

Available online at www.sciencedirect.com



Food Chemistry

Food Chemistry 103 (2007) 420-427

www.elsevier.com/locate/foodchem

# Chemical and biochemical changes of hybrid catfish fillet stored at 4 °C and its gel properties

Channarong Chomnawang <sup>a</sup>, Kasem Nantachai <sup>a,\*</sup>, Jirawat Yongsawatdigul <sup>b</sup>, Supawan Thawornchinsombut <sup>a</sup>, Somjintana Tungkawachara <sup>a</sup>

<sup>a</sup> Department of Food Technology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand <sup>b</sup> School of Food Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhonratchasima 30000, Thailand

Received 30 January 2006; received in revised form 13 July 2006; accepted 13 July 2006

# Abstract

Chemical and biochemical changes of aquacultured hybrid catfish fillet (*Clarias macrocephalus* × *Clarias gariepinus*) and its gelforming ability as affected by age and sex of fish along with storage time were investigated. Fillets were stored at 4 °C for 0, 3, 6, 9, 12 and 15 days. There was no significant effect of sex and age of fish as well as storage time on fat, moisture and ash contents (P > 0.05). The total protein, water soluble protein, and salt soluble protein contents of the fillets significantly decreased with storage time (P < 0.05). On the other hand, pH, total volatile base nitrogen (TVB-N) and autolytic degradation products (ADP) increased as storage time continued (P < 0.05). Decreases in Ca<sup>2+</sup>-ATPase activity and gel properties were observed as storage time increased. However, there was no significant effect of either sex or age of fish on textural properties of gel (P > 0.05). Hybrid catfish fillet stored at 4 °C should be processed within 6 days.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Hybrid catfish (Clarias macrocephalus × Clarias gariepinus); Fish fillet; Ca<sup>2+</sup>-ATPase; TVB-N; Gel properties

# 1. Introduction

Hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) farming has been developed and cultured more than other catfish species in Thailand because of its better taste and faster growth (Chinabut, 2002; Somsiri, Tompson, Dumrongphol, & Panbanpeaw, 1999). The annual production of cultured catfish in Thailand is estimated to be 95 600 tonnes with a value of 72.4 million US\$ in 2000 (Department of Fisheries, 2004). Majority of cultured catfish is sold in raw and some are exported in chilled and frozen fillets, being around 184 tonnes in 2004 (The Customs Department, 2005). Therefore, studies on chemical and biochemical changes of hybrid catfish fillet and textural properties of its gel during cold storage are necessary. The results could be possibly used for appropriate post-

0308-8146/\$ - see front matter @ 2006 Elsevier Ltd. All rights reserved. doi:10.1016/j.foodchem.2006.07.039

harvest hybrid catfish fillets cold storage management for human consumption and the possibility of utilizing hybrid catfish mince and surimi for value-added products such as fish ball, fish sausage, fish hams, fish burgers, extruded products and imitation seafood products such as crab legs, shrimp, scallop and lobster tail, among others.

Chilling is a means to preserve fish before processing or consumption. The growth of bacteria and changes of enzymatic as well as chemical reactions are factors affecting fish and fishery product qualities, which are greatly influenced by storage temperature. When fish is stored at low temperature, both enzymatic and chemical reactions are slowed down. The biochemical and physicochemical properties of fish and textural properties of its gel changes during cold storage have previously been reported (Benjakul, Seymour, Morrissey, & An, 1997; El Marrakchi, Bennour, Bouchriti, Hamama, & Tagafatit, 1990; Pacheco-Aguilar, Lugo-Sanchez, & Robles-Burgueno, 2000; Yongsawatdigul & Park, 2002). The objective of this study was to investigate

<sup>\*</sup> Corresponding author. Tel.: +66 43 362132; fax: +66 43 362131. *E-mail address:* kasem@kku.ac.th (K. Nantachai).

the chemical and biochemical changes of hybrid catfish fillet and textural properties of its mince gel as affected by age and sex of the fish and storage time at  $4 \,^{\circ}$ C.

### 2. Materials and methods

## 2.1. Chemicals

Adenosine 5'-triphosphate,  $\beta$ -mercaptoethanol, N,N, N',N'-tetramethylethylenediamine, acrylamide and N,N'methylene-bis-acrylamide were purchased from Fluka Company (Buchs, Switzerland). Bovine serum albumin and Fiske and Subbarow reducer were purchased from Sigma Chemical Company (St. Louis, Mo, USA). Trichloroacetic acid (TCA) was purchased from Carlo erba Reactifs (Rodano, Italy). Sodium dodecyl sulphate (SDS) was purchased from Ajex Finechem (NSW, Australia). Other chemicals were analytical grade and purchased from Fluka Company (Buchs, Switzerland) and Carlo erba Reactif (Rodano, Italy).

# 2.2. Samples preparation and cold storage study

Hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) were obtained from an aquatic farm in Kalasin province, Thailand. Fish weighed about 150–175, 180–215 and 230–255 g when 6, 8 and 10 months old, respectively. Fish were transported to a fish processing plant (Kalasin sausage Co., Ltd.) within 20 min and kept alive for 12 h before being processed. Fish were filleted, deskinned and eviscerated by experienced workers. Samples were packed in polyethylene bags, placed in plastic boxes and filled with crushed ice, and immediately transported to the Khon Kaen University Food Processing Laboratory within 2 h. The fillets were repacked in polyethylene bag of 1.5 kg each. Fish fillets were kept at 4 °C for 0, 3, 6, 9, 12 and 15 days for analyses.

# 2.3. Proximate analysis

Crude protein, fat, ash, and moisture contents of fillets were determined by AOAC (1999). The pH value of sample was determined according to the method of Yongsawatdigul and Park (2002).

# 2.4. Determination of water soluble protein (WSP) and salt soluble protein (SSP)

WSP and SSP were determined by the method of Dyer, French, and Snow (1950) with a slight modification. The sample for WSP analysis was prepared by homogenization of fish mince (5 g) and 95 ml of distilled water for 3 min at high speed using a laboratory blender (Waring Commercial, New Hartford, CT, USA). The sample for SSP was prepared by homogenizing fish mince (5 g) with 95 ml of 5% NaCl (sodium chloride solution buffered with 0.02 M NaHCO<sub>3</sub>, pH 7.2) for 3 min at high speed using a laboratory blender. WSP and SSP were extracted by stirring in an ice bath for 2 h and then centrifuged at  $8000 \times g$  for 15 min at 4 °C (Beckman Instruments, Inc., Palo Alto, CA, USA). Protein content of supernatant was assayed using biuret method (Gornall, Bardawill, & David, 1949).

# 2.5. Determination of total volatile basic nitrogen (TVB-N)

TVB-N was determined by the method of Suvanich, Jahncke, and Marshall (2000) with a slight modification. Fish mince (10 g) was homogenized in 30 ml of 7.5% TCA solution for 2 min. The mixtures were filtered through a Buchner funnel using Whatman No. 1 filter paper (Whatman Int. Ltd., Mildstone, England). The filtrate (25 ml) was transferred to a distillation apparatus (Kjeltech System, Hoganas, Sweden) and 10 ml of 20% NaOH were added to it. Distillation was continued until a final volume of 50 ml was obtained in a 150 ml conical flask containing 25 ml of 2% boric acid with methyl red-bromocresol green indicator. The distillate was titrated with 0.1 N sulphuric acid solution. The concentration of total volatile bases nitrogen was expressed as mg nitrogen/100 g sample as described by Hasegawa (1987).

#### 2.6. Determination of autolytic degradation products (ADP)

ADP was determined as described by Benjakul et al. (1997). Fish muscle (3 g) was homogenized in 27 ml of 5% (w/v) TCA. The homogenate was kept on ice for 1 h and centrifuged at  $5000 \times g$  for 5 min. Tyrosine in the supernatant was measured as an index of autolytic degradation and expressed as µmol tyrosine/g muscle using Lowry method (Lowry, Rosebrough, Fan, & Randall, 1951).

# 3. Determination of Ca<sup>2+</sup>-ATPase activity

# 3.1. Actomyosin preparation

Actomyosin was prepared according to the method described by Benjakul et al. (1997). Sample (8 g) was homogenized in 80 ml chilled (4 °C) 0.6 M KCl, pH 7.0 for 4 min. The extract was centrifuged at 9000 × g for 30 min at 0 °C. Three volumes of chilled deionized water were added to precipitate actomyosin. Actomyosin was collected by centrifugation at 9000 × g for 20 min at 0 °C and the pellet was dissolved in an equal volume of chilled 1.2 M KCl, pH 7.0 in iced bath and stirred for 30 min. Undissolved material was removed by centrifugation at 9000 × g for 20 min at 0 °C.

# 3.2. Actomyosin Ca<sup>2+</sup>-ATPase activity

ATPase activity was determined using the modified method described by Benjakul et al. (1997). The prepared actomyosin was diluted to 2.5–4 mg/ml with 0.6 M KCl,

pH 7.0 and 1 ml of the diluted solution was added to 0.6 ml of 0.5 M Tris-maleate, pH 7.0. To the mixture, 10 mM CaCl<sub>2</sub> was then added to a total volume of 9.5 ml. To the assay solution, 0.5 ml of 20 mM ATP was added to initiate the reaction. The reaction was conducted for exactly 8 min at 25 °C and stopped by adding 5 ml of chilled 15% (w/v) TCA. The reaction mixture was then centrifuged at  $3500 \times g$  for 5 min, and the inorganic phosphate liberated in the supernatant was measured using a spectrophotometer (Perkin–Elmer Inc., Uberlingen, Germany) according to the method of Fiske and Subbarow (1925). Specific activity was expressed as µmoles inorganic phosphate (Pi) released/mg protein/min.

# 3.3. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-polyacrylamide gel electrophoresis was carried out according to the method of Laemmli (1970) with slight modifications as described by Yongsawatdigul and Park (2002). Sample (3 g) was homogenized with 5% (w/v) SDS in a final volume of 30 ml. The homogenate was incubated at 85 °C for 1 h to dissolve total proteins and centrifuged at 5000 × g for 10 min. The supernatant was used for SDS-PAGE analysis, using 4% stacking gel and 10% separating gel with applied protein of 25 µg. Electrophoresis was performed at a constant voltage of 75 V using electrophoresis apparatus (Pharmacia LKB Biotechnology, Uppsala, Sweden). Proteins were stained in 0.125% Coomassie brilliant blue R-250 and destained in 25% ethanol and 10% acetic acid. A high molecular weight standard was used (BioLabs Inc., New England, USA).

# 3.4. Minced fish gel preparation

Fish flesh was chilled at 0 °C for 2 h. Fish flesh (500 g) was chopped using a blender (Matsushita Electric Industrial Co., Ltd., Selangor Darul Ehsan, Malaysia) for 15 min with 5 stops for 0.5 min to scrape the bowl. Sodium chloride and ice were added to obtain a sol with a composition of 2% salt and 80% moisture content. During chopping, the temperature of sol was maintained below 15 °C. The sol was vacuum-packed (Supervac; Busch, West Germany) in a plastic bag and stuffed into 2.3 cm. diameter stainless steel tube using a stuffer (Dick; D73728, Esslingen, Germany), then submerged in a 90 °C water bath (Thermo Haake, Karlsruhe, Germany) for 30 min. After cooking the gels were immediately chilled in ice water for 20 min and kept at 4 °C overnight before texture measurement.

# 3.5. Texture analysis

Puncture test was carried out using Texture Analyzer (Stable Micro System, Surrey, UK) with 6 mm cylindrical probe (P/6) using 5 kg load cell, at a test speed of 1.0 mm/s. Five samples were cut into 2.3 cm long. Puncture force (g) and puncture deformation (mm) were recorded.

# 3.6. Experimental design

A split–split–plot design was used to collect all data. There were 3 factors consisting of age of fish (6, 8 and 10 months; (A), storage time at 4 °C (0, 3, 6, 9, 12 and 15 d; (B), and sex of fish (male and female; (C). Main plot was factor A, the first split–plot was factor B and the split–split–plot was factor C. There was one split–split–plot randomization restriction since factors A and B were necessary to run on the different time. The order in the sub-subplot was randomized as completely randomized design (CRD) with three replications. Independent variables included proximate compositions, water soluble protein, salt soluble protein, total volatile basic nitrogen, autolytic degradation products, Ca<sup>2+</sup>-ATPase activity of fillet and textural properties of gel.

# 3.7. Statistical analysis

General linear model and correlation analysis for sex, age and storage time of fish fillets were conducted using the SPSS computer program version 11.5 for windows (SPSS Inc., Chicago, USA). All experiments were conducted in triplicate. Comparison of means was determined using Duncan's multiple-range test. The level of significance was set at 0.05.

#### 4. Results and discussion

# 4.1. Chemical composition of fillets change during storage

There was no significant ( $P \ge 0.05$ ) effect of sex, age and storage time of fish fillet on fat, moisture and ash contents. Fat, moisture and ash contents were 2.90-3.03, 73.71–75.68 and 1.16–1.18%, respectively (Table 1). Total protein, water soluble protein (WSP) and salt soluble protein (SSP) of fillets decreased with storage time ( $P \le 0.05$ ) (Tables 1 and 2). Total protein, SSP and WSP content decreased from an initial value of about 18.68-17.25%, 124.02–95.25 mg/g sample and 54.86–40.34 mg/g sample at the end of storage, respectively. Decrease in total protein content of fish flesh was possibly due to a decrease in SSP and WSP. A significant reduction in WSP content was also observed during the storage of Atlantic salmon in ice; whereas no significant change in SSP was noted (Hultmann & Rustad, 2004). Munasinghe, Ohkubo, & Sakai (2005) reported that SSP of yellowtail minced stored at 4 °C decreased from 123.93 to 119.40 mg/g tissue in 10 days of storage. The SSP level in channel catfish frame mince also decreased during storage at 4 °C. Suvanich et al. (2000) suggested that long storage time and high temperature contribute to the loss of SSP. Loss of these proteins was due to autolytic deterioration associated with actions of endogenous enzymes. These changes were followed by the growth of microorganisms. Generally, the rates of both autolysis and microbial spoilage were dependent upon the storage temperature (Garthwaite, 1997).

Table 1	
Chemical composition changes of hybrid catfish fillets during chilled storage at 4 °C for	15 days

Storage	Total protein content $(\%)^{A}$	Water soluble protein $(ma/a \text{ somm} a)^{\hat{A}}$	Fat content $(\%)^A$	Moisture content $(\%)^A$	Ash content (%) <sup>A</sup>
time (day)	content $(\%)^{A}$ (mg/g sample) <sup>A</sup>				
0	$18.68\pm0.32^{\rm a}$	$54.86\pm1.37^{\rm a}$	$3.03\pm0.07^{\rm a}$	$75.68 \pm 1.09^{\mathrm{a}}$	$1.17\pm0.01^{\rm a}$
3	$18.41\pm0.50^{\rm a}$	$53.35\pm1.53^{\rm b}$	$2.98\pm0.02^{\rm a}$	$75.21\pm1.00^{\rm a}$	$1.18\pm0.03^{\rm a}$
6	$18.36\pm0.65^{\rm a}$	$49.58\pm1.52^{\rm c}$	$2.90\pm0.02^{\rm a}$	$75.08 \pm 1.11^{\mathrm{a}}$	$1.18\pm0.01^{\rm a}$
9	$17.70 \pm 0.40^{\rm b}$	$45.86 \pm 1.76^{\rm d}$	$2.96\pm0.03^{\rm a}$	$74.14\pm1.36^{\rm a}$	$1.16\pm0.01^{\rm a}$
12	$17.40 \pm 0.46^{\rm bc}$	$43.13 \pm 1.30^{\rm e}$	$2.96\pm0.03^{\rm a}$	$74.14\pm1.36^{\rm a}$	$1.17\pm0.02^{\rm a}$
15	$17.25 \pm 0.55^{\rm c}$	$40.34\pm1.06^{\rm f}$	$2.95\pm0.03^{\rm a}$	$73.71\pm1.08^{\rm a}$	$1.18\pm0.04^{\rm a}$

<sup>A</sup> Values are given as mean value from all sex and age of fish  $\pm$  standard deviation from triplicate observations. Values in the same column with different letters are significantly different (P < 0.05).

Table 2 Changes of pH and salt soluble protein of hybrid catfish fillets during chilled storage at  $4 \,^{\circ}$ C for 15 days

Storage time (day)	pH value <sup>A</sup>	Salt soluble protein (%) <sup>A</sup>
0	$6.41\pm0.34^{\rm a}$	$124.02 \pm 5.93^{\rm a}$
3	$6.41\pm0.40^{\rm a}$	$119.08 \pm 5.30^{\mathrm{b}}$
6	$6.53\pm0.24^{\rm a}$	$106.42 \pm 4.13^{\circ}$
9	$6.72\pm0.13^{\rm b}$	$99.01\pm3.60^{\rm d}$
12	$6.88\pm0.08^{\rm c}$	$96.15 \pm 2.21^{e}$
15	$7.07\pm0.08^{\rm d}$	$95.25\pm3.62^{\rm f}$

<sup>A</sup> Values are given as mean  $\pm$  standard deviation from six observations. Values in the same column with different letters are significantly different (*P* < 0.05).

Loss of SSP resulted in inferior gel-forming ability of minced fish. The pH value of hybrid catfish fillets increased as storage time was extended (P < 0.05). The initial pH value increased from about of 6.4-7.1 at the end of storage (Table 2). Huss (1988) suggested that the initial post mortem pH of fish varies with species, catching ground and season. Usually pH decreases due to anaerobic formation of lactic acid during the first hour after death. Later, pH increases slightly because of the formation of alkaline compounds. The increase of pH throughout 15 days of storage might be attributed to the formation of basic decomposition products, such as ammonia and trimethylamine. These compounds are produced by endogenous enzymes and bacterial spoilage (Ruiz-Capillas & Moral, 2001; Villemure, Simard, & Picard, 1986). Ruiz-Capillas & Moral (2001) found that pH value for hake stored in ice increased progressively throughout 25 days of storage. Yongsawatdigul & Park (2002) reported a gradual increase in pH of threadfin bream stored in ice, from 6.50 to 6.74 after 12 days of storage. Wang, Liceaga-Gesualdo, & Li-Chan (2003) found that the pH value of young Atlantic salmon muscle increased from 6.32 to 6.58 and to 6.80 at the end of storage at 0 and 4 °C, respectively. Hultmann & Rustad (2004) also reported that pH value of Atlantic salmon stored in ice increased significantly during the storage. Suvanich et al. (2000) suggested that pH affected the denaturation rate of myofibrillar proteins, which are important in gel-forming ability of minced fish. Ruiz-Capillas & Moral (2001) suggested that pH changes during refrigerated storage of fish differed according to the species of fish and other factors, such as degree of microorganism contamination and storage condition.

#### 4.2. Biochemical properties changes during storage

There was no difference (P > 0.05) in total volatile basic nitrogen (TVB-N), autolytic degradation products (ADP) and  $Ca^{2+}$ -ATPase activity among sex and age of fish (Figs. 1, 2 and 4). The values for TVB-N increased as storage time progressed (P < 0.05) (Fig. 1). TVB-N content of filleted fish progressively increased to 32.75 mg N/100 g muscle at day 9 and to 44.37 mg N/100 g muscle at day 15. TVB-N content in albacore (Perez-Villareal & Pozo, 1990), hake (Ruiz-Capillas & Moral, 2001) and lizardfish (Benjakul, Visessanguan, & Tueksuban, 2003) stored in ice and channel catfish frame mince (Suvanich et al., 2000) during chilled and iced storage increased with storage time. Özogul, Polat, & Özogul (2004) reported that the TVB-N value of gutted sardine stored at 4 °C was higher than 35 mg N/100 g muscle. Fagan, Gormley, & Mhuircheartaigh (2003) found that TVB-N of raw whiting fillet, mackerel fillet and salmon portions chilled at 4 °C increased for 13.7, 15.9 and 17.0 mg N/ 100 g muscle on day 0 to 25.0, 23.1 and 22.7 mg N/100 g

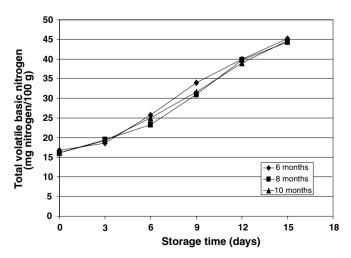


Fig. 1. Total volatile basic nitrogen (TVB-N) content in fish fillets during storage at 4  $^{\circ}$ C for 15 days. Numbers in the legends indicate age of fish in month.

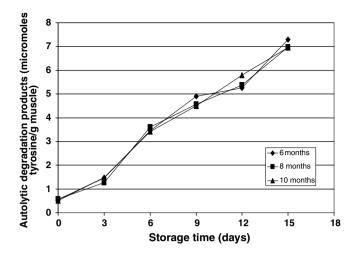


Fig. 2. Autolytic degradation products (ADP) content in fish fillets during storage at 4  $^{\circ}$ C for 15 days. Numbers in the legends indicate age of fish in month.

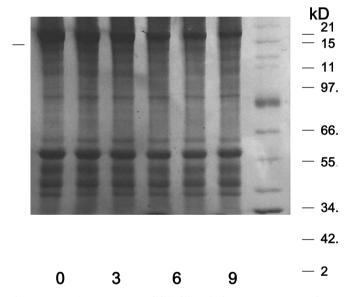


Fig. 3. SDS–PAGE patterns of fish fillets during storage at  $4 \,^{\circ}$ C for 15 days; numbers designate storage time (days) and S designate protein standard.

muscle at day 3, respectively. Yamanaka (1990) reported that TVB-N of saury pike stored at 5 °C was 5.5 mg TVB-N/100 g flesh during storage period on day 0 and increased to 64.1 mg TVB-N/100 g flesh after 11 days of storage. Oehlenschlager (1992) studied cod and haddock stored at refrigerated temperature (4–6 °C); the TVB-N value increased slowly in the first 7–9 days of the storage period, and increased rapidly afterwards. From this result, the TVB-N content of fish samples on day 0 was in the range of 15.73 and 16.91 mg N/100 g muscle. Other researchers (above) have also reported that the TVB-N content of many fish species at day 0 of storage to be in the range of 5.5–17.0 mg N/100 g muscle, suggesting that fish muscle possibly underwent some deterioration during handling (Benjakul et al., 2003). The formation of TVB-N is generally

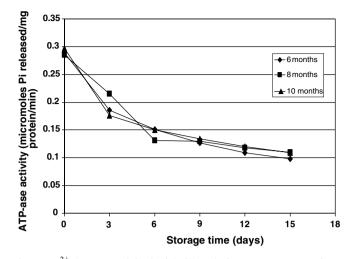


Fig. 4.  $Ca^{2+}$ -APTase activity in fish fillets during storage at 4 °C for 15 days. Numbers in the legends indicate age of fish in month.

associated with activity of micro-organisms during later stages of deterioration that can be used as an indicator of spoilage (Benjakul et al., 2003; Özogul & Özogul, 2000). Our results showed that both TVB-N content and pH values of chilled fish increased during storage. Hultmann & Rustad (2004) reported that the changes in TVB-N content during storage period may be related to changes in pH. Connell (1990) suggested that the recommended level of TVB-N for rejection is 20 mg N/100 g flesh for fatty fish. Connell (1975) & Oehlenschlager (1992) concluded that when the concentration of TVB-N exceeded 30 mg N/100 g flesh, the fish should be considered unfit for consumption. However, a TVB-N value of 30 to 40 mg/100 g was reported to be the limit for acceptability of cold and temperate water fish (Benjakul et al., 2003). Horner (1997) suggested that TVB-N is insensitive to freshness, which means that it can not be used as a freshness indicator. However, it relates well with unfitness for human consumption as it is a good spoilage indicator. Our results showed that TVB-N content of all samples exceeded 30 mg N/100 g flesh after day 9 of storage in accordance with slime on the surface of fillets from visual observation and putrid smell. These results indicated that fillets were unfit for consumption after day 9 of storage. However, further studies are required to evaluate the correlation between TVB-N content and sensory properties of fish fillet during cold storage. Results can be used to limit the TVB-N level for acceptability of hybrid catfish fillet during chilled storage.

Autolytic degradation products (ADP) were detected throughout the storage period, suggesting autolytic degradation of fish protein (Benjakul et al., 2003). Initial ADP of 0.49–0.60 µmol tyrosine/g muscles on day 0 indicated the endogenous oligopeptides in muscle plus the degradation products accumulated during post-harvest handling (Benjakul et al., 1997). The values of ADP increased with storage time (P < 0.05) and sharply increased to 7.29 µmol tyrosine/g muscle in fillet stored at day 15 (Fig. 2). Our results showed that ADP content was affected by storage time. Fish fillet contained the highest amount of ADP on day 15, suggesting that proteolysis of hybrid catfish fillet continuously occurred at 4 °C. Degradation of myofibrils occurred at 0 °C by endogenous proteinases, though the hydrolysis rate was low (Benjakul et al., 1997; Tokiwa & Matsumiya, 1969). Pacheco-Aguilar et al. (2000) reported that during the first day of sardine storage in ice, endogenous enzymes played an important role in the loss of freshness. SDS–PAGE patterns revealed that the intensity of myosin decreased as the storage time increased, corresponding to an increased ADP content (Figs. 2 and 3). A decrease in myosin heavy chain for all samples confirmed proteolysis caused by endogenous proteinases at 4 °C.

Ca<sup>2+</sup>-ATPase activities of all samples decreases as the storage time increased (P < 0.05).  $Ca^{2+}$ -ATPase activity decreased from 0.287 to 0.293 µmoles inorganic phosphate (Pi) released/mg protein/min at day 0 to 0.103-0.108 µmoles inorganic phosphate (Pi) released/mg protein/min at day 15 (Fig. 4). Ca<sup>2+</sup>-ATPase activity is a measure of muscle tissue ability to hydrolyze adenosine triphosphate (ATP) in the presence of Ca<sup>2+</sup>ions. The myofibrillar adenosine triphosphatase (ATPase) is located in the myosin head region (Chan, Gill, Thompson, & Singer, 1995). Thus, Ca<sup>2+</sup>-ATPase activity is a good indicator of the integrity of myosin molecule (Roura & Crupkin, 1995). A decrease of Ca<sup>2+</sup>-ATPase activity implied conformational changes of myosin (Yongsawatdigul & Park, 2002). The result indicated that myosin underwent some conformational changes during chilled storage at 4 °C. In addition, the loss in Ca<sup>2+</sup>-ATPase activity was possibly associated with the proteolysis of myosin molecule (Benjakul et al., 2003). Yongsawatdigul & Park (2002) found that freshness of threadfin bream kept in ice decreased slightly during 12 days and actomyosin underwent conformational changes after storage in ice for 3 days. Benjakul et al. (2003) reported that lizardfish underwent rapid physico-chemical changes by both denaturation and proteolytic degradation during iced storage. Therefore, storage time was found to be a key factor governing the biochemical changes of filleted fish during chilled storage at 4 °C.

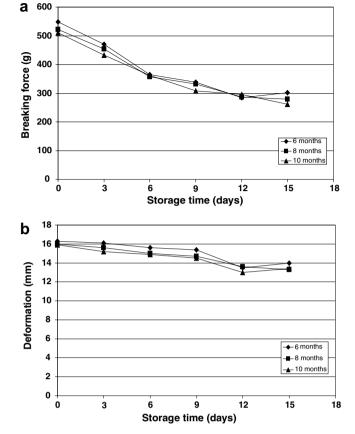
# 4.3. Textural properties of gel during storage

Breaking force and deformation of mince gel decreased as storage time progressed (P < 0.05) (Fig. 5). There was no significant (P > 0.05) effect of both sex and age of fish. Deformation of gel decreased approximately 15.5% throughout 15 days of storage. Breaking force value decreased about 46.7% on day 15. This was possibly due to a decrease in salt soluble proteins (SSP) and myosin contents as shown in SDS-PAGE patterns. Based on these results, breaking force of mince gel decreased more than 38.1% when gel was prepared from fillet stored over 6 days. Therefore, to maintain high quality of gel, hybrid catfish should be processed within 6 days of storage at 4 °C. Degradation of myosin resulted in an inferior gel network forFig. 5. Breaking force (a) and deformation (b) of mince gel prepared from fillets stored at  $4 \,^{\circ}$ C for 15 days. Numbers in the legends indicate age of fish in month.

mation, causing a lower elasticity with poor water-holding capacity in gel matrix (Benjakul et al., 2003). McDonald, Lelievre, & Wilson (1990) found that strength of gels prepared from minces of hoki (Macruronus novaezelandiae) stored in ice decreased throughout 21 days of storage. However, gel quality of hoki surimi was acceptable after holding on ice up to 10 days. Breaking force and deformation of gel prepared from lizardfish (Saurida tumbil) stored in ice also decreased with storage time (Benjakul et al., 2003). Yongsawatdigul & Park (2002) reported that threadfin bream should be processed to surimi within 3 days when stored in ice to maintain high quality of gel. From the results, there was no significant effect of both sex and age of hybrid catfish on breaking force and deformation of mince gel. Therefore, sex of hybrid catfish both male and female and age of fish at 6 months or higher maturity could be processed to obtain the same textural properties of mince gel. However, fish at 6 months is quite difficult to fillet because of small size.

# 5. Conclusions

Sex and age of hybrid catfish as well as storage period had no effect on fat, moisture and ash contents of hybrid catfish, whereas total protein and water soluble protein



contents decreased with storage time. A decrease in salt soluble protein and an increase in pH were found in different age of fish and storage time. Age and sex of fish had no effect on biochemical properties of fillet and textural properties of its gel. Storage time was found to be a prime factor determining the biochemical properties of fillets and textural properties of gel. Hybrid catfish fillet should be processed within 6 days to maintain high textural properties when stored at 4 °C, whereas fillet should be consumed within 9 days. In addition, hybrid catfish at 6 months or higher maturity for both male and female could be processed to obtain the same textural properties of mince gel.

#### Acknowledgements

Authors would like to thank Boon Rawd Brewery Co., Ltd. and Postgraduate Education and Research Development Project in Postharvest Technology for the financial supports.

# References

- AOAC (1999) (16th ed.). Official methods of analysis of association of official analytical chemists (Vol. II). Maryland: AOAC International.
- Benjakul, S., Seymour, T. A., Morrissey, M. T., & An, H. (1997). Physicochemical changes in pacific whiting muscle proteins during iced storage. *Journal of Food Science*, 62(4), 729–733.
- Benjakul, S., Visessanguan, W., & Tueksuban, J. (2003). Changes in physico-chemical properties and gel-forming ability of lizardfish (*Saurida tumbil*) during post-mortem storage in ice. *Food Chemistry*, 80, 535–544.
- Chan, J. K., Gill, T. A., Thompson, J. W., & Singer, D. S. (1995). Herring surimi during low temperature setting, physicochemical and textural properties. *Journal of Food Science*, 60(6), 1248–1253.
- Chinabut, S. (2002). Jaundice disease in catfish, a case study demonstrating a decline in incidence as a result of research output. In: J.R. Arthur, M.J. Phillips, R.P. Subasinghe, M.B. Reantaso, I.H. MacRae, Primary aquatic animal health care in rural, small-scale, aquaculture development (pp. 77–80). FAO Fish Tech Pap No. 406.
- Connell, J. J. (1975). *Control and fish quality*. Survey, UK: Fishing News (Books) LTD.
- Connell, J. J. (1990). Control of fish quality (3rd ed.). Survey, UK: Fishing News (Books) LTD.
- Department of Fisheries. (2004). Fisheries statistical research and analysis group. [Cited on April 15, 2004] Available from: <a href="http://www.fisheries.go.th/it/stat/">http://www.fisheries.go.th/it/stat/</a>.
- Dyer, W. J., French, H. V., & Snow, J. M. (1950). Proteins in fish muscle. 1. Extraction of protein fraction in fresh fish. *Journal of the Fish Research Board of Canada*, 7, 585.
- El Marrakchi, A., Bennour, M., Bouchriti, N., Hamama, A., & Tagafatit, H. (1990). Sensory chemical and microbiological assessments of Moroccan sardine (*Sardina pilchardus*) stored in ice. *Journal of Food Protection*, 53(7), 600–605.
- Fagan, J. D., Gormley, T. R., & Mhuircheartaigh, M. U. (2003). Effect of freeze-chilling, in comparison with fresh, chilling and freezing, on some quality parameters of raw whiting, mackerel and salmon portion. *Lebensmittel-Wissenschaft und- Technologie, 36*, 647–655.
- Fiske, C. H., & Subbarow, Y. (1925). The colorimetric determination of phosphorus. *Journal of Biological Chemistry*, 66, 375–400.
- Garthwaite, G. A. (1997). Chilling and freezing of fish. In G. M. Hall (Ed.), *Fish processing technology* (first ed., pp. 93). UK: Blackie Academic and Professional.

- Gornall, A. G., Bardawill, C. S., & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry*, 177, 751–766.
- Hasegawa, H. (1987). Laboratory manual on analytical methods and procedures for fish and fish products. Marine Fisheries Research Department. Southeast Asian Fisheries Development Center, Singapore.
- Horner, W.F.A. (1997). A study on the health quality of fish products. Final report to directorate general for fisheries DG XIV commission of the European Community. University of Hull: HIFI. Unpublished manuscript, pp. 9–57.
- Hultmann, L., & Rustad, T. (2004). Iced storage of Atlantic salmon (Salmo salar)-effects on endogenous enzymes and their impact on muscle portions and texture. Food Chemistry, 87, 31–41.
- Huss, H.H. (1988). Fresh fish- quality and quality changes. Training Manual Rome: United Nations. Food and Agriculture Organization and Denish International Development Agency: FAO/DANIDA.
- Laemmli, U. K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature*, 227(5259), 680–685.
- Lowry, O. H., Rosebrough, N. J., Fan, A. L., & Randall, R. J. (1951). Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry*, 193, 256–275.
- McDonald, G. A., Lelievre, J., & Wilson, N. D. C. (1990). Strength of gels prepared from washed and unwashed minces of hoki (*Macruronus* novaezeandiae) stored in ice. Journal of Food Science, 55(4), 976–978.
- Munasinghe, D. M. S., Ohkubo, T., & Sakai, T. (2005). The lipid peroxidation induced changes of protein in refrigerated yellowtail minced meat. *Fisheries Science*, *71*, 462–464.
- Oehlenschlager, J. (1992). Evaluation of some well established and some underrated indices for the determination of freshness and/or spoilage of ice stored wet fish. In H. H. Huss (Ed.), *Quality assurance in the fish industry* (pp. 339–351). Netherland: Elsevier Science Publishers B.V.
- Özogul, F., & Özogul, Y. (2000). Comparison of methods used for determination of total volatile basic nitrogen (TVB-N) in rainbow trout (Oncorhynchus mykiss). Turk Journal of Zoology, 24, 113–120.
- Özogul, F., Polat, A., & Özogul, Y. (2004). The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (*Sardina pilchardus*). Food Chemistry, 85, 49–57.
- Pacheco-Aguilar, R., Lugo-Sanchez, M. E., & Robles-Burgueno, M. R. (2000). Postmortem biochemical and functional characteristic of monterey sardine muscle stored at 0 °C. *Journal of Food Science*, 65(1), 40–47.
- Perez-Villareal, B., & Pozo, R. (1990). Chemical composition and iced spoilage of albacore (*Thunnus alalunga*). Journal of Food Science, 55, 678–682.
- Roura, S. I., & Crupkin, M. (1995). Biochemical and functional properties of myofibrils from pre and post-spawned hake (*Merluccius hubbsi Marini*) stored on ice. *Journal of Food Science*, 60, 269–272.
- Ruiz-Capillas, C., & Moral, A. (2001). Correlation between biochemical and sensory quality indices in hake stored in ice. *Journal of Food Science*, 34, 441–447.
- Somsiri, T., Tompson, M., Dumrongphol, Y., Panbanpeaw, A. (1999). Study of Bacterial Loading in Macrophage Cells of Hybrid Catfish. In: Papers presented at Fourth Symposium on Diseases in Asian Aquaculture held in Cebu City, Philippines during 22–26 November 1999.
- Suvanich, V., Jahncke, M. L., & Marshall, D. L. (2000). Changes in selected chemical quality characteristics of channel catfish frame mince during chill and frozen storage. *Journal of Food Science*, 65(1), 24–29.
- The Customs Department. (2005). Export statistic. [Cited on September 20, 2005] Available from: <a href="http://www.customs.go.th/statistic/statisticIndex.jsp">http://www.customs.go.th/statistic/statistic/statisticIndex.jsp</a>.
- Tokiwa, T., & Matsumiya, J. (1969). Fragmentation of fish myofibril. Effect of storage condition and muscle cathepsin. Bulletin of the Japanese Society of Fisheries Science, 35, 1099–1109.

- Villemure, G., Simard, R. E., & Picard, G. (1986). Bulk storage of cod fillets and gutted cod (*Gadus morhua*) under carbon dioxide atmosphere. *Journal of Food Science*, 51(2), 317–320.
- Wang, H., Liceaga-Gesualdo, A. M., & Li-Chan, E. C. Y. (2003). Biochemical and physicochemical characteristics of muscle and natural actomyosin isolated from young Atlantic salmon (*Salmo salar*) fillets stored at 0 and 4 °C. *Journal of Food Science*, 68(3), 784–789.
- Yamanaka, H. (1990). Polyamines as potential indices for freshness and decomposition of saury pike (*Cololabis saira*) and scallop (*Patinopecten yessoensis*). In Torry Research Station, *Chilling and freezing of new fish products* (pp. 123–129). Aberdeen.
- Yongsawatdigul, J., & Park, J. W. (2002). Biochemical and conformational changes of actomyosin from threadfin bream stored in ice. *Journal of Food Science*, 67(3), 985–990.