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Effect of Chilled-frozen Storage on the Physico-chemical, Microbial and Sensory Quality of Farmed Bighead Carp (*Hypophthalmichthys nobilis*)

Laila D. Latip, Wahidu Zzaman and Tajul A. Yang

Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 Pulau Pinang, Malaysia

Corresponding Author: Tajul A. Yang, Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 Pulau Pinang, Malaysia Tel: 604-6532224 Fax: 604-6573673

ABSTRACT

Aquaculture and its further processing of Bighead Carp has become an growing economic importance in Malaysia. The present study was carried out to know the effects of chilled-frozen storage on the quality of Bighead Carp (*Hypophthalmichthys nobilis*). The quality changes in Bighead Carp were evaluated by conducting proximate analysis, oxidative stability determination (TBA Test), microbiological analysis, texture profile analysis, colour measurement and sensory evaluation on fish stored in ice for 1, 4 and 7 days before 4 weeks of frozen storage. The results showed that there was a significant increase ($p < 0.05$) in moisture content on the seventh day of ice storage before freezing. The content of protein and ash decreased significantly and TBA value of both red and white fish muscle increased significantly ($p < 0.05$) on the seventh day of ice storage before freezing. There were no significant different in fat, texture, yeast, aerobic plate (APC) and mould count (PDA). The sensory evaluation also showed that there were no significant differences on overall acceptability among fish ice stored for 1, 4 and 7 days before freezing. The study concluded that the quality of Bighead Carp can be maintained after 4 days of ice storage before 4 weeks of frozen storage.

Key words: Bighead carp, physico-chemical, microbial, sensory evaluation, chilled-frozen storage

INTRODUCTION

Bighead Carp is a species that have fast growth rate, easy cultivation and high feed efficiency ratio which causes demand in commercial interest for this species becomes increasing important due to its beneficial features (Bell *et al.*, 2003). In recent years, the increasing production of this species as an aquaculture product has made it more available to consumers. Along with black carp, grass carp and silver carp, Bighead carp is one of the four main cultured fish species grown in China (Wang *et al.*, 2008). Since the processing or retail places are located far from the fish farm and large amount of fish harvested per batch and lack of freezing equipments in fish farm, therefore, there is need to store the fish in ice to preserve its freshness and quality before freezing or processing. Preservation by chilling and freezing are widely accepted by consumers, even though other methods for preservation such as salting, drying, canning and smoking are applied (Mehta *et al.*, 2011). Chilling does not prevent spoilage but reducing the body temperature will help to prolong the storing quality of fish (Pigott and Tucker, 1990). The colder the fish the better storing process,

because it will help in minimize activities of microbial or enzymatic spoilage (Tawari and Abowei, 2011). It is necessary that a sufficiently cold surrounding must be supplied to effect this change (Davies, 2006). Biochemical changes are identified to take place, as for example changes in the lipid and protein fractions during fish chilled and frozen storage. By enzymatic action, sugar is formed with the amino acids and forms the brownish or yellowish colour (Azeza, 1976). It has great impact on the nutritious value of fish and the health of consumers for deterioration of fish during refrigerator storage (Gandotra *et al.*, 2012). There is no information about the effects of ice and frozen storage on quality of Bighead Carp. Only few studies have done on the effects of ice storage before frozen storage which one of the studies was for scad (Smith *et al.*, 1978) but no study for Bighead Carp.

The main objective of this research is to analyze the effects of chilled-frozen storage (undergo ice storage before freezing and frozen storage) on the quality of Bighead Carp in terms of proximate composition, degree of rancidity, total microbial load, texture, colour and sensory.

MATERIALS AND METHODS

Design of experiment: Randomized Complete Block Design (RCBD) was applied to run the experiment due to the limited raw material and time. The fish were arranged into 2 groups randomly of more or less uniform experimental units. RCBD can reduce the experimental error as small as possible which is the variability between among the fish as well as provide estimation of error from the experiments (Montgomery, 2001).

Sample preparation: After the 1 year-old fish were harvested in the morning; the fish were transported in ice to the laboratory 10 h after catching. The fish specimens were neither headed nor gutted and were directly placed in ice at $4\pm 1^{\circ}\text{C}$. The length of the specimens was in the length of 85-90 cm and the weight was 3-3.5 kg. In the afternoon of the same day, the fish were taken out from the ice storage, wrapped individually in aluminium foil and being quick frozen in air blast freezer at -40°C for 1 h before they were stored in a freezer (-18°C) for 4 weeks. The same method was applied to other fish after 4 days and 7 days of ice storage. During ice storage, the ice was replenished at least once per day. After 4 weeks (counted starting from the 1st day the fish were caught), all the fish were taken out from the freezer and thawed for overnight at 4°C for the analyses. All analyses were performed in triplicates unless stated.

Firstly, the skin were gutted and cut into 4 logs described by Espe *et al.* (2004). Then, each log will be trimmed by hand to remove skin, bones, fins off and the red muscle was collected prior to the preparation of white muscle samples. The white muscle from the 1st log and 4th log was used for oxidative stability determination (TBA test) and proximate analysis respectively. Only the red muscle from block 2 was used for oxidative stability determination (TBA test). Whereas the white muscle from 2nd and 3rd log was trimmed into 15 muscle blocks of approximately equal size. Then muscle cubes from 2nd log were used for sensory test while small fish cubes from 3rd log were used for colour and texture analysis.

Proximate analysis: Proximate analysis was conducted on white muscle of fish for both sample A and sample B which included analysis of moisture, crude protein, crude fat and ash. The moisture content of fish from sample A and B was determined by using direct oven method (AOAC, 1990). Protein content of fish from sample A and B was determined by using Micro Kjeldahl Method (AOAC, 1990). Fat content was determined using Soxhlet Extraction Method (AOAC, 1990). Ash content was determined using Atmosphere Oven Drying Method (AOAC, 1990).

The oxidative stability determination of fish samples was conducted based on 2-thiobarbituric acid test (TBA test) which was described by Tarladgis *et al.* (1964) to determine the extent of lipid oxidation in fish sample using UV-Vis spectrophotometer (Model-UV-1601PC, *Shidmazu*, Japan) at 530 μm and the result was expressed as mg malonaldehyde per kg sample.

Microbiological analysis: Microbial analysis was conducted by referring the standard method described in Bacteriological Analysis Manual 7th edition. In sample preparation for microbial analysis, modification was made based on standard method whereby 10 g of sample was used instead of 25 g due to the limitation of samples.

The total Plate count, Yeast and Mould count were counted using colony counter (Suntex Model 560). The results were expressed as CFU g^{-1} of fish flesh.

Texture profile analysis: Texture profile analysis of fish samples was conducted by using texture analyser (TA-XT2 Texture Analyzer, UK), interfaced with microcomputer and equipped with 25 kg load cell and 5 mm cylinder compression probe. Determination of textural parameter was calculated from TPA curves. The parameters measured in this analysis were hardness (kg), cohesiveness, elasticity (mm), gumminess (kg) and chewiness (kg mm).

Colour measurement: Colorimeter (*Minolta spectrophotometer*, Model CM-3500d, Osaka, Japan) was used to measure the colour of the white muscle of fish samples. The instrument was calibrated first using the zero calibration box (CM-A120) and white calibration plate (CM-A128).

Sensory evaluation: Sensory evaluation for steamed fish samples were conducted by 15 panellists (non-trained) by using a nine-point scale Hedonic Test. The attributes studied were colour, odour, flavour, juiciness, texture and overall acceptability.

Statistical analysis: All the data were analyzed statistically by means and one-way ANOVA (for comparing more than 2 means) at 5% significant level. Duncan test was also conducted to perform comparison between treatments at 5% significant level. For determination of significant differences between 2 blocks, univariate of general linear model was conducted at 5% significant level. All statistical analyses were conducted by using SPSS 12.0 for Windows (SPSS Inc., *Chicago, Illinois*).

RESULTS AND DISCUSSION

Proximate analysis: The proximate composition depends on a few factors such as genetic factor, feeding, fish size and environmental conditions. However, these factors can be controlled during the production of farmed fish. During fish chilled storage, biochemical changes such as changes in protein and lipid fractions will occur.

Moisture content: Moisture content of Bighead Carp did not show significant difference between Block 1 and 2. Moisture content of Bighead Carp showed significant increase ($p < 0.05$) on 7th day of ice stored before freezing. According to Table 1, the moisture content dropped on the 4th day of ice storage before freezing but the value was not significantly difference between 1st and 4th days. However, it rose again on the 7th day of ice storage until it reached the highest. According to Kirschnik *et al.* (2006), moisture content was constant for 14 days in samples of tail meat of the giant river prawn (*Macrobrachium rosenbergii*) stored without direct contact in ice while in

Table 1: Moisture content (dry basis) of bighead carp after chilled-frozen storage

| Parameters | Block | Iced 1 day | Iced 4 days | Iced 7 days |
|--------------|-------|-------------------------|-------------------------|-------------------------|
| Moisture (%) | 1 | 78.76±0.23 ^a | 77.67±1.00 ^a | 80.38±0.14 ^b |
| | 2 | 79.26±0.45 ^a | 78.75±0.19 ^a | 80.46±0.04 ^b |

Values with different letters are significantly different at $p = 0.05$

Table 2: Fat content (dry basis) of bighead carp after chilled-frozen storage

| Parameters | Block | Iced 1 day | Iced 4 days | Iced 7 days |
|------------|-------|------------------------|-------------------------|------------------------|
| Fat (%) | 1 | 1.62±0.79 ^a | 1.02±0.28 ^{ab} | 0.53±0.12 ^b |
| | 2 | 0.84±0.21 ^a | 0.82±0.10 ^a | 0.54±0.37 ^a |

Values with different letters are significantly different at $p = 0.05$

samples stored in direct contact with ice it increased approximately 6% revealing absorption of water through exposed surface of meat and decrease in solid content (crude protein) during early stages of storage in ice.

Fat content: Bighead Carp is a kind of lean fish since its fat content in muscle is lower than 2% (Johnsen and Lloyd, 1992). Comparison between moisture and fat content in Bighead Carp showed a correlation between the results which correlate well with the expected inverse ratio between water and lipid matter as it had been previously described (Aubourg *et al.*, 2005). Increasing in water content may increase the rate of lipid hydrolysis, thus reduce the fat content.

During chilled-frozen storage, the fat content in Bighead Carp was decreasing (Table 2). Arannilewa *et al.* (2005) calculated decreasing in total lipid content in Tilapia after storing it in freezer compartment and associated the changes in fat content during frozen storage with the oxidation of fat. Siddique *et al.* (2011) observed decreasing in total lipid content while assessing the effect of freezing time on nutritional value of *Puntius sophore*, *P. sarana* and *P. gonionotus* during the frozen storage at -5°C of 20 days. This was due to both lipid oxidation and hydrolysis had taken place during ice and cold storage.

However, the decrease was significant ($p < 0.05$) in Block 1 but not significant in Block 2 until 7 days of ice storage before freezing. Fat content in flesh showed an inverse correlation with total microbial load. According to Shewan (1961), at the initial stage of microbial spoilage, large amount of lipases enzyme was produced which it break down lipids to form fatty acids, glycerol and other compounds. Therefore, the significant decrease of fat content in Block 1 may due to the higher total microbial load. Aerobic plate count for samples in both Block 1 and 2 on seventh day of ice storage before freezing were 8.4×10^4 and 4.8×10^4 CFU g^{-1} , respectively whilst the yeast and mould count for samples in Block 1 on seventh day of ice storage before freezing was 6.8×10^8 CFU g^{-1} while for Block 2 was 1.5×10^8 CFU g^{-1} .

Protein content: The protein content in Bighead Carp showed a significant decrease ($p < 0.05$) throughout chilled-frozen storage (Table 3). During investigation decreasing trend was observed in muscle of *Mystus seenghala* in chilled sample and frozen samples (Gandotra *et al.*, 2012). This decrease was rapid and higher in flesh stored at -10°C compared with those stored at -30°C . Besides, the decrease in protein solubility could be due to protein denatured and protein aggregation induced by frozen storage. The stabilisation of myofibrillar proteins is directly related to better fish quality (Martinez *et al.*, 2001). Thus the decreasing in protein content showed that there was a decreasing in fish quality during ice and cold storage.

Table 3: Protein content (dry basis) of Bighead Carp after chilled-frozen storage

| Parameters | Block | Iced 1 day | Iced 4 days | Iced 7 days |
|-------------|-------|-------------------------|-------------------------|------------------------|
| Protein (%) | 1 | 18.38±0.75 ^a | 12.58±1.84 ^b | 5.39±2.75 ^c |
| | 2 | 17.68±4.75 ^a | 10.94±2.15 ^b | 6.71±2.34 ^b |

Values with different letters are significantly different at $p = 0.05$

Table 4: Ash content (dry basis) of Bighead Carp after chilled-frozen storage

| Parameters | Block | Iced 1 day | Iced 4 days | Iced 7 days |
|------------|-------|------------------------|------------------------|------------------------|
| Ash (%) | 1 | 0.88±0.10 ^a | 0.87±0.06 ^a | 0.57±0.12 ^b |
| | 2 | 0.78±0.05 ^a | 0.76±0.05 ^a | 0.50±0.04 ^b |

Values with different letters are significantly different at $p = 0.05$

The type of protein change following freezing and storage in the frozen state is made up from a partial decomposition of molecular and the formation of new primary and secondary linkages. Long term frozen storage normally results in a marked hardening of the flesh, considered to be due to crossing-over of the fibrillary proteins.

Protein content in flesh also showed an inverse correlation with microbial load and degree of oxidation (TBA value) during chilled-frozen storage. According to Kandeepan and Biswas (2007) the lower protein content of chilled meat was due to increased microbial growth resulted from higher water activity (a_w) and enzymatic autolysis moreover on frozen storage the protein content was decreased due to protein denaturation and proteolysis induced by enzymatic activities of psychrotrophic microbial growth.

Ash content: During chilled-frozen storage, the ash content in Bighead Carp showed a significant decrease (Table 4). Okeyo *et al.* (2009) observed that the ash context of the frozen raw Nile perch decreases with storage time. Ash may lose during ice storing by leaching out through the skin to melting ice. Besides, Garcia-Arias *et al.* (2003) suggested that during thawing, decreasing in ash content was caused by lixiviation (leaching).

Oxidative stability determination (TBA test): TBA is a good indicator of the quality of the fish, commonly used for evaluation of oil stability and monitoring of deterioration during storage whether it was frozen, chilled or stored with ice (Tarladgis *et al.*, 1960; Vareltzis *et al.*, 1988). According to Scormuller (1969), the maximum TBA value used to indicate the good quality of the fish frozen, chilled or stored with ice is 5 mg malonaldehyde/kg while the fish may be consumed up to level of 8 mg malonaldehyde/kg.

TBA value of white muscle did not different significantly between Block 1 and 2. Table 5, showed the TBA values for white muscle which had been stored in ice for 1 and 4 days before freezing were within the good quality limits which is below 5 mg malonaldehyde/kg. However, the white muscle which had been stored in ice for 7 days before freezing showed a TBA value higher than 8 mg malonaldehyde/kg. Thus, it was regarded as poor in quality according to the standards. This result did not correlate with the result from sensory evaluation where the odour, flavour and overall acceptability by consumers did not show significant different among sample stored for 1, 4 and 7 days in ice before freezing. On the other hand, the quality of red muscle which had been stored in ice for 4 and 7 days before freezing was regarded as poor as their TBA values were exceed 8 mg malonaldehyde/kg as shown in Table 5. TBA test had only done on red muscle in Block 2 due

Table 5: TBA value (mg malonaldehyde/kg) of Bighead Carp after chilled-frozen storage

| Parameters | Block | Iced 1 day | Iced 4 days | Iced 7 days |
|--------------|-------|------------------------|-------------------------|-------------------------|
| White muscle | 1 | 2.10±1.23 ^a | 4.75±2.72 ^a | 12.19±1.81 ^b |
| | 2 | 2.05±0.50 ^a | 4.49±0.80 ^a | 12.97±3.28 ^b |
| Red muscle | 2 | 6.24±1.15 ^a | 11.80±1.17 ^b | 22.58±1.92 ^c |

Values with different letters are significantly different at $p = 0.05$

Table 6: Aerobic plate count (APC) and yeast and mould count (PDA) in fish white muscle of bighead carp after chilled-frozen storage

| Parameters | Block | Iced 1 day | Iced 4 days | Iced 7 days | Significance |
|----------------------------|-------|----------------------|---------------------|---------------------|--------------|
| APC (CFU g ⁻¹) | 1 | 3.20×10 ⁴ | 6.6×10 ⁴ | 8.4×10 ⁴ | 0.136 |
| | 2 | 1.80×10 ⁴ | 4.5×10 ⁴ | 4.8×10 ⁴ | 0.738 |
| PDA (CFU g ⁻¹) | 1 | 1.00×10 ³ | 2.0×10 ³ | 6.8×10 ³ | 0.577 |
| | 2 | 0.58×10 ³ | 1.0×10 ³ | 1.5×10 ³ | 0.245 |

to insufficient red muscle sample for Block 1. When compared with the starting fish sample (first day in ice storage), a significant increase ($p < 0.05$) was obtained at seventh day of ice storage before freezing in white muscle and at fourth day of ice storage before freezing in red muscle. Comparison between the two kinds of muscle showed higher TBA value ($p < 0.05$) for the red muscle.

The increase in TBA value showed a correlation with the results from previous study where Boran *et al.* (2005) reported that TBA value of horse mackerel oil stored at 4°C significantly increased from 2.15 to 2.75 after 15 days. Whereas, the horse mackerel oil stored at -18°C significantly increased from 2.15-2.45 after 30 days.

The TBA value showed a correlation with total microbial load where increasing in aerobic plate count and yeast and mould count resulted in high TBA value.

Microbiological analysis: Bacterial growth is the main cause of fish spoilage; therefore it is logical to use bacterial number as an index of fish quality (Mohammed and Hamid, 2011). Block 1 and 2 did not show any significant difference plate count (APC) and yeast and mould count (PDA). The results in Table 6 showed that the colony forming units per gram wet sample (CFU g⁻¹) for both aerobic plate count (APC) and yeast and mould count (PDA) were increasingly steadily but not significant during chilled-frozen storage. The yeast and mould count was smaller in numbers than aerobic plate count.

These results were supported by the works of Ross and Morris (1965) and Koburger *et al.* (1975) where yeasts are reported to be present in small numbers on fish while moulds are rarely reported to be associated with marine life. Therefore, bacteria and pathogen poses greater importance in spoilage and safety of fish. Their relative importance varying with species of fish (size, lipid content, maturate stage, etc.), environmental conditions (feeding availability, temperature, microbial load, etc.), method of slaughter and postmortem handling and storage procedures and processing conditions (Medina *et al.*, 2011).

The International Commission on Microbiological Specification for Food (ICMSF) recommends that the flesh APC should not exceed 10⁶ CFU g⁻¹ wet weights. This recommendation was met by our samples which indicated that the fish retaining their quality in terms of microbiological after the chilled-frozen storage.

There is correlation between sensory analysis and bacterial count where high bacteria count in the flesh will contribute to decrease in overall acceptability for the cooked fish in sensory analysis.

Table 7: Textural parameters values of Bighead Carp after chilled-frozen storage

| Parameters | Block | Iced 1 day | Iced 4 days | Iced 7 days |
|-------------------|-------|------------------------|-------------------------|------------------------|
| Hardness (kg) | 1 | 0.30±0.07 ^a | 0.24±0.05 ^a | 0.23±0.08 ^a |
| | 2 | 0.29±0.06 ^a | 0.29±0.06 ^a | 0.23±0.07 ^a |
| Cohesiveness | 1 | 0.36±0.18 ^a | 0.29±0.24 ^a | 0.21±0.12 ^a |
| | 2 | 0.24±0.05 ^a | 0.20±0.08 ^a | 0.20±0.07 ^a |
| Elasticity (mm) | 1 | 4.87±1.47 ^a | 4.47±2.25 ^a | 4.23±1.45 ^a |
| | 2 | 5.44±1.58 ^a | 4.06±1.87 ^a | 4.60±1.38 ^a |
| Gumminess (kg) | 1 | 0.08±0.04 ^a | 0.08±0.07 ^a | 0.05±0.03 ^a |
| | 2 | 0.07±0.02 ^a | 0.06±0.02 ^{ab} | 0.05±0.02 ^b |
| Chewiness (kg mm) | 1 | 0.41±0.26 ^a | 0.28±0.16 ^a | 0.24±0.17 ^a |
| | 2 | 0.39±0.16 ^a | 0.25±0.16 ^a | 0.25±0.16 ^a |

Values with different letters are significantly different at $p = 0.05$

Textural profile analysis: All textural parameters did not show significant difference between Block 1 and 2. The results in Table 7 showed that the hardness of fish white muscle in Block 1 decreased but not significantly during chilled-frozen storage and Block 2 showed significant decrease on seventh day of ice storage before freezing. Cohesiveness was a measure of muscle elasticity since it describes the ability of the muscle to recover from deformation and its resistance to subsequent deformation. Mahmoudzadeh *et al.* (2010) had showed that sensory parameters (color, texture, taste and general acceptability) for two groups decreased significantly during storage.

The damaged tissues lose their desirable appearance and other important characteristics of a foodstuff (Cardoso *et al.*, 2009). Different spoilage mechanisms reported to be involved in this quality loss include microbiological development, endogenous enzyme activity, non-enzymatic lipid oxidation and browning and enzymatic browning (Aubourg *et al.*, 2005; Aubourg, 2008).

Colour measurement: All colour parameters did not show significant difference between Block 1 and 2. Figure 1 and 2 showed the results of colour measurement where the lightness of fish white muscle decreased significantly ($p < 0.05$) as time of ice storage increased before freezing while the yellowness and redness of fish muscle varied during chilled-frozen storage but the differences were not significant ($p > 0.05$). The variation in yellowness and redness may due to the individual variation in fish instead of the effect of chilling and freezing.

According to Fagan *et al.* (2003), freeze-chilling (freezing followed by chilling) promoted some yellowing in whiting filets. During thawing of fish for sample preparations, discolouration may also occur due to acceleration of pigments oxidation at higher temperature.

The data obtained from colour measurements showed a correlation with the colour scores obtained from sensory evaluation where the colour scores from sensory evaluation were vary and no significant difference ($p > 0.05$) among fish ice stored for 1, 4 and 7 days before freezing.

Sensory evaluation: The sensory qualities of cooked Bighead Carp were given score where 1 represent dislike extremely and 9 represent like extremely in terms of colour, odour, flavour, juiciness, texture and overall acceptability.

All sensory parameters did not different significantly between Block 1 and 2. Base on the results showed there was no significant different ($p > 0.05$) in colour, odour, flavour, juiciness, texture and overall acceptability among fish ice stored for 1, 4 and 7 days before freezing. The panellists

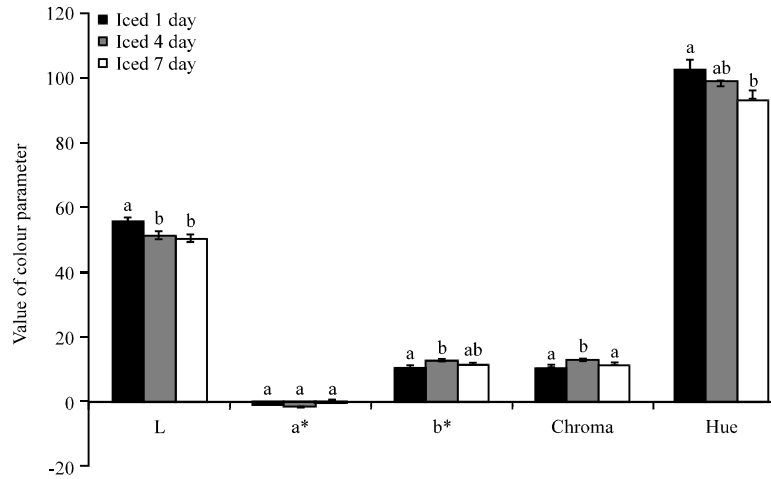


Fig. 1: Values of colour parameters for each parameter (L, a*, b*, chroma and hue) for Block 1 fish white muscle after chilled-frozen storage. Bars denote standard deviation of the mean (n = 3)

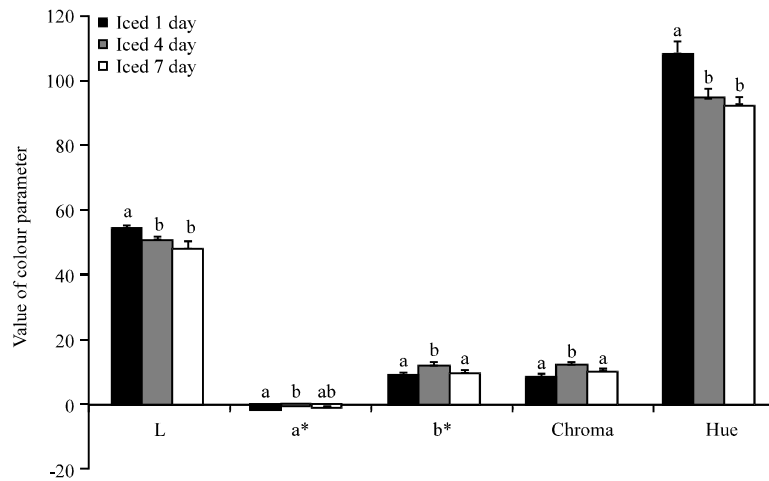


Fig. 2: Values of colour parameters for each parameter (L, a*, b*, chroma and hue) for Block 2 fish white muscle after chilled-frozen storage. Bars denote standard deviation of the mean (n = 3)

accepted the fish with the microbial load did not exceed the ICMSF (1986) limit of $CFU\ g^{-1}$ throughout ice storage. However, the TBA value showed a value higher than 8 mg malonaldehyde/kg on seventh day of ice storage before freezing which made it regarded as poor quality according to the standards. Therefore, the sensory evaluation was not a good measure to evaluate the fish quality since it greatly depends on the personal experience and preference of Bighead Carp and the panellists involved in this sensory evaluation were untrained about the characteristics of Bighead Carp.

CONCLUSION

The results from microbiological analysis showed that the fish was safe to be consumed after 7 days of ice storage before freezing. However, the TBA value showed that the fish was only in good quality which is lower than 5 mg malonaldehyde/kg on fourth day of ice storage before freezing. To decide the quality and the acceptability for consuming Bighead Carp, both total microbial load and TBA value should be taken into account. Therefore, it can be concluded that Bighead Carp can be ice stored for 4 days with assured quality before freezing.

The quantity of TMA found in fish is used as an index of spoilage. In fresh fish, the TMA-N value is about 1 mg/100 g; in spoiled samples it is above 8 mg/100 g (ICMSF, 1986). Protein denaturation and the loss of quality of the frozen fish have been associated with the formation of formaldehyde (Aubourg, 2008).

E. coli, total *Coliforms* and *Staphylococcus aureus* can be analyzed aside from aerobic plate count to assure the safe consuming of Bighead Carp more accurately. Besides, the sensory evaluation shall involved trained panellists to evaluate the fish more precisely.

Other than that, the analysis can be conducting after 1, 4, 5 and 6 days of ice storage before freezing of the fish. With this, we can decide more accurately which day the fish are still in good quality. In the analysis, the kind of chemical and the concentration of the chemical to be added into the ice should also be determined.

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