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Effect of irradiation on properties and storage stability of Som-fug produced from bigeye snapper

Siriporn Riebroy [a], Soottawat Benjakul [a,*], Wonnop Visessanguan [b], Munehiko Tanaka [c], Ulf Erikson [d], Turid Rustad [e]

[a] Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, 15 Kanchanawanich Road, Hat Yai, Songkhla 90112, Thailand
[b] National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Paholyothin Rd., Klong 1, Klong Luang, Pathumthani 12120, Thailand
[c] Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Konan 4, Minato, Tokyo 108-8477, Japan
[d] SINTEF Fisheries and Aquaculture, Processing Technology, 7465 Trondheim, Norway
[e] Department of Biotechnology, Faculty of Natural Science and Technology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway

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Abstract

Effects of irradiation at different doses (0, 2 and 6 kilogray (kGy)) on the microbiological, chemical and physical properties of Som-fug, a Thai fermented fish mince, were investigated. Lactic acid bacteria (LAB), yeast and mould counts in samples irradiated at 6 kGy were not detectable throughout the storage of 30 days at 4 °C, whereas no growth was found in the sample irradiated at 2 kGy within the first 10 days. Generally, greater carbonyl contents of lipid and protein, as well as thiobarbituric acid-reactive substances (TBARS), were noticeable in the irradiated samples, than in the non-irradiated sample ($p < 0.05$). The carbonyl contents and TBARS increased with increasing storage time and the rate of increase was more pronounced in samples irradiated at higher dose ($p < 0.05$). With increasing storage time, Som-fug irradiated at 6 kGy showed greater decreases in hardness, adhesiveness, springiness and cohesiveness, than did non-irradiated samples and those irradiated at 2 kGy ($p < 0.05$). L^* value of all samples decreased, whereas a^* and b^* values increased throughout storage ($p < 0.05$). Lower acceptance in all attributes was observed in the samples irradiated at 6 kGy, than in other samples, particularly when storage time increased ($p < 0.05$). However, samples irradiated at 2 kGy showed no changes in acceptability within 20 days. The results revealed that irradiation at high dose (6 kGy) might induce lipid and protein oxidation, though the growth of microorganisms was inhibited. Therefore, the irradiation at low dose (2 kGy) could be used to control the overfermentation of Som-fug up to 20 days at 4 °C without adverse effects on quality and acceptability.

Keywords: Som-fug; Bigeye snapper; Irradiation; Storage stability

1. Introduction

Since global demand for fishery products is increasing, there is a need for efficient preservative methods (Rahman, 1999). The major problem with respect to distribution of seafood or fishery products is their susceptibility to spoilage, mainly due to the contamination of spoilage and pathogenic microorganisms (Gram & Huss, 1996). Refrigerated foods are prone to the growth of psychrophilic spoilage bacteria and pathogens. Papadima and Bloukas (1999) reported that fermented Greek sausage, stored at 13–15 °C, showed a rapid increase in lactic acid bacteria (LAB) count and had lower pH than did those stored at 3–7 °C. However, Østergaard et al. (1998) found that chilled storage could extend the shelf-life of Som-fug, a Thai fermented fish mince, to at least 18 days.

Food irradiation is a food processing technology that exposes certain types of food to a source of ionizing energy. Gamma irradiation at low doses (1–3 kGy) is a process,

* Corresponding author. Tel.: +66 7428 6334; fax: +66 7421 2889.
E-mail address: soottawat.b@psu.ac.th (S. Benjakul).

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which has potential for shelf-life extension and hygienization of fishery products (Venugopal, Doke, & Thomas, 1999). With the observation of the Joint Expert Committee of Food and Agriculture Organization/World Health Organization/International Atomic Energy Agency (FAO/WHO/IAEA), irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard. There has been worldwide interest in using this technology for preservation of various foods, including fishery products (IAEA, 1989; WHO, 1994). Although irradiation is an effective means for extending the shelf-life of fishery products, indirect effects, including the acceleration of lipid oxidation and hydrolysis, still limit its application in some food products (Ghadi & Venugopal, 1991; Rahman, 1999).

Som-fug has received much attention due to its unique delicacy. The short shelf-life of Som-fug, mainly caused by over-fermentation, is a drawback of this product. The growth of LAB and the changes in Som-fug properties still occurs during storage (Valyasevi & Rolle, 2002). Therefore, irradiation at the appropriate dose can be a promising way to control the overfermentation of Som-fug, as well as to extend its shelf-life. However, no information regarding the use of irradiation in Som-fug has been reported. The objective of this study was to investigate the effect of irradiation at different doses on microbiological, chemical and physical changes and the storage stability of Som-fug produced from bigeye snapper during storage at 4 °C.

2. Materials and methods

2.1. Chemicals

Plate count agar (PCA), Man Rogasa Sharpe (MRS) agar, trichloroacetic acid (TCA), sodium hydroxide, potassium hydroxide, and potassium chloride were purchased from Merck (Darmstadt, Germany). Guanidine hydrochloride, 2-thiobarbituric acid (TBA), and Coomassie G-25 were procured from Sigma Chemical Co. (St. Louis, MO, USA). Guanidine thiocyanate and malonaldehyde were purchased from Fluka (Buchs, Switzerland). 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB), 2-nitro-5-thiosulfobenzoate (NTSB) and 2,4-dinitrophenylhydrazine (DNPH) were obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan).

2.2. Som-fug preparation

Bigeye snapper (*Priacanthus tayenus*), caught from Songkhla-Pattani Coast along the Gulf of Thailand and off-loaded approximately 48 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 washed and fish mince was prepared and used for surimi preparation as described by Benjakul, Visessanguan, Riebroy, Ishizaki, and Tanaka (2002). Bigeye snapper surimi stored at −18 °C was thawed to obtain the core temperature of 0–2 °C and used for Som-fug production. Som-fug samples were prepared according to the method of Riebroy, Benjakul, Visessanguan, and Tanaka (2006). 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. The fermentation was conducted until the pH of Som-fug reached 4.60. After the fermentation was completed, samples were then subjected to irradiation.

2.3. Gamma irradiation

Irradiation was carried out at the Office of Atoms for Peace, Ministry of Science and Technology, Bangkok, Thailand. Som-fugs were irradiated in a ^{60}Co source irradiator (point source Gamma beam 650, MDS Nordion International Co. Ltd., Ottawa, Canada). The samples were placed in the uniform part of the radiation field and arranged to minimize any differences in the radiation dose. The source strength was approximately 48,860 Ci. A constant dose rate (2 kGy/h) at room temperature was used. The dosimiters used were radiochromic films (FWT-60-00, South Kellogg Goleta, CA, USA), which were placed on the top and bottom of one package per lot. Two target doses (2 kGy and 6 kGy) were applied. Actual absorbed doses were measured by spectrophotometer (UV-1301 PC, Shimadzu, Kyoto, Japan). Immediately after irradiation, the samples were transferred to the cold room (4 °C). Samples without irradiation were used as the control.

2.4. Microbiological analyses

Total viable count (TVC) and lactic acid bacteria (LAB) count were determined using plate count agar (PCA) and De Man Rogasa and Sharpe (MRS) agar according to the method of AOAC number 966.23 and 947.04, respectively (AOAC, 2000). The samples (25 g) were 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 each dilution (0.1 ml) were plated in duplicate on MRS agar and PCA and incubated at 30 °C for 1–2 days. Yeast or mould counts were measured in yeast and malt extract (YM) agar incubated at 30 °C for 3–4 days. TVC, LAB and yeast or mould counts were reported as log CFU/g sample.

2.5. Chemical analyses

2.5.1. pH

The pH of sample was determined according to the method of Benjakul, Seymour, Morrissey, and An

(1997). Sample (5 g) was mixed with 50 ml of deionised water (w/v) and the mixture was homogenised at 11,000 rpm for 1 min using an IKA homogeniser (Model T25, Selangor, Malaysia). The pH of homogenate was measured using a pH meter (CG842 Schott, Mainz, Germany).

2.5.2. Determination of total sulfhydryl (SH) and disulphide bond contents

Total SH content of natural actomyosin (NAM), prepared as described by Benjakul et al. (1997), was determined using 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) according to Ellman (1959) as modified by Benjakul et al. (1997). NAM (1 mg/ml) was treated with 9 ml of 0.2 M Tris–HCl, pH 6.8, containing 8 M urea, 2% SDS, and 10 mM EDTA. A 4 ml aliquot of the mixture was taken and 0.4 ml of 0.1% (w/v) DTNB solution was added. The reaction mixture was incubated at 40 °C for 25 min. Absorbance was measured at 420 nm with a spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). A blank was prepared by replacing the NAM with 0.6 M KCl, pH 7.0. Total SH content was calculated, using the molar extinction of 13,600 $M^{-1}\,cm^{-1}$, and expressed as mol/10^5 g protein.

Disulphide bond content in NAM was determined by using 2-nitro-5-thiosulfobenzoate (NTSB) assay, according to the method of Thannhauser, Konishi, and Scheraga (1987). To 0.5 ml of NAM sample (4 mg/ml), 3.0 ml of freshly prepared NTSB assay solution were added. The mixture was incubated in the dark at room temperature (25–27 °C) for 25 min. Absorbance at 412 nm was measured. Disulphide bond content was then calculated, using a molar extinction coefficient of 13,900 $M^{-1}\,cm^{-1}$, and expressed as mole/10^5 g protein.

2.5.3. Carbonyl content of proteins and lipids

Carbonyl content of NAM extracted was determined according to the method of Liu, Xiong, and Butterfield (2000). NAM solution (0.5 ml, 4 mg/ml) was reacted with 2.0 ml of 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2 N of HCl for 1 h at room temperature. After incubation, 2 ml of 20% TCA were added to precipitate protein. The pellet was washed twice with 4.0 ml of ethanol:ethylacetate (1:1, v/v) mixture to remove unreacted DNPH, blow-dried, and dissolved in 1.5 ml of 0.6 M guanidine hydrochloride in 20 mM potassium phosphate (pH 2.3). Absorbance of protein was measured at 370 nm. A molar absorptivity of 22,400 $M^{-1}\,cm^{-1}$ was used to calculate protein-carbonyl content. An aliquot of the protein solution was reacted with Coomassie protein assay reagent to measure its protein concentration according to the method of Bradford (1976).

The carbonyl content of lipids was determined by a colorimetric method (Lappin & Clark, 1951) as slightly modified by Rababah et al. (2004). Sample (1 g) was homogenised with 20 ml of chloroform/methanol (1:2) using a homogeniser (IKA Labortechnik, Selangor, Malaysia) at a speed of 11,000 rpm for 2 min. The homogenate was shaken, using a shaker (Unimax 1010, Heidolph, Germany) at a speed of 250 rpm for 30 min. The sample was filtered through Whatman filter paper No. 4. The solvent was removed and dried using a stream of nitrogen gas. Then, the dried sample was dissolved in 1 ml of carbonyl-free methanol. One millilitre of saturated 2,4-DNPH in methanol and 0.05 ml of HCl was added. The mixture was immediately vortexed, heated at 50 °C for 30 min and cooled in cold water (4 °C) for 15 min. After the sample was cooled, 5 ml of 10% KOH was added and the mixture was filtered through Whatman filter paper No. 4 to obtain a clear solution. A blank was made using 1.0 ml of chloroform/methanol (1:2) instead of the sample. The absorbance of the solution was read with a model UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) at 480 nm. The calculation was made from the observed absorbance of samples, using an equation of a standard curve of acetophenone as follows:

$$\text{Carbonyl content (mmol acetophenone/1 g sample)} = [0.00255 + (0.2993 \times A)] \times 0.001,$$

where A = absorbance at 480 nm

2.5.4. Determination of thiobarbituric acid reactive substances (TBARS)

TBARS were determined according to the method of Buege and Aust (1978). Samples (5 g) were 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 a boiling water (95–100 °C) to develop a pink colour. Then the mixture was cooled with running water and centrifuged at $5500 \times g$ for 25 min. The absorbance of the supernatant was measured at 532 nm, using a model UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). TBARS value was calculated from the standard curve of malonaldehyde and expressed as mg malonaldehyde/kg sample.

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

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 $10{,}000 \times g$ for 15 min at room temperature. The protein concentration of supernatant was determined by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 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, California, USA). The electrophoresis was carried out at 15 mA. After separation, protein bands were stained using 0.2% Coomassie Brillant Blue R-250 in 25% methanol and 10% acetic acid. Destaining was carried out using 40% methanol and 10% acetic acid.

2.6. Physical analyses

2.6.1. Determination of weight loss

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

2.6.2. Determination of released water and expressible water contents

The percentage of water released from sample was determined according to the method of Nakao et al. (1991). The sample in package was weighed (A) and 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:

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

Expressible water 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 was determined as the weight loss after the compression of sample. Samples were cut into cylindrical form (2.0 cm height × 2.0 cm diameter), placed between double layers of filter papers (Whatman No. 4) and subjected to compression using a texture analyzer (Stable Micro Systems, Surrey, England) with a cylindrical aluminium probe (50 mm diameter). The measurement was performed with a cross-head speed of 3 mm/s to 70% strain for 60 s. Samples were subjected to moisture analysis by AOAC method number 950.46 (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 equations:

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

where $\text{Apparent expressible water content} = 100 \times (W_{\text{before}} - W_{\text{after}})$, W_{before} = weight before compression; W_{after} = weight after compression.

2.6.3. Texture profile analysis

Texture profile analysis (TPA) was performed using a TA-XT2i texture analyser (Stable Micro Systems, Surrey, England) with a cylindrical aluminium probe (50 mm diameter). The samples were cut into cylinders (30 mm high × 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: cross-head 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. TPA analyses were defined and calculated as described by Bourne (1978). Hardness, adhesiveness, springiness and cohesiveness were calculated from the force–time curves generated for each sample.

2.6.4. Determination of 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, Reston, VA, USA). L^*, a^*, and b^* indicate lightness, redness/greenness, and yellowness/blueness, respectively.

2.7. Acceptability test

Som-fug samples, without and with irradiation, were evaluated for acceptance by an untrained 50-member panel. The panellists were graduate students in the Food Technology program, of age ranging from 22 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 like nor dislike and 9 = like extremely, was used for evaluation (Meilgaard, 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. Individual samples of each Som-fug sample 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. 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, colour, texture, taste, flavour, and overall liking.

2.8. Statistical analysis

One-way analysis of variance (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 Windows (SPSS Inc. Chicago, IL).

3. Results and discussion

3.1. Effect of irradiation on microbiological changes of Som-fug during refrigerated storage

Changes in TVC and LAB counts of Som-fug irradiated at 0 (control), 2 and 6 kGy, during storage at 4 °C for 30 days, are depicted in Fig. 1. At day 0, the control sample

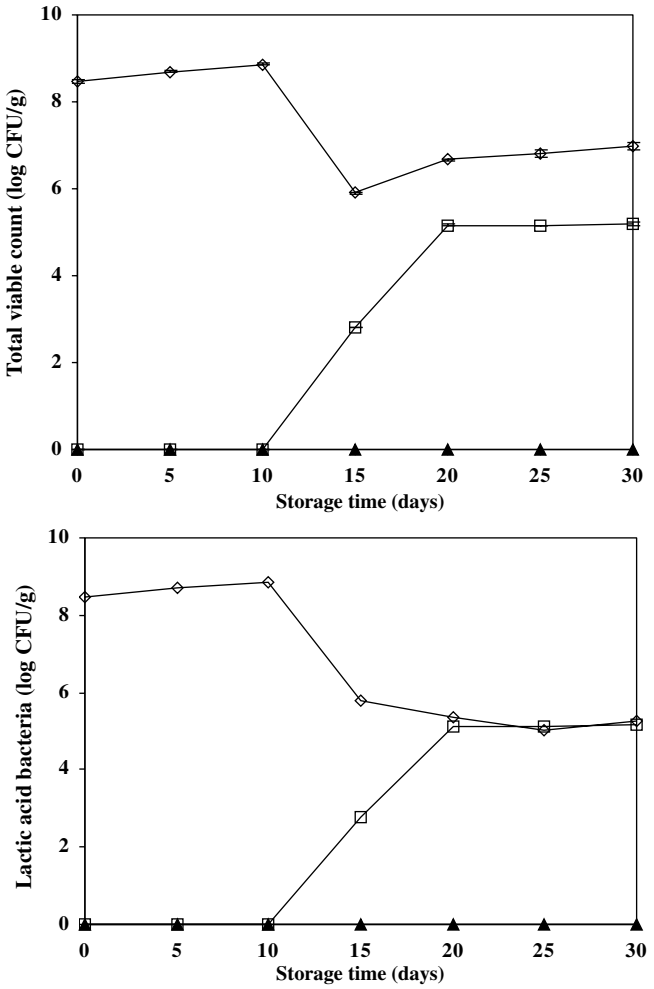

Fig. 1. Changes in total viable and lactic acid bacteria counts of Som-fug irradiated at 0 (◇), 2 (□), and 6 kGy (▲) during storage at 4 °C. Bars represent the standard deviations from triplicate determinations.

had a TVC of 2.9×10^8 CFU/g and LAB of 2.8×10^8 CFU/g. On the other hand, no TVC or LAB were observed in the samples irradiated at either 2 or 6 kGy. Total viable counts are mainly reduced by gamma irradiation (Loaharanu, 1995). The results indicated that gamma irradiation showed high efficacy in inhibiting bacterial growth in Som-fug. During storage, TVC and LAB counts of the control increased slightly within the first 10 days, with a marked decrease at day 15. This result suggested that bacteria in Som-fug might be inhibited or killed by lactic acid produced with sufficient exposure time. Additionally, bacteriocin from LAB might function as a bacteriocidal agent, leading to a lowered count. For the sample irradiated at 2 kGy, sharp increases in TVC and LAB count were noticeable after 10 days of storage and reached a plateau after 20 days of storage. However, no TVC or LAB were detectable in the sample irradiated at 6 kGy throughout the storage of 30 days at 4 °C. Gamma irradiation has been considered as an interesting method of preservation to extend the shelf life of chilled, stored fish and also to reduce, qualitatively and quantitatively, the microbial population in fish and fishery products (Abu-Tarboush et al., 1996). Ionisation irradiation affects microorganisms, such as bacteria, yeasts, and moulds, by causing lesions in the genetic material of the cell, effectively preventing it from carrying out the biological processes necessary for its continued existence (Rahman, 1999). Gram-negative bacteria seem to be more sensitive to irradiation than do Gram-positive, such as Lactobacillus and Micrococcus (Lebepe, Molins, Charven, Farrar, & Skowronski, 1990). The major effect of irradiation on microorganisms is due to the charged particles generated by irradiation, which are able to break deoxyribonucleic acid (DNA) (Rahman, 1999). In the presence of water, cell injury during irradiation is due to both, direct damage to cell DNA and indirect damage through reactivity of the radiolytic products with cell components (Grecz, Rowley, & Matsuyama, 1983). However, some of the constituents, such as proteins, are believed to compete with the bacterial cell for interaction with free radicals, thereby reducing the net effect of radiation damage and making the organism more resistant (Urbain, 1986). Lee, Ahn, Jo, Yook, and Byun (2002) reported that combination of low salt concentration and gamma irradiation reduced the growth of microorganisms during fermentation of shrimp. Blank and Cumming (2000) found that the irradiation process at low dose (0.4–2.5 kGy) can be considered as cold pasteurisation, implying that the process kills microorganisms without participation of heat. Typical doses from 0.4 to 2.5 kGy are employed during treatment of foods in order to substantially reduce specific spoilage microorganisms and/or to delay ripening and senescence, thereby extending its usefulness or keeping quality (Rahman, 1999). Therefore, irradiation effectively reduced the initial microbial level and retarded microbial growth of Som-fug during storage for 30 days. Generally, LAB were predominant in fermented Som-fug (Paludan-Müller, Valyasevi, Huss, & Gram, 2002; Riebroy et al., 2006; Saisithi et al., 1986). The continuous growth of LAB normally contributes to overfermentation. Thus, the inhibition or retardation of their growth by irradiation could prevent such a problem. All samples had no yeast or mould after fermentation and irradiation or during storage (data not shown). Saisithi et al. (1986) reported that yeasts, tentatively identified as *Pichia* spp. and *Candida* spp, increased in Som-fug after fermentation was completed (pH < 4.5) and were considered as the spoilage organisms in this product. Additionally, yeasts with the range of $0–2 \times 10^2 \log_{10}$CFU/g indicated overfermentation of Som-fug (Saisithi et al., 1986).

3.2. Effect of irradiation on chemical changes of Som-fug during refrigerated storage

3.2.1. pH and total acidity

Changes in pH and total acidity of Som-fug with and without irradiation during storage at 4 °C are shown in Fig. 2. Regardless of irradiation, the same pH values were observed among all samples at day 0 ($p > 0.05$). Riebroy,

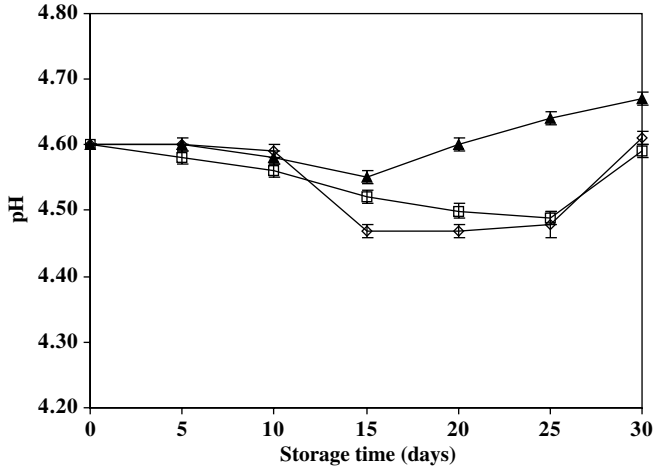

Fig. 2. Changes in pH of Som-fug irradiated at 0 (◇), 2 (□), and 6 kGy (▲) during storage at 4 °C. Bars represent the standard deviations from triplicate determinations.

Benjakul, Visessanguan, and Tanaka (2005) reported that the pH of fermented Som-fug produced from bigeye snapper surimi was 4.5–4.6. The pH lowering in fermented Som-fug might be due to lactic acid produced by LAB (Riebroy et al., 2005; Saisithi et al., 1986). During storage, the pH values of all samples decreased gradually during the first 15 days of storage, compared with those stored at day 0 ($p < 0.05$). The decrease in pH during storage was probably due to an increased amount of organic acids. These organic acids, particularly lactic acid, were responsible for the flavour of Som-fug (Østergaard et al., 1998). It was found that a greater decrease in pH was noticeable in the control (without irradiation) ($p < 0.05$). Irradiation, particularly at a higher dose, might cause the death of some LAB. As a result, a smaller amount of lactic acid was produced, as indicated by the higher pH of irradiated samples during storage. After 25 days of storage, the pH value of the control and the sample irradiated at 2 kGy increased ($p < 0.05$). This might be due to the growth of spoilage bacteria, in which some volatile bases were produced. For the sample irradiated at 6 kGy, a continuous increase in pH was observed after 15 days of storage.

3.2.2. Total sulfhydryl and disulphide bond contents

Changes in total sulfhydryl (SH) and disulphide bond contents of Som-fug, without and with irradiation during the storage at 4 °C, are shown in Fig. 3. After irradiation, samples irradiated at 6 kGy had the lowest total SH content ($p < 0.05$). However, no differences in SH content between the control and samples irradiated at 2 kGy were observed ($p < 0.05$). Total SH content of all samples decreased gradually throughout 30 days of storage ($p < 0.05$). A greater decrease in total SH content was observed in the sample irradiated at 6 kGy, than in other samples ($p < 0.05$). The decrease in SH group most likely resulted from the formation of disulphide bonds through oxidation of SH groups or disulphide interchanges (Hayakawa & Nakai, 1985). SH_a, the sulfhydryl group on the light meromyosin region

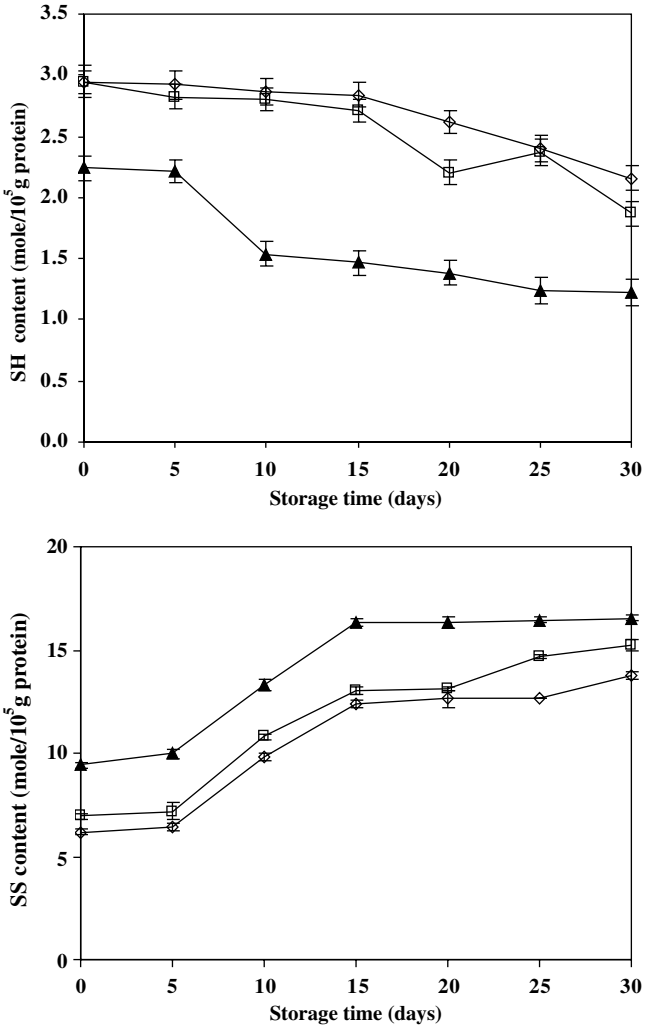

Fig. 3. Changes in total sulfhydryl and disulphide bond contents of Som-fug irradiated at 0 (◇), 2 (□), and 6 kGy (▲) during storage at 4 °C. Bars represent the standard deviations from triplicate determinations.

of the myosin molecule, is susceptible to oxidation during iced storage of fish actomyosin (Benjakul, Visessanguan, & Leelapongwattana, 2002; Sompongse, Itoh, & Obatake, 1996). From the result, the irradiation might cause unfolding of protein molecules, leading to the availability of more reaction sites (Rahman, 1999). Apparently, peptide linkages were generally not attacked by irradiation, and the main effects were concentrated around sulphur linkages and hydrogen bonds (Graham, 1980). The sequence of protein bonds attacked by ionizing radiation is as follows: –S–CH_3, –SH, imidazole, indol, alpha-amino, peptide, and proline. From the results, decrease in SH groups was more pronounced with higher doses of irradiation, suggesting that the SH groups might undergo more oxidation. The decrease in SH content was coincidental with the increase in disulphide bond content (Fig. 3). Therefore, the oxidation of SH groups induced by irradiation might contribute to the denaturation of myofibrillar proteins. With extended storage time, the protein molecules, especially those with the exposed reactive groups induced by irradiation, were more prone to oxidation of SH groups as evidenced by the fewer

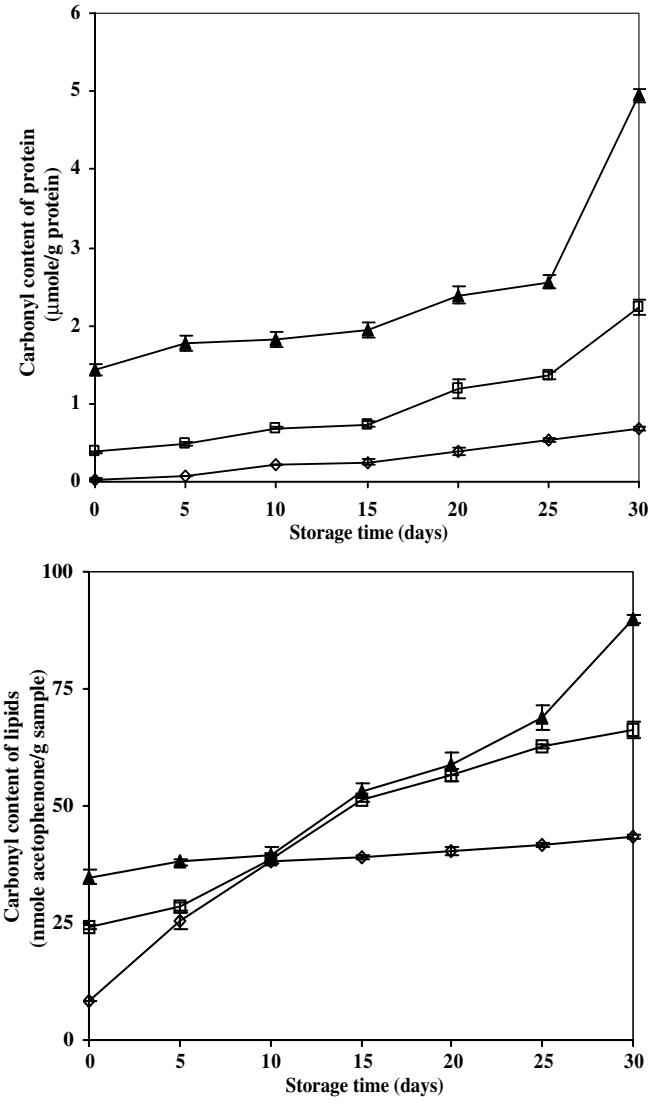

Fig. 4. Changes in carbonyl content of protein and lipid of Som-fug irradiated at 0 (◇), 2 (□), and 6 kGy (▲) during storage at 4 °C. Bars represent the standard deviations from triplicate determinations.

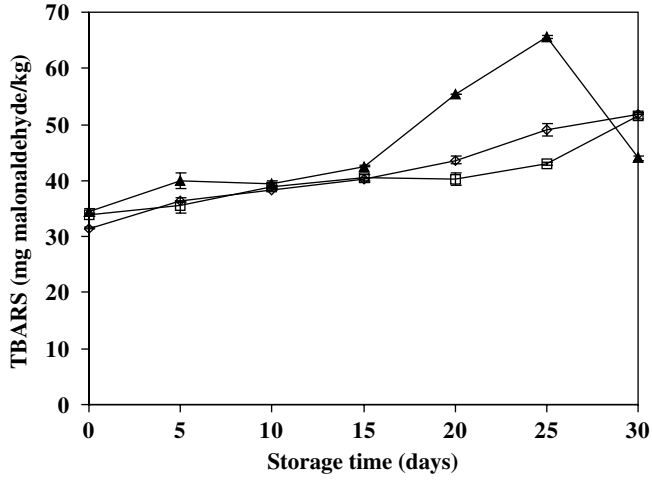

Fig. 5. Changes in TBARS of Som-fug irradiated at 0 (◇), 2 (□), and 6 kGy (▲) during storage at 4 °C. Bars represent the standard deviations from triplicate determinations.

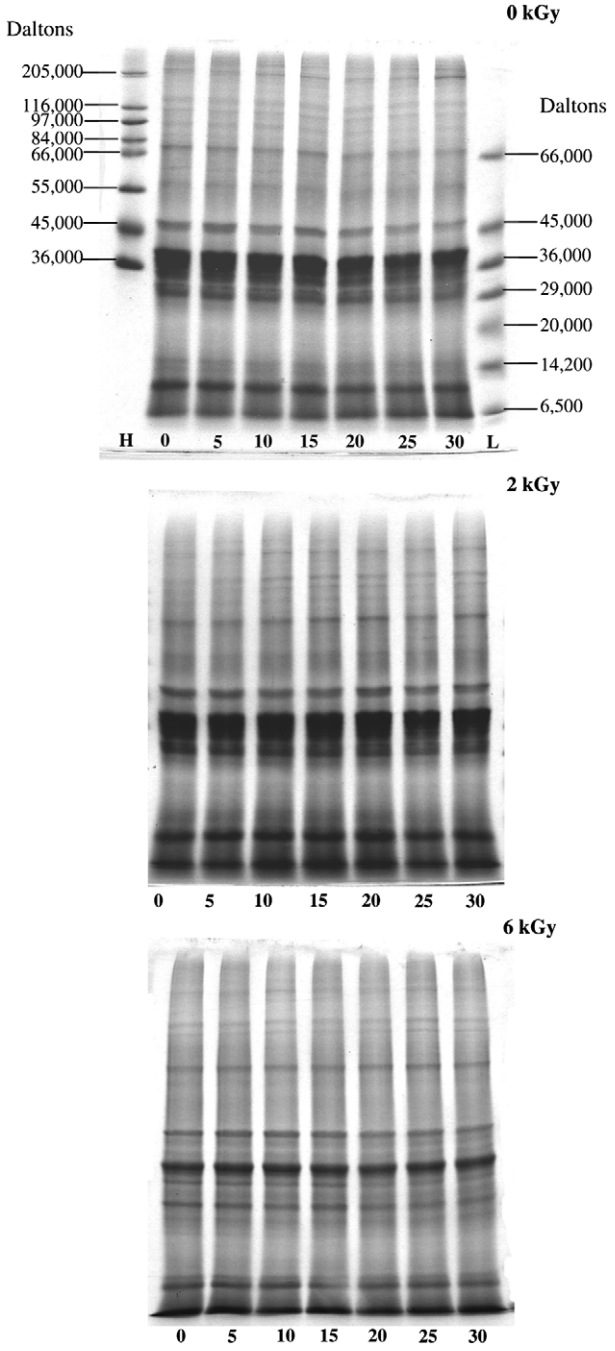

Fig. 6. SDS-PAGE patterns of muscle proteins in Som-fug irradiated at 0, 2, and 6 kGy during storage at 4 °C. H and L denote high- and low-molecular-weight protein standards, respectively. Numbers designate the storage time (days).

SH groups, with concomitant increase in disulphide bond content throughout the storage of 30 days ($p < 0.05$), except the control, which had a constant disulphide bond after 15 days ($p > 0.05$).

3.2.3. Carbonyl content

As shown in Fig. 4, the irradiated Som-fug samples showed marked increases in the protein-carbonyl content, compared with the control ($p < 0.05$). Samples irradiated

at 6 kGy had the highest initial carbonyl contents, compared with other samples. The formation of carbonyl is one of the most salient changes in oxidized proteins (Butterfield & Stadtman, 1997; Stadtman & Berlett, 1997). While protein carbonyl can be generated by oxidative attack on protein side-chains or peptide bonds, exogenous carbonyl may also be introduced into proteins by the reactions of lysine residues with oxidized ascorbate (dehydroascorbate) or by Michael-type assertion of 4-hydroxy-2-nanenal (HNE), a product of lipid oxidation, to cysteine-sulfhydryl groups, lysine-amino groups, or a histidine-imidazole moiety (Butterfield & Stadtman, 1997). In general, protein oxidation occurred within the first 10 days, and thereafter no additional carbonyl was formed, or extra carbonyls reacted with nucleophiles, such as amines, making them undetectable (Liu et al., 2000). Myofibrillar proteins are sensitive to oxidation by irradiation (Rahman, 1999). However, the activity can vary with amino acids. The amino acid residues most susceptible to oxidation are methionine, cysteine, tryptophan, and histidine. From the results, protein oxidation in Som-fug was pronounced when irradiation was used. The radicals produced might contribute to the induction of the oxidation of proteins or amino acids in the fish muscle.

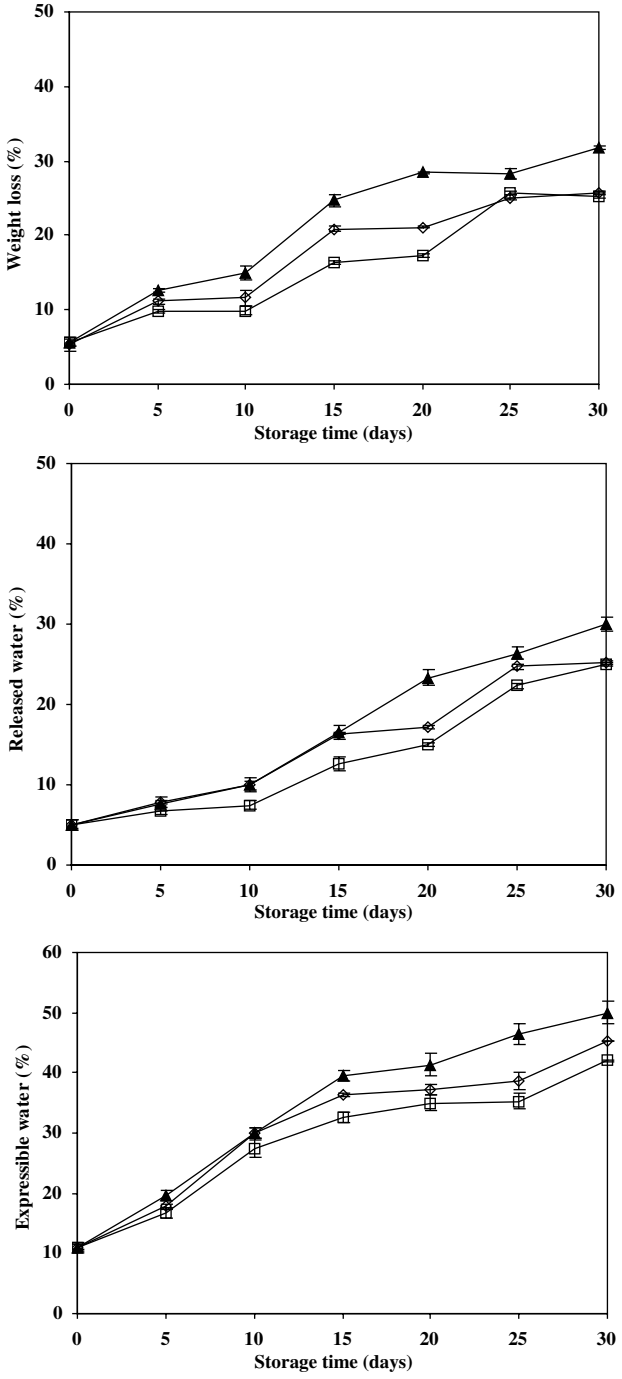

Fig. 7. Changes in weight loss, released water and expressible water contents of Som-fug irradiated at 0 (◇), 2 (□), and 6 kGy (▲) during storage at 4 °C. Bars represent the standard deviations from triplicate determinations.

Carbonyl contents of lipid, in all samples during storage, are shown in Fig. 4. After irradiation, an increase in carbonyl content was found in Som-fug. A higher carbonyl content was noticeable in the samples irradiated at 6 kGy, suggesting that irradiation induced lipid oxidation in Som-fug. Among the carbonyls, the aldehydes have very low-threshold olfactory concentrations and therefore they contribute to the off-flavours or rancidity. Irradiation of lipids induces the production of free radicals, which react with oxygen, leading to the formation of carbonyls. This result indicated that irradiation at a higher dose had an adverse effect on the quality of Som-fug via the induction of lipid oxidation.

3.2.4. TBARS values

Effect of irradiation on the lipid oxidation of Som-fug during the storage at 4 °C is monitored using TBARS, as depicted in Fig. 5. After fermentation was accomplished, the TBARS value of Som-fug was 30.2 mg malonaldehyde/g sample. Irradiated samples had higher TBARS values than had the control ($p < 0.05$). In general, the rate of increase in TBARS values was greater in samples irradiated with higher doses (6 kGy) during the first 25 days of the storage, than in control or samples irradiated at 2 kGy. During storage, TBARS values in all samples increased continuously ($p < 0.05$). The highest TBARS value was observed in Som-fug irradiated at 6 kGy when stored for 25 days ($p < 0.05$). Thereafter, a decrease in TBARS value was noticeable. This was postulated to be due to the loss of volatile oxidation compounds from Som-fug stored for a longer time. Irradiation has been known to initiate the normal process of lipid oxidation, which gives rise to rancid off-flavours (Ahn & Nam, 2004). Highly unsaturated fatty acids are more readily oxidized than are less unsaturated fatty acids. In lipids, particularly those containing unsaturated fatty acids, radiolytic decomposition occurs and induces the formation of some volatile compounds responsible for off-odours. Ionizing radiation produces free radicals that can accelerate oxidative processes and produce radiolytic products from meat components (Woods & Pikaev, 1994). Generally, marine fish have higher unsaturated

fatty acid contents than have freshwater fish (Sikorski, Kolakowska, & Pan, 1990). Hence, Som-fug, produced from marine fish, was more prone to lipid oxidation than was that produced from freshwater fish (Riebroy et al., 2005, 2006). From the results, lipid oxidation proceeded during the extended storage, especially with the irradiated samples. The increases in TBARS values were coincidental with the increase in carbonyl content (Fig. 4). The damage of the muscle structure by radicals produced, exposing the fatty acids to oxygen and catalysing factors, such as iron and heme, might be associated with the accelerated lipid oxidation in samples irradiated at higher doses (Morrisey, Sheehy, Galvin, Kerry, & Buckley, 1998). Lipid oxidation is responsible for reduction in nutritional quality as well as changes in flavour (Aguirreźabal, Meteo, Domínguez, & Zumalacárregui, 2000). From the results, it appears that lipid oxidation in Som-fug samples could be stimulated by irradiation at the higher dose.

3.2.5. SDS-PAGE pattern

Electrophoretic patterns of non-irradiated and irradiated Som-fug samples during storage at 4 °C are shown in Fig. 6. Similar protein patterns of Som-fug without irradiation and those irradiated at 2 kGy were found. Only small myosin heavy chain (MHC) bands were remained in Som-fug, suggesting intense hydrolysis caused by both endogenous and microbial proteinases during fermentation. Both indigenous and microbial proteases contributed to the degradation of muscle proteins (Hughes et al., 2002). From the results, protein with the MW of 38 kDa was found to be the dominant protein. However, actin was also retained as the second predominant protein in Som-fug, regardless of irradiation. Nevertheless, slight differences in protein patterns of Som-fug from 6 kGy-irradiated samples were noticeable, compared to those of the non-irradiated and 2 kGy-irradiated samples. No smear bands below the MW of 38 kDa were found in the sample irradiated at 6 kGy. This might be due to the cross-linking of those proteins induced by irradiation. Those protein or protein fragments might be oxidized and disulfide bond could be formed. Therefore, irradiation somehow affected the protein pattern of Som-fug, particularly when the higher dose was applied. Riebroy, Benjakul, Visessanguan, Kijrongrojana, and Tanaka (2004) reported that protein band with the MWs of 205, 116, 45, and 36 kDa generally occurred in commercial Som-fug produced from freshwater fish. During a storage of 30 days, no changes in protein patterns were found with any irradiated samples. The results suggested that no further degradation occurred in the irradiated sample. However, a lower band intensity of protein

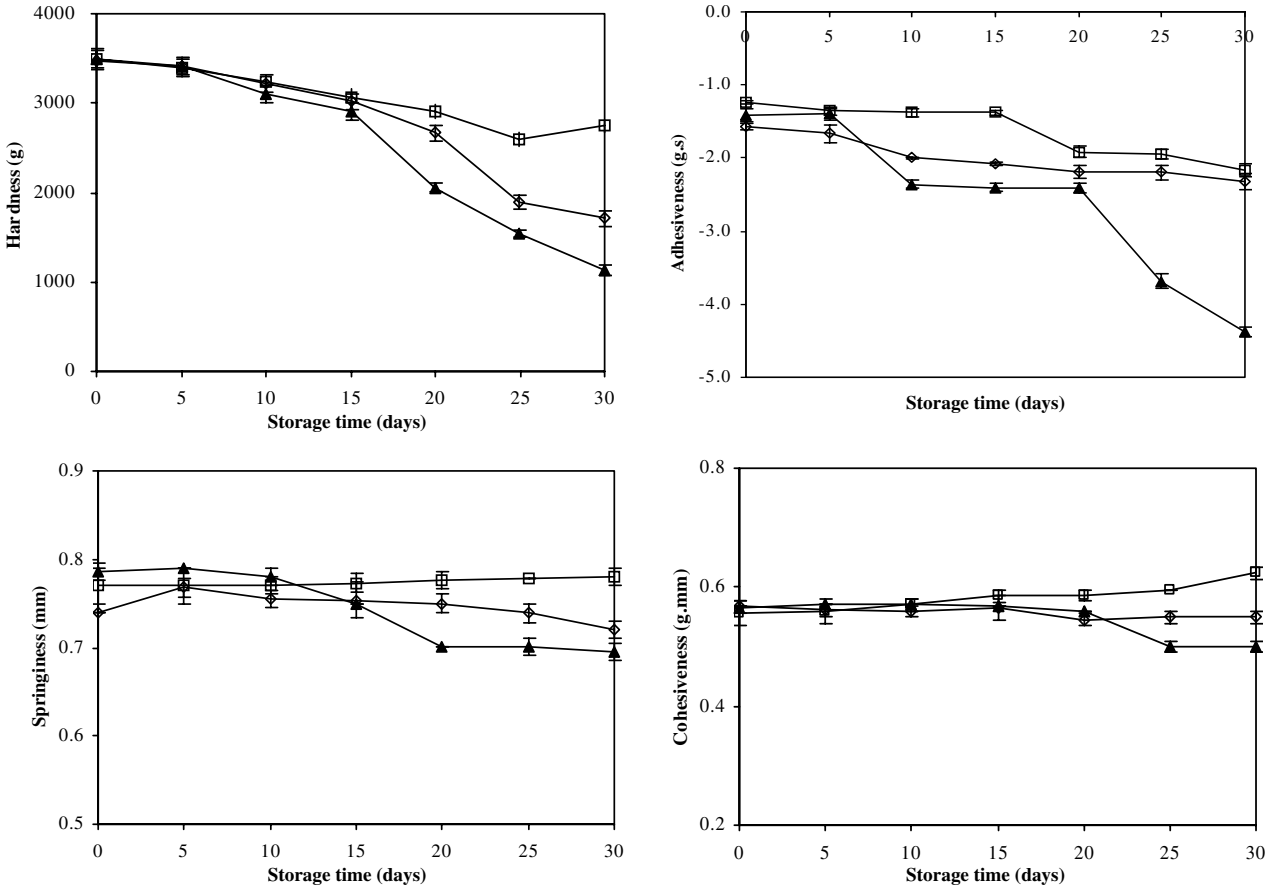

Fig. 8. Changes in textural properties of Som-fug irradiated at 0 (◇), 2 (□), and 6 kGy (▲) during storage at 4 °C. Bars represent the standard deviations from five determinations.

with the MW of 38 kDa was noticeable at days 25 and 30 of storage. This might be due to proteolysis, mainly caused by microorganism, which is associated with the increase in TVC of the control sample during storage for a longer time (Fig. 1).

3.3. Effect of irradiation on physical properties of Som-fug during refrigerated storage

3.3.1. Weight loss, released water and expressible water contents

Weight loss, released water and expressible water contents of non-irradiated and irradiated Som-fug during storage at 4 °C are shown in Fig. 7. At day 0, no differences in any parameters were found among any samples, with and without irradiation ($p > 0.05$). During storage, non-irradiated Som-fug samples showed poorer water-holding capacities, than did samples irradiated at 2 kGy ($p < 0.05$). However, irradiation at higher dose (6 kGy) caused a detrimental effect on water-holding capacity of Som-fug, as indicated by the increases in weight loss, released water and expressible water contents. 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 (Liu et al., 2000). From the results, the increases in weight loss, released water and expressible water contents found in the samples irradiated at 6 kGy might be associated with more denaturation of muscle proteins in these samples, as evidenced by the higher carbonyl contents of protein. The denatured protein had a lower water-binding capacity. As a result, the Som-fug network could not imbibe more water via the reactive hydrophilic groups of proteins, which were more likely oxidized and became less reactive. Thus, irradiation resulted in an increase in free water or exudates. Accumulated exudation is not attractive to the consumer and water is also important for the texture (Foegeding, Laneir, & Hultin, 1996).

3.3.2. Textural properties

With the irradiation, springiness and adhesiveness of Som-fug slightly increased, compared with those of the control samples ($p < 0.05$). Hardness and adhesiveness of non-irradiated and irradiated Som-fug decreased when storage time increased ($p < 0.05$) (Fig. 8). Also, springiness and cohesiveness of Som-fug irradiated at 6 kGy and the control decreased with increasing storage time ($p < 0.05$). However, no changes in springiness in samples irradiated at 2 kGy were noticeable throughout the storage ($p > 0.05$). Additionally, samples irradiated at 2 kGy had a slight increase in cohesiveness when storage time increased ($p < 0.05$). After 10 days of storage, Som-fug irradiated at 2 kGy generally had higher hardness, adhesiveness, springiness, and cohesiveness than had other samples ($p < 0.05$). Generally, Som-fug produced from bigeye snapper had higher cohesiveness, springiness, and adhesiveness than had those from largehead hairtail, barracuda, obtuse barracuda, lizardfish, and threadfin bream (Riebroy et al., 2006). 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 water (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., 2005; Visessanguan

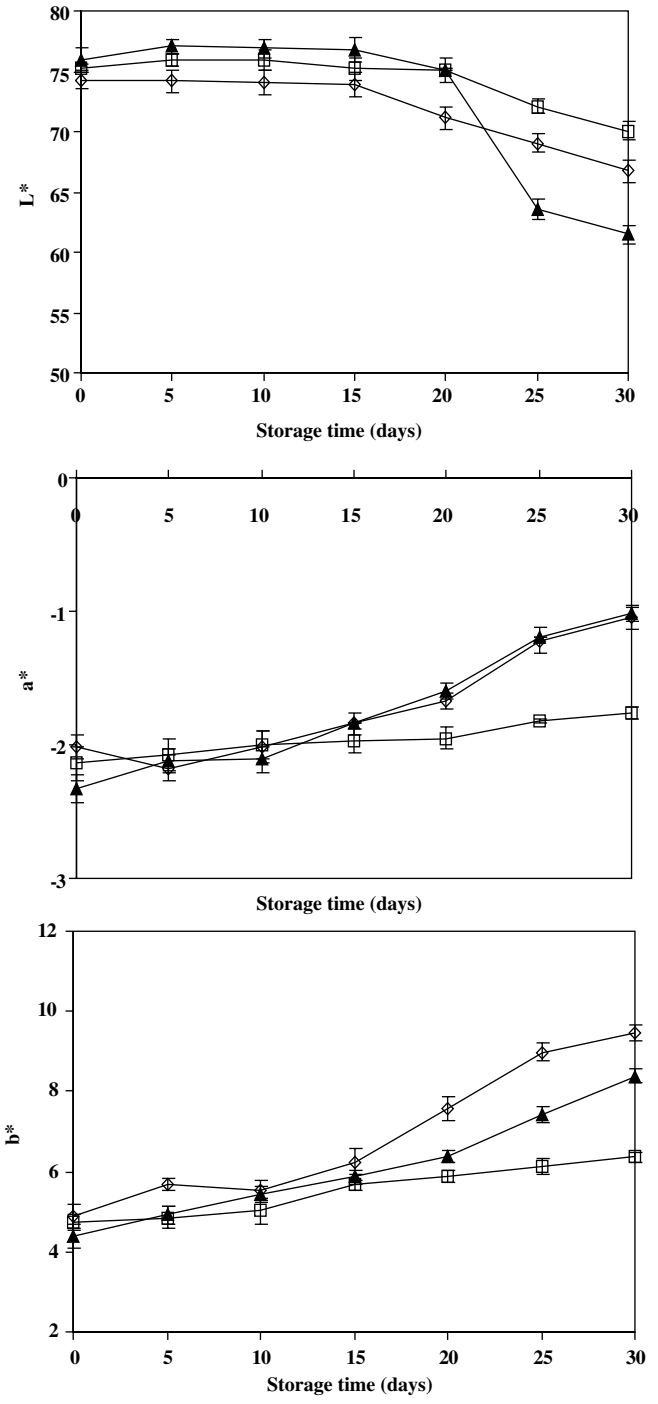

Fig. 9. Changes in L^*, a^* and b^*-values of Som-fug irradiated at 0 (◇), 2 (□), and 6 kGy (▲) during storage at 4 °C. Bars represent the standard deviations from five determinations.

et al., 2004). Among all samples, the Som-fug samples irradiated at 6 kGy showed the greatest decrease in all textural parameters tested. A marked decrease was generally found with increasing storage time. The accelerated oxidation of both lipids and proteins in the irradiated sample might contribute to the lowered textural properties. The oxidized proteins could not form a uniform and fine network of Som-fug. As a consequence, a poor integrity of protein network associated with the poor water holding capacity (Fig. 8) was obtained, particularly with a longer storage time, in which more oxidation took place. Irradiation induced a decrease in gel strength of washed mince of red hake (*Urophycis chuss*) (Dymszu et al., 1990). Diehl (1995) reported that the decrease in viscosity of irradiated fibrous proteins in solution was due to the scission of C–N bonds of the polypeptide backbone and also to splitting of disulfide bridges. From the results, the lowered textural properties of Som-fug samples were induced by irradiation at high dose (6 kGy). However, irradiation at 2 kGy could retard these changes and the resulting sample exhibited superior textural properties to the control sample throughout the storage of 20 days.

3.3.3. Colour

L^*, a^*, and b^* of non-irradiated and irradiated Som-fug sample stored at 4 °C for 30 days are shown in Fig. 9. Generally, L^* of irradiated Som-fug samples was higher than that of the control during the first 20 days of the storage ($p < 0.05$). During storage, L^* of all samples decreased and a sharp decrease was found in samples irradiated at 6 kGy after 20 days ($p < 0.05$). During storage, a^* and b^* values of all samples increased continuously ($p < 0.05$). Samples irradiated at 2 kGy had the smallest changes in a^* and b^* values. For b^*-value, the control sample showed the greatest rate of increase. The marked increase in b^*-value with the coincidental decrease in L^*-value, in samples irradiated at 6 kGy and stored for more than 20 days, might be associated with a greater formation of carbonyl compounds, either from lipid or protein oxidation. Those compounds might react with amino groups, leading to the formation of Maillard reaction products with a yellowish brown colour. The colour of irradiated samples was slightly more brownish than that of the control, which could be due to radiation-induced oxidation of myoglobin (Venugopal, 1981).

3.4. Effect of irradiation on acceptability of Som-fug during the refrigerated storage

The acceptance scores of non-irradiated and irradiated Som-fug samples, stored at 4 °C, are shown in Table 1. No differences in overall liking of any samples were noticeable after irradiation ($p > 0.05$). Appearance, colour, texture and flavour likeness of both non-irradiated and irradiated Som-fug samples generally decreased when the storage time increased ($p < 0.05$). However, the degree of decrease varied with the samples. Non-irradiated samples presented slimy surfaces at day 30 of storage as a result of mould growth (data not shown). However, the sample irradiated at 2 kGy was acceptable until 25 days of storage, whereas the sample irradiated at 6 kGy was unacceptable at day 20 of storage, especially in flavour liking, texture lik-

Table 1
Acceptance score of Som-fug affected by different irradiation dose during storage at 4 °C

Attributes	Samples (kGy)	Storage time (days)						
		0	5	10	15	20	25	30
Appearance	0	8.56 ± 1.12[*] a[**] A[***]	8.16 ± 1.41aB	7.46 ± 1.28bB	7.46 ± 1.45bB	6.06 ± 2.01cC	6.01 ± 1.00cB	5.36 ± 1.03 dB
	2	8.68 ± 1.04aA	8.65 ± 0.95aA	8.54 ± 0.99abA	8.02 ± 1.01bA	7.85 ± 1.12cA	7.66 ± 1.18cA	7.35 ± 1.11dA
	6	8.50 ± 1.20aA	8.02 ± 0.99bB	7.29 ± 1.01cB	7.12 ± 1.32 dB	7.00 ± 1.21eB	5.03 ± 1.18fC	3.38 ± 1.62gC
Colour	0	8.73 ± 1.01aA	8.46 ± 1.22bA	7.93 ± 1.05cA	7.30 ± 1.88dA	6.73 ± 1.14eB	6.11 ± 0.86fB	6.08 ± 0.97fA
	2	8.70 ± 0.97aA	8.12 ± 0.98bA	7.93 ± 1.14cA	7.68 ± 1.06dA	7.35 ± 1.09eA	7.12 ± 1.15fA	6.09 ± 0.92gA
	6	8.60 ± 1.01aA	7.95 ± 1.12bB	7.21 ± 0.54cB	7.00 ± 1.25 dB	6.08 ± 0.99eC	5.11 ± 0.97f C	5.10 ± 1.00gB
Texture	0	8.33 ± 1.08aA	8.16 ± 1.36bA	7.06 ± 1.29cB	7.03 ± 0.97cA	5.86 ± 1.02 dB	5.50 ± 0.59eB	5.12 ± 0.99fA
	2	8.36 ± 1.16aA	8.33 ± 1.02aA	8.03 ± 1.09bA	7.25 ± 1.11cA	7.01 ± 0.97dA	6.00 ± 0.97eA	5.92 ± 1.09fA
	6	8.30 ± 0.99aA	8.12 ± 1.09bA	6.21 ± 1.11cC	5.02 ± 1.09 dB	4.28 ± 0.99eC	1.00 ± 0.28fC	1.00 ± 0.06fB
Taste	0	8.53 ± 1.45aA	8.46 ± 1.28aA	8.20 ± 0.98bA	7.13 ± 1.25cA	7.03 ± 1.00cA	6.84 ± 1.52dA	6.25 ± 1.02eA
	2	8.53 ± 0.95aA	8.50 ± 1.00aA	8.22 ± 1.04bA	7.10 ± 1.22cA	6.98 ± 1.09dA	6.61 ± 1.07eA	6.34 ± 0.99fA
	6	8.50 ± 1.02aA	7.98 ± 1.04bB	7.01 ± 1.11cB	6.32 ± 0.99 dB	5.00 ± 0.92eB	1.00 ± 0.09fB	1.00 ± 0.02fB
Flavour	0	8.46 ± 1.37aA	8.40 ± 1.05aA	7.23 ± 0.65bB	7.16 ± 0.98bcB	6.03 ± 1.25 dB	5.63 ± 1.02eB	5.10 ± 1.06fB
	2	8.69 ± 1.15aA	8.22 ± 1.22bA	8.14 ± 0.99cA	8.06 ± 1.06dA	7.04 ± 1.08eA	7.00 ± 1.12fA	6.38 ± 1.23gA
	6	8.54 ± 1.01aA	8.03 ± 0.85bB	7.11 ± 0.99cB	6.92 ± 0.40dC	4.28 ± 0.18eC	2.12 ± 0.09fC	1.00 ± 0.08gC
Overall liking	0	8.60 ± 0.96aA	8.56 ± 1.08abA	8.36 ± 1.02bA	7.26 ± 0.99cB	6.20 ± 1.68 dB	6.04 ± 1.03eB	6.00 ± 0.90fB
	2	8.62 ± 1.28aA	8.54 ± 1.52aA	8.39 ± 1.15bA	8.15 ± 1.01cA	7.97 ± 1.37dA	7.05 ± 1.66eA	7.01 ± 1.09eA
	6	8.60 ± 0.97aA	7.89 ± 0.95bB	7.02 ± 0.90cB	6.58 ± 0.99dC	4.39 ± 0.46eC	2.44 ± 0.09fC	1.09 ± 0.08gC

[*] Mean ± SD from 40 determinations.
[**] Different letters in the same row indicate significant differences ($p < 0.05$).
[***] Different capital letters within the same attribute in the same column indicate significant differences ($p < 0.05$).

ing, and overall liking. In general, the irradiated sample at 6 kGy showed the lowest scores for all the characteristics evaluated. These samples were very soft and could not hold the water within the sample. Additionally, samples subjected to irradiation at 6 kGy had the lowest flavour likeness, possibly due to greater lipid oxidation. Generally, flavour liking is the characteristic, which mostly affects the sensory quality of foods, and is strongly related to lipids in fermented meat products (Naes, Holock, Axelesson, Andersen, & Bloom, 1994). The off-odour of irradiated products was influenced much more by sulphur-volatiles, such as dimethyl sulphide, dimethyl disulphide, and dimethyl trisulphide, than by lipid oxidation-dependent volatiles such as aldehydes (Ahn, Jo, Du, Olson, & Nam, 2000). Jo and Ahn (2000) reported that the radiolytic degradation of amino acids, especially sulphur amino acids, was the main mechanism of off-odour production in irradiated meat. Therefore, both lipid oxidation products and radiolytic S-volatiles possibly contributed to the overall off-flavour of the irradiated Som-fug. From the results, it can be concluded that the use of irradiation at 2 kGy seems to be useful for keeping quality and extending the shelf-life of Som-fug.

4. Conclusion

Shelf-life of Som-fug produced from bigeye snapper could be extended by using irradiation at 2 kGy in combination with the refrigerated storage. Gamma irradiation could retard microbial growth in Som-fug, leading to the retardation of spoilage and overfermentation. However, irradiation caused lipid oxidation and exudates loss in Som-fug, which need to be tackled. Therefore, the Som-fug industry can use irradiation, in combination with effective means for preventing the oxidation, to obtain a prime quality product with prolonged shelf-life.

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