.48±0.16 <sup>ef</sup> <sub>A</sub>	5.52±0.20 <sup>g</sup> <sub>A</sub>
0°C	0.28±0.00 <sup>a</sup> <sub>A</sub>	0.32±0.02 <sup>a</sup> <sub>A</sub>	0.52±0.09 <sup>a</sup> <sub>A</sub>	1.00±0.05 <sup>bc</sup> <sub>B</sub>	2.22±0.14 <sup>d</sup> <sub>A</sub>	3.23±0.04 <sup>e</sup> <sub>A</sub>	3.92±0.03 <sup>f</sup> <sub>B</sub>
-20°C	0.28±0.00 <sup>a</sup> <sub>A</sub>	0.33±0.00 <sup>a</sup> <sub>A</sub>	0.43±0.08 <sup>a</sup> <sub>A</sub>	1.30±0.11 <sup>c</sup> <sub>A</sub>	2.25±0.10 <sup>d</sup> <sub>A</sub>	2.37±0.07 <sup>d</sup> <sub>B</sub>	3.52±0.13 <sup>ef</sup> <sub>B</sub>

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

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649 TABLE 6.  
 650 CHANGES IN THE HARDNESS (N), COHESIVENESS, SPRINGINESS (CM),  
 651 GUMMINESS (KGF), CHEWINESS (KGF.MM), AND STIFFNESS (KGF/MM) OF  
 652 BARRAMUNDI (*LATES CALCARIFER*) FILLETS BEFORE FREEZING (BF) AND PRE-  
 653 BLAST FREEZING TREATMENTS AT 0°C, 5°C AND, -20°C FOR 0H, 1H, 1 DAY, 2 DAYS,  
 654 4 DAYS, 8 DAYS AND 16 DAYS.

Hardness (N)							
Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	1.98±0.04 <sup>a</sup> <sub>A</sub>	1.76±0.01 <sup>b</sup> <sub>A</sub>	1.68±0.02 <sup>bc</sup> <sub>A</sub>	1.66±0.00 <sup>bc</sup> <sub>B</sub>	1.59±0.02 <sup>cc</sup> <sub>B</sub>	1.46±0.01 <sup>d</sup> <sub>C</sub>	1.46±0.02 <sup>de</sup> <sub>B</sub>
0°C	1.98±0.04 <sup>a</sup> <sub>A</sub>	1.72±0.02 <sup>b</sup> <sub>A</sub>	1.71±0.00 <sup>b</sup> <sub>A</sub>	1.71±0.01 <sup>b</sup> <sub>B</sub>	1.64±0.02 <sup>b</sup> <sub>B</sub>	1.60±0.02 <sup>bc</sup> <sub>B</sub>	1.48±0.00 <sup>c</sup> <sub>B</sub>
-20°C	1.98±0.04 <sup>a</sup> <sub>A</sub>	1.79±0.02 <sup>b</sup> <sub>A</sub>	1.75±0.01 <sup>b</sup> <sub>A</sub>	1.84±0.0 <sup>b</sup> <sub>A</sub>	1.84±0.01 <sup>b</sup> <sub>A</sub>	1.84±0.01 <sup>b</sup> <sub>A</sub>	1.76±0.01 <sup>b</sup> <sub>A</sub>
Cohesiveness							
Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	0.08±0.00 <sup>a</sup> <sub>A</sub>	0.07±0.00 <sup>a</sup> <sub>A</sub>	0.06±0.00 <sup>a</sup> <sub>A</sub>	0.01±0.00 <sup>b</sup> <sub>B</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>
0°C	0.08±0.00 <sup>a</sup> <sub>A</sub>	0.07±0.00 <sup>a</sup> <sub>A</sub>	0.05±0.00 <sup>ab</sup> <sub>A</sub>	0.03±0.00 <sup>bc</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>
-20°C	0.08±0.00 <sup>a</sup> <sub>A</sub>	0.04±0.01 <sup>b</sup> <sub>A</sub>	0.01±0.00 <sup>bc</sup> <sub>A</sub>	0.00±0.00 <sup>c</sup> <sub>B</sub>	0.00±0.00 <sup>c</sup> <sub>A</sub>	0.00±0.00 <sup>c</sup> <sub>A</sub>	0.00±0.00 <sup>c</sup> <sub>A</sub>
Springiness (cm)							
Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	0.08±0.00 <sup>a</sup> <sub>A</sub>	0.03±0.00 <sup>b</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>
0°C	0.08±0.00 <sup>a</sup> <sub>A</sub>	0.04±0.00 <sup>b</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>
-20°C	0.08±0.00 <sup>a</sup> <sub>A</sub>	0.02±0.00 <sup>b</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>A</sub>
Gumminess (kgf)							
Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	0.10±0.00 <sup>a</sup> <sub>A</sub>	0.05±0.02 <sup>ab</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>
0°C	0.10±0.00 <sup>a</sup> <sub>A</sub>	0.07±0.02 <sup>a</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>
-20°C	0.10±0.00 <sup>a</sup> <sub>A</sub>	0.07±0.02 <sup>a</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>	0.00±0.00 <sup>b</sup> <sub>A</sub>
Chewiness (kgf.mm)							
Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	0.04±0.00 <sup>a</sup> <sub>A</sub>	0.02±0.00 <sup>ab</sup> <sub>A</sub>	0.02±0.00 <sup>ab</sup> <sub>A</sub>	0.01±0.00 <sup>ab</sup> <sub>B</sub>	0.01±0.00 <sup>b</sup> <sub>B</sub>	0.01±0.00 <sup>b</sup> <sub>A</sub>	0.01±0.00 <sup>b</sup> <sub>A</sub>
0°C	0.04±0.00 <sup>ab</sup> <sub>A</sub>	0.03±0.00 <sup>ab</sup> <sub>AB</sub>	0.05±0.00 <sup>a</sup> <sub>A</sub>	0.05±0.00 <sup>a</sup> <sub>A</sub>	0.03±0.00 <sup>ab</sup> <sub>A</sub>	0.01±0.00 <sup>b</sup> <sub>A</sub>	0.01±0.00 <sup>b</sup> <sub>A</sub>
-20°C	0.04±0.00 <sup>a</sup> <sub>A</sub>	0.03±0.01 <sup>ab</sup> <sub>A</sub>	0.02±0.00 <sup>ab</sup> <sub>A</sub>	0.02±0.00 <sup>ab</sup> <sub>B</sub>	0.01±0.00 <sup>b</sup> <sub>B</sub>	0.01±0.00 <sup>b</sup> <sub>A</sub>	0.01±0.00 <sup>b</sup> <sub>A</sub>
Stiffness (kgf/mm)							
Treatment	0h	1h	1day	2days	4days	8days	16days
5°C	0.25±0.01 <sup>a</sup> <sub>A</sub>	0.06±0.00 <sup>b</sup> <sub>B</sub>	0.06±0.00 <sup>b</sup> <sub>B</sub>	0.04±0.00 <sup>bc</sup> <sub>A</sub>	0.01±0.00 <sup>c</sup> <sub>B</sub>	0.02±0.00 <sup>bc</sup> <sub>A</sub>	0.01±0.00 <sup>bc</sup> <sub>A</sub>
0°C	0.25±0.01 <sup>a</sup> <sub>A</sub>	0.07±0.00 <sup>b</sup> <sub>AB</sub>	0.07±0.00 <sup>bc</sup> <sub>AB</sub>	0.05±0.01 <sup>bcd</sup> <sub>A</sub>	0.03±0.00 <sup>cd</sup> <sub>AB</sub>	0.02±0.00 <sup>d</sup> <sub>A</sub>	0.02±0.00 <sup>d</sup> <sub>A</sub>
-20°C	0.25±0.01 <sup>a</sup> <sub>A</sub>	0.10±0.00 <sup>b</sup> <sub>A</sub>	0.09±0.00 <sup>b</sup> <sub>A</sub>	0.06±0.01 <sup>bc</sup> <sub>A</sub>	0.04±0.00 <sup>c</sup> <sub>A</sub>	0.03±0.00 <sup>c</sup> <sub>A</sub>	0.03±0.00 <sup>c</sup> <sub>A</sub>

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