Research Article

Effect of Cryoprotectants on Quality of Desalted Jellyfish Subjected to Multiple Freeze-Thaw Cycles

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Abstract
A freeze-thaw cycle in frozen products occurs when the temperature fluctuates during storage or transportation, causing drip loss, changes in ice crystal reformation, and textural protein. In practical freezing, using cryoprotectants in frozen products aids in delaying the physicochemical changes. The problem has been found in commercial frozen jellyfish with sesame oil, causing the separating oil and water derived from drip loss of thawed jellyfish protein. This study aimed to select an appropriate cryoprotectant and concentration for frozen jellyfish products. Therefore, this research compared the changes in the physical and textural properties of desalted jellyfish collagen protein soaked in inulin, sucrose, or sorbitol at 1, 5, and 10% and subjected to three freeze-thaw cycles. Results showed increased concentration of each cryoprotectant increased soaking yield. The maximum soaking yields of desalted jellyfish were 2.49 ± 0.54, 2.79 ± 0.82, and 2.78 ± 0.51%, and each cryoprotectant content was 7.18 ± 0.01, 7.54 ± 0.00, and 8.58 ± 0.32% when using static soaked in inulin, sucrose, and sorbitol at 10%. During the freeze-thaw cycle, the retardation of the denatured jellyfish protein from ice crystals increased when desalted jellyfish were immersed in inulin, sucrose, or sorbitol at the maximum concentration of 10%, displaying the drip losses at 27.88 ± 0.45, 29.45 ± 0.35, and 28.56 ± 0.73% that lowered than the control at 56.54 ± 0.64%. The increased repeated freeze-thaw cycles increased the compact structure of thawed jellyfish collagen, supported by microstructure analysis. In summary, inulin at 10% appears to have a cryoprotective effect similar to sucrose and sorbitol and will be a choice for commercial frozen jellyfish-based food menu development.

Keywords: Cryoprotectant, Circular economy, Edible jellyfish, Freeze-thaw cycle

1 Introduction

Freezing and frozen storage are often used to prolong the shelf-life of seafood products. However, temperature fluctuation during storage and transportation, known as the freeze-thaw effect, leads to the deterioration of frozen products affecting the water-holding and physical properties of a thawed sample, resulting in free water (drip loss) and the degradation of textural properties [1], [2].

Cryoprotectants have been used in frozen seafood products to reduce the ice crystal growth rate and alter ice crystal shapes [3], [4], resulting in reduced protein denaturation during frozen storage and maintenance of functional properties such as water-holding capacity and solubility of proteins [5]. Commercially, the major use of cryoprotectants of phosphate and sorbitol are in frozen shrimp [5]–[7] and surimi products [2], [8]. The drawback of phosphate is associated with the translucent appearance and crispy texture of shrimp [6] and the slightly sweet taste of sugar alcohol applied [2]. To date, many cryoprotectants of polysaccharides (pectin, xanthan gum, carboxymethyl cellulose) [9], sugar alcohols (xylitol, sorbitol, erythritol, lactitol, mannitol, maltitol, and iso-maltitol) [2] and sugar (trehalose) [9] has been reported. Another group of cryoprotectants, including amino acids [10], proteins of soy protein concentrate and hydrolyzed fish proteins [11], and antifreeze peptides [8] have also been reported. Sucrose is a widely utilized cryoprotectant due to its low price and general commercial availability as sugar compared to all commercial cryoprotectants [12]. However, sucrose has caloric values and sweetness, increasing the sweetness in the food products, which might impact consumer acceptability. As a result, finding cryoprotectants with low sweetness and low calories has increased attention.

Inulin is one of the interesting carbohydrate moieties and prebiotic compounds in which the fructose polymer has a degree of polymerization between 4–150 [13]. Its sweetness level is approximately 10 percent sucrose [14]. Inulin is used commercially as a low-calorie sweetener, fat replacer, fiber enricher, prebiotic, and cryoprotectant. Ye *et al.* [15], reported that adding inulin to rice starch gel increased the gel's water-holding capacity, strengthening the gel network's microstructure and the decline in the gels' viscoelastic properties over seven freeze-thaw cycles. Cao *et al.* [16], investigated the effects of inulin on the silver carp surimi qualities. The promising results showed that inulin can alter water distribution, decrease freezing temperature, and prevent the formation of ice crystals. For five freeze-thaw stability tests, inulin at 8% reduced the deterioration of surimi quality on thawing water loss, superior to commercial cryoprotectant (sucrose mixed with sorbitol). These results suggest the potential use of inulin in the frozen surimi industry. Based on the research results, with the growth of diversified frozen seafood products, varying cryoprotectants for specific commercial seafood products, like jellyfish, are needed.

Jellyfish cuisine has been known in Asia thanks to its unique jelly, crunchy texture, and high protein content, especially collagen [17]–[19]. For using jellyfish as a food ingredient in the Chinese method, the preparation has specialized for cooked or uncooked jellyfish by shredding, scalding, and serving with various dressing sauces [20]. The cooking of jellyfish must be carefully elaborated for delicious cuisine. Serving jellyfish mixed with sesame oil is well-known as a fresh meal. This menu has a problem scaling up to commercial frozen jellyfish with sesame oil. Separating oil and water released from desalted jellyfish makes the product less acceptable. No information has been reported regarding freezing desalted jellyfish with any cryoprotectants and subjected to multiple freeze-thaw cycles. Therefore, the objectives of this study were to compare the changes in the physical and textural properties of desalted jellyfish collagen protein soaked in inulin, sucrose, or sorbitol and to investigate the effect of multiple freeze-thaw cycles on the quality of thawed jellyfish.

2 Materials and Methods

2.1 *Materials*

Inulin was purchased from Fuji Nihon Thai Inulin Co., Ltd., sorbitol from Union Chemical 1986 Co., Ltd., sucrose from Mitr Phol Sugar Corporation, Ltd., and salted jellyfish (Lobonema smithii) from Chock Dee Sea Products Co., Ltd., Samutsongkhram, Thailand.

2.2 *Preparation of desalted jellyfish*

As previously described, the desalted jellyfish were

washed using a washing machine, as previously described [21], [22]. Each 10 kg of the umbrella part of salted jellyfish was briefly washed with tap water at a 1:40 (w/v) ratio for two cycles. Each cycle was set for 15 min and mechanically agitated at 100 rpm. The total washing time of the salted jellyfish was 30 min.

The desalted jellyfish was cut into string-shaped pieces with a size of 0.4 × 10 cm, drained for 15 min, packed in sealed polyethylene bags, transported to our laboratory, and kept refrigerated at 10 °C until use.

2.3 *Comparative study of the static and agitated condition of desalted jellyfish in cryoprotectant solutions*

Each sample of desalted jellyfish (1 kg; approximately 91.86% moisture content) was soaked in distilled water (control), inulin, sucrose, or sorbitol, at concentrations of 1, 5, and 10%. The soaking was kept static or agitated by stirring the jellyfish sample and solution at 100 rpm for 3 h. After that, the samples were drained for 15 min and then analyzed.

2.4 *Preparation of multiple freeze-thaw cycles of soaked jellyfish*

Each sample of 1 kg of jellyfish soaked in inulin, sucrose, or sorbitol was packed in a sealed polyethylene bag and subjected to a cabinet freezer operated at –25 °C until the internal temperature was –18 °C. The frozen samples were kept at –20 °C and were then thawed until the internal temperature of the sample was 4 °C. The thawed samples were placed in a plastic basket and drained for 15 min at room temperature. The thawed samples were subjected to freezing for the second and third freeze-thaw cycles until the internal temperature reached –18 °C and kept for 3 days before thawing and refreezing, as described above.

2.5 *Analysis*

2.5.1 Soaking yield (%)

Desalted jellyfish samples (1 kg; M_1) soaked in each cryoprotectant solution described above were placed in a plastic basket, drained for 15 min, and weighed (M_2). The calculation of soaking yield (%) was performed by Equation (1), as shown below:

$$Soaking\ yield\ (\%) = \left(\frac{M_2 - M_1}{M_1}\right) \times 100 \quad (1)$$

where M_2 is the drained weight (kg) of the soaked sample, and M_1 is the initial weight of the jellyfish sample (1 kg).

2.5.2 Total soluble solids

The total soluble solids of the jellyfish sample were measured after blending 5 g of the sample with 45 mL distilled water and filtering through Whatman No. 1 paper. Soluble solids were determined using a refractometer (ATAGO, RHBS-28ATC, Japan) [21].

2.5.3 Determination of inulin, sucrose, and sorbitol content

The desalted jellyfish was soaked with inulin, sucrose, or sorbitol at 10% under static conditions and were analyzed by the Institute of Nutrition, Mahidol University, Thailand, using high-performance liquid chromatography (HPLC) (Prominence, LC-20AD, Shimadzu, Japan) according to the AOAC [23].

2.5.4 Texture analysis

The texture was measured by a Texture Analyzer (Stable microsystem, TA.XT plus, USA) equipped with an HDP/BS* blade set. Pre-test speed, test speed, and post-test speed were set at 2, 2, and 2 mm/s, respectively, with a distance of 25 mm. The cutting force (CF) of each sample was measured. The calculation is presented in Equation (2) to compare changes in the cutting force of thawed jellyfish.

$$Degree\ of\ change\ in\ CF\ (\%) = \left(\frac{CF_{FT0} - CF_{FTx}}{CF_{FT0}}\right) \times 100 \quad (2)$$

where CF_{FT0} is the cutting force value of the control or zero freeze-thaw cycle sample, and CF_{FTx} is the cutting force value of the freeze-thawing cycle number sample.

2.5.5 Measurement of thickness

The jellyfish thickness was measured at the center area with a Vernier caliper (Mitutoyo, series 530, Japan).

For each measurement, five different samples were measured for thickness. The calculation for comparing changes in the thickness of thawed jellyfish after freeze-thaw cycles is presented in Equation (3).

$$Degree\ of\ change\ in\ TN\ (\%) = \left(\frac{TN_{FT0} - TN_{FTx}}{TN_{FT0}}\right) \times 100 \quad (3)$$

where TN_{FT0} is the thickness of the control or zero freeze-thaw cycle sample, and TN_{FTx} is the thickness of the freeze-thawing cycle number sample.

2.5.6 Drip loss

Frozen jellyfish samples, each soaked with one of the three cryoprotectants (M_3), were thawed at refrigerated temperature (10 °C), drained, and weighed (M_4). Drip loss was calculated by Equation (4), shown below:

$$Drip\ loss\ (\%) = \left(\frac{M_3 - M_4}{M_3}\right) \times 100 \quad (4)$$

where M_4 is the drained weight (kg) of frozen product, and M_3 is the initial weight of desalted jellyfish after soaking in a cryoprotectant solution.

The calculation for determining changes in drip loss (DL) between freeze-thaw (FT) cycles is shown in Equation (5).

$$Degree\ of\ change\ in\ DL\ (\%) = \left(\frac{DL_{FT0} - DL_{FTx}}{DL_{FT0}}\right) \times 100 \quad (5)$$

where DL_{FT0} is the drip loss (%) of the control or zero freeze-thaw cycle sample, and DL_{FTx} is the drip loss (%) of the freeze-thawing cycle number sample.

2.5.7 Color

The color of the sample was measured using a Hunter Laboratory Color Quest (Hunter Lab, USA). The results are displayed as L* (lightness 0–100), a* (+ redness, – greenness), and b* (+ yellowness, – blueness). The total color difference (ΔE^*) was calculated by Equation (6), shown below:

$$\Delta E^* = \sqrt{(L^*_1 - L^*_0)^2 + (a^*_1 - a^*_0)^2 + (b^*_1 - b^*_0)^2} \quad (6)$$

When the desalted sample was subjected to several freeze-thaw (FT) cycles, the degree of changes in ΔE^* between freeze-thaw cycles was calculated in Equation (7)

$$Degree\ of\ change\ in\ \Delta E^*\ (\%) = \left(\frac{\Delta E^*_{FT0} - \Delta E^*_{FTx}}{\Delta E^*_{FT0}}\right) \times 100 \quad (7)$$

where ΔE^*FT0 refers to the control or zero freeze-thaw cycle, and ΔE^*FTx is the freeze-thawing cycle number.

2.5.8 Microstructure analysis

Samples of thawed jellyfish soaked in inulin, sucrose, or sorbitol at 10% under the static state were selected for scanning electron microscope (SEM) analysis. The sample preparation was slightly modified, as previously described [5]. Each jellyfish sample was cut to 1 × 1 cm with a razor blade. The prepared jellyfish samples were fixed in 2.5% glutaraldehyde in a 0.2 M phosphate buffer, pH 7.2 at 4 °C for 3 h. All specimens were washed twice with deionized water for 15 min. The samples were then dehydrated with a serial concentration of 30% to 100% ethanol for 15 min. All specimens were 100% gold (Sputterm coater SPI-Module, PA., USA). The microstructure was visualized using scanning electron microscopy (JEOL, JSM-IT500HR, Tokyo, Japan).

2.6 *Statistical analysis*

All experiments were carried out in triplicate. The measurements of color and texture were performed five times. The data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test for statistical significance (p-value < 0.05) using SPSS (SPSS 22.0 for Windows, SPSS Inc., Chicago, IL USA).

3 Results and Discussion

3.1 *Effect of concentration and type of cryoprotectants (inulin, sucrose, and sorbitol) on desalted-soaked jellyfish*

3.1.1 Soaking yield

Desalted jellyfish soaked in different concentrations and types of cryoprotectant showed different soaking yields. The soaking yield (%) of desalted jellyfish

soaked in a static condition with inulin at concentrations of 1%, 5%, and 10% were 0.11 ± 0.56, 1.79 ± 0.45, and 2.49 ± 0.54, respectively, and soaked in an agitated condition were 0.16 ± 0.43, 1.79 ± 0.60, and 2.50 ± 0.55, respectively. The soaking yield (%) of desalted jellyfish soaked in a static condition with sucrose at concentrations of 1%, 5%, and 10% were 0.21 ± 0.67, 2.20 ± 0.33, and 2.79 ± 0.82, respectively, and soaked in an agitated condition were 0.21 ± 0.50, 2.17 ± 0.68, and 2.78 ± 0.71, respectively. The soaking yield (%) of desalted jellyfish soaked in a static condition with sorbitol at concentrations of 1%, 5%, and 10% were 0.23 ± 0.11, 2.23 ± 0.94, and 2.78 ± 0.51, respectively, and soaked in an agitated condition were 0.51 ± 0.27, 2.09 ± 0.81, and 2.79 ± 0.59, respectively. Desalted jellyfish soaked in different concentrations and types of cryoprotectant showed different soaking yields. Soaking yield (%) increased with increased concentration of cryoprotectants, regardless of cryoprotectant type or shaking method. Typically, in Thailand, a mixture of salts containing sodium chloride, aluminium potassium sulfate, and sodium bicarbonate has been used to preserve jellyfish [24]. After the preservation stage, jellyfish protein, mainly collagen, changes the jelly-like texture to elastic. The replacement of salt mixtures caused denatured collagen protein to tighten their bundles and release water from their structure [17], [18], [24], [25]. Then, the irreversibly denatured jellyfish collagen changed from a transparent breakable jelly-like texture to a rigid and elastic sheet with a salty taste [24]. For cooking as food, the salted jellyfish must be washed several times to remove salts from the collagen. In washing, the water interacts with the desalted jellyfish collagen, resulting in a moist elastic appearance. In this study, soaking in water alone slightly limited the water-binding capacity of denatured collagen. Agitation was used to facilitate the penetration of each cryoprotectant into the binding collagen. The results show no differences in water binding between the static and agitated conditions.

Compared to soaking in the three cryoprotectants of inulin, sucrose, or sorbitol, the water binding to the collagen structure facilitates via hydroxy groups of inulin, sucrose, sorbitol, and amino acids groups of jellyfish collagen. Amino acids in the collagen of desalted jellyfish (Lobonema smithii) protein have negative and positive charges. The amount of negatively charged amino acids in the jellyfish protein, consisting of aspartic and glutamic acid, was 32.36 and 28.03 mg/100 g, respectively. The amount of positively charged amino acids in the jellyfish protein, consisting of lysine and arginine, was 11.53 and 17.55 mg/100 g, respectively [18]. Despite the differences in marine samples and cryoprotectants, the soaking yield results were lower than those of fresh shrimp, each soaked in amino acid solutions of glycine, proline, lysine, and arginine, which increased soaking yield from 6.55–14.37 g/100 g [10].

3.1.2 Total soluble solids

Increasing the concentration of each cryoprotectant increased total soluble solids in soaked desalted jellyfish. However, no differences in total soluble solids were obtained from static or agitation conditions at the same cryoprotectant concentration. The total soluble solids of desalted jellyfish soaked in static and agitated conditions with different types of cryoprotectant (inulin, sucrose, and sorbitol) at concentrations of 1%, 5%, and 10% were 0.80 ± 0.01°Brix, 4.26 ± 0.11°Brix, and 8.46 ± 0.11°Brix, respectively. Although inulin, sucrose, and sorbitol are water-binding, they bind mainly to the collagen surface in an aqueous solution [26]. The penetration of inulin, sucrose, or sorbitol and the binding to the collagen structure might be minimal but remain on the surface of desalted jellyfish instead. The remaining cryoprotectants in the samples soaked with a maximum concentration (10%) of each cryoprotectant were subjected to HPLC analysis.

Results showed that inulin, sucrose, and sorbitol concentrations in soaked desalted jellyfish were 7.18 ± 0.01%, 7.54 ± 0.00%, and 8.58 ± 0.32%. The high sorbitol concentration in soaked desalted jellyfish might cause strong binding and stability of complex collagen with sugars containing fewer carbon atoms. The binding stability in the collagen helix decreases as the presence of carbon atoms increases [27]. Inulin, sucrose, and sorbitol quantities differed slightly from the total soluble solid results. At the same concentration level, the inulin content in the jellyfish appeared lowest compared to sucrose and sorbitol, possibly due to the different migration of cryoprotectants based on weight, viscosity, and charge [27]. Sorbitol has the lowest molecular weight (MW of 182) compared to sucrose (MW of 342), and inulin, in this study, has a degree of polymerization (DP) of 14 and MW of

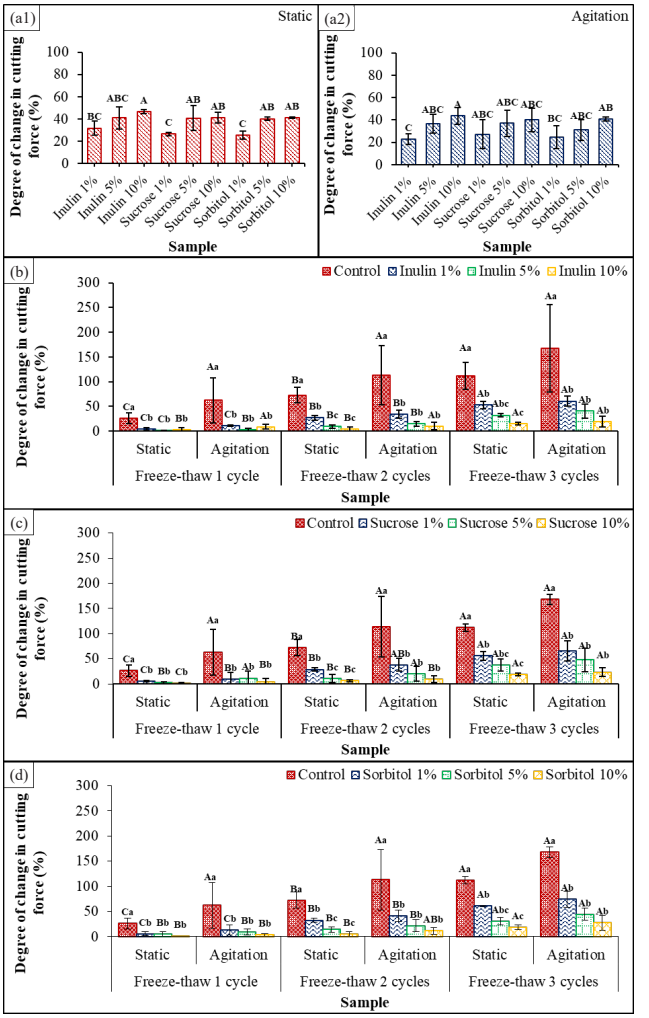

Figure 1: Changes in the cutting force of soaked jellyfish protein in different cryoprotectant conditions (a) Before freezing (b)–(d) At 1–3 Freeze-thaw cycles. Different superscripts (a)–(c) in the same condition of cryoprotectant mean significant differences (p-value < 0.5). Different superscripts (a)–(d) mean significant differences compared to the same freeze-thaw cycle and the same type of cryoprotectant (p-value < 0.5).

2,448. Moreover, 10% sorbitol has the lowest viscosity (175 cP) compared to 10% sucrose (190 cP) and 10% inulin (250 cP). As a result, inulin exhibits the lowest water migration.

3.2 *Comparative effect of inulin, sucrose, and sorbitol on desalted jellyfish subjected to three freeze-thaw cycles*

3.2.1 Cutting force

The toughness texture of desalted jellyfish measured by the texture analyzer's cutting force showed changes in the jellyfish soaked in each of the three cryoprotectant solutions (Figure 1). When calculating the change (%) compared to the control (a sample soaked with distilled water), the concentration of each cryoprotectant increased toughness or cutting force. Interestingly, the samples had lower cutting force values when the desalted sample was agitated in any of the cryoprotectant solutions. The agitation condition might accelerate the penetration of each cryoprotectant into the desalted jellyfish with a force, thereby causing slight damage

to the jellyfish surface and lowering the cutting force values. In most cases, desalted jellyfish soaked in 5% of each cryoprotectant yielded similar cutting force results compared to samples soaked in 10% cryoprotectant.

When the number of freeze-thaw cycles increased, the thawed jellyfish protein became less elastic but tighter and displayed a hardened texture, thus increasing cutting force values. The frozen-thawed jellyfish collagen tightens its structure and limits water absorption. When the freezing cycle was increased, the thawed jellyfish protein became less elastic but tighter and displayed a hardened texture, thus increasing cutting force values [Figure 1(b)–(d)]. When comparing the change in cutting force from zero to three freeze-thaw cycles, results showed that changes in cutting force increased. These results agree with a previous report of white shrimp treated with salt, STPP, and amino acids in which the freezing and thawing resulted in increased denatured white shrimp and cutting force [10]. Increasing the freeze-thaw cycle also increased the cutting force in tiger shrimp (*Penaeus monodon*) with no use of cryoprotectant [28]. However, in this study, the factors of soaking condition (static or agitation state) or type of cryoprotectant did not affect cutting force values at the same number of freeze-thaw cycles. Increased inulin, sucrose, or sorbitol concentrations to 10% significantly reduced thawed jellyfish protein's toughness for all freeze-thaw cycles. As a result, the cryoprotectant effectiveness of inulin compared to sucrose and sorbitol is similar. However, inulin has less sweetness than sucrose at approximately 10% [14], and having prebiotic activity appears to be a potential cryoprotectant for freezing jellyfish.

3.2.2 Thickness

In general, the texture of desalted jellyfish is dense and elastic-like, with unique crunchiness. Sample thickness increased when desalted jellyfish were soaked with increased inulin, sucrose, or sorbitol (Figure 2). The control was 0.15 ± 0.00 cm. The thickness slightly increased as the concentration increased in samples soaked in each cryoprotectant. The static and agitation conditions did not significantly differ in thickness. However, when calculating the degree of changes in thickness, results show the changes in the thickness of desalted jellyfish soaked in inulin at 1, 5, and 10% increased by 10.71, 26.33, and 36.83% under the static condition and 17.66, 27.16, and 20.83% under agitation. The thickness of soaked jellyfish in sucrose and sorbitol increased as the sucrose and sorbitol concentrations increased. With the maximum increase in thickness, each cryoprotectant's concentration at 10% was chosen for the freeze-thaw experiment. The jellyfish soaked in an increased concentration of each solution from 1, 5–10% retarded the decrease of jellyfish thickness in each freeze-thaw cycle. However, the static and agitation conditions did not impact the jellyfish protein's thickness. The increased number of freeze-thaw cycles of soaked jellyfish reduced the degree of change in the thickness of the jellyfish sample [Figure 2(b)–(d)]. The reduced thickness of thawed jellyfish could be due to the denatured protein structure with ice crystals in repeated freezing and thawing.

3.2.3 Drip loss

The use of cryoprotectants in frozen desalted jellyfish reduces drip loss during thawing. Results showed the drip losses of the control (without using any cryoprotectant) soaked in static condition were 26.18 ± 0.84, 33.22 ± 0.53, 50.35 ± 0.16, and 56.54 ± 0.64%. The drip losses of control soaked in agitated condition were similar, showing at 25.88 ± 0.49, 33.00 ± 0.26, 50.13 ± 0.56, and 56.31 ± 0.52% at the freeze-thaw cycles of 0, 1, 2, and 3. The increased concentration of each solution, from 1, 5, and 10%, decreased drip loss with no significant differences among the three cryoprotectants when increased multiple freeze-thaw cycles. Results showed at the static condition of 10% of inulin, the drip losses were at 3.15 ± 0.28, 6.95 ± 0.58, 16.45 ± 0.10, and 27.88 ± 0.45%, while at the agitated condition, the losses were 2.58 ± 0.18, 6.34 ± 0.45, 15.87 ± 0.48, and 27.35 ± 0.68% when the increased cycles from 0, 1, 2, and 3. Similar results were obtained when using 10% of sucrose, displaying at the static condition losses of 2.98 ± 0.41, 7.93 ± 0.27, 17.37 ± 0.19, and 29.45 ± 0.35%. In the samples soaked at the agitated condition, the losses were 2.42 ± 0.68, 6.50 ± 0.78, 15.84 ± 0.55, and 27.47 ± 0.57%. For static soaking 10% sorbitol, the drip losses were 2.98 ± 0.41, 7.93 ± 0.27, 17.37 ± 0.19, and 29.45 ± 0.35%. The losses of samples were 2.42 ± 0.68, 6.50 ± 0.78, 15.84 ± 0.55, and 27.47 ± 0.57% when applied under the agitated condition. It is noted that the degree of change in drip loss increased when

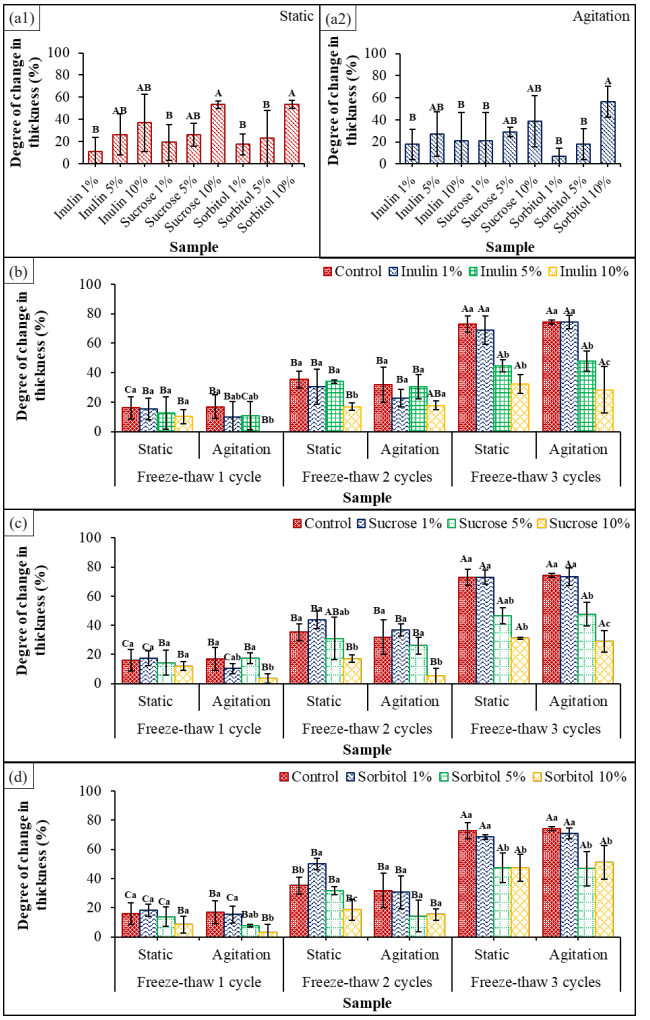

Figure 2: Changes in thickness of soaked jellyfish protein in different cryoprotectant conditions (a) Before freezing (b)–(d) At 1–3 Freeze-thaw cycles. Different superscripts (a)–(c) in the same condition of cryoprotectant mean significant differences (p-value < 0.5). Different superscripts (a)–(d) mean significant differences compared to the same freeze-thawed cycle and the same type of cryoprotectant (p-value < 0.5).

samples were subjected to several freeze-thaw cycles (Figure 3). In this study, factors of cryoprotectant type or soaking state (static and agitation) did not affect drip loss. Increasing the number of freeze-thaw cycles of soaked desalted jellyfish increased drip loss percentage despite using cryoprotectants due to repeated thawing and ice crystals reforming in multiple freeze-thaw cycles. The reforming ice crystals might interrupt the collagen structure and increase the thawing loss [28], [29]. However, inulin, sucrose, or sorbitol concentration at 10% reduced the drip loss more than other concentrations (1% and 5%) for all freeze-thaw cycles.

The results of this study were consistent with the study of frozen white shrimp, which showed that sorbitol reduces drip loss of frozen white shrimp [2]. Increased concentrations of sucrose and sorbitol (1:1) at 0, 2, 4, and 8% reduced drip loss in surimi products [30]. The mechanism of cryoprotectant compounds in this study, which prevents jellyfish collagen denaturation, could be due to the retardation of the growth rate of ice crystals and changes in the ice crystal shape during the freeze-thaw cycles [4]. Thus, the absorbed solubilized

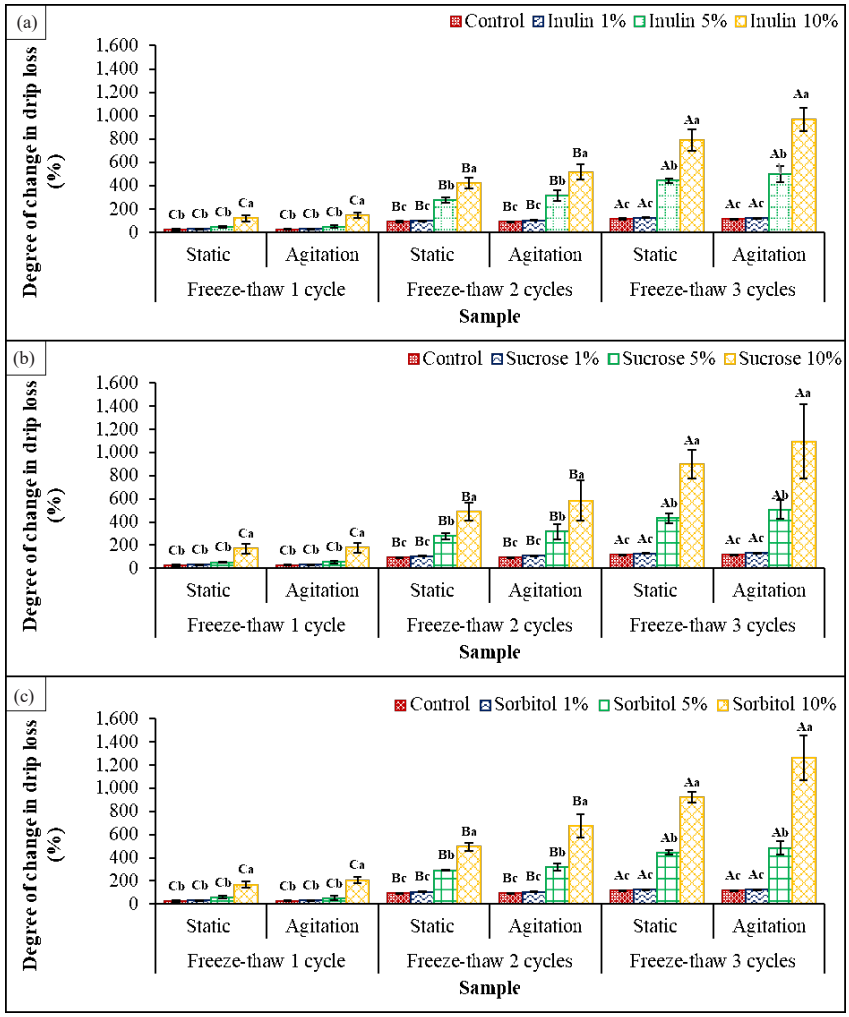

Figure 3: Changes in drip loss of frozen-thawed desalted jellyfish in various cryoprotectant solutions (a)–(c) At 1–3 Freeze-thaw cycles. Different superscripts (a)–(c) in the same condition of cryoprotectant mean significant differences (p-value < 0.5). Different superscripts (a)–(d) mean significant differences compared to the same freeze-thawed cycle and the same type of cryoprotectant (p-value < 0.5).

cryoprotectant compounds in the sample benefit from reduced drip loss in frozen samples during the freeze-thaw cycle.

3.2.4 Color

The color of desalted jellyfish is affected by factors associated with static and agitation conditions, type of cryoprotectant, and concentration (Figure 4). The color of desalted jellyfish soaked in cryoprotectants appeared light creamy and differed from the color of the control desalted sample, which showed light pale color. After soaking in inulin at all concentrations, the samples' total color difference (ΔE^*) was higher in ΔE^* than with other cryoprotectants. Agitation reduced the values of ΔE^* of jellyfish samples soaked in sorbitol (1, 5, and 10%) but increased the values of ΔE^* samples soaked in inulin (1, 5, and 10%). When calculating the degree of changes in ΔE^*, the dominant changes were found in the sample soaked in inulin under static conditions. The color of thawed desalted jellyfish changed after the freeze-thaw cycles.

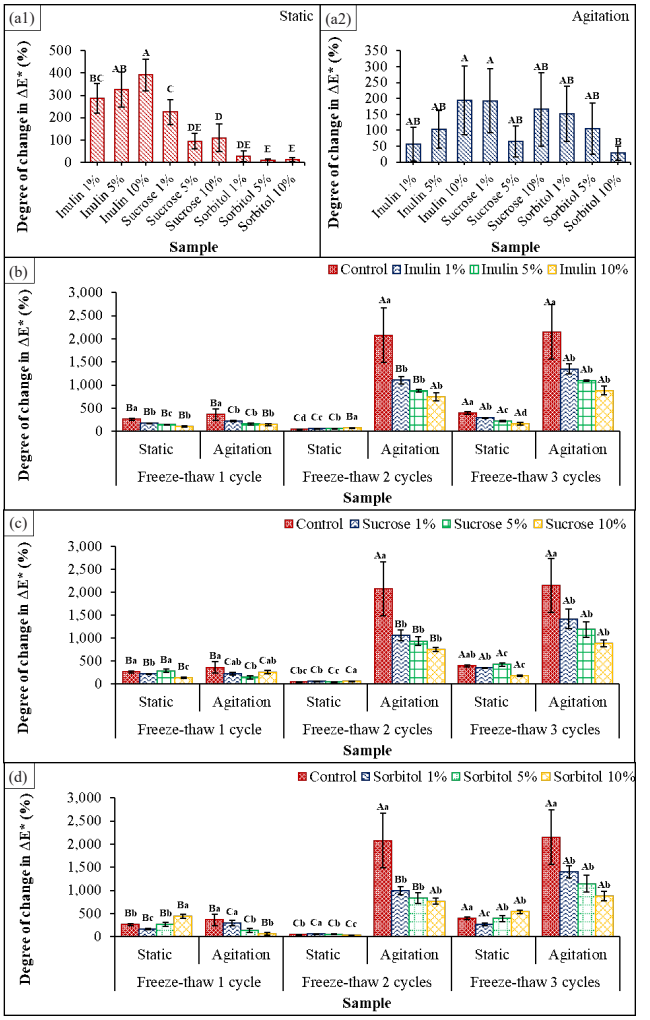

Figure 4: Changes in total color differences of thawed jellyfish (a) Before freezing; (b)–(d) At 1–3 Freeze-thaw cycles. Different superscripts (a)–(c) in the same condition of cryoprotectant mean significant differences (p-value < 0.5). Different superscripts (a)–(d) mean significant differences compared to the same freeze-thawed cycle and the same type of cryoprotectant (p-value < 0.5).

When the number of freezing cycles was increased, the degree of changes in values of ΔE^* of samples after each freeze-thaw cycle increased. At the same number of freeze-thaw cycles, factors of soaking condition (static or agitation state) did not affect the total color differences of thawed desalted jellyfish. Increased inulin, sucrose, and sorbitol resulted in significant changes in ΔE^* of samples, mostly when the concentration of these cryoprotectants was at 10%. The desalted jellyfish soaked with cryoprotectant and subjected to multiple freeze-thaw cycles could cause instability of water molecules within the jellyfish collagen structure and retarded collagen fiber contraction. The contraction of collagen protein caused the lightness of the jellyfish to increase, and ΔE^* of samples after the freeze-thaw cycles increased.

3.2.5 Microstructure

The microstructure of soaked jellyfish collagen in each cryoprotectant solution observed by SEM is displayed in Figure 5(a)–(d). The control sample (desalted

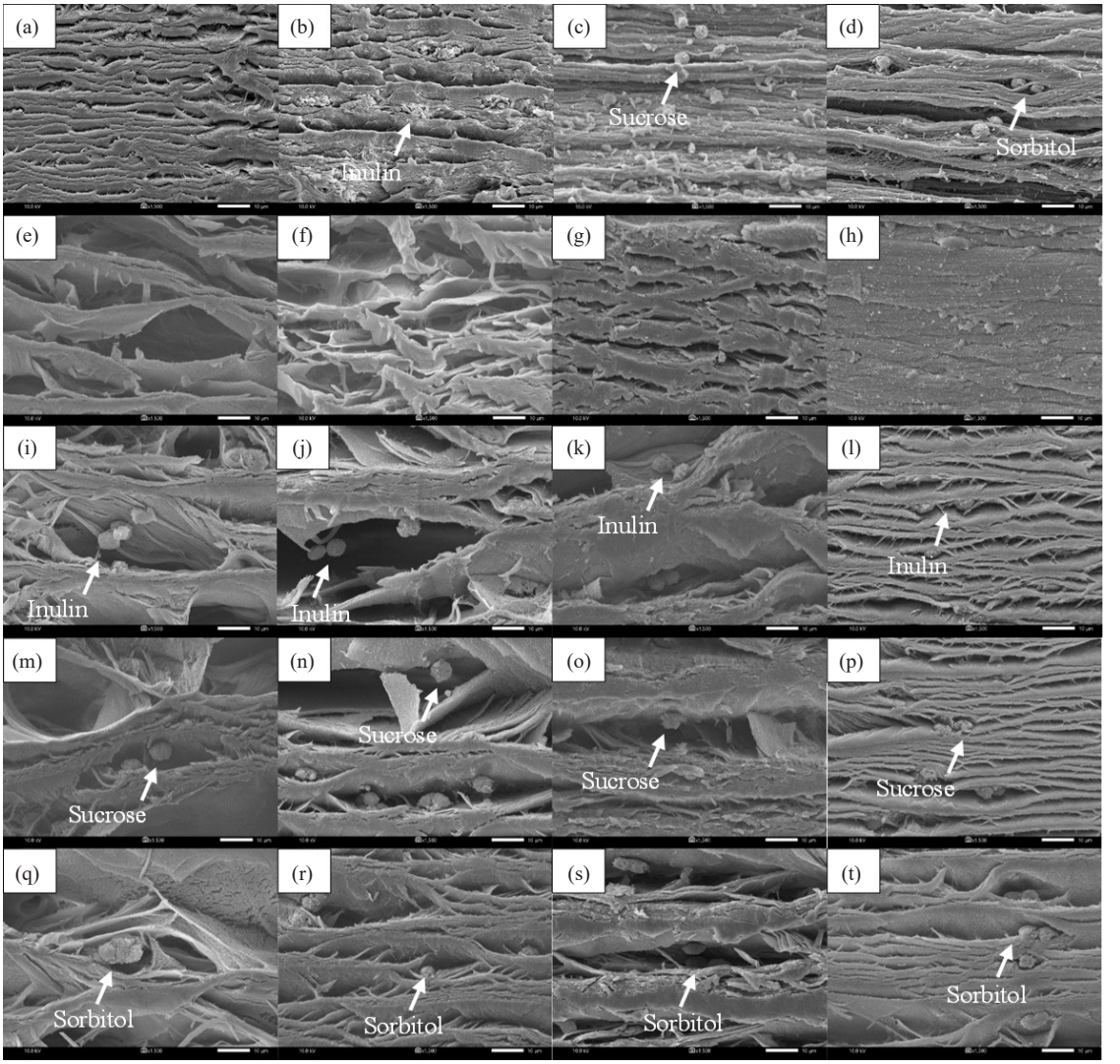

Figure 5: Cross-section of desalted jellyfish soaked in various cryoprotectant solutions before and after thawing, magnified at 1,500 times Before freezing (a) Control (b) Inulin 10% (c) Sucrose 10% (d) Sorbitol 10% Freeze-thaw cycle (e) Control 0 cycle (f) Control 1 cycle (g) Control 2 cycles (h) Control 3 cycles (i) 10% Inulin 0 cycle (j) 10% Inulin 1 cycle (k) 10% Inulin 2 cycles (l) 10% Inulin 3 cycles (m) 10% Sucrose 0 cycle (n) 10% Sucrose 1 cycle (o) 10% Sucrose 2 cycles (p) 10% Sucrose 3 cycles (q)10% Sorbitol 0 cycle (r) 10% Sorbitol 1 cycle (s) 10% Sorbitol 2 cycles and (t) 10% Sorbitol 3 cycles.

jellyfish in water) exhibited intensely packed protein bundles due to the previous effect of salt preservation, causing dehydration and denaturation of the jellyfish collagen protein. Thus, the denatured protein absorbed water slightly in small amounts. When the denatured jellyfish protein was in the presence of inulin, sucrose, or sorbitol solutions, these cryoprotectant compounds penetrated and attached themselves to the collagen fibril structure [Figure 5(b)–(d)]. After soaking the cryoprotectant and then freezing, the accumulation of crystal solutes, including inulin, sucrose, or sorbitol, are effective stabilizers of jellyfish collagen protein by displaying between collagen protein bundles. The structure differences between inulin and sucrose are

that the fructose polymers are joined by a β (2→1) glycosidic bond, sucrose consisting of glucose and fructose connected via α-1, β-2 glycosidic linkage is a nonreducing sugar, and sorbitol is a sugar alcohol. The presence of cryoprotectant might shorten the protein structure via H-bonding [27], [31], [32]. After soaking, cryoprotectants remained in the desalted jellyfish due to the interaction of both negatively and positively charged amino acids in protein structure and hydroxyl groups of cryoprotectant and water [27], [31].

When subjected to freeze-thaw cycles, changes in microscopic jellyfish protein structures of soaked desalted jellyfish occurred and are displayed in Figure 5(e)–(t). Increasing freeze-thaw cycles affected the denatured jellyfish collagen fiber structure, resulting in closer adhesion. When the samples were thawed, the jellyfish collagen protein's water affected the protein's conformation and aggregation. The desalted jellyfish soaked in each cryoprotectant and freeze-thawed for three cycles revealed inulin, sucrose, or sorbitol inserted into the jellyfish collagen structure. Cryoprotectants strengthen the bridge interaction between polypeptide chains to stabilize the triple helix by replacing the water molecules. With many carbon atoms, inulin could reduce instability in the collagen complex. Therefore, using inulin on frozen-thawed jellyfish resulted in minimal collagen structure changes.

4 Conclusions

The effect of multiple freeze-thaw cycles on drip loss and physicochemical changes of desalted jellyfish was minimized when using cryoprotectants of sucrose, sorbitol, or inulin at all concentrations. The optimal use of cryoprotectant is 10%. Soaking desalted jellyfish in either static or agitated conditions of cryoprotectants found similar results in most parameters such as soaking yield, thickness, cutting force, and drip loss. For repeated freeze-thaw cycles, the physical changes in the thawed samples showed pale but hardened and shrunk texture with increased cutting force and ΔE^*. The microscopic results displayed the reduced thickness and tightened jellyfish collagen bundle with each cryoprotectant penetrated and attached to the protein structure. These results show that using inulin with additional prebiotic benefits has a cryoprotective effect similar to sucrose and sorbitol, which could be a choice for future frozen jellyfish-based food menu development.

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Author Contributions

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Conflicts of Interest

The authors declare no conflict of interest.

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