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Thermal-Induced Autolysis Enzymes Inactivation, Protein Degradation and Physical Properties of Sea Cucumber, *Cucumaria frondosa*

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Abstract: The main objective is to effectively denature the autolysis enzymes of *C. frondosa* on the premise of avoiding the quality deterioration caused by overheating. The effects of the different thermal treatments (blanching at 40–80 °C for 45 min, boiling and steaming at 100 °C for 15–120 min) on the cooking yield, moisture content, protein degradation, texture, and enzyme inactivation were studied, and the inner relationship was investigated by multivariate analysis. The autolysis enzymes of *C. frondosa* were thermally stable and cannot be denatured completely by blanching. Boiling and steaming could efficiently inactivate the enzymes but overheating for 60–120 min reduced the cooking yield and texture quality. Boiling at 100 °C for 45 min was suitable for pre-treatment, with cooking yield of 70.3% and protein content of 78.5%. Steaming at 100 °C for at least 30 min was preferable for long-term storage and instant food, in which the relative activity was only 3.2% with better palatability.

Keywords: *Cucumaria frondosa*; autolysis enzyme; inactivation; thermal processing; protein denaturation; texture

1. Introduction

Sea cucumber is an invertebrate animal belonging to the phylum Echinodermata. There are about 1100 species of sea cucumbers around the world, but only 40 of them are edible [1], mainly including *Stichipus japonicus* [2], *Apostichopus japonicus* [3], *Holothuria forskali* [4], *Holothuria polii*, *Holothuria tubulosa* [5], etc. Sea cucumber is very popular among Asian countries. In 2020, the output value of the whole industry chain in China reached USD 9.5 billion [6]. Since the sea cucumber sulfated polysaccharide was proved to have strong inhibitory activity on SARS-CoV-2 [7], consumer demand surged.

Cucumaria frondosa is an inhabitant of the North Atlantic Ocean, not only a valuable food source consisting of high quantity of nutrients [8,9], but also has health care functions, such as anti-inflammatory, antimicrobial, antioxidant, and anti-cancer functions [10]. Fisheries for this species have been well-established since 1988 [11,12]. According to FAO statistics, the annual production quantity of *C. frondosa* has increased from 1411 tons to 2655 tons from 2008 and 2015. However, *C. frondosa*, similar to *Stichipus japonicus*,

Apostichopus japonicus, and other species of sea cucumber, has autolysis particularity. The protein degradation caused by autolysis enzymes, mitochondrial apoptosis caused by ultraviolet light and temperature, and microbial degradation were the main reasons for quality deterioration of sea cucumber. Dong [13] monitored the activities of 13 endogenous enzymes, and found that leucine aminopeptidase, alkaline protease, phosphohydrolase, and glycosylhydrolase had higher activities during the sea cucumber heating process, which may play a leading role in body wall degradation. Even though producers have attempted a variety of treatment methods, including freezing quickly after capture, blood-letting, and freezing, etc., enzymatic autolysis is still a reoccurring problem during shipping and storage. The sea cucumber industry in the North Atlantic has been seriously hampered as a result of the huge financial losses.

Heating treatments are commonly used methods for enzyme deactivation. A few studies have investigated the enzyme thermal stability of sea cucumber. Hernandez-Samano [14] extracted metalloproteases from *Isostichopus fuscus* ventral muscle and found that more than 74% of residual activity was still retained after heating at 80 °C for 1 h. The enzymes showed higher heat-resistant properties than those in Holothurians [15], in which the enzymes could be inactivated at 60 °C for 55 min. High hydrostatic pressure and high-pressure steam were utilized for autolysis enzyme deactivation of *Apostichopus japonicus*, but quality deterioration took place during storage because the autolysis enzymes were not fully denatured [16,17]. This indicates that although the thermal stability of autolysis enzymes is different among species, autolysis is still a widespread issue in this field. Heating treatment not only inactivates enzymes, but also changes the physicochemical quality of sea cucumber. Gao et al. [2] studied the heating effects of different durations at 70 °C on the rheological properties of *Stichipus japonicus* sea cucumbers and observed that the collagen in the body wall was converted into gelation within 1 h and both the weight and rupture strength of sea cucumbers decreased continuously during thermal treatments. Moreover, Dong et al. [18] observed that inappropriate low-temperature heating caused decrease in hardness, as well as high drip loss.

Currently, studies on *C. frondosa* only focus on nutritional and bioactive compounds [10,19,20]. However, the effect of heating treatments on physicochemical quality and enzyme inactivation of *C. frondosa* is unknown. To be noticed, *C. frondosa* is extremely big and tough with a thick body wall and tendons, and the nutritional compounds are different from many other sea cucumber species [9]. It may result in tremendous differences compared with other species, not only in physicochemical quality properties, but also in heat transfer resistance. This research intends to provide a promising strategy for *C. frondosa* autolysis enzyme thermal inactivation while guaranteeing physicochemical quality. The effects of blanching, boiling, and steaming on the physicochemical quality and enzyme inactivation of *C. frondosa* were investigated. The results give insights into the physicochemical properties altering mechanism of *C. frondosa* from the perspective of protein degradation.

2. Materials and Methods

2.1. Raw Materials and Pre-Treatment

C. frondosa was caught on the east coast of Iceland, out of Stöðvarfjörður by dredge (2.4 m wide), which was dragged for a maximum of 25 min each time. The raw materials were stored at −25 °C within a month and thawed at 4 °C for 18 h before use. Individuals with an average weight of 490 ± 50 g (n = 140) were selected for the experiment. The guts and crown of the oral tentacles were meticulously removed. The body wall was cleaned with cold water and placed on ice until thermal treatments. The raw materials (n = 140) were divided into 14 groups of 10 sea cucumbers each.

2.2. Thermal Treatments

For the blanching and boiling cooking, an electro-thermostatic water bath (Julabo B33, JULABO GMBH, Seelbach, Germany) was used. The water bath was preheated to specific temperatures. The samples were immersed in water (sample:water = 1:2), and blanched at

40, 60, and 80 °C for 45 min (abbreviated to B40 °C, B60 °C, and B80 °C, respectively), and heated at 100 °C for 15, 30, 45, 60, and 120 min (abbreviated to B15, B30, B45, B60, and B120, respectively). For the steaming cooking, an electro-steam oven (Convostar, Convotherm Elektrogerate GmbH, Eglfing, Germany) was used and preheated to 100 °C. The samples were arranged on trays tidily with the entocoele downwards and steamed at 100 °C for 15, 30, 45, 60, and 120 min (abbreviated to S15, S30, S45, S60, and S120, respectively). After cooking, the samples were placed on ice with a plastic membrane until analyzed.

2.3. Cooking Yield

The raw and cooked samples were weighed accurately before (W_b) and after heating (W_a) at room temperature. The cooking yields were calculated according to the following equation (n = 5).

$$\text{Cooking yield} = \frac{W_a}{W_b} \times 100\% \quad (1)$$

2.4. Water Content and Water Holding Capability (WHC)

Approximately 30 g of tissue was taken from every individual. These tissue samples (300 g per group) were collected and homogenized (8000 rpm) in an Ultra Turrax mixer (T25, IKA-Labortechnik, Staufen, Germany) for 10 min prior to the following tests.

Approximately 5 g of minced sea cucumber samples were dried in a ceramic bowl at 105 °C for 4 h. The water content of the sea cucumber tissue was calculated based on the weight changes according to ISO 6496 1999 (n = 3).

The WHC was measured according to Zhang et al. [21], with slight modification. Approximately 2 g of the sea cucumber samples were weighed precisely into a vial and centrifuged (TJ 25 centrifuge, Beckman coulter, Pasadena, CA, USA) at 210× g-force (1500 rpm) and at 2–5 °C for 5 min. The WHC (%) was calculated as the ratio of water in the sample after centrifugation to the water in the sample before centrifugation (n = 3).

2.5. Protein Characteristics

2.5.1. Crude Protein Content

The total nitrogen (TN) content of the *C. frondosa* sea cucumbers was measured by the micro-Kjeldahl method (Kjeltec 8400 Tecator Line, Foss Analytic Hilleroed, Hilleroed, Denmark) according to Mizuta et al. (n = 2).

2.5.2. Trichloroacetic Acid (TCA)-Soluble Nitrogen

Approximately 20 g of minced sea cucumber were weighed accurately into a 250 mL flask, and 50 mL of 10% (w/w) trichloroacetic acid (TCA) were added. It was mixed for 4 min in an Ultra Turrax mixer (T25, IKA-Labortechnik, Staufen, Germany) and centrifuged (TJ 25 centrifuge, Beckman coulter, CA, USA) at 4 °C for 30 min at the speed of 2300× g-force. Then, 10–12 g of the supernatant were used for the determination of TCA-soluble nitrogen by the Kjeldahl method (Kjeltec KT 200 Labtec Line, Foss Analytic, Hilleroed, Denmark). The ratio of TCA-soluble nitrogen (TCA-N) to total nitrogen on a dry basis was calculated to represent the protein degradation during the heating process. Due to the ease with which TCA-N dissolves into water, the TCA-N in water was also measured (n = 2).

2.5.3. Electrophoresis SDS-PAGE

Protein degradation was analyzed with sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). The proteins were extracted by solution (20 mM Tris–HCl, pH 7.4, 150 mM NaCl, 2 mM ethylenediaminetetraacetic acid disodium salt, 10 mM dithiothreitol, 1% Triton X- 100, 0.1% SDS) on ice for 20 min [22] with a ratio of minced sample to extraction solution 1:3 (w/v). Extracts were centrifuged (Centrifuge 5415 D, Eppendorf, Hamburg, Germany) at 4 °C for 15 min at the speed of 12,000× g-force, 30 µL of supernatants were then added to 10 µL of sample buffer (277 mM Tris-HCl, pH 6.8, 44.4% glycerol, 4.4% Lithium Dodecyl Sulfate). A Bradford colorimetric assay (Colorimet-

ric Protein Determination with Coomassie Blue, Bio-Rad Laboratories Ltd., Stockholm, Sweden) was adopted to obtain a protein concentration of 2 mg/mL. After blanching the diluted sample solution for 5 min, 20 µL of the blanched solution was analyzed with 10% polyacrylamide gel electrophoresis, and a running buffer of Tris/Tricine/SDS using a Mini Protein system (Bio-Rad Laboratories Ltd., Stockholm, Sweden). The cooking liquid was collected carefully and subjected to freeze drying (Alpha 2-4 LSC plus, Martin Christ, Osterode am Harz, Germany). The dried materials from cooking liquids were analyzed using the same procedure as described before.

2.6. Texture Profile Analysis (TPA)

The raw and cooked *C. frondosa* were cut into 3 × 3 × 1 cm portions and analyzed with a cylindrical probe P/50. (Texture analyzer TA.XT Plus, Stable MicroSystems, London, UK). The pre-test speed, test speed, and post-test speed were set as 1, 0.5, and 1 mm/s, respectively. The deformation rate was set to 60%, in agreement with the description of Bi et al. [23]. The hardness, springiness, adhesiveness, springiness, cohesiveness, and resilience of the samples were assessed from the obtained texture profiles (n = 5).

2.7. Relative Enzymes Activity

The sea cucumber body wall is mainly composed of protein, and proteases showed high activity in the thermal processing, so protease activity was measured to represent the autolysis enzymes inactivation [24]. Approximately 6 g of minced sample were added to 30 mL of phosphate buffer solution. The mixture was shaken for 40 s and left at 4 °C for 6 h. The solution was centrifuged (TJ 25 centrifuge, Beckman coulter, CA, USA) at 4000 rpm for 8 min. Then, 3.0 mL of supernatants were analyzed with 3.0 mL of distilled water taken as a blank control. Samples were placed in a water bath (Julabo 19, JULABO GMBH, Seelbach, Germany) at 30 °C, and then 2.0 mL of 0.5% casein solution were added to each sample. Mixtures were heated for 10 min accurately, and 2.0 mL of 10% trichloroacetic acid (TCA) solution were added to terminate the protein denaturation reaction. In a subsequent step, the solution was centrifuged at 4000 rpm for 8 min, and 1.0 mL of supernatant was added with 0.5 mL of 0.55 mol/L Na_2CO_3 solution and 0.5 mL of Folin-Phenol reagent. The solution was shaken and subjected to a water bath at 30 °C for 15 min. The absorbance was measured by a UV spectrophotometer (Ultrospec 3000 pro, Amersham pharmacia biotech Biochrom, Cambridge, UK) at 650 nm (n = 6).

The relative enzymes activity (REA) was calculated as follows,

$$\text{REA} = \frac{Activity_{sample} / Activity_{control}}{Protein\ content_{sample} / Protein\ content_{control}} \times 100\% \quad (2)$$

2.8. Statistical Analysis and PCA

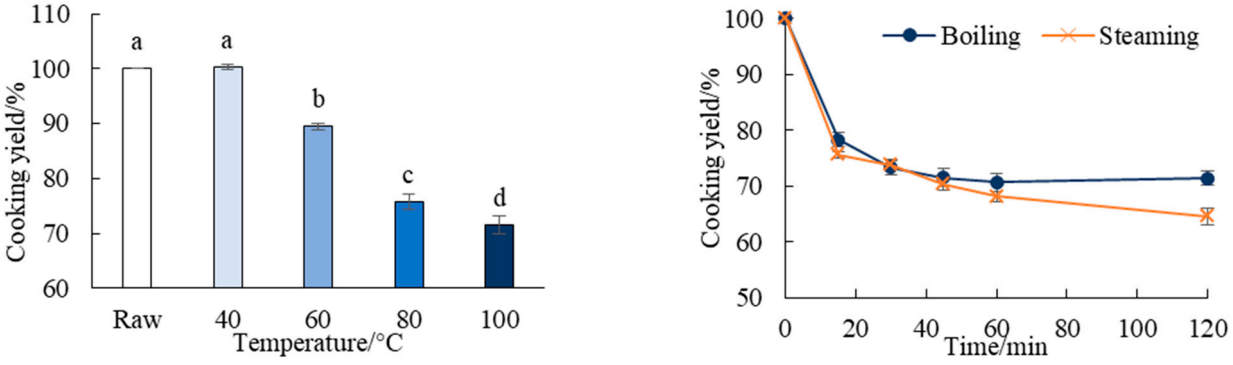
The data were processed by Microsoft Office Excel 2016 (Microsoft Inc., Redmond, Washington, DC, USA), the analysis of variance (ANOVA) was performed in the IBM SPSS Statistics 22 software. Principal component analysis (PCA) was completed in Origin pro 2019b (Origin Lab Corporation, Northampton, MA, USA).

3. Results

3.1. Effects of Thermal Treatments on Cooking Yield

The cooking yield of *C. frondosa* after various thermal treatments is illustrated in Figure 1. The cooking yield reduced significantly to 89.5 ± 0.5% when the temperature was up to 60 °C, and the drip loss was increased with the heating temperature and duration. When cooked at 100 °C, the first 15 min contributed most to the drip loss. In comparison, the cooking yield decreased steadily throughout the steaming treatment, but it was relatively steady in boiling groups. As a result, as high as 35.4 ± 1.5% and 28.6 ± 1.2% of the raw material was lost in the drip during the heating process by steaming and by boiling within 120 min. The average yield rate of the boiling cooking was 3.5% higher than that of the

steaming cooking, which was in line with the finding on crab meat by Crapo et al. [25]. Therefore, the cooking yield could be improved by choosing boiling instead of steaming treatment when the heating time exceeded 30 min at 100 °C. Salomina et al. [26] considered that the cooking yield was mostly a function of moisture loss. In this study, all the cooking liquid turned from transparent to dark brown, indicating that the ash, saponin, chondroitin sulfate, and other soluble components may release from *C. frondosa* to the cooking liquid. Furthermore, the dry matter content of cooking liquid was measured to be 3.2 ± 0.1% in group S120 and 1.9 ± 0.2% in group B120. It confirmed that the nutrients of *C. frondosa* were also released to the ambient liquid lost as drip.



(**a**) (**b**)

Figure 1. Cooking yield of *C. frondosa* after various heat treatments, (**a**) comparison of cooking yield during cooking for 45 min at different temperatures (40, 60, 80, and 100 °C), and (**b**) comparison of cooking yield after boiling and steaming at 100 °C for 0–120 min. Different letters (a, b, c, d) indicate significant differences between groups as determined via one-way ANOVA ($p < 0.05$).

3.2. Effects of Thermal Treatments on the Water Content and WHC

The water content and WHC of the raw and cooked *C. frondosa* is shown in Figure 2. Water content is an important factor that affects economic efficiency, and the water inside collagenous tissue is crucial for the preservation of mechanical properties [27]. When the temperature exceeded 80 °C, the water content decreased significantly in comparison to the raw material ($p < 0.05$). There was a negative correlation between steaming time and water content at 100 °C, owing to the drip loss. It reduced significantly from 85.7% to 79.7% after steaming for 120 min. While the water content remained within the range of 83.1 ± 0.5% to 85.7 ± 0.2%, it did not decline continuously with boiling duration. As a consequence, the water content of the boiled sea cucumber was 2.3 ± 1.5% higher than that of the steamed group in average, which partially explained the yield advantages.

Interestingly, blanching of the *C. frondosa* at 40 and 60 °C resulted in higher moisture contents than in the raw sea cucumber. Xue [28] reported that an expansion of apertures among muscle fibrils in *Stichopus japonicus* was formed by hydrogen bond rupture during heating at 40 °C. Therefore, the rise in cooking yield and water content in group B40 °C may be attributed to the amplified space within the muscle fibrils which absorbed moisture from the blanching liquid. However, the absorbed water was easy to lose, leading to a low WHC in the B40 °C group. The cooked sea cucumber shrank obviously after blanching at 60 °C, resulting in smaller apertures between fibrils and it may explain the high WHC in the B60 °C group. In conjunction with the results of the cooking yield, it was obvious that the loss of nutrients surpassed the absorption of water during boiling at 60 °C, indicating the initiation of serious protein denaturation. Prolonged cooking time also exerted significant effects on the WHC ($p < 0.05$) of the *C. frondosa* dermis. When heated at 100 °C, the WHC was increased in the early 15 min, but declined successively to 84.1 ± 1.5% by steaming and 77.0 ± 1.2% by boiling 120 min later. WHC reflects the juiciness and tenderness of the product, and is significantly associated with quality loss, shrinkage, and compactness. The

thermal-induced decrease in WHC also indicated that the microstructure of the *C. frondosa* dermis degraded during heating, which is consistent with Bi et al. [23].

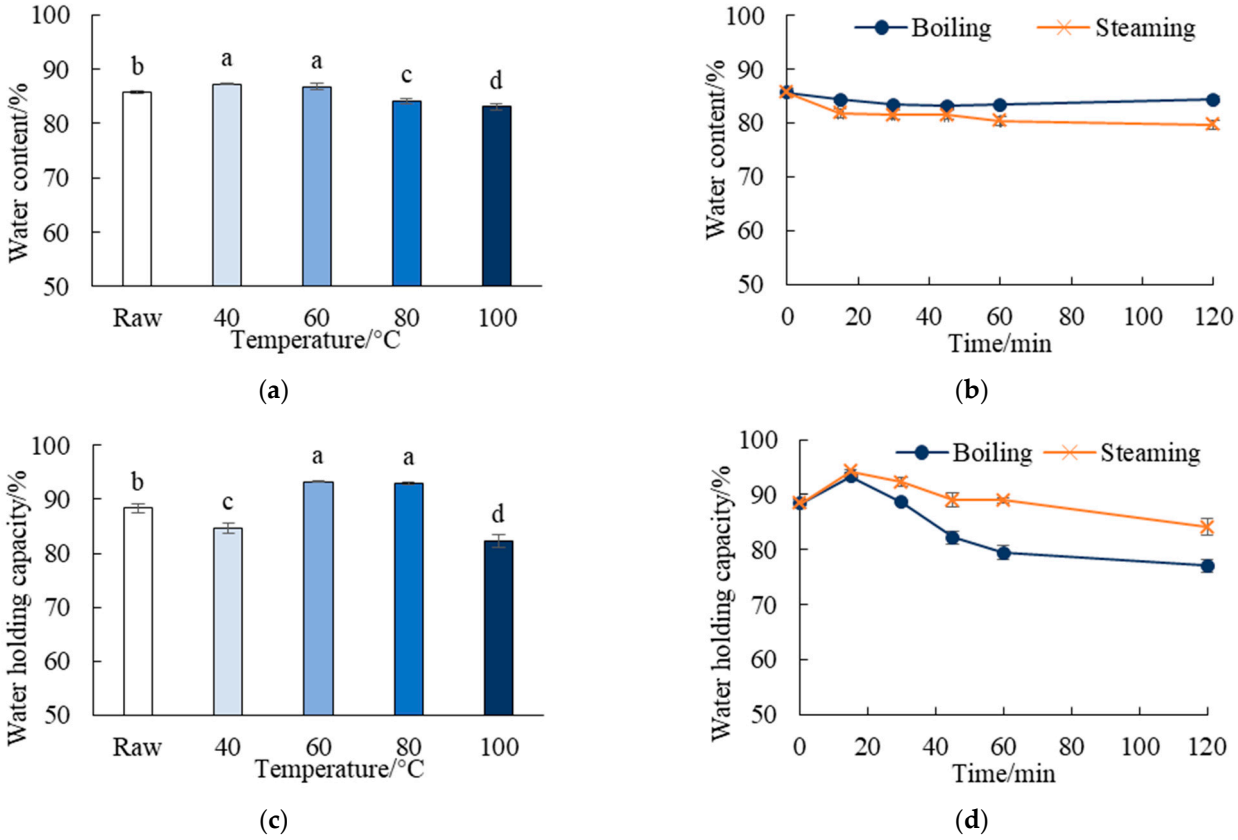


Figure 2. Water content and WHC of *C. frondosa* as affected by various heat treatments, (**a**) comparison of water content (**a**) and WHC (**c**) during cooking for 45 min at different temperatures (40, 60, 80, and 100 °C), and comparison of water content (**b**) and WHC (**d**) after boiling and steaming at 100 °C for 0–120 min. Different letters (a, b, c, d) indicate significant differences between groups as determined via one-way ANOVA ($p < 0.05$).

3.3. Effects of Thermal Treatments on Protein Denaturation

3.3.1. Crude Protein and TCA-Soluble Nitrogen

The contents of crude protein and TCA-soluble nitrogen are presented in Figure 3. Heating treatments under specific circumstances could increase the crude protein content per unit weight of sea cucumber, owing to the loss in moisture and water-soluble components during prolonged cooking. However, the crude protein content decreased when cooked for more than 30 min at 100 °C, implying that the thermal-induced protein denaturation took place during heating. It was consistent with Damodaran [29], who expounded that the collagen fibrils were degraded into small fractions and released into cooking liquid while being heated. Remarkably, the protein level on the dry basis of the boiled *C. frondosa* was consistently higher than that of the steamed sea cucumber, with a maximum difference of 11.8%. However, the content of crude protein on the wet basis in steaming groups was higher than that in boiling groups. It means that if the sea cucumber is dehydrated, boiling is more suitable than steaming in terms of protein content.

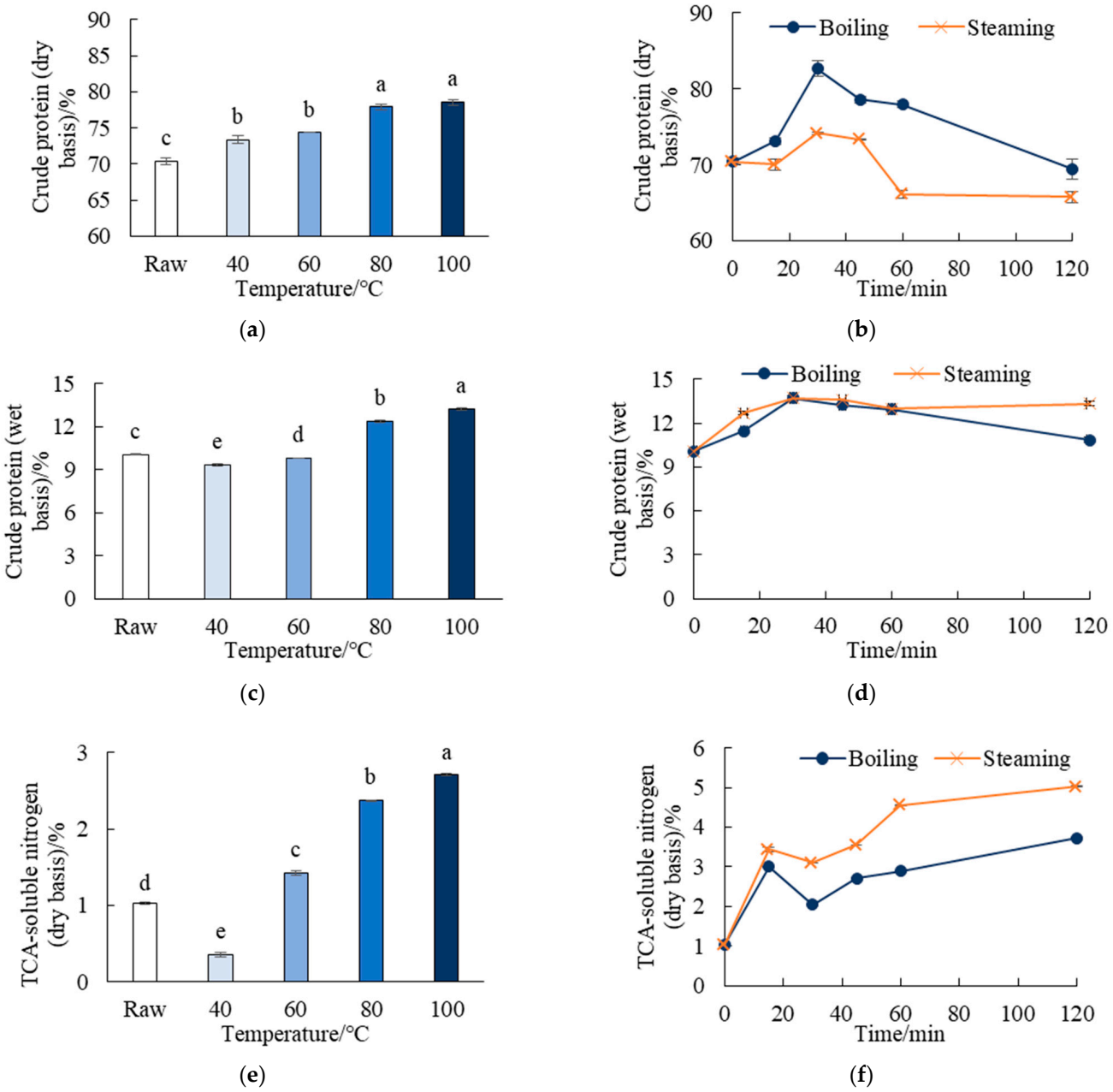
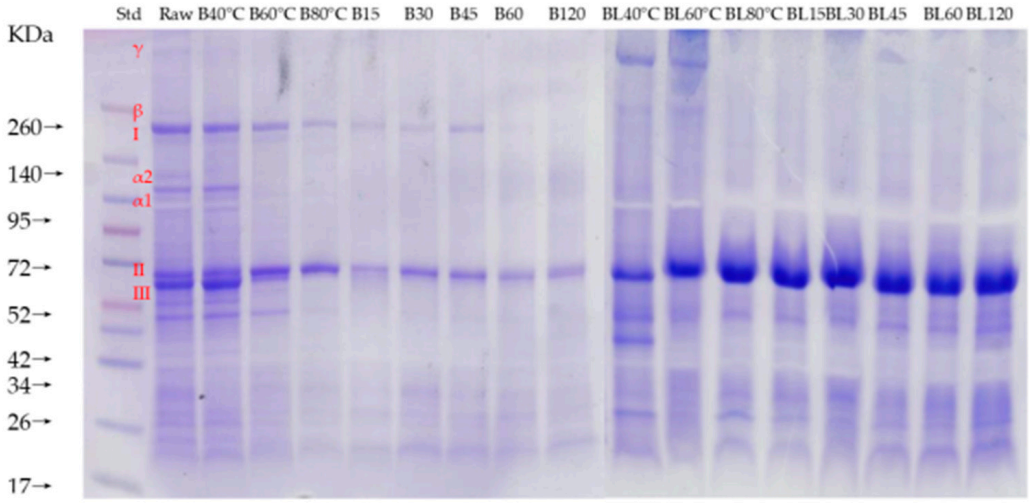


Figure 3. Content of crude protein and TCA-soluble nitrogen of *C. frondosa* after various heat treatments, comparison of crude protein on dry basis (**a**) crude protein on wet basis (**c**) and TCA-soluble nitrogen (**e**) during cooking for 45 min at different temperatures (40, 60, 80, and 100 °C), and comparison of crude protein on dry basis (**b**) crude protein on wet basis (**d**) and TCA-soluble nitrogen (**f**) after boiling and steaming at 100 °C for 0–120 min. Different letters (a, b, c, d, e) indicate significant differences between groups as determined via one-way ANOVA ($p < 0.05$).

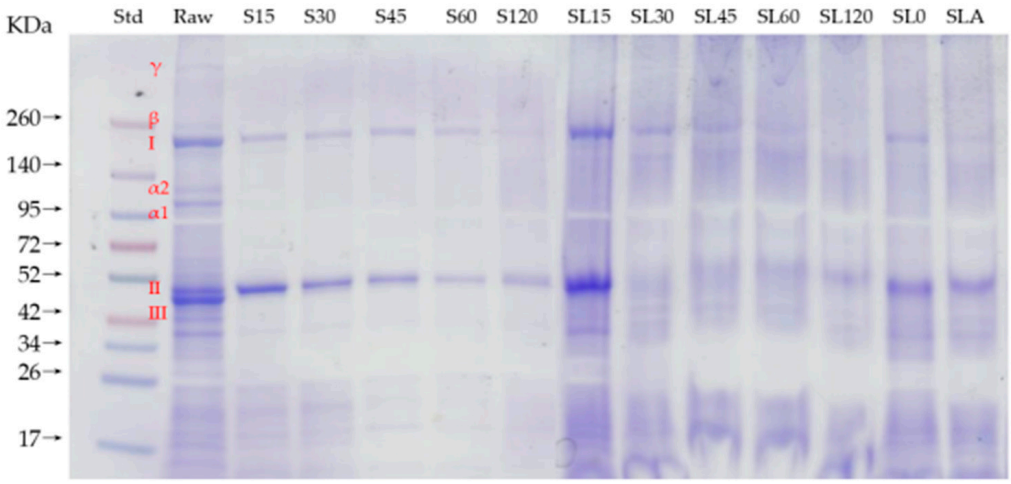
TCA-soluble nitrogen refers to amino acids and small peptides which can be released during protein degradation [30]. It was apparent that the TCA-soluble nitrogen was generated when temperatures exceeded 60 °C and increased both with the cooking temperature and duration. It is in accordance with previous reports, which states that the collagen denatured temperature of *Stichopus japonicus* to be in the range from 49.7 [23] to 57 °C [2].

3.3.2. Protein Distribution Pattern

As a means of investigating the thermal-induced protein degradation of *C. frondosa*, protein distribution patterns were analyzed by electrophoresis SDS-PAGE (Figure 4). The SDS-PAGE of the raw sea cucumber samples revealed three major proteins with molecular weights of 200 (Band I), 50 (Band II), and 44 kDa (Band III). Bands I and III were assumed to be the two same structural proteins observed in *Stichopus japonicus*, i.e., major yolk protein (MYP, Band I) and actin (Band III) [22,31]. Band II was not familiar among other sea cucumber species, whereas the protein molecular weight approximated troponin in fishes [32]. Considering that troponin is unavailable in the majority of echinoderms, further studies are needed to identify the protein composition of Band II.



(**a**)



(**b**)

Figure 4. SDS-PAGE analysis of blanched and boiled samples (**a**) and steamed samples (**b**). BL samples correspond to the cooking liquid of the boiled sea cucumber samples (B), respectively. SL samples represent the steaming liquid from 0–15 min, 15–30 min, 30–45 min, 45–60 min, 60–120 min, 0–60 min, 0–120 min, respectively, of the steamed sea cucumber samples (S).

A variety of sea cucumber collagen proteins have been reported, including those of *Stichopus japonicus* [33–35], *Parastichopus californicus* [36], *Stichopus monotuberculatus* [37], and *Cucumaria frondosa* [38]. However, the collagen structure of sea cucumbers is still controversial. Hou et al. [33] and Cui et al. [34] reported the collagen structure of *Stichopus japonicus* sea cucumber as $[\alpha_1(I)]_3$, which is consistent with Trotter [38], who concluded that the

collagen of *C. frondosa* belonged to type I collagen with α_1 trimers molecular composition. Saito et al. [35], on the other hand, classed the collagen structure of *Stichopus japonicus* as $[\alpha_1(I)]_2(\alpha_2(I))$. According to the current study, the collagen of *C. frondosa* contains two different α-chains (α_1, α_2), one β-chain and one γ-chain (Figure 4). The concentration of band α_1 was close to double that of the α_2, meaning that the collagen was more likely to be comprised of two α_1-chain units, and one unit of α_2-chain ($[\alpha_1(I)]_2(\alpha_2(I))$, which was in line with the observations of Saito et al. [35]. Similar patterns have been observed in other animals, including sea urchins [39], cephalopods [40], and fish [41].

The molecular weight of the α-chain in collagen of sea cucumber has earlier been reported in the range of 135–146 kDa [33,42]. The molecular weight of α-chains of the *C. frondosa* species were close to these reports, but slightly smaller than that of *Stichopus japonicus* and *Apostichopus japonicus*.

As seen in Figure 4a, the α_2-chain began to degrade during cooking at 40 °C, and the β-chain and γ-chain vanished together with the α_1-chain at 60 °C due to collagen fibrils unscrewing. Actin (Band III) also began to degrade at 60 °C, and completely disappeared at 80 °C. The MYP (Band I) of *C. frondosa* was relatively steady, and did not degrade until boiled at 100 °C for 120 min. Troponin (Band II) was highly thermostable during the boiling treatments, which is consistent with the studies by Chen et al. [43], who found the porcine troponin was stable during heat treatment up to 126 °C for 120 min. Considering that Band II has not been reported among other sea cucumber species yet, it indicates the differences not only in body wall protein composition, but also in the physicochemical properties during heating of *C. frondosa* from other reported sea cucumbers. In the boiling liquid, only Troponin could be clearly recognized for temperatures over 60 °C, and by then a new band appeared above it, presumably as an oxidized polymerization [44]. This was in line with a previous investigation of TCA-soluble nitrogen, which showed an increase in the fractions of small molecules during cooking.

In the raw materials, the collagen structural characteristics of the α_1-chain, α_2-chain, β-chain, and γ-chain were clear, but completely degraded in all steamed groups, indicating that the typical triple-helical structure of collagen was destroyed once steamed at 100 °C. Compared to earlier findings, these results were not unexpected. As reported by Wang et al. [45], the collagen fibrils in the body wall of the sea cucumber are sensitive to temperature change. The nonspecific bond strength of the collagen fibrils of the sea cucumber body wall was increased in the heating process of 60–90 °C, but the ionic bond, hydrogen bond, hydrophobic bond, and disulphide bond decreased. In consequence, the collagen fibrils degraded completely after heating at 90 °C for 60 min. Dong et al. [46] revealed that the thermal treatments resulted in conversion of α-helixes to β-sheets, and affected N–H bending, coupled with C–N stretching during heating. Moreover, the MYP was relatively intact during steaming until 120 min, which is consistent with the results of the boiling groups.

3.4. Effects of Thermal Treatments on Texture

As shown in Figure 5, the raw *C. frondosa* was thick, firm, and sticky. The texture characteristics (in Table 1) revealed an extremely high value of hardness and adhesiveness, and low springiness and chewiness. These results were similar to the TPA of *Stichopus japonicus* [23]. After heating, the dermis tightened and rolled inward, making the steamed sea cucumber crimpier.

Figure 5. Dermis of *C. frondosa* ((**I–III**) represent the raw, boiled, and steamed samples on the ventral side, (**IV–VI**) represent them on the dorsal side).

Table 1. Texture of *C. frondosa* after various heating treatments. Different letters (a, b, c, d, e, f, g, h) indicate significant differences between groups as determined via one-way ANOVA ($p < 0.05$).

Group Abbreviation	Group Description	Hardness/g	Adhesiveness/g.s	Springiness	Cohesiveness	Chewiness/g	Resilience
Raw	Raw	8895.19 ± 501.37 b	−331.97 ± 10.97 d	0.73 ± 0.05 d	0.33 ± 0.03 e	1679.57 ± 295.47 g	0.13 ± 0.02 g
B40 °C	Blanch 40 °C 45 min	12,532.65 ± 288.70 a	−668.44 ± 37.01 e	0.54 ± 0.05 e	0.28 ± 0.04 f	2020.45 ± 249.92 f g	0.15 ± 0.03 g
B60 °C	Blanch 60 °C 45 min	7416.39 ± 612.14 c	−143.01 ± 68.51 c	0.80 ± 0.02 c	0.67 ± 0.04 d	4453.01 ± 576.48 a	0.36 ± 0.01 f
B80 °C	Blanch 80 °C 45 min	5955.04 ± 423.38 d	−30.88 ± 7.38 a	0.86 ± 0.02 a, b	0.78 ± 0.01 b c	3995.48 ± 930.18 a, b	0.44 ± 0.01 e
B15	Boil 100 °C 15 min	6504.57 ± 405.68 d	−41.72 ± 14.55 a, b	0.84 ± 0.01 b c	0.81 ± 0.01 a, b	4176.96 ± 300.27 a	0.47 ± 0.01 d
B30	Boil 100 °C 30 min	6394.92 ± 283.05 d	−27.89 ± 10.46 a	0.89 ± 0.02 a, b	0.82 ± 0.02 a	4030.32 ± 665.58 a, b	0.50 ± 0.02 b, c
B45	Boil 100 °C 45 min	4958.39 ± 627.12 e, f	−28.77 ± 12.12 a	0.90 ± 0.03 a	0.83 ± 0.01 a	3301.81 ± 465.00 c	0.51 ± 0.01 b
B60	Boil 100 °C 60 min	4542.24 ± 426.70 f	−37.07 ± 6.38 a	0.89 ± 0.01 a, b	0.82 ± 0.01 a	3024.04 ± 608.18 c, d	0.53 ± 0.01 a, b
B120	Boil 100 °C 120 min	3693.17 ± 558.31 g	−30.23 ± 8.47 a	0.84 ± 0.06 b, c	0.81 ± 0.03 a, b	2788.71 ± 566.11 c, d, e	0.55 ± 0.01 a
S15	Steam 100 °C 15 min	5162.88 ± 199.91 e	−72.37 ± 8.58 b	0.86 ± 0.03 a, b	0.77 ± 0.02 c	3415.58 ± 242.77 b, c	0.43 ± 0.01 e
S30	Steam 100 °C 30 min	4739.95 ± 450.27 e, f	−51.52 ± 12.91 a, b	0.89 ± 0.03 a b	0.81 ± 0.03 a b	3398.32 ± 114.26 b, c	0.48 ± 0.04 c, d
S45	Steam 100 °C 45 min	3459.53 ± 430.16 g, h	−44.11 ± 5.17 a b	0.88 ± 0.06 a, b	0.81 ± 0.03 a, b	2654.81 ± 254.46 d, e, f	0.47 ± 0.03 d
S60	Steam 100 °C 60 min	3443.25 ± 215.68 g, h	−46.29 ± 11.27 a b	0.88 ± 0.04 a, b	0.81 ± 0.01 a	2514.34 ± 262.32 d, e, f	0.48 ± 0.03 c d
S120	Steam 100 °C 120 min	3090.11 ± 256.76 h	−20.27 ± 3.43 a	0.89 ± 0.02 a, b	0.82 ± 0.01 a	2260.60 ± 238.41 e, f, g	0.52 ± 0.01 b

After blanched at 40 °C, the hardness of the *C. frondosa* was increased sharply, while adhesiveness and springiness decreased significantly. It may be because of the aggregation and contraction of fibrous tissue induced by the degeneration of muscle fibrous protein, as documented by Xue [28]. When the temperature was increased to 60 °C, the hardness was reduced and the adhesiveness, springiness, and chewiness were significantly increased. It may be because the triple-helical structure of collagen and the actin deteriorated, so the body wall of *C. frondosa* was slightly loose and elastic. When the heating temperature exceeded 80 °C, the cohesiveness and resilience was improved, and the hardness and

chewiness was reduced effectively; this was in line with Gao et al. [47], who reported that the collagen gelation occurred and the solubility of collagen fibrils increased. The dissolved collagen lubricated in muscle fibrils, Furthermore, with the increase in temperature and heating duration, collagen fibrils were dissolved continuously, and myofibrils were broken, causing the texture to be further tenderized.

Hardness and springiness are the main factors of texture evaluation of the sea cucumber. Considering that the collagen gelation contributes much to palatability [48], it is wise to blanch above 80 °C. The hardness decreased dramatically from 8895 ± 501 g to 3090 ± 257~3693 ± 558 g after boiling for 120 min or steaming for 45–120 min. The changing trend was consistent with previous reports [47], in which the rupture strength of *Stichipus japonicus* decreased from 130 ± 13 N (raw material) to 17 ± 3 N after heating for 2 h. By contrast, the toughness of the *C. frondosa* body wall was relatively high, and difficult to tenderize.

3.5. Thermal Inactivation Kinetics of Autolysis Enzymes

Figure 6 depicts the variations in relative enzyme activity (REA) caused by various heating temperatures and durations. The enzymes activity was significantly affected by the temperature and time. The autolysis enzymes were relatively stable at 40 °C. Similar findings were confirmed by Zeng et al. [16], who found that the autolysis enzyme of sea cucumber was even activated at 40 °C and proposed the processing temperature should be controlled below 10 °C or higher than 60 °C for enzyme inactivation. The REA was only decreased to 79.0 ± 0.4% when blanched at 60 °C. It is not congruent with the research on Holothurians [15], of which 46% REA was denatured at 40–60 °C for 10 min and the enzyme was almost fully inactivated after heating at 60 °C for 55 min [15], and the alkaline protease could be inactivated after heating at 80 °C for 1 h [13]. Even though 58.5% of REA were inactivated in the temperature range of 60–80 °C, the heat-resistant enzymes survived up to a temperature of 100 °C, and only when the cooking time was more than 45 min by boiling or 30 min by steaming could the REA reduce to below 10%, which was reported to be safe for storage as well as avoiding overheating [49]. The autolysis enzymes of *C. frondosa* are more thermally stable than previously reported species, which means the common processing methods of sea cucumber are not applicable for *C. frondosa* from an enzyme inactivation point of view.

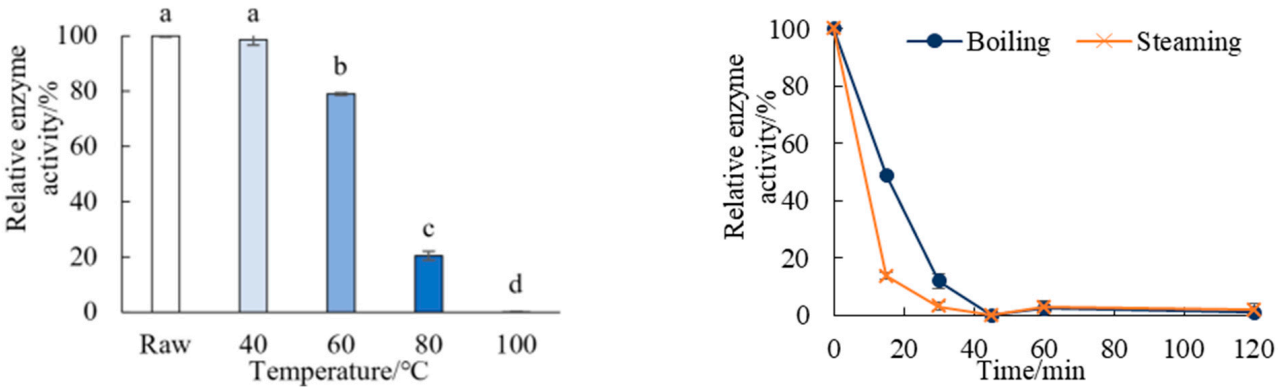


Figure 6. Relative enzyme activity of *C. frondosa* after various heat treatments, (**a**) comparison of relative enzyme activity during cooking for 45 min at different temperatures (40, 60, 80, and 100 °C), and (**b**) comparison of relative enzyme activity after boiling and steaming at 100 °C for 0–120 min. Different letters (a, b, c, d) indicate significant differences between groups as determined via one-way ANOVA ($p < 0.05$).

3.6. Multivariate Analysis

In order to further clarify the relationship between the analyzed physicochemical properties of the *C. frondosa* and to improve its processing characteristics, a principal component analysis (PCA) of all variables was performed (Figure 7). The cumulative contribution of the first two principal components accounted for 84.4%, meaning that they explained 84.4% of the variation effects between the thermal treatments.

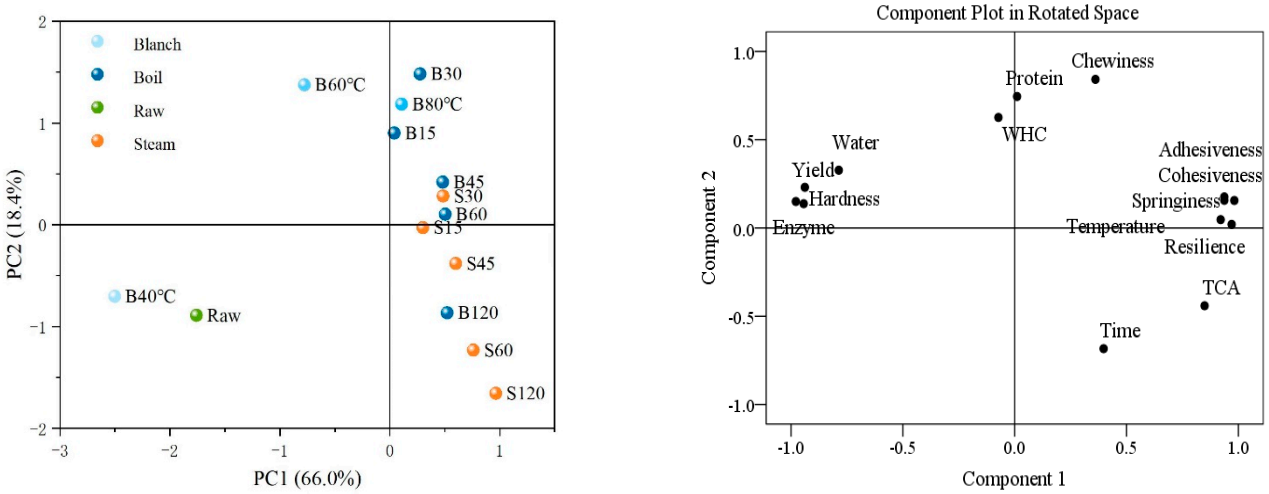


Figure 7. Principal component PC1 and PC2 scores plot (**a**) and component plot in rotated space from the principal component analysis of *C. frondosa* samples (**b**) assessing the effects of blanching, boiling, and steaming at different temperatures and cooking durations. Raw is sample without heating treatment.

The body wall of the raw *C. frondosa* had high mechanical strength (hardness). During the blanching process, 60 °C was a key temperature for the physicochemical quality of *C. frondosa*. When the blanching temperature was below 60 °C, the water content and autolysis enzymes activity were comparable to that of the raw sea cucumber. When above 60 °C, the protein denatured but crude protein content increased because of drip loss. It resulted in the increase in chewiness, and the decrease in hardness, hence improving the palatability of *C. frondosa*. However, since the enzyme was not fully inactivated, the blanched sea cucumber was not stable for long-term preservation because the enzyme not fully inactivated.

When boiled and steamed at 100 °C, the collagen fibrils of the *C. frondosa* were fractured and dissolved, resulting in the formation of TCA-soluble nitrogen along with heating. However, the texture was further improved in consideration of the increased springiness and decreased hardness with increased heating duration. The autolysis enzymes were fully inactivated at 100 °C, considering that the cooking yield was reduced during long-time cooking, 30–45 min was sufficient for enzyme deactivation and benefitted texture enhancement. Additionally, the cooking yield, protein content, and moisture content of the boiled *C. frondosa* were greater than that of the steamed samples when heated for the same duration. However, the steamed sea cucumber was superior in terms of palatability because of the lower hardness and chewiness.

4. Conclusions

The study demonstrated that the *C. frondosa*'s enzyme activity and protein denaturation were significantly affected by various heating treatments, which, in turn, influenced the cooking yield, water holding capacity, and texture quality.

The autolysis enzyme of *C. frondosa* was thermally stable, which cannot be denatured completely by blanching. High temperature may deactivate enzymes but also leads to rapid

product quality deterioration in WHC and protein content. Taking the physicochemical quality and enzyme inactivation into consideration, boiling at 100 °C for 45 min was more suitable for pre-treatment of subsequent processing (such as salting, drying, or freezing), with cooking yield of 70.3% and protein content of 78.5%. Steaming at 100 °C for at least 30 min is preferable for long-term preservation and instant food, in which the relative activity was only 3.2% with better palatability.

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