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Effect of processing method on quality, texture, collagen and amino acid composition of sandfish (*Holothuria scabra*)

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Abstract

Textural properties and collagen and amino acid contents of fresh (raw) and processed sea cucumbers (sandfish, *Holothuria scabra*) were compared. Several processing procedures using different salting methods (brine and kench salting) were tested, and the resulting processed products (bêche-de-mer, BDM) were compared with partially processed tissue and BDM processed without salting and by smoke drying. Weight and length based recovery rates did not differ significantly across salting treatments or from the non-salted control treatment. There was a general trend of decreasing collagen content with increasing brine strength in the brining treatments, and sequential increases in the force required to shear reconstituted BDM processed with increasing brine strength. This has implication for BDM processing because the quality of reconstituted BDM is judged primarily by texture, not flavor, with softness and elasticity being of prime importance. BDM from most treatments was significantly less firm than cooked, partially processed tissue. The most abundant protein-bound amino acids in sandfish BDM were glycine, glutamic acid, proline, arginine, aspartic acid, alanine and hydroxyproline, but their levels did not vary significantly across treatments. Our results provide a basis for improvements to sandfish processing that optimize textural properties of resulting BDM.

Key words: Sea cucumber; processing; *Holothuria scabra*; Sandfish; Amino acids; Collagen; Bêche-de-mer.

1. Introduction

Over 1200 species of sea cucumbers are known of which 58 are commercially exploited (Ferdouse, 2004; Purcell, Samyn, & Conand, 2012). Sea cucumbers are usually processed into a dried product called bêche-de-mer (*iriko* in Japanese, *hai-som* in Chinese or *trepang* in Indonesian) (Bumrasarinpai, 2006; Ferdouse, 1999; McElroy, 1990) that is consumed as a delicacy and for perceived medicinal benefits in S.E. Asian countries (Bordbar, Anwar, & Saari, 2011; Esmat, Said, Soliman, El-Masry, & Badiea, 2013), particularly in China, Hong Kong, Taiwan, Singapore and Malaysia (Eriksson & Clarke, 2015; Ferdouse, 2004) where well-dried 'A' grade product may command $US 70-190 kg^{-1} depending on the species used, size and quality (Purcell et al., 2012). At least 15,000 tonnes of bêche-de-mer (BDM) are traded annually in S.E. Asia (Qao, Xu, & Yang, 2011).

BDM processing results in a product that is non-perishable if stored in dry, dark conditions. Processing steps include first boiling, slitting and gutting, second boiling, smoking and finally sun-drying (Purcell, 2014a; Ram, Chand, & Southgate, 2014; Ram, Chand, Zeng, & Southgate, 2016). For example, the processing steps used for sandfish (*Holothuria scabra*) are shown in Fig. 1. Each contributes to the resulting quality of the final product which determines the suitability of processed product for Asian markets (Conand, 1990; Purcell, 2014b; Sachithananthan, Osman, Mlay, & Schoemaker, 1985; SPC, 1994) and their value. Processing BDM may also involve a 'salting' step (Fig. 1) that uses salt to draw water from sea cucumber tissues (Lavitra, Rachelle, Rasolofonirina, Jangoux, & Eeckhaut, 2008) in order to facilitate dehydration and shrinking. Salting can be achieved by immersing sea cucumber tissue in a brine solution (brining), or by dry salting where the tissue is covered in coarse salt and developing brine is

allowed to drain away (kenching) (Sampels, 2015). If BDM processing includes salting, salt soluble proteins are generally leached from the tissues during the process, while salt enters the tissues and binds to the triple-helix collagen structure (Duerr & Dyer, 1952; Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011), where it contributes to the weight of the final BDM product (Bao et al., 2010; Dong, Jiang, Sun, & Zhu, 2008; Dong et al., 2011), protects the product from spoilage and prolongs shelf life.

The major factors affecting the quality and value of BDM are species used, appearance, odour, colour, thickness of the body wall and market demand (Ram, 2008). Nutrient content and nutritional quality are not considered when BDM is assessed for quality and value (Wen, Hu, & Fan, 2010) because these are generally unknown. Body wall thickness of BDM is a key determinant of quality and value (Skewes et al., 2004) because thicker body wall flesh provides better texture and improved eating quality (Lo, 2004). The body wall of sea cucumbers contains a high proportion of collagen (Xia & Wang, 2015) that has a major influence on BDM firmness and texture quality. Collagen molecules are composed of three α–chains that are stabilized by intra- and inter- chain hydrogen bonds forming the collagen triple helix (Zhang, Olsen, Grossi, & Otte, 2013). It contains a repeat of the amino acids glycine, proline and hydroxyproline (Ichikawa et al., 2010). In beef tissue, collagen content is generally influenced by the age of the animal because, as the animal matures, less collagen is synthesized and more mature crosslinks occur in the collagen fibrils (Weston, Rogers, Pas, & Althen, 2002). The nature of the bonds changes over time as these reducible crosslinks are replaced by mature thermally-stable and less soluble crosslinks (Weston et al., 2002). In livestock, the tissues of older animals develop more mature crosslinks that generate greater tension resulting in increased red meat toughness (Marsh,

1977; Weston et al., 2002), however, similar information on the influence of age on the structure of body wall collagen has not been documented in sea cucumbers. Although a number of prior studies have reported collagen contents of seafood such as fish (Liu, Li, & Guo, 2007 ; Matmaroh, Benjakul, Prodpran, Encarnacion, & Kishimura, 2011; Pati, Adhikari, & Dhara, 2010; Singh, Benjakul, Maqsood, & Kishimura, 2011), similar reports on collagen content of sea cucumber (Dong et al., 2011; Zhong, Chen, Hu, & Ren, 2015) and the effect of processing on collagen composition and texture in sea cucumbers (Chen, Peng, Lu, Li, & Hou, 2015) are few. Prior studies have shown that sea cucumber collagen is denatured around 60-100^{o}C (Dong et al., 2008; Dong et al., 2011) and the collagen fibrils were observed to be thicker and broken. But the influence of variations in processing methods on collagen content of resulting BDM, and the resulting influence on product texture and quality, has not previously been reported.

The flavours of aquatic foods are derived from non-protein nitrogen compounds including free amino acids, salts and minerals (Bremner, 2012). The flavor of BDM is generated by a range of amino acids including glycine, glutamic acid, lysine and aspartic acid that give sea cucumbers their unique taste (Zunying, Yicheng, & Mingyong, 2011). However, amino acids are readily soluble in water and can be leached from tissue during processing (Bremner, 2012) and such losses are likely to be increased if a salting step is included in BDM processing, because this process draws water from sea cucumber tissues (Lavitra et al., 2008) to facilitate dehydration. Furthermore, excessive cooking at a temperature above 100^{o}C can also result in excessive loss of amino acids during sea cucumber processing (Dong et al., 2008). However, information on losses of amino acids from BDM during process, and the effects of variation in processing method on such losses is very limited and has not previously been reported for tropical species.

A number of studies have reported the nutrient composition of both fresh (Haider et al., 2015; Lee et al., 2012; Omran, 2013) and processed (Ozer, Mol, & Varlık, 2004; Wen et al., 2010) sea cucumbers, but we are aware of only one prior study that determined the effects of variations in processing method on nutrient composition of resulting BDM. It reported on the effects of inclusion of a salting step, and variations in salting method, on the proximate and fatty acid compositions of resulting BDM processed from sandfish, *Holothuria scabra* (Ram, Francis, & Southgate, 2017). However, no prior study has reported the effects of various methods. This study examined changes in yield and texture, as well as collagen and amino acid compositions, of BDM processed from sandfish, *Holothuria scabra*, using variations in processing method. The influences of brine and kench salting, as well as brine strength and kenching duration, were determined and results provide a greater understanding of the influence of processing method on BDM composition.

2. Materials and Methods

This study was conducted at the post-harvest processing facility of the University of the South Pacific, Fiji, and the Food Science Precinct, Department of Agriculture and Fisheries, Queensland Government, Australia.

Holothuria scabra (Sandfish) were collected from Tavua Bay, Fiji Islands (17°26'29.4"S 177°51'44.4"E). After collection, they were left for 5 min before length and weight was determined. Individuals were gutted and held in an insulated fish box containing ice. A total of 36 individuals were collected and subsamples of three sea cucumbers were each subject to nine

different processing treatments. The remaining nine individuals were used for collagen and amino acid analysis of fresh *H. scabra* tissues (3), and for partial processing through cooking at 80-90^{o}C for 15 min (3) and cooking then kenching for 48 h only (3). Sea cucumber processing followed the general method normally used by BDM processors in Fiji as outlined by Purcell (2014a). Sea cucumbers were laid on a table for approximately five minutes to allow them to relax before the length and weight of each was determined to the nearest 10 g. All sea cucumbers were first cooked at a water temperature of 45^{o}C for 10 min before the water temperature was gradually increased to 80^{o}C (Fig. 1). Sea cucumbers were cooked for a further 10 min until they were hard and springy indicating completion of the first cook. Cooked sea cucumbers were then immersed in a 3 g/100 mL saline solution for 36 h to allow the outer layer of spicules to disintegrate (Purcell, 2014a). Sea cucumbers with spicules removed were then subject to seven different salting treatments using grade 11 coarse solar salt:

1) Kenching for 24 h prior to further processing;
2) Kenching for 36 h prior to further processing;
3) Kenching for 48 h prior to further processing;
4) Kenching for 72 h prior to further processing;
5) Immersion in a 15 g/100 mL saline solution for 48 h prior to further processing;
6) Immersion in a 18 g/100 mL saline solution for 48 h prior to further processing; and
7) Immersion in a 25 g/100 mL saline solution for 48 h prior to further processing.

After salting the sea cucumbers were cooked a second time for 15-25 min (at 45^{o}C rising to 96^{o}C) and were then solar dried for at least 2 to 3 weeks. Any salt crystals that formed on the surface of the sandfish during the drying period were washed off. After 2 to 3 weeks, a third

cook for 5-15 min (at 45^{o}C rising to boiling) was then followed by shape correction (straitening and closure of body cut) to assist market acceptability, before the product was finally dried using solar drying. Sea cucumbers in two other treatments were not salted and served as controls. Following removal of the spicules, these sea cucumbers were not salted, but proceeded directly to the second cooking step and were then dried as follows:

8) Solar dried; and
9) Smoke dried using smoke machine for 48 h.

2.1. *Recovery rates*

On completion of processing, recovery rate for length (RRL) and recovery rate for weight (RRW) were calculated for BDM resulting from each treatment as:

RRL = *Mean length after processing/mean fresh length x 100*

RRW = *Mean weight after processing/mean fresh weight x 100*

2.2.Texture determination

Prior to texture determination and amino acid analysis of BDM, processed dried BDM samples were reconstituted in fresh water for five days at 4^{o}C (Fukunaga, Matsumoto, Murakami, & Hatae, 2004). Once reconstituted, the samples along with partially processed cooked samples were crushed using the Retch-MM301 mixer mill and lyophilized.

Texture analysis of processed and reconstituted BDM, as well as cooked (80-90^{o}C) Sea cucumber tissue was performed using an Instron Penetrometer (Model no 5543, Instron Corporation, 825 University Avenue, Norwood MA, USA) with a 500 N load cell with a Kramer-Shear cell modified to have two blades instead of five.

2.3. Amino acid analysis

The amino acid analysis procedure employed pre-column derivatisation with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) and ultra-performance liquid chromatography (UPLC) analysis after first performing acid hydrolysis on the sea cucumber samples to release the protein-bound amino acids. Each sample was run in duplicate. The methodology used has been described in detail by Truong et al. (2015).

The samples were mixed till uniform, then 40-50 mg was hydrolysed by adding 5.0 mL of 20 ml/100 mL HCl in a 10 mL hydrolysis vial, flushing with nitrogen then incubated at 110°C for 24 h. An internal standard (norvaline) was added to the hydrolysate and it was diluted 1 part in 25 with MQ water prior to derivatization.

Hydrolysed samples and amino acid standards (Standard H, ThermoFisher Scientific) were derivatised with AQC reagent using the AccQ-Tag Ultra derivatisation kit (Waters Corporation, Milford, MA, USA) according to the manufacturer's instructions.

Chromatographic separation and quantitation of the reported 17 acid hydrolysate amino acids was performed on an ACQUITY UPLC system (Waters Corporation). The column was a BEH RP C18 (2.1 x 100 mm, 1.7 µm, Waters Corporation). The separation of amino acids was run

with a binary gradient flow rate of 0.7 mL min^{-1} at 60^{o}C and UV detection was at 260 nm with a 10.2 min. analysis time per sample. Data was acquired and quantified using Empower software (Waters) using prepared 2.5, 10 and 50 pmol per µL injection analytical standards and an internal standard calibration procedure.

BDM samples from each treatment (n=3) were analysed for their proximate amino acid compositions. For amino acid analysis including hydroxyproline and taurine determination, the samples were hydrolysed in 6 mol/L HCl at 110^{o}C for 24 h. Cysteine and tryptophan were not analysed using this method. All amino acids were analysed using a Waters AccQTag Ultra Chemistry on a Waters Acquity UPLC system. Since the limit of reporting using this method was 1 mg/g, taurine was not detected in the samples.

2.4. Collagen analysis

Hydroxyproline content of the BDM was used to estimate collagen content following application of a conversion coefficient (Chen et al., 2015). Collagen analysis was carried out using the ISO method (3496: 1978). BDM was reconstituted for 3-5 days and cut into small pieces before being milled using Retch-MM301 mixer mill and lyophilised. Approximately 250 mg of dried sample was measured into hydrolysis jar and filled with 10 mL of 6 mol/L HCl and hydrolysed at 115 ^{o}C for 18 h. Hydrolysates were filtered using Whatman No. 1 filter paper and serially diluted to the ratio of 1:100 and 1:10. A 2 mL aliquot of the 1:10 diluent was transferred to a glass test tube for analysis of hydroxyproline and diluted where necessary in duplicates and 1 mL chloramine-T reagent was added, mixed and left at room temperature for 20 minutes. An aliquot of 1 mL colour reagent was added and mixed thoroughly and heated at 60°C ± 0.5 °C for 20 min. The

tubes were cooled under tap water for 3 min and left at room temperature for a further 30 min. Absorbance was read at 558 nm against a deionized water blank. Hydroxyproline content was calculated from the absorbance using a standard curve, taking into account the dilution factors. Amino acid analysis (*section 2.3*) determined that hydroxyproline contributed 64 reissues per 1000 amino acids within sandfish BDM tissues (i.e. 6.4% of all amino acids (Table 2). On this basis, estimation of collagen content based on hydroxyproline equivalents, employed a conversion factor of 6.4 in this study.

2.5. Statistical analysis

All analyses were conducted in triplicate. Results are expressed as mean values ± standard error mean (SEM) and one-way analysis of variance (ANOVA) was carried out using the SPSS Version 24 statistical software. Differences in the nutrient contents of BDM across treatments were tested with ANOVA followed by the multiple comparison test (Tukey HSD) after Levene's test for homogeneity of variance was not significant ($p>0.05$). Data for collagen content and texture (firmness and hardness) were similarly analysed, but following a significant Levene's test ($p<0.05$), data were $\log_{10}$ transformed and a non-parametric (Kruskal Wallis) test was conducted. A Pearson's correlation test between collagen content and the firmness reconstituted BDM processed using a salting step (kench and brining) was conducted. Differences were considered to be significant when $p<0.05$.

3. Results

Weight and length based recovery rate (RRW and RRL, respectively) of BDM from all treatments is shown in Fig. 2. Mean RRL ranged from 20.70 ± 0.02% to 26.40 ± 3.50% across all salting treatments but none of these values differed significantly from that of the control treatment (25.9 ± 1.30%) ($p>0.05$). Smoke dried BDM, however, had a significantly greater RRL (35.10 ± 2.80%) than all other treatments ($p<0.05$). Mean RRW ranged from 4.50 ± 1.10% (brining 25 g/100 mL) to 9.70 ± 2.20% (kenching 24 h) across salting treatments but did not differ significantly among treatments or from those of control (3.80 ± 0.60%) and smoked product (6.90 ± 1.60%) (Fig. 2; $p>0.05$).

Crude collagen contents and textural measurements of BDM from all treatments are shown in Table 1. Collagen content of BDM ranged from 81.90 ± 4.30 mg/g (brining 25 g/100 mL) to 108.20 ± 8.80 mg/g (brining 15 g/100mL) across salting treatments. These values did not differ significantly among treatments or from those of control (126.70 ± 24.20 mg/g) and smoked product (110.90 ± 17.20 mg/g) (Table 1; $p>0.05$). Kruskal Wallis test for collagen content (($X^2(8, N=27) = 6.466$, $p=0.595$; $p>0.05$) across all treatments was not significant. Partial processing of *H. scabra* by cooking sea cucumbers at 80-90^{o}C, and cooking at 80-90^{o}C followed by salting for 48 h, produced tissue that had lower crude collagen contents (38.25 ± 1.36 and 78.04 ± 15.11 mg/g dry weight, respectively) than those of BDM from any of the treatments. Increasing the duration of kenching or the strength of brine during the salting step of BDM processing did not have any significant influence on collagen content of the product.

Textural parameters for cooked and cooked and kenched sandfish, and for BDM processed using various methods assessed in this study are shown in Table 1. The results show that 87.37 ± 3.32

N g^{-1} force and 180.19 ± 19.27 mJ g^{-1} of energy was required to shear sandfish flesh that has been cooked at 80-90^{o}C for 15 min. Processed, dried and reconstituted BDM showed no significant differences ($p<0.05$) for either of the textural parameters across treatments (Table 1); but BDM from all treatments, with the exception of 25 g/100 mL brining, was significantly less firm than flesh cooked at 80-90^{o}C. Despite the fact that dried and reconstituted BDM resulting from the 48-h and 72-h kenching, and 18 g/100 mL and 25 g/100 mL brining treatments, showed around half the hardness of sandfish flesh that was cooked at 80-90^{o}C, these differences were not significant ($p>0.05$). However, subsequent non-parametric analysis (Kruskal Wallis test) showed a significant differences between treatment for firmness ($X^2(9, N=30) = 19.942$, $p=0.018$) and hardness ($X^2(9, N=30) = 17.473$, $p=0.042$) (Table 1). There was a significant correlation (Pearson's correlation (*2-tailed*), $p=0.04$) between collagen content and firmness in reconstituted BDM produced across salting treatments.

The protein-bound amino acid contents of fresh sandfish flesh, cooked (80-90^{o}C) sandfish and cooked and salted sandfish are shown in Table 2 along with those of normally dried and smoke dried BDM, and BDM processed with 48 h kenching. Similar data for free amino acids are shown in Table 3. The most abundant protein-bound amino acids in fresh sandfish tissue were glycine, glutamic acid, proline, arginine, aspartic acid, alanine and hydroxyproline which made up 10.22 ± 0.80, 9.91 ± 0.51, 5.81 ± 0.48, 5.53 ± 0.36, 5.39 ± 0.32, 5.31 ± 0.37 and 3.82 ± 0.33 mg/g of tissue weight, respectively. The same amino acids dominated the BDM samples and there were no significant differences ($p>0.05$) in levels of these seven major protein-bound amino acids across the three BDM treatments. Furthermore, there were no significant differences in the levels of any of the 17 protein-bound amino acids reported between the three BDM

products, with the exception of serine content that differed between BDM processed using smoked drying and 48-h kenching. The most abundant free amino acids in fresh, cooked, and process sandfish tissues were glycine, glutamic acid, proline, alanine, aspartic acid and arginine (Table 3). Again there were no significant differences in the levels of any of the 17 free amino acids reported between the three BDM products, with the exception of serine content that differed between BDM processed using smoked drying and 48-h kenching.

4. Discussion

This study focused on the influence of processing method on yield, collagen content and textural properties, and amino acid content of BDM processed from Sandfish using different methods, particularly the influence of salting and salting procedure.

Literature on the processing yield of sea cucumbers relates primarily to recovery rates determined for fishery management purpose (Purcell, Gossuin, & Agudo, 2009; Ram et al., 2016; Skewes et al., 2004). Yield of BDM is generally expressed as a 'recovery rate' that determines the relationship (usually percentage) between fresh weight of sea cucumbers and the dry weight of resulting BDM (Skewes et al., 2004), but the relationship between fresh length and processed length is also meaningful (Ram et al., 2016). We are unaware of any previous research that has reported recovery rates for any species of sea cucumber to assess the effects of variations in BDM processing method. This study determined a maximum yield by weight (RRW) of 9.7% and of 25.6% by length (RRL) for Sandfish. This RRW value for Sandfish is higher than previously reported values of 5.0% (Conand, 1979), 5.1% (Skewes et al., 2004) and 8.1% (Ram et al., 2016). The maximum RRL value reported here was lower than the previous reported value

for sandfish of 32.6% (Ram et al., 2016). Use of a salting step during sea cucumber processing facilitates entry of salt into the tissues where it binds to the triple helix structure and contributes to the weight of the final BDM product (Bao et al., 2010; Dong et al., 2008; Dong et al., 2011). Prior research in this laboratory reported that the ash content of BDM produced from sandfish was significantly higher when a salting (kenching or brining) steps was included on processing (Ram et al., 2017), presumably as a result of the uptake of salt. It is surprising therefore that BDM produced by salting did not have increased RRW values compared to the control treatment and that increased kenching duration or brine strength did not produce sequential gains in RRW in such treatments.

About 60% of the sea cucumber body wall is composed of water (SPC, 1994) and most of this is lost during processing. The remainder is composed primarily of soluble and insoluble proteins that accounts for the high protein content of BDM (Dong et al., 2011). Much of the protein content of the body wall of sea cucumbers, and resulting BDM, is composed of collagen, that provides sea cucumbers with their body shape and form, and assists during feeding, respiration, burrowing and in defence (Yamada, Tamori, Iketani, Oiwa, & Motokawa, 2010). The potential effect of processing on the crude collagen composition of sandfish has not previously been reported. The collagen content of fresh tissue of sandfish was less than that of 8.16 ± 1.14% reported for *Astichopus japonicus* (Chen et al., 2015) and 10.9% reported for snapper (*Priacanthus tayenus*) skin (Kittiphattanabawon, Benjakul, Visessanguan, Nagai, and Tanaka (2005). The temperature used in processing sea cucumbers can have considerable effect on structure of collagen fibrils, resulting in shortening of fibrils and complete denaturing around 90-100^{o}C (Dong et al., 2008; Dong et al., 2011).

However, despite BDM from the normal dry control and smoke-dry treatments having the two highest (respectively) collagen contents, there were no significant difference between these levels and those of BDM prepared using brine or kench salting. There was however a general trend of decreasing collagen content with increasing brine strength in the brining treatments, although these differences between treatments were non-significant. On this basis our results do not show salting-out of collagen, with increasing salt concentration, in contrasts to the results of Duan, Li, Li, and Li (2013) who worked with the collagen from fresh calf skin. Generally, higher salt strengths during brining result in a higher degree of protein denaturisation in fish flesh (Gallart-Jornet et al., 2007) and this may have been evident in the results of this study where we recorded sequential decreases in collagen content with increasing brine strength.

Our results also indicate sequential increases in the force required to shear reconstituted BDM processed with increasing brine strength. The force required for BDM processed with 15 g/100 mL brine (6.91 n g^{-1}) was almost six-times less than that of BDM prepared using 25 g/100 mL brine solution. Similar results have been reported for fish tissues where, for example, the hardness of fresh Atlantic salmon (*Salmo salar*) tissue increased and elasticity decreased with increasing brine concentration (Gallart-Jornet et al., 2007). This probably results because protein denaturation increases with higher brine strength (Sampels, 2015; Thorarinsdottir, Arason, Bogason, & Kristbergsson, 2004; Thorarinsdottir, Arason, Sigurgisladottir, Gunnlaugsson, et al., 2011; Thorarinsdottir, Arason, Sigurgisladottir, Valsdottir, & Tornberg, 2011), resulting in a harder product (Barat, Rodríguez-Barona, Andrés, & Fito, 2002, 2003). Reconstituted BDM is preferred when it has softer texture (Akamine, 2007; Fukunaga et al., 2004) and, on this basis, our results indicate that inclusion of a salting step during BDM processing, and choice of salting

method, does influence the quality of resulting BDM, and that lower brine strengths are likely to result in improved product quality. Further research could determine the effect of brine strength on BDM texture at a finer scale than used in the present study (15, 18 and 25 g/100 mL), with a view to optimizing conditions for processing sandfish.

Sea cucumbers processed with a salting step lose around 90% of their original moisture content after drying (Ram et al., 2016). Resulting BDM is rehydrated for as long as 7 – 10 days prior to consumption (Fukunaga et al., 2004). However, the quality of reconstituted BDM is judged primarily by texture, not flavor, with softness and elasticity being of prime importance (Fukunaga et al., 2004). Our results show that there were no differences in the texture (firmness and hardness) of reconstituted sandfish BDM across the salting treatments used in this study. However, all salt processed BDM was soft compared to sandfish tissue that was only cooked (80 – 90^{o}C) and not fully process and reconstituted. The softer texture that results from dehydration and reconstitution of sea cucumbers is probably the reason that rehydrated dried sea cucumbers are preferred to fresh or cooked sea cucumbers in SE Asians countries. The softer texture of reconstituted BDM may result from a greater degree of damage to the crosslinks of the collagen fibril of the tissue after rehydration (Fukunaga et al., 2004). In other animal proteins, toughness and tenderness of the meat depends on collagen crosslink maturity (Weston et al., 2002), but research in this field with sea cucumbers is lacking and the changes that occur in tissue protein as a result of processing are unknown. However, during cooking of red meat the shear forces (toughness) increases in 2 phases: (1) as temperature increases to 40-50^{o}C denaturation of the contractile proteins, actin and myosin, and an increase in fluid loss takes place; and (2) when temperature increases to 64-68^{o}C, collagen denaturation results from shrinkage in fibrils and

383 excessive fluid loss generated by the pressure exerted during thermal contraction (Weston et al.,
384 2002). Similar knowledge of changes in tissue proteins during sea cucumber processing would
385 provide a basis for optimizing BDM production methods, and is a key component of future
386 research in this field.
387

388 Together with appropriate texture, the taste of sea cucumbers is an important factor contributing
389 to product quality and value (Akamine, 2007). Amino acids play an important role in giving rise
390 to sweetness, bitterness, sourness and umami taste in sea cucumbers (Sicuro et al., 2012). In this
391 study we found that glycine, glutamic acid, proline, arginine, aspartic acid, alanine and
392 hydroxyproline were the most abundant amino acids found in BDM processed from sandfish.
393 Prior studies with other species of sea cucumbers have similarly reported that glycine, glutamic
394 acid, aspartic acid were the dominant characteristic amino acid (Bechtel, Oliveira, Demir, &
395 Smiley, 2012; Haider et al., 2015; Lee et al., 2012; Omran, 2013; Sicuro et al., 2012), and higher
396 glutamic acid levels are considered responsible for the distinctive flavor (Zhong, Khan, &
397 Shahidi, 2007). Omran (2013) reported values for glycine, alanine and aspartic acid of 18.38 ±
398 1.10%, 6.52 ± 1.05% and 4.81 ± 0.80% of total amino acid, respectively, for fresh tissue of
399 sandfish, and these are higher than the values reported here for the same species. Despite that
400 fact that levels of all seven dominant amino acids (glycine, glutamic acid, proline, arginine,
401 aspartic acid, alanine and hydroxyproline) in sandfish BDM were higher in product that was
402 processed with kenching for 4 h than in normal dried and smoked dried BDM, these differences
403 were not significant. Our results show that inclusion of a kenching step in sandfish processing
404 does not negatively affect levels of tissue amino acids that contribute to product quality. This is
405 surprising because prior research in this laboratory has that salting significantly reduced the

protein content of resulting BDM processed from sandfish compared to non-salted BDM (Ram et al., 2017) and it is reasonable to assume that such protein loss would result in reduced tissue amino acid content when expressed on a dry tissue weight basis as it was in this study. Loss of protein as a result of salting has also been reported for fish tissues (Jittinandana, Kenney, Slider, & Kiser, 2002; Nketsia-Tabiri & Sefa-Dedeh, 1995), with protein extraction from tissue being a function of salt concentration attributed to the protein denaturing effect of salt (Sampels, 2015; Thorarinsdottir et al., 2004; Thorarinsdottir, Arason, Sigurgisladottir, Gunnlaugsson, et al., 2011; Thorarinsdottir, Arason, Sigurgisladottir, Valsdottir, et al., 2011).

Conclusions

The influences of brine and kench salting, as well as brine strength and kenching duration, on texture, collagen and amino acid contents of BDM processed from sandfish were determined for the first time in this study. Major amino acids in sandfish BDM were glycine, glutamic acid, proline and arginine. BDM collagen content decreased with increasing brine strength while firmness increased. Results provide a greater understanding of the influence of processing method on BDM composition and a basis to improve BDM processing to optimize textural properties.

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620

Table 1. Crude collagen contents and texture characteristics (mean ± SE) of fresh, cooked and processed sandfish.

Processing Method	**Crude collagen content (mg/g) dry tissue weight (except fresh)**	**Shear force (N/g) Firmness**	**Energy (mJ/g) Hardness**
Unprocessed fresh	4.34 ± 0.63	na	na
Cooked at 80-90^{o}C	38.25 ± 1.36	87.36 ± 3.32^{b}	180.19 ± 19.27^{b}
Cooked at 80-90^{o}C and salted for 48 h	78.04 ± 15.11	na	na
Normal dry (control)	126.72 ± 24.16^{a}	20.73 ± 4.48^{a}	51.39 ± 12.50^{a}
Smoked	110.87 ± 17.25^{a}	39.16 ± 17.05^{a}	90.87 ± 41.92^{ab}
Kenching 24 h	103.28 ± 22.45^{a}	8.24 ± 2.05^{a}	34.11 ± 4.72^{a}
Kenching 36 h	83.72 ± 2.49^{a}	22.99 ± 11.47^{a}	83.98 ± 42.08^{ab}
Kenching 48 h	83.20 ± 5.04^{a}	31.71 ± 8.05^{a}	96.89 ± 19.22^{ab}
Kenching 72 h	99.06 ± 16.89^{a}	8.48 ± 2.24^{a}	29.40 ± 3.97^{a}
Brining 15 g/100 mL saline	108.19 ± 8.78^{a}	6.91 ± 1.68^{a}	29.04 ± 7.66^{a}
Brining 18 g/100 mL saline	102.88 ± 15.29^{a}	26.23 ± 7.06^{a}	88.53 ± 31.74^{ab}
Brining 25 g/100 mL saline	81.89 ± 4.27^{a}	40.49 ± 17.62^{ab}	83.05 ± 27.81^{ab}

Means in the same column with different superscripts are significantly difference ($p < 0.05$)

Table 2. Tissue amino acid content (mg/g ± SEM) of fresh and cooked sandfish, and normal dried, smoke dried and kench salted BDM. Means in the same row with a different superscript are significant different ($p < 0.05$)

Amino Acid residue in tissue (mg/g tissue)	Fresh	Cooked (80-90°C)	Cooked (80-90°C) and salted	Normal dried	Smoked	Kench salted 48 h
Hydroxyproline	3.82 ± 0.33	14.12 ± 0.29	4.43 ± 0.33	44.13 ± 0.56[a]	45.53 ± 1.09[a]	50.25 ± 3.34[a]
Histidine	0.35 ± 0.01	1.35 ± 0.04	0.37 ± 0.02	4.35 ± 0.12[a]	4.05 ± 0.13[a]	4.12 ± 0.26[a]
Serine	2.08 ± 0.12	7.10 ± 0.12	2.16 ± 0.15	23.78 ± 0.19[ab]	23.09 ± 0.42[a]	24.77 ± 0.24[b]
Arginine	5.53 ± 0.36	19.58 ± 0.42	6.03 ± 0.43	63.19 ± 0.34[a]	62.32 ± 1.57[a]	68.94 ± 2.32[a]
Glycine	10.22 ± 0.80	36.74 ± 0.81	11.53 ± 0.86	116.02 ± 1.10[a]	117.66 ± 3.15[a]	131.79 ± 7.00[a]
Aspartic acid	5.39 ± 0.32	18.53 ± 0.39	5.70 ± 0.39	60.36 ± 0.74[a]	60.26 ± 1.44[a]	63.59 ± 0.54[a]
Glutamic acid	9.91 ± 0.51	30.35 ± 0.71	9.36 ± 0.67	99.93 ± 1.09[a]	99.43 ± 3.09[a]	107.62 ± 2.37[a]
Threonine	2.63 ± 0.17	9.17 ± 0.19	2.82 ± 0.20	30.77 ± 0.22[a]	29.99 ± 0.72[a]	32.11 ± 0.52[a]
Alanine	5.31 ± 0.37	18.75 ± 0.41	5.85 ± 0.42	59.74 ± 0.50[a]	60.33 ± 1.74[a]	66.86 ± 3.00[a]
Proline	5.81 ± 0.48	21.00 ± 0.44	6.59 ± 0.50	67.57 ± 0.39[a]	67.63 ± 1.71[a]	75.90 ± 3.23[a]
Lysine	1.15 ±0.01	3.97 ± 0.13	1.11 ± 0.07	13.91 ± 0.51[a]	12.13 ± 0.62[a]	12.74 ± 1.07[a]
Tyrosine	1.21 ± 0.07	4.18 ± 0.11	1.28 ± 0.08	14.60 ± 0.21[a]	14.13 ± 0.14[a]	14.52 ± 0.30[a]
Methionine	0.78 ± 0.04	2.64 ± 0.08	0.78 ± 0.05	8.91 ± 0.14[a]	8.42 ± 0.27[a]	8.95 ± 0.18[a]
Valine	2.16 ± 0.12	7.37 ± 0.14	2.21 ± 0.14	25.06 ± 0.20[a]	24.25 ± 0.53[a]	25.37 ± 0.16[a]
Isoleucine	1.29 ± 0.06	4.32 ± 0.12	1.26 ± 0.08	15.03 ± 0.30[a]	14.06 ± 0.47[a]	14.43 ± 0.61[a]
Leucine	2.29 ± 0.10	7.84 ± 0.19	2.33 ± 0.15	26.74 ± 0.36[a]	25.46 ± 0.83[a]	26.71 ± 0.53[a]
Phenylalanine	1.25 ± 0.06	4.18 ± 0.08	1.22 ± 0.08	14.63 ± 0.21[a]	13.76 ± 0.29[a]	14.02 ± 0.54[a]

Table 3. Free amino acid content (mg/g ± SEM) of fresh and cooked sandfish, and normal dried, smoke dried and kench salted BDM. Means in the same row with a different superscript are significant different ($p < 0.05$)

Free Amino Acid (mg/g tissue)	Fresh	Cooked (80-90°C)	Cooked (80-90°C) and salted	Normal dried	Smoked	Kench salted 48 h
Hydroxyproline	4.42 ± 0.39	14.06 ± 0.29	5.13 ± 0.38	51.16 ± 0.65a[a]	52.78 ± 1.27[a]	58.25 ± 3.87[a]
Histidine	0.40 ± 0.01	1.31 ± 0.04	0.42 ± 0.02	4.92 ± 0.14[a]	4.58 ± 0.15[a]	4.67 ± 0.30[a]
Serine	2.51 ± 0.14	7.36 ± 0.12	2.60 ± 0.18	28.70 ± 0.23[ab]	27.87 ± 0.51[a]	29.89 ± 0.29[b]
Arginine	6.17 ± 0.41	18.75 ± 0.40	6.73 ± 0.48	70.48 ± 0.38[a]	69.51 ± 1.75[a]	76.89 ± 2.59[a]
Glycine	13.45 ± 1.05	41.52 ± 0.92	15.17 ± 1.13	152.67 ± 1.45[a]	154.83 ± 4.14[a]	173.41 ± 9.21[a]
Aspartic acid	6.23 ± 0.36	18.41 ± 0.39	6.59 ± 0.45	69.80 ± 0.86[a]	69.69 ± 1.66[a]	73.54 ± 0.63[a]
Glutamic acid	10.38 ± 0.58	29.70 ± 0.70	10.66 ± 0.76	113.87 ± 1.24[a]	113.30 ± 3.52[a]	122.63 ± 2.70[a]
Threonine	3.10 ± 0.20	9.28 ± 0.19	3.32 ± 0.23	36.25 ± 0.26[a]	35.33 ± 0.85[a]	37.83 ± 0.61[a]
Alanine	6.66 ± 0.46	20.19 ± 0.45	7.33 ± 0.53	74.88 ± 0.63[a]	75.62 ± 2.18[a]	83.80 ± 3.76[a]
Proline	6.88 ± 0.57	21.38 ± 0.45	7.81 ± 0.59	80.10 ± 0.46[a]	80.18 ± 2.03[a]	89.97 ± 3.83[a]
Lysine	1.31 ± 0.01	3.89 ± 0.13	1.27 ± 0.08	15.87 ± 0.58[a]	13.84 ± 0.70[a]	14.54 ± 1.22[a]
Tyrosine	1.35 ± 0.07	3.99 ± 0.10	1.42 ± 0.09	16.21 ± 0.23[a]	15.69 ± 0.16[a]	16.12 ± 0.33[a]
Methionine	0.89 ± 0.05	2.57 ± 0.07	0.89 ± 0.06	10.14 ± 0.16[a]	9.58 ± 0.31[a]	10.18 ± 0.20[a]
Valine	2.55 ± 0.14	7.48 ± 0.15	2.62 ± 0.17	29.62 ± 0.25[a]	28.66 ± 0.63[a]	29.99 ± 0.19[a]
Isoleucine	1.50 ± 0.07	4.30 ± 0.12	1.46 ± 0.09	17.42 ± 0.35[a]	16.30 ± 0.55[a]	16.73 ± 0.71[a]
Leucine	2.65 ± 0.12	7.80 ± 0.19	2.70 ± 0.17	30.99 ± 0.41[a]	29.51 ± 0.96[a]	30.97 ± 0.61[a]
Phenylalanine	1.40 ± 0.07	4.03 ± 0.08	1.37 ± 0.09	16.42 ± 0.24[a]	15.45 ± 0.32[a]	15.74 ± 0.61[a]

Table 4. Major amino acids reported for various sea cucumber species. Samples reported in the table are for fresh sea cucumbers

Sea cucumber species	Amino acid	Amino acid content (%)	References
Holothuria scabra	Glycine	18.38	Omran (2013)
	Aspartic acid	4.81	Omran (2013)
	Alanine	6.52	Omran (2013)
	Hydroxyproline	6.30	This study
	Arginine	9.16	This study
	Glycine	16.90	This study
	Aspartic acid	8.93	This study
	Glutamic acid	15.11	This study
	Alanine	8.79	This study
	Proline	9.59	This study
Parastichopus californicus	Hydroxyproline	2.6	Bechtel (2012)
	Glycine	12.3	Bechtel (2012)
	Aspartic acid	11.8	Bechtel (2012)
	Glutamic acid	13.1	Bechtel (2012)
	Alanine	5.6	Bechtel (2012)
	Proline	6.7	Bechtel (2012)
Astichopus japonicus	Aspartic acid	10.8 – 11.3	Lee et al. (2012)
	Glutamic acid	4.53 – 5.12	Lee et al. (2012)
Holothuria aernicola	Aspartic acid	15.71	Haider (2015)
	Glycine	17.33	Haider (2015)
Actinopyga mauritiana	Aspartic acid	10.83	Haider (2015)
	Glycine	21.70	Haider (2015)
Holothuria tubulosa	Glycine	10.36	Sicuro (2012)
	Aspartic acid	5.60	Sicuro (2012)
	Glutamic acid	8.03	Sicuro (2012)
Holothuria polii	Glycine	7.42	Sicuro (2012)
	Aspartic acid	4.49	Sicuro (2012)
	Glutamic acid	6.18	Sicuro (2012)

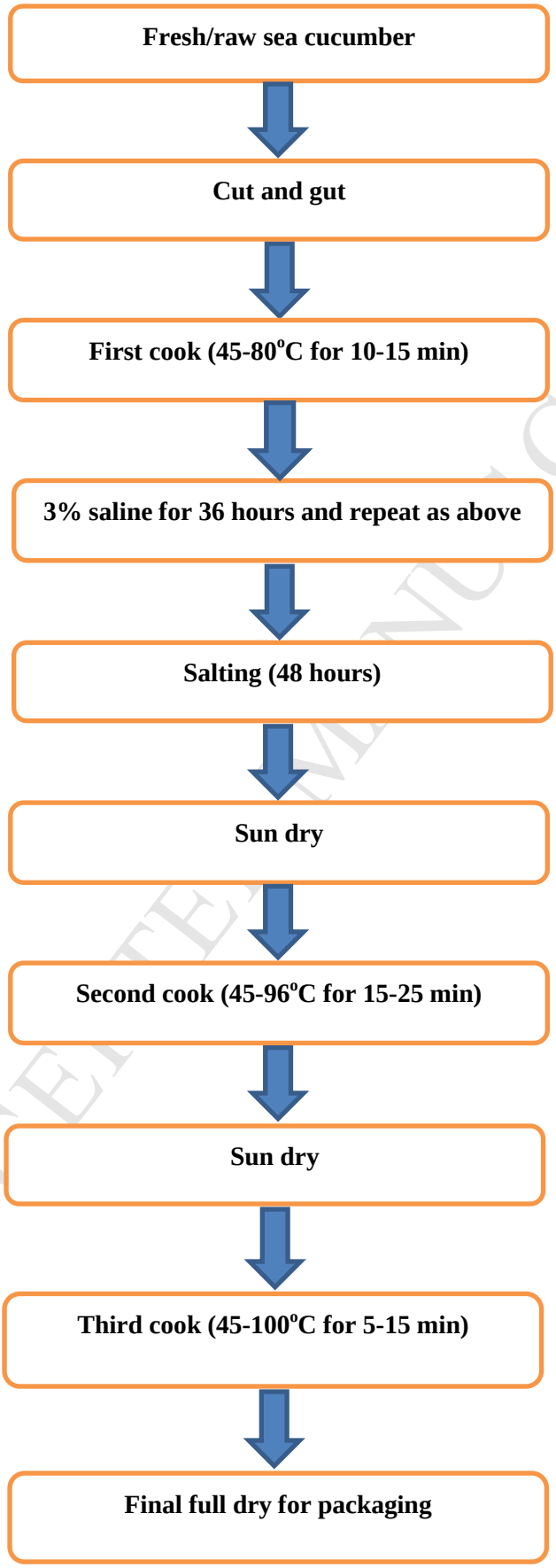

Figure 1. Steps used to process sandfish.

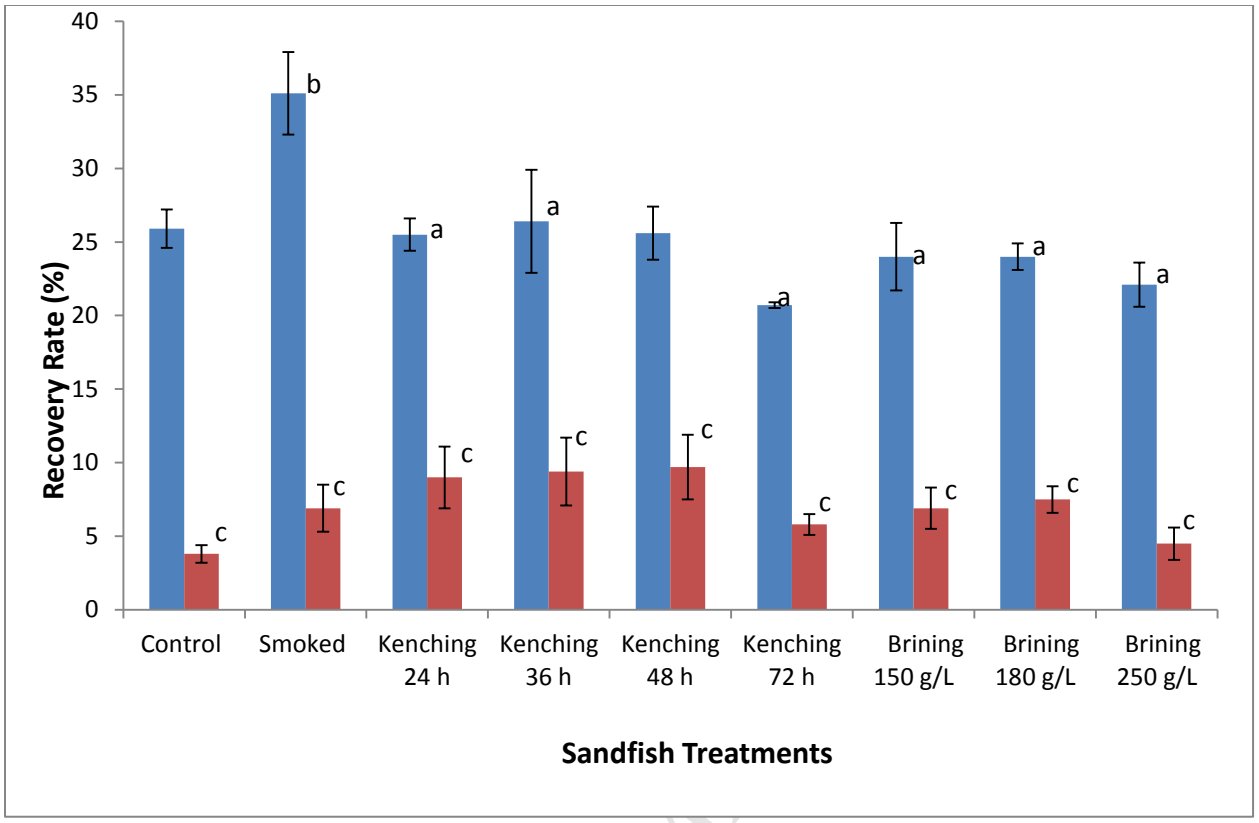

Figure 2. Recovery rates (RRL RRW) for bêche-de-mer processed from sandfish using different methods. Means with the same superscript are not significantly different ($p>0.05$)

Highlights:

- Salting method affected texture and composition of sandfish bêche-de mer (BDM)
- BDM collagen content decreased and firmness increased with increasing brine strength
- Major amino acids in sandfish BDM were glycine, glutamic acid, proline and arginine
- Textural properties of BDM can be improved by more appropriate processing methods