

Available online at www.sciencedirect.com



Food Chemistry 88 (2004) 527-535

Food Chemistry

www.elsevier.com/locate/foodchem

Some characteristics of commercial Som-fug produced in Thailand

Siriporn Riebroy^a, Soottawat Benjakul^{a,*}, Wonnop Visessanguan^b, Kongkarn Kijrongrojana^a, Munehiko Tanaka^c

^a Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai 90112, Thailand

^b National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Paholyothin Road,

Klong1, Klong Luang, Pathumthani 12120, Thailand

^c Department of Food Science and Technology, Tokyo University of Fisheries, Konan 4, Minato, Tokyo 108-8477, Japan

Received 15 October 2003; received in revised form 28 January 2004; accepted 28 January 2004

Abstract

Seven commercial brands of Som-fug were analysed for chemical composition, biogenic amines and acceptance. Commercial Som-fug contained 11.4–16.2% protein, 1.13–1.56% lipid, 69.66–77.08% moisture, 2.45–3.60% ash and 3.37–4.90% salt. The pH values ranged from 4.56 to 4.60 and total acidity varied from 1.42% to 2.35%. Lactic acid was the major organic acid in all samples, but citric acid, succinic acid and pyruvic acid were found only in some brands. Differences in the trichloroacetic acid soluble peptides and lipid oxidation, among all samples, were observed. The proteolysis of myosin heavy chain was observed on SDS–PAGE analysis. Additionally, the formation of biogenic amines, including tryptamine, putrescine, cadaverine, histamine and tyramine, was observed in commercial Som-fug, but β -phenylethylamine was not detectable in any samples. Different Som-fug samples showed different acceptabilities, especially for taste and appearance. Therefore, raw materials, ingredients and process should be factors for determining the characteristics of Som-fug products.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: Som-fug; Fermented fish; Composition; Protein; Biogenic amines; Acceptance

1. Introduction

Som-fug is Thai traditional fermented minced fish, which is composed of fish mince, salt (2.5%), ground steamed rice (2–12%) and minced garlic (4%). The mixture is tightly packed in banana leaves or plastic bags and left to ferment for 2–5 days at 30 °C (Saisithi et al., 1986). Som-fug can be served either as a main dish or as a snack with vegetables. Som-fug is highly nutritious and is an excellent source of protein. The fish used are mainly fresh water fish rather than marine fish (Phitakpol, 1993; Saisithi et al., 1986). The fish species include giant snake-head fish (*Ophicephalus micropeltes*), Rohu (*Labeo rohita*), spotted featherback (*Notopterus*) (Østergaard et al., 1998; Phitakpol, 1993). Physical, microbiological and biochemical changes, involving tissue enzymes as well as microbial enzymes, take place in food during fermentation. These changes are influenced by the process conditions (Beriain, Peña, & Bello, 1993). The fermentation process depends on different parameters, such as the proportions and the quality of raw material, the use of starter cultures, temperature and relative humidity and microbial flora (Rosello, Barbas, Berna, & Lopez, 1995). Proteolysis is one of the most important biochemical changes occurring during the ripening of fermented sausage (Visessanguan, Benjakul, Riebroy, & Thepkasikul, 2004). It influences both texture and flavour development due to the formation of several low molecular weight compounds, including peptides, amino acids, aldehydes, organic acids and amines (Díaz, Fernández, Garcia de Fernando, de la Hoz, & Ordóñez, 1993). Lipid changes are manifested as adverse changes in flavour, colour, and nutritive value (Morrisey, Sheehy, Galvin, Kerry, & Buckley, 1998).

Biogenic amines in fermented foods are a potential risk for consumers (Shalaby, 1996). The formation of

^{*} Corresponding author. Tel.: +66-74-28-6334; fax: +66-74-212889. *E-mail address:* bsoottaw@ratree.psu.ac.th (S. Benjakul).

biogenic amines in fermented sausages has been reported (Hernandez-Jover, Pulido, Nougues, Font, & Vidal-Carou, 1997; Senöz, Isýklý, & Coksöyler, 2000). Biogenic amines, especially histamine, putrescine and cadaverine, have been suggested as indicators of spoilage of some foods, such as fresh fish, meat and vegetables. Biogenic amine formation is associated with the contaminating microbial flora (Bover-Cid, Miguélez-Arrizado, & Vidal- Carou, 2001). Due to the increasing demand for Som-fug in the market, a larger amount of Som-fug has been produced continuously. However, no information on chemical composition or properties of Som-fug produced commercially, has been reported. The aim of this study was to investigate the chemical composition, biogenic amines and acceptibility of commercial Som-fug produced in Thailand.

2. Materials and methods

2.1. Chemical reagents

Trichloroacetic acid (TCA), sodium bicarbonate, sodium hydroxide, and sulfuric acid were obtained from Merck (Darmstadt, Germany). β-Phenylethylamine hydrochloride, histamine dihydrochloride, cadaverine dihydrochloride, putrescine dihydrochloride, tryptamine hydrochloride, tyramine hydrochloride, 1,7-diamino heptane and malonaldehyde were obtained from Sigma (St. Louis, MO, USA). Butyric acid was obtained from Fluka (Buchs, Switzerland). Acetone, acetronitile, and methanol, of HPLC grade, were purchased from Lab-Scan (Ireland). All chemicals for electrophoresis were obtained from Bio-Rad (Richmond, CA, USA).

2.2. Sample collection

Seven commercial brands of Som-fug from different fermented fish companies were randomly purchased from supermarkets in Songkhla and Bangkok, Thailand. Prior to analyses, the samples were incubated at 30 °C until pH decreased to 4.60. Two batches were used for this study and all analyses of each batch were performed in triplicate. In order to prepare the samples for analysis, the casings were removed. Samples were cut up and ground in a meat grinder (MX-T2G National, Tokyo, Japan) for 1 min and kept at 4 °C for further analysis.

2.3. Proximate analysis

Samples were analysed for moisture and ash contents (AOAC, 2000). Total lipid was determined by an extraction technique with petroleum ether (AOAC, 2000). Protein was analysed according to the Kjeldahl method (AOAC, 2000). The factor 6.25 was used for conversion of nitrogen to crude protein content.

2.4. Determination of salt content

Salt content in samples was measured by the method of AOAC (2000). Sample (1 g) was treated with 10 ml of 0.1 N AgNO₃ and 10 ml of HNO₃. The mixture was boiled gently on a hot plate until all solids except AgCl₂ were dissolved (usually 10 min). The mixture was then cooled using running water; 50 ml of distilled water and 5 ml of ferric alum indicator were added. The mixture was titrated with standardized 0.1 N KSCN until the solution became permanent brownish-red. The salt content was then calculated and expressed as % NaCl.

2.5. Determination of pH and total acidity

The pH of the sample was measured using a pH meter equipped with an electrode for solid samples (CG842 Schott, Germany). The tritratable acidity was determined by the method of AOAC (2000). To the sample (5 g), 40 ml of boiling 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 3000g 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 standard 0.1 N NaOH, using phenolphthalein as an indicator. The total acidity was calculated as lactic acid and expressed as % total acidity (w/w).

2.6. Determination of organic acids

Organic acids in commercial Som-fug were extracted according to the method of Nassos, Schade, King, and Stafford (1984) with a slight modification. Sample (15 g) was treated with 35 ml of nanopure distilled water and the mixture was homogenised at 8000 rpm for 1 min using an Ultra Turrax homogenizer (IKA Labortechnik, Selangor, Malasia). The homogenate was centrifuged at 4500g for 15 min (5416 Eppendorf, Germany). The supernatant (0.9 ml) was mixed with 0.1 ml of 5% (w/v) butyric acid as an internal standard. To the mixture, 2 ml of 0.5 N perchloric acid were added. The mixture was allowed to stand for 5 min prior to centrifugation at 12,000g for 30 min (5403 Eppendorf, Germany). The supernatant was filtered through 0.45 µm membrane (Minisart RC-15, Satorious, Goettingen, Germany). Prepared filtrate was subjected to organic acid analysis by HPLC system (2690 Alliance, Waters, USA) using a Waters 996 Photodiode Array Detector, an Aminex HPX-87H column (300×7.8 mm i.d.) (Bio-Rad, USA), and a computer containing a Millenium 32 package programme-based integrator (Waters, Milford, MA, USA). Sample (20 µl) was injected into the HPLC. A mobile phase (0.01 N H₂SO₄) was run in isocratic mode at 0.6 ml/min. The temperature around the column was maintained at 50 °C. The running time, for all organic acids, was 40 min.

2.7. Determination of TCA-soluble peptides

TCA-soluble peptides were determined according to the method described by Greene and Babbit (1990). Sample (3 g) was homogenised with 27 ml of 5% TCA (w/v). The homogenate was kept in ice for 1 h and centrifuged at 12,000g for 5 min. The soluble peptides in the supernatant were measured by the method of Lowry, Rosebrough, Farr, and Randall (1951) and expressed as µmole tyrosine/g sample.

2.8. Determination of thiobarbituric acid reactive substances (TBARS)

TBARS were determined according to the method of Buege and Aust (1978). 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 5500g for 25 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (UV-1601, Shimadzu, Japan). TBARS value was calculated from the standard curve of malonaldehyde and expressed as µg malonaldehyde/g sample.

2.9. Determination of biogenic amines

Biogenic amines in commercial Som-fug were extracted according to the procedure of Koutsoumanis, Lampropoulou, and Nychas (1999) with a slight modification. Sample (4 g) was added with 10 ml of 10% TCA solution and homogenised at 13,500 rpm using an Ultra Turrax homogeniser (IKA Labortechnik, Selangor, Malasia) for 2 min. The homogenate was centrifuged at 12,000g for 10 min at 4 °C using a Sorvall Model RC-5B Plus centrifuge (Newtown, CT, USA). The pellet was extracted with 10 ml of 10% TCA solution and the supernatants were combined and made up to 25 ml with 10% TCA solution. Derivatisation of extracted sample was performed according to the method of Senöz et al. (2000). The extract (0.5 ml) was treated with 50 μ l of 1,7 diaminoheptane (3.0 mg/ml) as the internal standard. The solution was then mixed with 100 µl of 2 N NaOH and 150 µl of saturated sodium bicarbonate. One ml of dansyl chloride (10mg/ml) was added to each sample, mixed very well, and then incubated for 45 min at 40 °C. Residual dansyl chloride was removed by adding 50 µl of 25% ammonia and centrifuged at 2500g for 30 min and the supernatant was filtered (0.45 µm); 20 µl of filtrate was then injected into the HPLC (600E Waters, Massachusetts, USA). Quantitative analysis of amines was carried out in a Waters HPLC system (Water Associates, Miliford, MA, USA). Separation was achieved using a column of Hypersil BDS C18 (ThermoHypersil, Hercules, USA). A gradient elution programme was used with the mobile phase of 100% methanol (solvent A) and nanopure distilled water (solvent B), starting with 55% solvent A and 45% solvent B and finishing with 100% solvent A and 0% solvent B after 45 min. The flow rate was 1.5 ml/min. Data were processed and calculated with a CSW32 programme-based integrator.

2.10. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Samples (3 g) were 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 centrifuging at 10,000g for 15 min at room temperature. The protein concentration of supernatant was determined by the Lowry et al. (1951) method. 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, California., 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.

2.11. Acceptibility test

The fermented Som-fug samples were evaluated for acceptance by a untrained 30-member panel. The panellists were graduate students in the Food Technology programme of age ranging from 20 to 35 years, Faculty of Agro-Industry, Prince of Songkla University. Panellists had sensorial acquaintance with Som-fug. A nine-point hedonic scale, in which a score of 1 =dislike extremely, 5 = neither liked nor disliked and 9 = like extremely, was used for evaluation (Chamber IV & Wolf, 1996). 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. Individual samples of each brand were placed on dishes (diameter 3.0 cm) and the samples were covered with aluminium foil. The samples were allowed to stand at room temperature for at least 30 min prior to analysis. A balanced incomplete block (BIB) design was used to group samples (Melgaard, Civille, & Carr, 1990). Each of thirty panellists was randomly assigned one block of four samples from the design. Samples were randomly selected and coded with three-digit random numbers and presented to the panellists at room temperature. During evaluation, the panellists were situated in private booths. Room temperature water was given to rinse the mouth between samples. The

panellists evaluated each sample for appearance, flavour, taste and overall liking.

2.12. Statistical analysis

All data were subjected to analysis of variance (AN-OVA) and differences between means were evaluated by Duncan's multiple range test (Steel & Torrie, 1980) using SPSS statistic programme (Version 10.0) (SPSS, 1.2, 1998) for data analysis.

3. Results and discussion

3.1. Chemical composition of commercial Som-fug

Chemical compositions of commercial Som-fug samples are shown in Table 1. Som-fug contained 11.4–16.2% protein, 1.13–1.56% lipid, 69.66–77.08% moisture and 2.63–4.29% ash. Som-fug proteins mainly originated from minced fish. Thus, variation of protein content was likely affected by the different proportions of minced fish used. The result indicated that commercial Som-fug was highly nutritious and could be an excellent source of protein with low fat content. Moisture content in the sample could be affected by the amounts of minced fish used (Saisithi et al., 1986). The different fish mince preparations e.g. partial removal of water

Table 1 Chemical composition (%) of commercial Som-fug^a

prior to processing, possibly contributed to different moisture contents of the products. The highest ash content was observed in brand E, followed by F, C, G, A, D and B, respectively. Salt content and other adjuncts may contribute to ash found in the samples. Salt content of samples ranged from 3.02% to 3.95%. The salt content of Som-fug has been reported to be 2.50-5.45% (Adams, Cooke, & Rattagool, 1985; Phitakpol, Varanyanond, Reungmaneepaitoon, & Wood, 1995; Saisithi et al., 1986). Sodium chloride is an important adjunct in fermented food due to its many beneficial functions. Sodium chloride is also essential in processed meat to promote flavour, to improve emulsifying capacity, and to lower water activity and thus to help control microbial growth (Seman, Olson, & Mandigo, 1980).

The pH of samples was 4.56–4.60, while the total acidity varied from 1.42% to 2.35% (Table 2). The lowered pH was generally associated with the organic acids formed. The initial pH observed in commercial Som-fug was different. Thus the incubation time to obtain the final pH designated (4.6) varied. Furthermore, the different buffering capacity of muscle proteins from different species used for Som-fug production contributed to varying rate of pH decrease (Owens & Mendoza, 1985). Lactic acid was a dominant organic acid in Somfug and acetic acid was also found in all samples, even at a low level (0.06–0.1%). Acid content of all samples did

Brands	Protein	Lipid	Moisture	Ash	Salt
А	$16.2\pm0.17^{\rm d}$	$1.56\pm0.04^{\rm c}$	$73.25\pm0.48^{\text{b}}$	$3.60\pm0.02^{\rm c}$	3.08 ± 0.05^{a}
В	$11.9\pm0.21^{\rm a}$	$1.19\pm0.05^{\rm b}$	$77.08\pm0.36^{\rm d}$	$3.08\pm0.05^{\rm a}$	$3.10\pm0.02^{a,b}$
С	$13.9\pm0.21^\circ$	$1.30\pm0.01^{\rm b,c}$	$74.88\pm0.38^{\circ}$	$3.81\pm0.02^{\rm e}$	$3.75\pm0.04^{\rm b}$
D	$13.6\pm0.06^{b,c}$	$1.13\pm0.04^{\rm a}$	$75.16\pm0.03^{\circ}$	$3.43\pm0.03^{\rm b}$	3.15 ± 0.06 ^b
E	$13.0\pm0.17^{\rm b}$	$1.20\pm0.01^{\rm b}$	69.66 ± 0.16^a	$4.29\pm0.02^{\rm g}$	$3.37\pm0.02^{\rm c}$
F	$11.4\pm0.09^{\rm a}$	$1.48\pm0.09^{b,c}$	$73.65\pm0.32^{\text{b}}$	$3.90\pm0.05^{\rm f}$	$3.95\pm0.08^{\text{e}}$
G	$11.6\pm0.09^{\rm a}$	$1.46\pm0.08^{b,c}$	$76.25\pm0.37^{\text{d}}$	$3.75\pm0.05^{\rm d}$	$3.50\pm0.06^{\rm d}$

Different superscripts in the same column indicate significant differences (p < 0.05).

^a Means \pm SD from six determinations.

Table 2					
pH, total a	acidity and	organic a	cids in c	commercial	Som-fug ^a

Brands	pН	Total acidity	Organic acids ((%)			
		(% lactic acid)	Lactic acid	Acetic acid	Citric acid	Succinic acid	Pyruvic acid
А	$4.58\pm0.01^{\text{b}}$	$2.00\pm0.04^{\rm c}$	$1.89\pm0.03^{\rm c}$	$0.10\pm0.00^{\rm c}$	ND^b	ND	ND
В	$4.59\pm0.01^{\rm b}$	$2.02\pm0.02^{\rm c}$	$1.75\pm0.01^{\rm b}$	$0.10\pm0.00^{\circ}$	0.01 ± 0.00	ND	ND
С	$4.60\pm0.01^{\rm b}$	$2.07\pm0.06^{\rm c}$	$2.03\pm0.06^{\rm d}$	$0.05\pm0.00^{\rm a}$	0.02 ± 0.00	0.01 ± 0.00	ND
D	$4.59\pm0.01^{\rm b}$	$1.79\pm0.04^{\rm b}$	$1.70\pm0.02^{\rm b}$	$0.06\pm0.00^{\rm a,b}$	ND	ND	ND
Е	4.56 ± 0.01^{a}	$1.42\pm0.03^{\rm a}$	$1.21\pm0.04^{\rm a}$	$0.06\pm0.00^{\rm a,b}$	ND	ND	0.002 ± 0.00
F	$4.58\pm0.03^{\rm b}$	$2.35\pm0.03^{\text{e}}$	$2.17\pm0.07^{\rm e}$	$0.07\pm0.00^{\rm b}$	ND	ND	ND
G	$4.58\pm0.01^{\text{b}}$	$2.11\pm0.08^{\rm d}$	$2.15\pm0.08^{\text{e}}$	$0.10\pm0.00^{\rm c}$	ND	0.01 ± 0.00	ND

Different superscripts in the same column indicate significant differences (p < 0.05).

^a Means \pm SD from six determinations.

^b ND: not detectable.

not correlate well with the decreased pH, probably due to the differences in buffering capacity of muscle proteins (Srikorski, Kolakowska, & Burt, 1990) or different indigenous microflora (Østergaard et al., 1998). 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, are responsible for the flavour of Som-fug (Østergaard et al., 1998). The acid content is a very important factor determining the acceptability of product. Som-fug containing 2.2–2.5% lactic acid was the most acceptable for consumers (Saisithi et al., 1986). Østergaard et al. (1998) also reported that Som-fug containing 2.4-2.5% lactic acid had the highest sensory score. The lactic acid bacteria (LAB) are the main preserving factor and the addition of salt and garlic in many Thai fermented fish products probably contributes to the preservation (Steinkraus, 1996). The major role of LAB is production of organic acids, primarily lactic acid, from carbohydrate. Rapid growth of LAB caused the decrease in pH to below 4.5 within two days (Paludan-Müller, Huss, & Gram, 1999). Østergaard et al. (1998) reported that the initial counts of LAB in the Som-fug produced from two fresh water fish at day 0 were different. Pediococcus sp. and Lactobacillus sp. have been identified as the dominant LAB in commercial Som-fug and those prepared in the laboratory (Kimhamanon, 1994; Saisithi et al., 1986; Tanasupawat, Okada, Suzuki, Kazaki, & Komagata, 1993). The amount of lactic acid produced during fermentation was related to the salt content. Som-fug with a low salt content showed high acid formation (Saisithi et al., 1986). Lactic and acetic acids play an important role in imparting a tangy acidic character and may enhance saltiness, possibly by masking other flavours (Visessanguan et al., 2004).

The TBARS values in commercial Som-fug were different among brands, indicating the different lipid oxidation among samples (Table 3). Som-fug had TBARS values ranging from 5.00 to 13.9 mg/kg. Lipid oxidation in different samples was associated with

Table 3	
TCA-soluble peptides and TBARS of commercial Som-fug ^a	

Brands	TCA-soluble peptides (μmole tyrosine/g)	TBARS (μg malonaldehyde/g)
А	6.07 ± 0.16^d	$11.9\pm0.07^{\rm c}$
В	$4.48\pm0.08^{\rm b}$	$12.4\pm0.07^{\circ}$
С	$5.53\pm0.18^{\rm c}$	$5.8\pm0.03^{\mathrm{a}}$
D	$3.33\pm0.09^{\rm a}$	$5.03\pm0.10^{\rm a}$
Е	$4.79\pm0.06^{\rm b}$	$13.9\pm0.19^{\rm d}$
F	$6.46\pm0.06^{\rm d}$	$10.2 \pm 0.12^{\rm b}$
G	$3.84\pm0.10^{\rm a}$	$5.00\pm0.15^{\rm a}$

Different superscripts in the same column indicate significant differences (p < 0.05).

^a Means \pm SD from six determinations.

different antioxidant and prooxidant in the samples (Lorenzo, Michinel, López, & Carballo, 2000). Furthermore, different lipid contents and fatty acid compositions among fish used for Som-fug production were also postulated. Fish have a high content of highly unsaturated fatty acids (22-48%) which are prone to oxidation (Foegeding & Laneir, 1996). Thus, different fish species used as raw material might contribute to varied oxidation among samples. Fanco, Prieto, Cruz, López, and Carballo (2002) reported that the lipid oxidation of Androlla sausage at 0 day was 0.17 mg malonaldehyde/kg and increased during fermentation. Moreover, lipid oxidation is accelerated by processes that damage the muscle structure, such as mincing and comminuting. These could expose the fatty acids to oxygen and catalysing factors, such as iron and heme (Gray, Gomma, & Buckly, 1996; Morrisey et al., 1998). Oxidation is necessary to develop the desirable flavour of some fermented foods (Chizzolini, Novelli, & Zanardi, 1998; Stahnke, 1995).

3.2. Protein degradation of commercial Som-fug

TCA-soluble peptides were observed in all samples, ranging from 3.33 to 6.46 µmole tyrosine/g (Table 3). Among all samples tested, brands A and F contained more TCA-soluble peptide than other brands. Differences in TCA-soluble peptides observed among samples were associated with the differences in initial raw mix ingredients and proteolysis induced by acidic condition (Matulis, Mekeith, Sutherland, & Susan, 1995; Visessanguan et al., 2004). The pH values of 4.5-5.0 were optimal for the activity of proteolytic enzymes, especially cathepsins (Beriain, Chasco, & Lizaso, 2000; Sherekar, Gore, & Ninjoor, 1988). An, Weerasinghe, Seymour, and Morrissey (1994) reported that the TCAsoluble peptides of Pacific whiting proteins, detected at day 0, were negligible. 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). Strong proteinase activity, produced by staphylococci and bacilli, is responsible for breakdown of fish protein into peptides and free amino acids, which may contribute to the unique flavour of the products (Valysevi & Rolle, 2002). SDS-PAGE electrophoregrams of muscle proteins in seven brands of commercial Som-fug are shown in Fig. 1. Protein bands, with apparent molecular weights of 205, 116, 45, and 36 kDa, were generally observed. Different intensities of those bands were found among different brands. From this result, it was noted

S. Riebroy et al. / Food Chemistry 88 (2004) 527-535

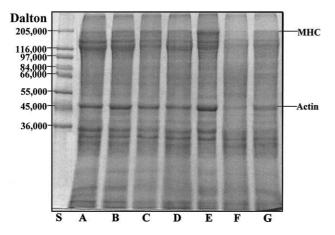


Fig. 1. SDS–PAGE pattern of muscle proteins in commercial Som-fug: S, molecular weight standard; MHC, myosin heavy chain; A–G, brands of commercial Som-fug.

that actin (MW of 45 kDa) was the dominant protein remaining in the samples, while myosin heavy chain (MW 205 kDa) appeared to a smaller extent. In general, myosin heavy chain is the major myofibrillar protein, constituting 55-60% (Xiong, 1997). From this result, the low myosin heavy chain band intensity indicated degradation, possibly during fermentation. Actin was more resistant to proteolysis, than were other proteins (An et al., 1994). Among all protein, myosin heavy chain was most susceptible to proteolysis (Benjakul, Seymour, Morrissey, & An, 1997). Therefore, it seems that myosin heavy chain underwent degradation during fermentation and this could be related to the characteristic of Somfug. Both indigenous muscle and microbial proteases contributed to the degradation of muscle proteins (Hughes et al., 2002). The differences in proteolytic phenomena found among commercial brands of Somfug were presumed to be due to the different fermentation technologies applied by each industry and to different raw materials (Chasco, Beriain, & Bello, 1993). From the results, it appears that proteolysis in Som-fug could be influenced by many factors, such as product formulation, indigenous microorganism, and processing condition.

		commercial	

The contents of tryptamine, β -phenylethylamine, putrescine, cadaverine, histamine and tyramine in commercial Som-fug are shown in Table 4. Putrescine and cadeverine were found in samples as the major biogenic amines. Highest contents of putrescine and cadaverine were observed in brands A and F. Lowest contents were found in brand G. Putrescine and cadaverine can be used as spoilage indices in fish (Koutsoumanis et al., 1999). From the result, putrescine and cadaverine contents were coincidental with TCA-soluble peptides. It appears that proteolysis might provide the nutrient for spoilage microorganisms, leading to the promoted growth of those microorganisms. Histamine content varied from 55.1 to 291 mg/kg. Nout (1994) pointed out that histamine contents should be in the range of 50-100 mg/kg in sausage processed according to "Good Manufacturing Practice". Among all samples tested, histamine levels were highest in brand G (291 mg/ kg). Şenöz et al. (2000) found that the histamine content in Turkish style sausages was between 6.72 and 362 mg/ kg. Tyramine content in Som-fug samples varied from 19.3 to 225 mg/kg. The allowable maximum level of tyramine in foods is 100-800 mg/kg and tyramine with a level of 1080 mg/kg is toxic (Shalaby, 1996). Tyramine is usually the major amine found in fermented products, such as fermented sausages. Its production is mainly associated with tyrosine-decarboxylase activity of LAB (Bover-Cid et al., 2001). In commercial Som-fug, tryptamine was a minor biogenic amine. High content of biogenic amines can be found in fermented products derived from raw materials with high protein content. The microbial growth often leads to high concentrations of biogenic amines (Silla Santos, 1996). The production of biogenic amines is a characteristic of several groups of microorganisms such as enterobacteriacece, Pseudomonas spp., micrococcaceae, enterococci and LAB (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994). Formation of biogenic amines in food generally occurs due to decarboxylation of amino acids by microbial substrate-specific enzymes. Therefore, biogenic

Table 4			
Biogenic amines	in	commercial	Som-fug ^a

Brands	Biogenic amine	(mg/kg)				
	Tryptamine	Phenylehtylamine	Putrescine	Cadaverine	Histamine	Tyramine
А	$33.6 \pm 1.88^{\rm c}$	ND^b	$219\pm11.0^{\rm e}$	$105\pm 6.98^{\circ}$	92.8 ± 4.14^{d}	$225\pm13.0^{\rm f}$
В	$38.3 \pm 1.96^{\rm d}$	ND	$104\pm3.07^{\circ}$	$328 \pm 12.49^{\rm f}$	$69.6\pm2.82^{\rm b}$	$40.8\pm1.61^{\rm c}$
С	$86.1\pm1.51^{\rm g}$	ND	$113\pm5.20^{\rm d}$	$271 \pm 7.72^{\rm e}$	$55.1\pm2.26^{\rm a}$	$19.3\pm1.03^{\rm a}$
D	$67.5 \pm 1.82^{\rm e}$	ND	$99.7 \pm 4.13^{\circ}$	$98.2\pm3.67^{\rm b}$	$71.6 \pm 1.12^{\circ}$	71.7 ± 3.63^{d}
Е	$18.6\pm0.21^{\rm a}$	ND	$58.6\pm2.00^{\rm b}$	$94.8\pm2.01^{\rm b}$	$111 \pm 3.68^{\text{e}}$	$36.3\pm1.55^{\text{b}}$
F	$70.8\pm2.17^{\rm f}$	ND	$275 \pm 11.9^{\rm f}$	$212\pm5.11^{\rm d}$	$149\pm2.35^{\rm f}$	$140 \pm 0.69^{\text{e}}$
G	29.6 ± 1.72^{b}	ND	$17.0\pm0.75^{\rm a}$	20.2 ± 0.79^a	$291\pm19.76^{\rm g}$	$76\pm1.39^{\rm d}$

Different superscripts in the same column indicate significant differences (p < 0.05).

^a Means \pm SD from six determinations.

^b ND: not detectable.

Brands	Attributes				
	Appearance Flavour		Taste	Overall liking	
A	$5.70\pm0.12^{\rm a}$	$6.18\pm0.39^{a,b}$	$4.60 \pm 1.10^{\mathrm{a}}$	$5.49\pm0.42^{a,b}$	
В	$7.20\pm1.05^{ m g}$	$6.26 \pm 0.47^{ m a,b}$	$5.40 \pm 1.05^{\rm b}$	$6.77\pm0.27^{\circ}$	
С	$6.40\pm0.12^{\rm b}$	$7.00\pm0.10^{\mathrm{b}}$	$7.10\pm1.38^{\rm g}$	7.19 ± 0.21^{d}	
D	$6.55\pm0.15^{\rm d}$	$5.28\pm0.01^{\mathrm{a}}$	$5.65 \pm 1.07^{\circ}$	$5.90\pm0.10^{\rm b}$	
E	$6.45\pm0.12^{\rm c}$	$5.20\pm0.58^{\rm a}$	$6.35\pm0.96^{\rm e}$	$4.94\pm1.05^{\rm a}$	
F	$7.05\pm1.12^{\rm f}$	$5.94 \pm 0.26^{a,b}$	$5.95\pm1.90^{\rm d}$	$6.10\pm0.16^{\rm b}$	
G	$6.75 \pm 1.05^{\rm e}$	$6.15\pm1.08^{\rm a,b}$	$6.40\pm1.24^{\rm f}$	$6.61 \pm 1.01^{\circ}$	

Different superscripts in the same column indicate significant differences (p < 0.05).

^a Means \pm SD from 18 determinations (nine determinations for each replication).

amines are also of concern in relation to food spoilage because they result from decarboxylase activity of spoiling microflora during the storage of food (Vidal-Carou, Izquierdo-Pulido, Martín-Morro, & Marine-Font, 1990). Maijala and Eerola (1993) concluded that contaminant LAB play an important role in tyramine and histamine formation during the fermentation of sausages. Putrescine is the major diamine produced by pseudomonas, whereas cadaverine is produced by enterobacteriaceae (Jay, 1996). Also, the higher proteolysis and the low pH of Som-fug samples might favour the decarboxylase activity of microorganisms (Kebary, El-Sonbaty, & Badawi, 1999).

3.4. Acceptibility of commercial Som-fug

The acceptibility evaluation of seven different commercial brands of Som-fug is shown in Table 5. Brand C had the highest score for flavour liking, taste liking and overall liking (p < 0.05). The lowest scores for flavour liking and overall liking were observed with brand E, whereas brand A had the lowest score for appearance liking and taste liking (p < 0.05). Generally, the flavour mainly affects the sensory quality of foods, and is strongly related to lipids in fermented food (Fernández, de la Hoz, Díaz, Cambero, & Ordóñez, 1995). The highest TBARS value (of brand E) probably affected the acceptability, especially flavour liking. Lipid oxidation products are responsible for the development of the undesirable taste and flavour. Different tastes were possibly caused by differences in proteolytic development (Molly et al., 1997). Most people prefer fully matured Som-fug, which is very sour, thereby masking the taste of salt (Saisithi et al., 1986). The differences in sensorial characteristics among samples could be influenced by the differences in fish used, ingredients, fermentation process and conditions (Beriain et al., 2000). Therefore, it is likely that chemical compositions and acidity probably contributed to the varied acceptibilities of different Som-fugs. Østergaard et al. (1998) reported that the lactic acid content of Som-fug needs to be above 2% with a pH below 4.5 and salt content of 2.5-4.5%.

Salt concentration was another important determinant of the overall quality of Som-fug (Saisithi et al., 1986; Seman et al., 1980). Valysevi and Rolle (2002) suggested that the acid production in Som-fug takes place most rapidly at low salt concentration (1.5%). Rapid acid production has been known to result in poor flavour development and poor overall liking of the product (Saisithi et al., 1986). Therefore, many factors, both intrinsic and extrinsic, could contribute to Som-fug characteristics with different acceptibilities.

4. Conclusion

The chemical composition of commercial Som-fug differed among brands. The organic acids, especially lactic acid, might contribute to acceptibility of commercial Som-fug. However, putrescine, cadaverine and also histamine were found. Therefore, improvement of process or formulation of Som-fug could be a promising means of minimizing the safety risk and maximizing the acceptability of Som-fug.

Acknowledgements

Authors thank Prince of Songkla University for financial support.

References

- Adams, M. R., Cooke, R. D., & Rattagool, P. (1985). Fermented fish products of South East Asia. *Tropical Science*, 25, 61–73.
- An, H., Weerasinghe, V., Seymour, T. A., & Morrissey, M. T. (1994). Cathepsin degradation of Pacific whiting surimi proteins. *Journal* of Food Science, 59, 1013–1017, 1033.
- AOAC (2000). Official methods of analysis, (17th ed.). Gaithersburg, MD: Association of Official Analytical Chemists.
- 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, 729–733.

- Beriain, J. A., Chasco, J., & Lizaso, G. (2000). Relationship between biochemical and sensory quality characteristics of different commercial brands of salichichon. *Food Control*, 11, 231–237.
- Beriain, J. A., Peña, M. P., & Bello, J. (1993). A study of the chemical components which characterize Spanish suacisson. *Food Chemistry*, 48, 31–37.
- Bover-Cid, S., Miguélez-Arrizado, M. J., & Vidal- Carou, C. (2001). Biogenic amine accumulation in ripened sausages affected by the addition of sodium sulphite. *Meat Science*, 59, 391–396.
- Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology*, 52, 302–304.
- Chamber IV, E., & Wolf, M. B. (1996). Sensory testing methods (2nd ed.). Philadelphia, USA: American Society for Testing and Materials.
- Chasco, J., Beriain, M. J., & Bello, J. (1993). A study of changes in the fat content of some varieties of dry sausage during the curing process. *Meat Science*, 34, 191–204.
- Chizzolini, R., Novelli, E., & Zanardi, E. (1998). Oxidation in traditional Mediterranean meat products. *Meat Science*, 49, S87– S89.
- Díaz, O., Fernández, M., Garcia de Fernando, G. D., de la Hoz, L., & Ordóñez, J. A. (1993). Effect of the addition of Pronase E on the proteolysis of dry fermented sausages. *Meat Science*, 34, 205–218.
- Fanco, I., Prieto, B., Cruz, J. M., López, M., & Carballo, J. (2002). Study of the biochemical changes during the processing of Androlla, a Spanish dry cured pork sausage. *Food Chemistry*, 78, 339–345.
- Fernández, M., de la Hoz, L., Díaz, O., Cambero, M. I., & Ordóñez, J. A. (1995). Effect of the addition of pancreatic lipase on the ripening of dry-fermented sausages-Part 2. Free fatty acids, short-chain fatty acids, carbonyls and sensory quality. *Meat Science*, 40, 351– 362.
- Foegeding, E. A., & Laneir, T. (1996). Characteristics of edible muscle tissues. In O. R. Fennema (Ed.), *Food chemistry* (pp. 879–942). New York, USA: Marcel Dekker.
- Gray, J. I., Gomma, E. A., & Buckly, D. J. (1996). Oxidative quality and shelf life of meats. *Meat Science*, 43, S111–S115.
- Greene, D. H., & Babbit, J. K. (1990). Control of muscle softening and protease–parasite interaction in arrowtooth flounder *Atheresthes* stomias. Journal of Food Science, 55, 579–580.
- Halász, A., Baráth, Á., Simon-Sarkadi, L., & Holzapfel, W. (1994). Biogenic amines and their production by microorganisms in food. *Trends in Food Science and Technology*, 5, 42–50.
- Hernandez-Jover, T. H., Pulido, M. I., Nougues, M. T. V., Font, A. M., & Vidal-Carou, M. C. (1997). Biogenic amine and polyamine contents in meat and meat products. *Journal of Agricultural and Food Chemistry*, 45, 2098–2102.
- Hughes, M. C., Kerry, J. P., Arendt, E. K., Kenneally, P. M., McSweeney, P. L. H., & O'Neill, E. E. (2002). Characterization of proteolysis during the ripening of semi-dry fermented sausage. *Meat Science*, 62, 205–216.
- Jay, J. M. (1996). Microorganisms in foods. In J. M. Jay (Ed.), Modern food microbiology (pp. 69–191). New York, USA: Chapman & Hall.
- Kebary, K. M. K., El-Sonbaty, A. H., & Badawi, R. M. (1999). Effects of heating milk and accelerating ripening of low fat Ras cheese on biogenic amines and free amino acids development. *Food Chemistry*, 64, 67–75.
- Kimhamanon, T. (1994). Microbial changes during production of fermented fish (Som-fug). Master of Science Thesis in Food Technology. Prince of Songkla University 2538.
- Koutsoumanis, K., Lampropoulou, K., & Nychas, G. E. (1999). Biogenic amines and sensory changes associated with the microbial flora of Mediterranean gilt-head sea bream (*Sparus aurata*) stored aerobically at 0, 8, and 15 °C. *Journal of Food Protection*, 62, 398– 402.

- Laemmli, U. K. (1970). Cleavage of structure protein during the assembly of head bacteriophage T4. *Nature*, 277, 680–685.
- Lorenzo, J. M., Michinel, M., López, M., & Carballo, J. (2000). Biochemical characteristics of two Spanish traditional dry-cured sausage varieties: Androlla and Botillo. *Journal of Food Composition and Analysis, 13*, 809–817.
- Lowry, Q. H., Rosebrough, N. J., Farr, L. A., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193, 256–275.
- Maijala, R., & Eerola, S. (1993). Contaminant lactic acid bacteria of dry sausages produce histamine and tyramine. *Meat Science*, 35, 387–395.
- Matulis, R. J., Mekeith, F. K., Sutherland, J. W., & Susan, B. M. (1995). Sensory characteristics of frankfurters as affected by fat, salt, and pH. *Journal of Food Science*, 60, 42–47.
- Melgaard, M., Civille, G. V., & Carr, B. T. (1990). Mutiple difference tests: Balanced Incomplete Block (BIB) Designs. In M. Meigaard, G. V. Civille, & B. T. Carr (Eds.), *Sensory evaluation techniques* (pp. 105–112). FL, USA: CRC Press.
- Molly, K., Demeyer, D., Johansson, G., Raemeakers, M., Ghistelinck, M., & Geenen, I. (1997). The importance of meat enzymes in ripening and flavour generation in dry fermented sausages. *Food Chemistry*, 59, 539–545.
- Morrisey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P., & Buckley, D. J. (1998). Lipid stability on meat and meat products. *Meat Science*, 49, S73–S86.
- Nassos, P. A., Schade, J. E., King, A. D., & Stafford, A. E. (1984). Comparion of HPLC and GC method for measuring lactic acid in ground beef. *Journal of Food Science*, 49, 671–674.
- Nout, M. J. R. (1994). Fermented foods and food safety. Food Research International, 27, 291–294.
- Østergaard, A., Emberk, P. K. B., Yamprayoon, J., Wedell-Neergarrd, W., Huss, H. H., & Gram, L. (1998). Fermentation and spoilage of som fak, a Thai low-salt fish product. *Tropical Science*, 38, 105– 112.
- Owens, J. D., & Mendoza, L. S. (1985). Enzymatically hydrolysed and bacterially fermented fishery products. *Journal of Food Technology*, 20, 273–293.
- Paludan-Müller, C., Huss, H. H., & Gram, L. (1999). Characterization of lactic acid bacteria isolated from Thai low-salt fermented fish product and the role of garlic as substrate for fermentation. *International Journal of Food Microbiology*, 46, 219–229.
- Phitakpol, B. (1993). Fish fermentation technology in Thailand. In C. H. Lee & K. H. Steinkraus (Eds.), *Fermentation technology* (pp. 155–166). Tokyo, Japan: United Nation University Press.
- Phitakpol, B., Varanyanond, W., Reungmaneepaitoon, S., & Wood, H. (1995). *The traditional fermented foods of Thailand*. Bangkok, Thailand: Institute of Food Research and Products Development, Kasetsart University.
- Rosello, C., Barbas, J. I., Berna, A., & Lopez, N. (1995). Microbial and chemical changes in Sobrassada during ripening. *Meat Science*, 40, 379–385.
- Saisithi, P., Wonkhalsung, C., Boonyaratanakornkit, M., Yongmanitchai, P., Chimanage, P., & Maleehuan, S. (1986). *Improvement of Thai traditional fermented fish product: Som-fug.* Bangkok, Thailand: Institute of Food Research and Product Development, Kasetsart University.
- Seman, D. L., Olson, D. G., & Mandigo, R. W. (1980). Effect of reduction and partial replacement of sodium on bologna characteristics and acceptability. *Journal of Food Science*, 45, 1116–1121.
- Şenöz, B., Işýklý, N., & Çoksöyler, N. (2000). Biogenic amines in Turkish sausages (sucuk). *Journal of Food Science*, 65, 764–767.
- Shalaby, A. R. (1996). Significance of biogenic amines to food safety and human health. *Food Research International*, 29, 675–690.
- Sherekar, S. V., Gore, M. S., & Ninjoor, V. (1988). Purification and characterization of cathepsin B from the skeletal muscle of fresh

water fish, *Tilapia mossambica. Journal of Food Science*, 53, 1018–1023.

- Silla Santos, M. H. (1996). Biogenic amines: Their importance in foods. *International Journal Food Microbiology*, 29, 213–231.
- Spanier, A. M., Flores, M., McMillin, K. W., & Bidner, T. D. (1997). The effect of post- mortem aging on meat flavor quality in Bragus beef. Correlation of treatment, sensory, instrument and chemical descriptors. *Food Chemistry*, 59, 531–538.
- Srikorski, Z. E., Kolakowska, A., & Burt, J. R. (1990). Postharvest biochemical and microbial changes. In Z. E. Sikorski (Ed.), *Seafood: Resources, nutritional composition and preservation* (pp. 55–76). Florida, USA: CRC Press.
- Stahnke, L. H. (1995). Dried sausages fermented with *Staphylococcus xylosus* at different temperatures and different ingredient levels-part II. Volatile components. *Meat Science*, 41, 193–209.
- Steel, R. G. D., & Torrie, J. H. (1980). Principle and procedure of statistics (2nd ed.). New York: McGraw-Hill.
- Steinkraus, K. H. (1996). *Handbook of indigenous fermented foods*. New York: Marcel Dekker.

- Tanasupawat, S., Okada, S., Suzuki, K., Kazaki, M., & Komagata, K. (1993). Lactic acid bacteria, particularly heterofermentative Lactobacilli, found in fermented foods in Thailand. *Bulletin Japan Federal Culture Collection*, 9, 65–78.
- Valysevi, R., & Rolle, R. J. (2002). An overview of small-scale food fermentation technologies in developing countries with special reference to Thailand: Scope for their improvement. *International Journal of Food Microbiology*, 75, 231–239.
- Vidal-Carou, M. C., Izquierdo-Pulido, M., Martín-Morro, M. C., & Marine-Font, A. (1990). Histamine and tyramine in meat products: Relationship with meat spoilage. *Food Chemistry*, 37, 239– 249.
- Visessanguan, W., Benjakul, S., Riebroy, S., & Thepkasikul, P. (2004). Changes in composition and functional properties of proteins and their contributions to Nham characteristics. *Meat Science*, 64, 579– 588.
- Xiong, Y. L. (1997). Structure-function relationships of muscle proteins. In S. Damodaran & A. Paraf (Eds.), *Food proteins and their applications* (pp. 341–392). NY, USA: Marcel Dekker.