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Effect of iced storage of bigeye snapper (*Priacanthus tayenus*) on the chemical composition, properties and acceptability of Som-fug, a fermented Thai fish mince

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Abstract

The effect of iced storage of bigeye snapper (*Priacanthus tayenus*) on the chemical composition, properties and acceptability of Som-fug was investigated. During 15 days of iced storage, total volatile base (TVB), trimethylamine (TMA) and TCA-soluble peptide contents as well as thiobarbituric reactive substances (TBARS) of fish muscle increased continuously after 3 days of storage ($p < 0.05$). It was suggested that deterioration, protein degradation and lipid oxidation proceeded with increasing storage time. Som-fug prepared from surimi of bigeye snapper stored in ice for different times had similar pH, acidity and lactic acid bacteria count at the end of the fermentation (30 °C, 48 h). Generally, higher content of TCA-soluble peptides and higher TBARS were found in fermented Som-fug produced from bigeye snapper stored in ice for a longer time ($p < 0.05$). Hardness, adhesiveness, springiness, cohesiveness, and resilience of fermented Som-fug decreased with a concomitant increase in weight loss, released water and expressible water contents when fish kept for a longer time were used ($p < 0.05$). L^*, a^*, b^*, whiteness and the likeness for appearance, colour, texture and flavour of Som-fug decreased when fish kept in ice for an extended time were used ($p < 0.05$). However, the taste likeness was not affected by iced storage time ($p > 0.05$). No differences in overall liking were noticeable when fish kept in ice for up to 12 days were used for Som-fug production ($p > 0.05$). Therefore, the quality of fish used as raw material should be an important factor in determining the characteristics of Som-fug.

Keywords: Som-fug; Bigeye snapper; Chemical properties; Physical properties

1. Introduction

Freshness is considered as the crucial factor determining fish quality. Deterioration of fish mainly occurs as a result of bacteriological activity, leading to loss of quality, and nutritive value (Ashie, Smith, & Simpson, 1996; Liston, 1980; Olafsdóttir, Martinsdóttir, & Oehlenschläger, 1997). The rate of deterioration is associated with many factors such as fish species, size, lipid content, state at the moment of capture, microbial load, and storage temperature. Fresh fish are extremely perishable and should be handled with great care. Low-temperature storage, especially iced storage, is one of the primary methods to maintain fish freshness (Benjakul, Visessanguan, Riebroy, Ishizaki, & Tanaka, 2002; Chapmen, 1990). However, changes in microbiological, chemical and physical properties of iced fish still occur (Benjakul et al., 2002; Chytiri, Chouliara, Savvaidis, & Kontominas, 2004).

Som-fug, an indigenous fermented fish mince of Thailand, has been paid increasing attention due to its unique delicacy. The mixture containing fish mince, cooked rice and minced garlic is tightly packed in banana leaves or

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plastic bags and left to ferment for 2–5 days at 30 °C (Saisithi et al., 1986). The ripened product is slightly sour and salty and is relatively firm and springy (Valyasevi & Rolle, 2002). Som-fug is highly nutritious and is an excellent source of protein. The freshwater fish are normally used as raw material rather than marine fish (Riebroy, Benjakul, Visessanguan, & Tanaka, 2005). However, Riebroy, Benjakul, Visessanguan, and Tanaka (2006a) reported that bigeye snapper (*Priacanthus tayenus*), a marine fish, can be used for Som-fug production. Fish with different quality or freshness may render the resulting Som-fug with varying characteristics and sensorial properties. Marine fish contain a high amount of trimethylamine oxide (TMAO) for osmoregulation (Barret & Kwan, 1985). TMAO can be reduced to trimethylamine (TMA) by microorganisms having TMAO reductase activity, particularly during inappropriate handling (Malle, Eb, & Tailliez, 1986; Sikorski, Kolakowska, & Burt, 1990). This leads to the fishy odour and lowered acceptability for the consumer (Gram & Huss, 1996). Recently, Riebroy, Benjakul, Visessanguan, and Tanaka (2006b) found that washing could improve the quality and acceptability of resulting Som-fug from bigeye snapper. Nevertheless, no basic information on the quality of Som-fug produced from marine fish with different postmortem storage times has been reported. The objective of this study was to investigate the chemical composition, properties and acceptability of Som-fug from bigeye snapper stored in ice for different times.

2. Materials and methods

2.1. Chemical reagents

Trichloroacetic acid, potassium carbonate, formaldehyde, sodium hydroxide and Man Rogosa Sharpe (MRS) medium were obtained from Merck (Darmstadt, Germany). Calcium carbonate was purchased from Fluka (Buchs, Switzerland). Thiobarbituric acid (TBA), L-tyrosine, and malonaldehyde were procured from Sigma (St. Louis, MO, USA). All chemicals for electrophoresis were obtained from Bio-Rad (Richmond, CA, USA).

2.2. Fish sample preparation and storage

Bigeye snapper (*P. tayenus*) were caught from Songkhla–Pattani coast along the Gulf of Thailand. The fish, off-loaded approximately 24 h after capture, were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 1 h. Upon arrival, fish were immediately washed with tap water and kept in a styrene foam box containing crushed ice. The fish were placed and distributed uniformly between the layers of ice using a fish/ice ratio of 1:2 (w/w). The box was kept at room temperature (27–30 °C) for up to 15 days. To maintain the ice content, the melted ice was removed and replaced with an equal amount of ice every 2 days. The fish were randomly taken every 3 days for analysis. Fish samples were also used to prepare surimi, which was used for Som-fug production. Two different lots of fish were used for this study.

2.3. Preparation of surimi

Bigeye snapper stored in ice for different times were used for surimi preparation according to the method of Benjakul et al. (2002). The fish were filleted and minced by mechanical mincer (CZ-112A Chuang Zong, Taipei, Taiwan) with an orifice diameter of 4 mm. Fish mince was washed with cold water (5 °C) using a water/mince ratio of 3:1 (v/w). The mixture was stirred gently for 5 min and the washed mince was filtered with a layer of nylon screen. Washing was performed twice. For the third washing, cold 0.2% NaCl solution was used. Finally, the washed mince was filtered, followed by centrifugation at 700*g* for 15 min using a basket centrifuge (Model CE 21K, Grandiumpiant, Belluno, Italy). To the washed mince, 4% sucrose and 4% sorbitol were added, mixed well and frozen using an air-blast freezer. Before use, samples were tempered overnight at 4 °C.

2.4. Preparation of Som-fug

Som-fug was prepared as described by Riebroy et al. (2006a, 2006b). Surimi (3 kg) was mixed with minced garlic (0.15 kg), ground steamed rice (0.45 kg), and salt (90 g) for 15 min using a mixer (Model EC-20 Crypto Peerless, Birmingham, England). The mixture was then stuffed into a polyethylene casing with a diameter of 2.0 cm, sealed tightly and incubated at 30 °C up to 72 h. The fermentation was conducted until the pH of Som-fug reached 4.60. After the fermentation was completed, five samples with the length of 10 cm were taken for microbiological, chemical and physical analyses.

2.5. Microbiological analysis

Lactic acid bacteria (LAB) count was determined using MRS medium according to the method of AOAC (2000). The sample (25 g) was aseptically transferred to a sterile plastic pouch and pummelled for 1 min in a stomacher Lab-blender 400 (Seward Medical, London, UK) with 225 ml of 0.1% sterile peptone water. Appropriate decimal dilutions of the samples were made using the same diluent. Aliquots of 0.1 ml of each dilution were plated in duplicate on MRS agar and incubated at 30 °C for 1–2 days. LAB count was reported as log cfu/g sample.

2.6. Chemical analyses

2.6.1. Determination of pH and total acidity

The pH of fish muscle was determined according to the method of Benjakul, Seymour, Morrissey, and An (1997). Sample was homogenised with 10 volumes of deionised

water (w/v), and the pH was measured using a pH meter (CG842 Schott, Germany). The total acidity was determined by the method of AOAC (2000). To the sample (5 g), 40 ml of CO_2-free distilled water were added and the mixture was homogenised at 11,000 rpm for 60 s using an IKA homogeniser (Model T25, Selangor, Malaysia). The homogenate was centrifuged at 3000*g* for 15 min using a Biofuge primo centrifuge (Sorvall, Hanau, Germany) at room temperature. The supernatant was filtered through a filter paper (Whatman No. 4). The filtrate was titrated with the standardised 0.1 N NaOH using phenolphthalein as an indicator. The total acidity was calculated as lactic acid and expressed as percentage (w/w).

2.6.2. Determination of total volatile base (TVB) and trimethylamine (TMA) contents

TVB and TMA contents were determined using the Conway microdiffusion assay as described by Ng (1987). A sample (2 g) was added to 8 ml of 4% trichloroacetic acid (TCA) (w/v) and homogenised with a homogeniser (IKA Labortechnik, Malaysia) at a speed of 11,000 rpm for 2 min. The homogenate was centrifuged at 3000*g* for 15 min using a Biofuge primo centrifuge (Sorvall, Hanau, Germany) room temperature. The supernatant referred to as 'sample extract' (1 ml) was placed in the outer ring of the Conway apparatus. The inner ring solution (1% boric acid containing the Conway indicator) was then pipetted into the inner ring. To initiate the reaction, K_2CO_3 (1 ml) was mixed with sample extract. The Conway unit was closed and incubated at 37 °C for 60 min. The inner ring solution was then titrated with 0.02 N HCl until the green colour turned to pink. Determination of TMA content was carried in the same manner except that 1 ml of 10% formaldehyde was added to the sample extract to fix ammonia present in the sample prior to the assay.

2.6.3. Determination of thiobarbituric acid reactive substances (TBARS)

TBARS was determined according to the method of Buege and Aust (1978). A sample (5 g) was homogenised with 25 ml of TBARS solution (0.375% TBA, 15% TCA, and 0.25 N HCl). The mixture was heated for 10 min in boiling water (95–100 °C) to develop a pink colour. Then the mixture was cooled with a running water and centrifuged at 5500*g* for 25 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). The TBARS value was calculated from the standard curve of malonaldehyde and expressed as mg malonaldehyde/kg sample.

2.6.4. Determination of TCA-soluble peptides

The TCA-soluble peptide content was determined according to the method of Green and Babbitt (1990). A sample (3 g) was homogenised with 27 ml of 15% TCA. The homogenate was kept in ice for 1 h and centrifuged at 12,000*g* for 5 min. The soluble peptides in the supernatant were measured by the method of Lowry, Rosebrough, Farr, and Randall (1951) and expressed as μmol tyrosine/g sample.

2.6.5. SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

A sample (3 g) was homogenised with 27 ml of solubilising agent (2% SDS, 8 M urea and 2% β-mercaptoethanol). The homogenate was heated at 85 °C for 1 h, followed by centrifugation at 10,000*g* for 15 min at room temperature. The protein concentration of supernatant was determined by the Lowry method (Lowry et al., 1951). SDS–PAGE was performed using 4% stacking gel and 10% running gel according to the method of Laemmli (1970) with a vertical gel electrophoresis unit (Mini-Protein II; Bio-Rad Laboratories, Richmond, CA, USA). The electrophoresis was carried out at 15 mA. After separation, protein bands were stained using Coomassie Brillant Blue R-250 (0.2%) in 25% methanol and 10% acetic acid. Destaining was performed using 40% methanol and 10% acetic acid.

2.7. Physical analyses

2.7.1. Determination of weight loss

Weight loss was determined as described by Nakao et al. (1991). A sample with casing (100 g) was accurately weighed before fermentation using an analytical balance (Model CP244S, Germany). During the fermentation process, Som-fug was taken and then reweighed. Difference in weight of Som-fug before and after fermentation was referred to as 'weight loss'.

2.7.2. Determination of released water content

The percentage of water released from sample was determined according to the method of Nakao et al. (1991). The sample with casing was weighed (A) and the sample was then removed from the casing. The water released on its surface was wiped with filter paper (Whatman No. 4) and the sample was then reweighed (B). The empty casing was weighed (C). The percentage of released water was calculated according to the following equation:

$$\text{Released water } (\%) = 100 \times \{(A - B) - C\}/(A - C).$$

2.7.3. Determination of expressible water content

The expressible water content of samples was measured according to the method of Funami, Yada, and Nakao (1998) as modified by Visessanguan, Benjakul, Riebroy, and Thepkasikul (2004). The expressible water content was determined as the weight loss after the compression of sample. Sample was cut into a cylinder form (2.0 cm height × 2.0 cm diameter), placed between double layers of filter papers (Whatman No. 4) and subjected to compression using the texture analyser (Stable Micro Systems, Surrey, England) with the cylindrical aluminum probe (50 mm diameter). The measurement was performed with crosshead speed of 3 mm/s to 70% strain for 60 s. Samples were subjected to moisture analysis by AOAC method

(AOAC, 2000). The expressible water content was calculated as the ratio of the apparent expressible water to the total moisture content of the Som-fug according to the following equation:

$$\text{Expressible water (\%)} = \frac{100 \times \text{Apparent expressible water content}}{\text{Total moisture content}}$$

where Apparent expressible water content $= 100 \times (W_{\text{before}} - W_{\text{after}})$

W_{before} = Weight before compression;

W_{after} = Weight after compression

2.7.4. Texture profile analysis (TPA)

TPA was performed using a TA-XT2i texture analyser (Stable Micro Systems, Surrey, England) with cylindrical aluminum probe (50 mm diameter). The samples were cut into cylinders (30 mm height × 20 mm diameter) and placed on the instrument's base. The tests were performed with two compression cycles. TPA textural parameters were measured at room temperature with the following testing conditions: crosshead speed 5.0 mm/s, 50% strain, surface sensing force 99.0 g, threshold 30.0 g, and time interval between first and second stroke 1 s. The Texture Expert version 1.0 software (Stable Micro Systems, Surrey, England) was used to collect and process the data. Hardness, adhesiveness, springiness, and cohesiveness were calculated from the force–time curves generated for each sample (Bourne, 1978).

2.7.5. Colour

The colour of sample was measured in the L^* a^* b^* mode of CIE (angle 10°, illuminant D65) using a HunterLab (ColorFlex, Hunter Associates Laboratory, Virginia, USA). L^*, a^*, and b^* indicate lightness, redness/greenness, and yellowness/blueness, respectively.

2.8. Acceptability test

The fermented Som-fug was evaluated for acceptance by an untrained 40-membered panel. The panelists were graduate students in Food Technology program of age ranging from 22 to 35 years, Faculty of Agro-Industry, Prince of Songkla University. Panelists had sensorial acquaintance with Som-fug. A nine-point hedonic scale, in which a score of 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely, was used for evaluation (Chamber IV & Wolf, 1996; Meigaard, Civille, & Carr, 1990). Samples were sliced perpendicular to the long axis to obtain the length of 2.0 cm. Acceptance evaluation was performed on raw samples without cooking. Som-fug samples were placed on dishes (diameter 3.0 cm) and the samples were covered with aluminum foil. The samples were allowed to stand at room temperature for at least 30 min prior to analysis. Samples were randomly selected and coded with three-digit random number and presented to the panelists at room temperature. During evaluation, the panelists were situated in private booths. Room temperature water was given to rinse the mouth between samples. The panelists evaluated each sample for appearance, colour, texture, taste, flavour, and overall liking.

2.9. Statistical analysis

One-way ANOVA was used and mean comparison was performed by Duncan's multiple range test (Steel & Torrie, 1980). Statistical analysis was carried out using SPSS statistic program (Version 10.0) for Window (SPSS Inc. Chicago, IL).

3. Results and discussion

3.1. Changes in bigeye snapper muscle during iced storage and the effect on the resulting surimi

3.1.1. Changes in pH

Changes in pH of bigeye snapper muscle and the resulting surimi during iced storage are shown in Fig. 1. Constant pH was observed during the first 3 days of iced storage. Thereafter, the pH of fish muscle and surimi increased with increasing storage time ($p < 0.05$). Surimi produced from bigeye snapper kept in ice exhibited a lower pH than muscle at the same storage time. Benjakul et al. (2002) reported that the post-mortem pH value of bigeye snapper (*P. tayenus*) muscle was approximately 6.6–6.7. The increase in pH was postulated to be due to an increase in volatile bases produced by either endogenous or microbial enzymes (Ababouch et al., 1996; Benjakul et al., 2002; Chang & Regenstein, 1997). Additionally, the decomposition of nitrogenous compounds causes an increase in pH in fish flesh (Sikorski et al., 1990). The changes in pH also depend on the liberation of inorganic phosphate and

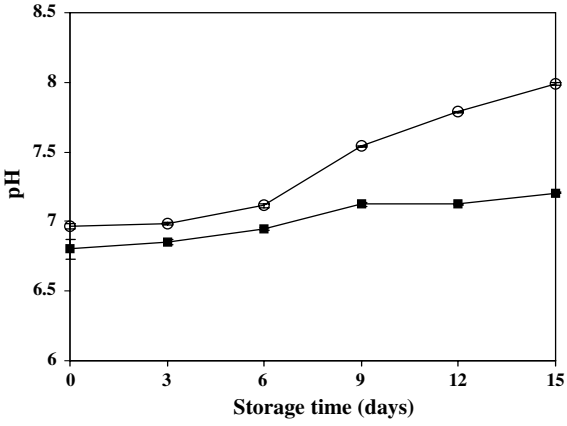

Fig. 1. Changes in pH of bigeye snapper muscle (○) and resulting surimi (■) during iced storage. Bars represent standard deviation from six determinations.

ammonia due to the enzymatic degradation of ATP (Sikorski et al., 1990).

3.1.2. Changes in TVB and TMA

TVB and TMA contents of bigeye snapper and the resulting surimi were monitored during iced storage as depicted in Fig. 2a and b, respectively. At day 0 of storage, the initial TVB content of fish muscle and resulting surimi were 5.53 mg N/100 g and 0.27 mg N/100 g, respectively (Fig. 2a). However, TMA in muscle and surimi was negligible at day 0 of iced storage. When storage time increased, both TVB and TMA contents in muscle and surimi increased ($p < 0.05$). TVB and TMA contents in surimi from fish stored in ice for all times studied were lower than those in muscle. These results indicated that TVB and TMA contents could be reduced by the washing process in surimi production. Benjakul et al. (2002) reported that initial values of TVB in bigeye snapper samples were 5.0–5.4 mg N/100 g. The presence of TVB and TMA indicated that some changes in the nitrogenous compounds occurred prior to iced storage (Fig. 2). The formation of TVB is generally associated with the growth of microorganisms and can be used as an indicator of spoilage (Benjakul et al., 2002). From the result, TVB content of bigeye snapper muscle increased slightly during the first 6 days of iced storage ($p < 0.05$). Thereafter, a sharp increase was found up to 15 days ($p < 0.05$). Generally, TVB comprises mainly TMA and ammonia, which are produced by both microbial and endogenous enzymes. A number of specific spoilage bacteria such as *Shewanella putrefaciens*, *Photobacterium phosphoreum*, *Vibrionaceae*, etc. typically use TMAO as an electron acceptor in anaerobic respiration, resulting in off-odour and off-flavour due to the formation of TMA (Gram & Huss, 1996; Huss, 1995). Therefore, the formation of TVB and TMA in fish muscle has been used as the indication of fish spoilage (Ryder, Buisson, Scott, & Fletcher, 1984). Since fish samples were kept in ice, the formation of TVB and TMA was probably mediated by psychrotropic bacteria (Sasajima, 1973, 1974). TVB content of 30 mg N/100 g is generally regarded as the fish acceptability limit (Sikorski et al., 1990). Up to 15 days, TVB content was lower than the limiting level (18.1 mg N/100 g). From the result, the increases in both TVB and TMA contents were in accordance with the increase in pH, especially when the storage time increased (Fig. 1).

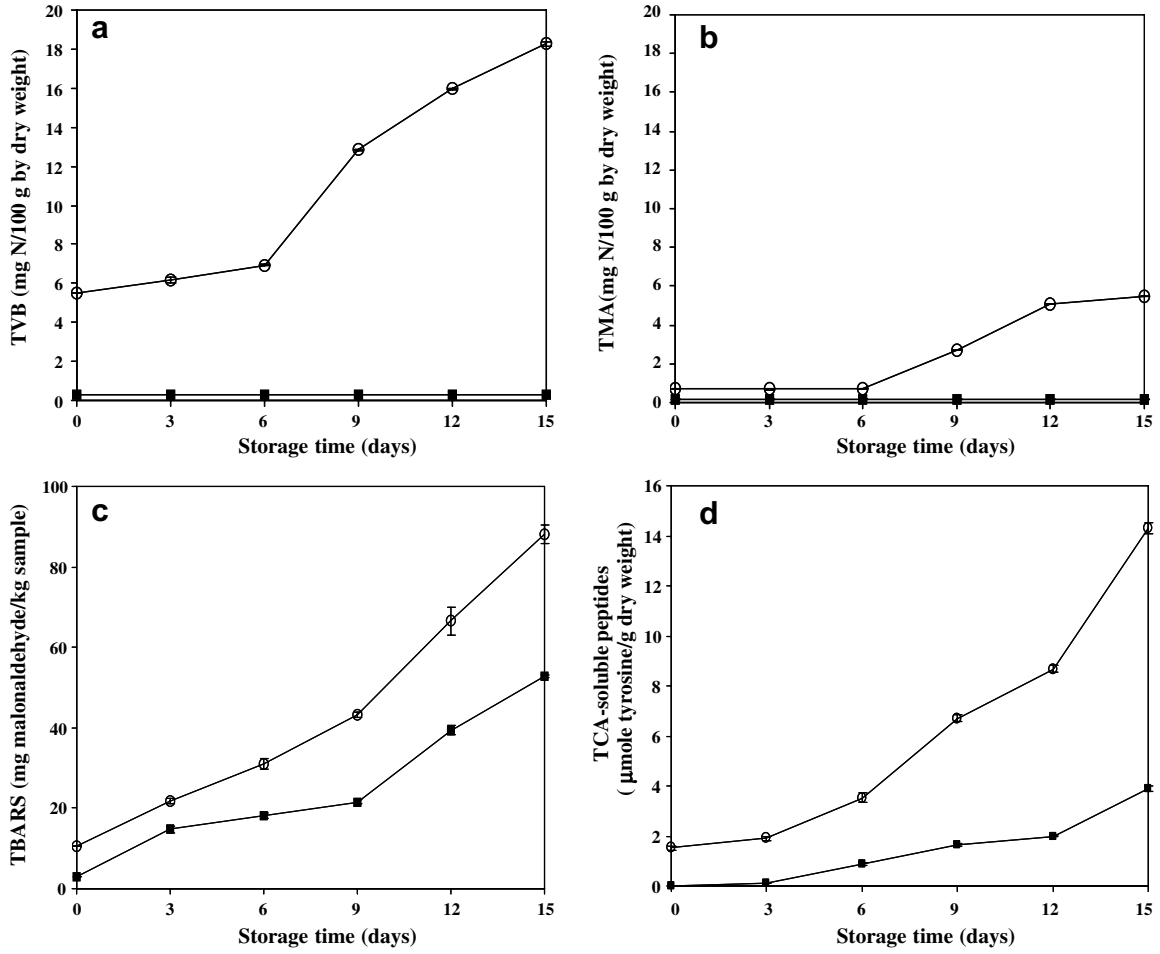

Fig. 2. Changes in TVB (a), TMA (b), TBARS (c) and TCA-soluble peptide contents (d) in bigeye snapper muscle (○) and resulting surimi (■) during iced storage. Bars represent standard deviation from six determinations.

3.1.3. Changes in TBARS

Changes in TBARS value of bigeye snapper muscle and the resulting surimi during iced storage are shown in Fig. 2c. TBARS of muscle increased continuously during storage ($p < 0.05$), indicating that lipid oxidation occurred during the extended iced storage. Fish muscle typically has a high content of polyunsaturated fatty acids and is consequently prone to oxidative reaction (Harris & Tall, 1994; Stamman, Gerdes, & Caporaso, 1990). TBARS has been widely used to indicate lipid oxidation in meat and meat products (Jo & Anh, 2000). The lipid oxidation can be initiated and accelerated by various mechanisms including the production of singlet oxygen, enzymatic and non-enzymatic generation of free radicals and active oxygen (Kubow, 1992). From the result, the storage time in ice was an important factor determining the lipid oxidation in bigeye snapper muscle. For surimi from bigeye snapper kept in ice for different times, the lower TBARS values were observed, compared with those of muscle ($p < 0.05$). This was probably due to the removal of some lipids as well as oxidation products from muscle during surimi production.

3.1.4. Changes in TCA-soluble peptides

The TCA-soluble peptide content in bigeye snapper muscle and the resulting surimi increased during iced storage (Fig. 2d). At day 0, the TCA-soluble peptide content in muscle and surimi were 1.523 and 0.004 μmol tyrosine/g sample, respectively. The tyrosine level detected at day 0 indicated the endogenous oligopeptides and free amino acids as well as degradation products accumulated during post-harvest handling (Benjakul et al., 1997). The TCA-soluble peptide content in surimi produced from ice-stored fish was lower than that of fish muscle at the same storage time ($p < 0.05$). Washing might remove the small peptides, resulting in the lowered TCA-soluble peptides in surimi. According to An, Weerasinghe, Seymour, and Morrissey (1994), the activity of cathepsin L was insignificant at 0–5 °C, whereas cathepsin B exhibited half of its maximal activity and cathepsin H retained about a fifth of its maximal activity. Therefore, cathepsins B and H might contribute to the degradation occurring at low-temperature storage. In addition, microbial proteases may also be a potential source of proteolytic degradation during iced storage. Protease from *Pseudomonas marinoglutinosa* was reported to hydrolyse actomyosin at 0–2 °C and the optimal pH was above 7.0 (Venugopal, Alur, & Lewis, 1983). From the result, a high TCA-soluble peptide content indicated greater hydrolysis of muscle proteins during iced storage.

3.2. Properties of Som-fug from surimi of bigeye snapper stored in ice for different times

3.2.1. Lactic acid bacteria count

LAB count in fermented Som-fug samples ranged from 8.06 to 8.11 log cfu/g and no differences in LAB count were observed among all fermented Som-fug samples produced from fish with different storage times (Table 1). Riebroy et al. (2006a) reported that the LAB count in Som-fug produced from six species of marine fish was approximately 10^8 cfu/g. The dominant LAB in commercial Som-fug including *Lactobacillus* sp. and *Pediococcus* sp. were identified (Tanasupawat, Okada, Suzuki, Kazaki, & Komagata, 1993).

3.2.2. pH and total acidity

Total acidity of fermented Som-fug produced from surimi of ice-stored bigeye snapper are shown in Table 1. There was no difference between the pH values of Som-fug produced from surimi of bigeye snapper stored in ice for different times ($p > 0.05$). Though the increase in pH of bigeye snapper muscle was found with increasing storage time, the meat was subsequently washed during surimi production. This led to the removal of volatile bases. Furthermore, a pH at 4.6 was used as the end point of fermentation of Som-fug. Nevertheless, the incubation time to obtain the final pH designated (4.6) varied with different samples (data not shown). The pH of Som-fug is generally regarded as an important factor to ensure the safety of these products and this is directly influenced by the production of organic acids, especially lactic acid. These organic acids, particularly lactic acid were responsible for the flavour of Som-fug (Østergaard et al., 1998). At the end of fermentation, all samples had total acidity ranging from 2.24% to 2.32%. The increase in total acidity was generally

Table 1
Lactic acid bacteria count and chemical compositions of fermented Som-fug produced from surimi of bigeye snapper stored in ice for different times

Samples	Lactic acid bacteria count (log cfu/g sample)	Total acidity (% lactic acid)	TCA-soluble peptides (μmol tyrosine/g sample)	TBARS (mg malonaldehyde/kg sample)
S-0	$8.08 \pm 0.07^{a\#,\dagger}$	2.24 ± 0.01^{b}	2.28 ± 0.05^{e}	48.67 ± 0.13^{e}
S-3	8.06 ± 0.12^{a}	2.25 ± 0.02^{b}	3.68 ± 0.04^{d}	57.87 ± 1.11^{d}
S-6	8.09 ± 0.09^{a}	2.28 ± 0.04^{a}	4.12 ± 0.02^{c}	65.96 ± 0.23^{c}
S-9	8.10 ± 0.10^{a}	2.30 ± 0.05^{a}	4.19 ± 0.01^{c}	67.54 ± 0.33^{c}
S-12	8.08 ± 0.05^{a}	2.30 ± 0.08^{a}	4.45 ± 0.01^{b}	70.91 ± 0.69^{b}
S-15	8.11 ± 0.06^{a}	2.32 ± 0.06^{a}	4.94 ± 0.01^{a}	72.71 ± 0.63^{a}

S-0, S-3, S-6, S-9, S-12 and S-15: fermented Som-fug produced from surimi of bigeye snapper stored in ice for 0, 3, 6, 9, 12, and 15 days, respectively.
[#] Mean ± SD from six determinations.
[†] Different superscripts in the same column indicate significant differences ($p < 0.05$).

accompanied by a decrease in the pH value. The results suggested that the lowering pH of fermented Som-fug could be achieved for all surimi produced from bigeye snapper, regardless of storage time in ice.

3.2.3. Protein degradation

TCA-soluble peptide contents in Som-fug from surimi of bigeye snapper kept in ice for various times were found to be different ($p < 0.05$). Som-fug samples from surimi of fish stored for a longer time consisted of a higher content of TCA-soluble peptide content ($p < 0.05$) (Table 1). Formation of TCA-soluble peptides in Som-fug suggested the degradation of muscle proteins by endogenous and microbial proteinases. The pH values of 4.5–5.0 might maximise the activity of proteolytic enzymes activated at the acidic pHs, especially cathepsins (Beriain, Chasco, & Lizaso, 2000). The peptides produced as a result of enzymatic degradation of proteins have an important influence on meat flavour development (Spanier, Flores, McMillin, & Bidner, 1997). The initial hydrolysis of muscle proteins is attributed mainly to endogenous cathepsin and is followed by the action of microbial peptidases, which further degrade the protein fragments to small peptides and free amino acids (Molly et al., 1997). From the result, the greater TCA-soluble peptide content in Som-fug from surimi of fish stored in ice for a longer time was possibly due to the higher susceptibility of shorter chain peptides in the starting material to the degradation (Fig. 2d). Though the washing could remove the smaller peptides in the fish to some extent, the remaining peptides generated during iced storage partially contributed to the greater TCA-soluble peptides in the resulting Som-fug. This result suggested that the proteolysis in Som-fug possibly occurred with different degrees, depending on the starting raw material used.

Electrophoretic patterns of fermented Som-fug of surimi prepared from fish kept in ice for different times are shown in Fig. 3. From the results, no MHC band was observed in Som-fug of surimi produced from fish kept for an extended time, especially for 15 days. This was presumed that Som-fug proteins from fish stored in ice for a longer time were more susceptible to proteolysis. Riebroy, Benjakul, Visessanguan, Kijrongrojana, and Tanaka (2004) reported that protein bands of commercial Som-fug with the molecular weights of 205, 116, 45, and 36 kDa were generally observed. Actin was the dominant protein remaining in the samples, while MHC appeared as a smaller band. Benjakul et al. (2002) reported that only small decrease in MHC band was found in bigeye snapper during ice storage up to 15 days. Thus, the low myosin heavy chain band intensity in Som-fug indicated that the degradation most likely occurred during fermentation. However, actin was more resistant to proteolysis. Among all proteins, MHC was most susceptible to proteolysis (Benjakul et al., 1997). Both indigenous muscle and microbial proteases contributed to the degradation of muscle proteins (Hughes et al., 2002). MHC underwent degradation during fermentation and this could be related to the characteristics of Som-fug (Riebroy et al., 2004).

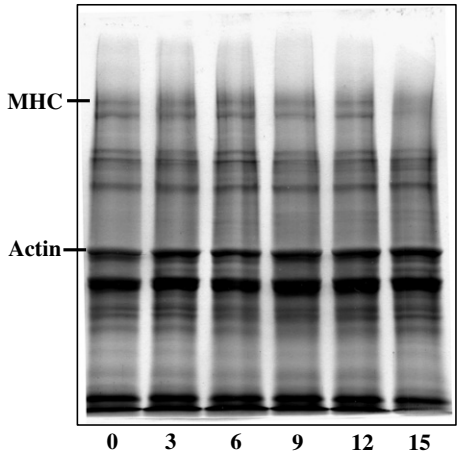

Fig. 3. SDS–PAGE patterns of muscle proteins in fermented Som-fug prepared from surimi of bigeye snapper kept in ice for different times. MHC: myosin heavy chain. Numbers designate the storage time in ice (days).

3.2.4. Lipid oxidation

Fermented Som-fug from surimi of fresh fish had the lowest TBARS value ($p < 0.05$) and Som-fug from surimi of fish with longer iced storage times showed the greater TBARS values ($p < 0.05$) (Table 1). The result suggested that lipid oxidation occurred in Som-fug from different starting raw materials with different degrees. Lipid oxidation in Som-fug produced from marine fish was also reported by Riebroy et al. (2006a). Generally, marine fish contain higher unsaturated fatty acid content than freshwater fish (Sikorski, Kolakowska, & Pan, 1990). Hence, Som-fug produced from marine fish was more prone to lipid oxidation than that produced from freshwater fish (Riebroy et al., 2005, 2006a). From the result, lipid oxidation might be accelerated by extended storage, which might cause the damage of the muscle structure by proteolysis, exposing the fatty acids to oxygen and catalysing factors, such as iron and heme (Morrisey, Sheehy, Galvin, Kerry, & Buckley, 1998). Lipid oxidation is responsible for a reduction in nutritional quality as well as changes in flavour (Aguirreźabal, Meteo, Domínguez, & Zumalacárregui, 2000). From the result, it was suggested that lipid oxidation in Som-fug samples could be influenced by the quality of the starting raw material, which was associated with postmortem storage time.

3.2.5. Weight loss, released water, and expressible water contents

Weight loss, released water and expressible water contents of Som-fug from surimi of bigeye snapper stored in ice for different times are shown in Table 2. Changes in weight loss were most likely associated with acidity. Som-fug from surimi of fish stored for an extended time showed the poorer water holding capacity. Degraded proteins in the fish with extended iced storage had lower affinity to

Table 2
Weight loss, released water, and expressible water contents of fermented Som-fug produced from surimi of bigeye snapper stored in ice for different times

Samples	Weight loss (%)	Released water (%)	Expressible water (%)
S-0	$19.47 \pm 0.44^{d\#,\dagger}$	5.78 ± 0.12^{f}	23.57 ± 0.30^{f}
S-3	19.91 ± 0.51^{d}	7.20 ± 0.04^{e}	26.97 ± 0.20^{e}
S-6	20.08 ± 0.53^{cd}	8.16 ± 0.12^{d}	27.85 ± 0.73^{d}
S-9	21.37 ± 0.25^{c}	10.51 ± 0.36^{c}	28.66 ± 0.33^{c}
S-12	22.94 ± 0.30^{b}	16.30 ± 0.81^{b}	31.02 ± 0.40^{b}
S-15	24.62 ± 0.11^{a}	24.86 ± 0.28^{a}	32.43 ± 0.14^{a}

S-0, S-3, S-6, S-9, S-12 and S-15: fermented Som-fug produced from surimi of bigeye snapper stored in ice for 0, 3, 6, 9, 12, and 15 days, respectively.
[#] Mean ± SD from six determinations.
[†] Different superscripts in the same column indicate significant differences ($p < 0.05$).

water. Water-holding capacity is directly correlated with the myofibrillar protein content (Smith, 1991). The extent of drip loss from meat is largely a function of changes, predominantly those affecting the ultimate pH value of the meat and the extent of changes in myofibrillar volumes. Denaturation of sarcoplasmic protein also contributed to the decreased water binding capacity of pork myofibrils (Wilson & Laack, 1999). The muscle proteins tend to denature as the pH falls. This leads to a reduction in their power to bind water. At the isoelectric point (p*I*), myosin and actin molecules have no net electrical charge and tend to lose the water normally bound to them, leading to exudation of muscle fibers (Visessanguan, Benjakul, Panya, Kittikun, & Assavanig, 2005). Differences in weight loss, released water and expressible water contents of commercial Som-fug samples indicated the difference in water-holding capacity (Riebroy et al., 2004, 2005). A weight decrease due to the loss of water is of economic importance. Accumulated exudation is not attractive to the consumer and water is also important for the texture (Foegeding, Laneir, & Hultin, 1996). From the results, the increases in weight loss, released water and expressible water contents were found in Som-fug from surimi of bigeye snapper kept in ice for longer time. Therefore, Som-fug produced from low quality fish gave the greater weight loss, released water and expressible contents, indicating the poorer quality of Som-fug.

3.2.6. Textural properties

Hardness, adhesiveness, springiness, cohesiveness, and resilience of fermented Som-fug decreased when surimi produced from fish stored for a longer time were used ($p < 0.05$) (Table 3). After the fermentation was accomplished, Som-fug from surimi of fresh fish had the highest hardness, adhesiveness, springiness, cohesiveness and resilience ($p < 0.05$). No significant differences in adhesiveness, springiness, cohesiveness, and resilience were observed in Som-fug from surimi of fish stored in ice up to 3 days of storage ($p > 0.05$). Thereafter, all parameters tested decreased with ice-storage time of starting raw materials. Generally, Som-fug produced from bigeye snapper mince showed the highest cohesiveness, springiness, and adhesiveness, compared with those from largehead hairtail, barracuda, obtuse barracuda, lizardfish, and threadfin bream (Riebroy et al., 2006a). The hardness of sausage is a measure of the degree of maturation, resulting from the denaturation and gelation of meat proteins, and the loss of waters (Gimeno, Astiasarán, & Bello, 1999). For Som-fug and Nham, texture formation is closely associated with fermentation, in which the mechanism of binding in Som-fug is an acid-initiated reaction (Riebroy et al., 2004, 2005; Visessanguan et al., 2004). Slow decrease in pH gradually induced aggregation of proteins, leading to the ordered formation of protein structure, which is associated with firmness (Fretheim, Egelandsdal, Harbitz, & Samejima, 1985). From the result, the decrease in textural parameters is coincidental with the degradation, deterioration as well as oxidation of muscle used as raw material for Som-fug production. The lowered textural properties of Som-fug from surimi of fish stored in ice for a longer time were also in accordance with the decrease in water holding capacity. Thus, the chain length of protein molecules mostly likely determined the texture of Som-fug.

3.2.7. Colour and whiteness

L^*, a^*, b^* and whiteness of fermented Som-fug from surimi of bigeye snapper stored in ice for different times are shown in Table 4. Generally, L^*, a^*, b^* and whiteness of fermented Som-fug from surimi of fish stored for an extended time were lower than those from surimi of fresh

Table 3
Textural properties of fermented Som-fug produced from surimi of bigeye snapper stored in ice for different times

Samples	Texture parameters				
	Hardness (g)	Adhesiveness (g s)	Springiness (mm)	Cohesiveness (g mm)	Resilience
S-0	$3434 \pm 89^{a\#,\dagger}$	-3.04 ± 0.04^{a}	0.82 ± 0.02^{a}	0.78 ± 0.01^{a}	0.35 ± 0.01^{a}
S-3	3301 ± 119^{b}	-3.00 ± 0.10^{a}	0.88 ± 0.03^{a}	0.77 ± 0.02^{a}	0.35 ± 0.00^{a}
S-6	3214 ± 69^{bc}	-3.38 ± 0.09^{b}	0.80 ± 0.02^{a}	0.75 ± 0.01^{b}	0.35 ± 0.00^{a}
S-9	3105 ± 88^{c}	-3.32 ± 0.04^{b}	0.76 ± 0.02^{b}	0.74 ± 0.01^{b}	0.35 ± 0.00^{a}
S-12	2961 ± 145^{d}	-3.49 ± 0.23^{b}	0.76 ± 0.02^{b}	0.74 ± 0.04^{b}	0.34 ± 0.01^{a}
S-15	2931 ± 38^{d}	-3.44 ± 0.25^{b}	0.76 ± 0.02^{b}	0.74 ± 0.03^{b}	0.29 ± 0.01^{b}

S-0, S-3, S-6, S-9, S-12 and S-15: fermented Som-fug produced from surimi of bigeye snapper stored in ice for 0, 3, 6, 9, 12, and 15 days, respectively.
[#] Mean ± SD from six determinations.
[†] Different superscripts in the same column indicate significant differences ($p < 0.05$).

Table 4
Colour and whiteness of fermented Som-fug produced from surimi of bigeye snapper stored in ice for different times

Samples	L^*	a^*	b^*	Whiteness
S-0	$74.78 \pm 0.03^{a\#,\dagger}$	-2.48 ± 0.06^{b}	7.47 ± 0.01^{a}	73.58 ± 0.02^{a}
S-3	74.42 ± 0.01^{bc}	-2.23 ± 0.06^{a}	6.09 ± 0.07^{b}	72.67 ± 0.02^{ab}
S-6	73.47 ± 1.09^{ab}	-2.47 ± 0.03^{b}	5.65 ± 0.07^{c}	72.76 ± 1.06^{ab}
S-9	73.11 ± 0.03^{ab}	-2.40 ± 0.02^{b}	5.40 ± 0.03^{c}	72.46 ± 0.02^{ab}
S-12	71.23 ± 0.50^{c}	-2.66 ± 0.08^{c}	6.15 ± 0.05^{b}	70.46 ± 0.40^{b}
S-15	71.09 ± 0.03^{c}	-2.88 ± 0.09^{d}	6.23 ± 0.08^{b}	70.28 ± 1.78^{b}

S-0, S-3, S-6, S-9, S-12 and S-15: fermented Som-fug produced from surimi of bigeye snapper stored in ice for 0, 3, 6, 9, 12, and 15 days, respectively.
Mean ± SD from six determinations.
† Different superscripts in the same column indicate significant differences ($p < 0.05$).

Table 5
Acceptance score of Som-fug produced from surimi of bigeye snapper stored in ice for different times

Samples	Attributes					
	Appearance	Colour	Texture	Flavour	Taste	Overall liking
S-0	$7.27 \pm 1.42^{a\#,\dagger}$	8.70 ± 1.12^{a}	7.85 ± 0.85^{a}	7.18 ± 1.39^{a}	6.55 ± 1.10^{a}	8.21 ± 0.92^{a}
S-3	7.20 ± 1.05^{a}	8.71 ± 1.55^{a}	7.83 ± 1.05^{a}	7.26 ± 1.27^{a}	6.52 ± 1.05^{a}	8.20 ± 1.27^{a}
S-6	7.04 ± 1.12^{b}	8.40 ± 0.95^{b}	7.69 ± 1.00^{ab}	7.00 ± 0.80^{b}	6.50 ± 1.38^{a}	8.19 ± 1.21^{a}
S-9	6.55 ± 1.85^{c}	8.45 ± 1.45^{b}	7.70 ± 1.05^{ab}	7.08 ± 0.97^{b}	6.53 ± 1.07^{a}	8.20 ± 0.50^{a}
S-12	6.45 ± 1.29^{c}	8.45 ± 0.62^{b}	7.65 ± 1.02^{b}	5.20 ± 0.58^{c}	6.55 ± 0.96^{a}	8.21 ± 1.05^{a}
S-15	6.05 ± 0.88^{d}	7.85 ± 1.12^{c}	7.05 ± 1.12^{c}	5.24 ± 1.26^{c}	6.50 ± 1.90^{a}	7.20 ± 0.16^{b}

S-0, S-3, S-6, S-9, S-12 and S-15: fermented Som-fug produced from surimi of bigeye snapper stored in ice for 0, 3, 6, 9, 12, and 15 days, respectively.
Mean ± SD from six determinations.
† Different superscripts in the same column indicate significant differences ($p < 0.05$).

fish ($p < 0.05$) (Table 4). Oxidation of myoglobin during storage might cause the adduction to the muscle, leading to the less removal of myoglobin during washing. Chaijan, Benjakul, Visessanguan, and Faustman (2005) found that the oxidation of myoglobin of sardine and mackerel muscles became intense with increasing storage time. Saisithi et al. (1986) reported that the colour of Som-fug produced from freshwater fish varied from white to brownish or reddish, depending on the kind of fish and the process used. However, some pigments, which are responsible for the colour in fish meat, might be removed by washing process of surimi production. The higher L^*, lightness, and whiteness might be associated with the denaturation of heme proteins under acidic condition and the exudates formed. Exudate possessed the light scattering property, resulting in whiter colour. The colour of Som-fug was reported to contribute to consumer acceptability (Riebroy et al., 2004, 2005).

3.2.8. Acceptability

The acceptance score of fermented Som-fug from surimi of bigeye snapper stored in ice for different times is shown in Table 5. Appearance, colour, texture and flavour likeness of fermented Som-fug generally decreased when fish kept in ice for a longer time were used as starting material ($p < 0.05$). However, the taste likeness was not affected by storage time ($p > 0.05$). No differences in overall liking of Som-fug were noticeable when fish kept in ice for up to 12 days were used ($p > 0.05$). During washing process, the low molecular weight compounds, especially TMA or ammonia as well as some oxidation products, might be removed to some extent. This might lead to the improvement of quality of the starting material. However, the storage of bigeye snapper for 15 days showed the detrimental effect on the acceptability of resulting Som-fug. Thus, the quality of raw material was found to have influence on the acceptance of the resulting Som-fug.

4. Conclusions

Characteristics and acceptance of Som-fug from bigeye snapper were affected by the quality of fish. Fish kept in ice for a longer time rendered Som-fug with lower water holding capacity, textural property and acceptance. Therefore, fresh fish gave Som-fug with the superior quality to those from poor quality fish.

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