Concentration of Aqueous Residue from Isada Krill Using
Freezing–Thawing Technique

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The concentrated aqueous residues from the recovery process of useful oil–soluble components from Isada krill were examined by freezing–thawing technique. The aqueous residue was completely frozen into spheres or cuboids of different sizes at −20°C or −80°C, and then thawed at room temperature (26 ± 2°C). The concentration index was defined as the ratio of the Brix concentration of the melted solution to that of the original solution. The changes in the cumulative fraction and concentration index of melted solution during the thawing process were measured. The freezing temperature had no significant effect on the thawing process. For all the frozen samples, the relationship between the cumulative fraction and concentration index of the melted solution was roughly represented by a single straight line, and the concentration index of the solution obtained by thawing half of the frozen sample was about 1.4. It was shown that the original solution could be concentrated twice when the freezing–thawing operation was repeated twice. However, further repeated operation had no effect on concentration.

Keywords: freezing–thawing, freeze concentration, Isada krill, aqueous residue

1. Introduction

Kril (Euphausia pacifica) inhabits the North Pacific Ocean and is called Isada in the Sanriku region of Japan. It is a marine zooplankton with the highest amount of resources after tococypods (Copepod) [1, 2]. Due to their high resource amount, krill is expected to be used as a food source [1]. In addition, it contains many functional ingredients such as eicosapentanoic acid (EPA), docosahexanoic acid (DHA), and astaxanthin [3]. However, the quality of the krill deteriorates within 1 to 2 h of fishing due to self-digestion and color change mediated by endogenous proteases and tyrosinases, respectively [1], and its use for food gets limited [4]. As described above, Isada contains EPA, DHA, and 8-hydroxyeicosapentanoic acid (8-HEPE), which is expected to have a stronger effect for decreasing plasma and hepatic triglycerides than EPA and DHA [5]. The process of extracting these functional lipids from Isada generates a large amount of aqueous residue. This aqueous residue cannot be used because of its fishy smell. In addition, the treatment is costly, thereby necessitating the need for the effective use of the aqueous residue.

Subcritical water refers to water in a liquid state under pressurized conditions between 100°C and 374°C. Because the subcritical water has a lower dielectric constant and higher ionic product than ambient water, its use as a reaction solvent and extractant has attracted attention [6, 7]. We have previously demonstrated that treatment of residual waste solution from Isada processing with subcritical water at 160–180°C reduced fishy odor and enhanced its shrimp–like flavor [8]. This method is believed to be applicable to the production of shrimp–like seasonings from the aqueous residue [8]. Considering the energy consumption, it is desirable to increase the solute concentration and reduce the volume of the solution prior to treatment under subcritical water conditions.

Methods for concentrating liquid solution are classified into three categories: evaporation, reverse osmosis, and freeze concentration [9]. Evaporation is a simple method that removes water by heating, but it may cause quality degradation [10]. Reverse osmosis is a concentration method using the difference in solute size. Although the method can be operated without heating, there are concerns about microbial contamination and difficulty in maintaining membrane performance [10]. Freeze concentration causes the least quality deterioration among the three methods [9], and it can suppress microbial
spoilage, degradation, or deterioration by endogenous enzymes, and release of flavor. Freeze-concentration includes the following four methods: freezing-thawing, progressive freeze-concentration, non-equilibrium crystallization, and equilibrium suspension crystallization [9]. Among these methods, freezing-thawing is the simplest one [9]. In the freezing-thawing method, the liquid sample was completely frozen and then gradually thawed [12]. A solution with a higher solute concentration than the original solution is obtained in the early stages of melting [9]. The application of this method to the concentration of low-value-added solutions such as food waste has been studied in past [13].

In this study, the freezing-thawing method was applied during the concentration of aqueous residue after recovering useful oil-soluble components from Isada krill. Furthermore, the effects of freezing temperature and shape of the frozen sample on the melting behavior and the relationship between the cumulative fraction and concentration index of melted solution were examined. The concentration index was defined as the ratio of the Brix concentration of the melted solution to that of the original solution.

2. Materials and Methods

2.1 Materials

After recovering useful oil-soluble components, such as EPA and 8-HEPE, the aqueous residue from Isada krill was supplied by Kokuyo (Ofunato, Japan). The aqueous residue was centrifuged (II-3Gα, Kokusan, Saitama) at 1500×g for 15 min to remove insoluble matter and the supernatant was used for performing freezing-thawing experiments.

2.2 Thawing of frozen sample

The aqueous residue was placed in spherical plastic balloons of different sizes (large: about 6.8 cm in diameter (TK-1329, Matsuno Industry, Osaka); medium: about 5.2 cm in diameter (Eikoh, Tokyo); and small: about 4.0 cm in diameter (Echo Kinzoku, Niigata), and completely frozen in VT-208HC and GX-823HC freezers (Nihon Freezer, Tokyo) at −80°C and −20°C, respectively. Aqueous residue was also placed in beverage paper containers (small: 2.7×4.2×4.9 cm; medium: 4.6×4.7×4.9 cm; large: 5.9×6.0×6.0 cm) and frozen completely at −80°C and −20°C to prepare cuboidal frozen samples.

After the aqueous residue samples were completely frozen, the spherical or cuboidal frozen samples obtained by cutting the balloons or containers were thawed at 26±2°C in an air-conditioned room or a stainless steel desiccator (SCD-1L, As One, Osaka), with a temperature controller and heating device. Although it has been reported that the concentration index is higher when a frozen sample is slowly melted at a lower temperature [14], it was thawed at room temperature in this experiment because of lesser productivity at low temperature owing to the longer melting time. The size of the frozen samples was measured with a digital caliper (Shinwa Sokutei, Niigata); the diameter of spherical samples and length, width, and height of cuboidal ones were measured. The thawing process of the frozen sample was measured using a method similar to the one described by Yee et al. [12]. The frozen sample was placed on a plastic funnel with a mesh (diameter 10 cm), and the melted drips were fractionated with approximately 2 g, 4 g, and 8 g each for small, medium, and large samples, respectively, in a 15 mL conical tube (Schaffhausen, Switzerland). The sampling time was recorded and the exact weight of the melted solution was measured with a balance (AG204, Shimadzu, Kyoto). The Brix concentration, salinity, and pH of the fractionated solution were measured. This operation was repeated until all of the frozen samples were melted.

2.3 Repeated freezing-thawing treatment

Approximately 500 mL of the aqueous residue was placed in a beverage PET bottle and frozen at −20°C. The bottle was then inverted and the frozen sample was thawed at room temperature. About half of the frozen sample was thawed and the Brix concentration, salinity, and pH of the melted solution were measured. The melted solution was frozen again at −20°C and about half of the frozen sample was thawed at room temperature. The Brix concentration, salinity, and pH of the melted solution were measured for the second time. This process was repeated 4 times.

2.4 Analysis

The Brix concentration, salinity, and pH of the melted solution were measured using the PAL-S pocket refractometer (Atago, Tokyo), PAL-ES1 pocket salinometer (Atago), and compact pH meter (B-71X, Horiba, Kyoto), respectively.
3. Results and Discussion

3.1 Thawing of spherical and cuboidal frozen samples

Figures 1 (a) and (b) show the changes in the cumulative fraction and concentration index of the melted solution in the thawing processes of large, medium, and small spherical samples frozen at −80°C and −20°C, respectively. The smaller the size of the frozen sample, the faster was the rate of its thawing. Samples frozen at −20°C began to thaw slightly earlier than those frozen at −80°C. Regardless of the size of the frozen sample, the Brix concentration of the melted solution was highest immediately following the start of thawing, and it gradually decreased as thawing progressed. These results show that the aqueous residue can be concentrated by the freezing–thawing method.

Figures 2 (a) and (b) show the changes in the cumulative fraction and concentration index of the melted solution and (closed symbols) concentration index of melted solution for cuboidal samples with different diameters, which were frozen at (a) −80°C and (b) −20°C. Diameters of (□, ■) small, (○, ◆) medium, and (◇, ●) large samples frozen at −80°C were 2.6×2.7×2.8 cm, 4.7×4.7×5.0 cm, and 5.8×6.0×6.0 cm, respectively, and those of the samples frozen at −20°C were 2.6×3.2×4.7 cm, 4.3×4.8×4.8 cm, and 5.1×6.0×6.0 cm. The solid curves were calculated by substituting the estimated μ and σ values into Eq. (1).

![Fig. 1 Changes in (open symbols) cumulative fraction of melted solution and (closed symbols) concentration index of melted solution for spherical samples with different diameters, which were frozen at (a) −80°C and (b) −20°C. Diameters of (□, ■) small, (○, ◆) medium, and (◇, ●) large frozen samples were 3.9, 5.4, and 6.9 cm, respectively, for both the freezing temperatures of −80°C and −20°C. The solid curves were calculated by substituting the estimated μ and σ values into Eq. (1).](image1)

![Fig. 2 Changes in (open symbols) cumulative fraction of melted solution and (closed symbols) concentration index of melted solution for cuboidal samples with different diameters, which were frozen at (a) −80°C and (b) −20°C. Diameters of (□, ■) small, (○, ◆) medium, and (◇, ●) large samples frozen at −80°C were 2.6×2.7×2.8 cm, 4.7×4.7×5.0 cm, and 5.8×6.0×6.0 cm, respectively, and those of the samples frozen at −20°C were 2.6×3.2×4.7 cm, 4.3×4.8×4.8 cm, and 5.1×6.0×6.0 cm. The solid curves were calculated by substituting the estimated μ and σ values into Eq. (1).](image2)

3.2 Melting curves

All the melting curves, which depict the changes in the cumulative fraction of melted solution, were sigmoidal. Therefore, the curves were empirically expressed by the error function (Eq. (1)).

\[
\text{Cumulative fraction of melted solution} = \frac{1}{2} \left[ 1 + \text{erf} \left( \frac{t - \mu}{\sqrt{2}\sigma} \right) \right]
\]

where \( \mu \) is the time for the fraction to reach 0.5, and \( \sigma \) is a parameter reflecting the spread of the melting curve.

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i.e., the melting velocity. Using Microsoft Excel® Solver, the values of $\mu$ and $\sigma$ were determined so that the residual sum of squares between the measured value and the value calculated by Eq. (1) was minimized. The solid curves in Figs. 1 and 2 were calculated by substituting the estimated $\mu$ and $\sigma$ values into Eq. (1).

In the thawing process, the heat supplied from the surrounding raises the sample temperature and is used as latent heat of fusion. Therefore, the parameters involved in the thawing process seem to depend on the volume-based specific surface area of the samples. Figure 3 shows the dependence of the $\mu$ and $\sigma$ values on the initial specific surface area of the frozen samples in units of cm$^2$/cm$^3$. The results for cuboids and spheres are not represented by a single line, but the effects of freezing temperature ($-80^\circ$C and $-20^\circ$C) on the parameters were small, and the spheres and cuboids are located on almost one straight line. The plots for spherical and cuboidal samples were found to lie on separate straight lines.

3.3 Relationship between concentration index and cumulative fraction of melted solution

Figure 4 shows the relationship between concentration index and cumulative fraction of the melted solution for all the results shown in Figs. 1 and 2. Most plots are located on a straight line with a slope of $-2$, which passes through the points (0, 2) and (1, 0). Therefore, regardless of the freezing temperature and shape of the frozen samples, a solution concentrated 1.4 times can be obtained by gathering the melted solution until half of the frozen sample is thawed. Yee et al. [12] also reported that the freezing temperature did not affect the concentration index. In addition, this relationship is not always represented by such a straight line [12,13].

3.4 Repeated freezing–thawing treatment

As mentioned in the previous section, when half of the frozen sample is thawed, a solution concentrated about 1.4 times can be attained. Subsequently, this solution obtained by thawing half of the frozen sample was frozen again and then the frozen sample was thawed half. This operation was repeated four times, and the values for the Brix concentration, salinity, and pH of the melted solution in each of the operations were measured (Fig. 5). After the first freezing–thawing operation, both the Brix concentration and salinity of the melted solution increased by 1.4 times. Furthermore, the Brix concentration and salinity of the melted solution further increased by about 1.4 times after the second freezing–thawing operation, which was about twice than those of the original solution. However, the Brix concentration and salinity of the melted solution hardly increased in the third and subsequent operations. For the concentration of sodium chloride and saccharides by the freezing–thawing technique in a single component system, Yee et al. [12]
reported that the concentration of the melted solution decreased when the concentration of the original solution was high, and that the thawing behavior depended on the molecular mass of the solute. However, in our experiments, the Brix concentration and salinity showed a similar behavior. Therefore, it can be suggested that there is no significant difference in the molecular mass between the substances measured with the refractometer and salinometer. The pH of the melted solution scarcely changed in the repeated freezing–thawing processes.

When the freezing–thawing treatment is repeated twice, a solution that is twice as concentrated can be obtained. From mass balance, the melted solution in the latter half of the second operation should have nearly the same concentration as the original solution. Therefore, the melted solution can be reused by mixing with the original solution and freeze–thawed. Reusing the melted solution can reduce the amount of aqueous solution to be discarded.

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イサダからの水溶性残渣液の凍結融解法による濃縮

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イサダ（学名ツノナシオキアミ、別名アミエビ）は2月～4月頃に三陸沿岸で漁獲される小型のアミ類で資源量は豊富である。しかし、内在性酵素により漁獲後短時間で品質が低下するため、食品への利用は限定的で、大半は釣りの撒き餌や養殖魚の餌として利用されている。一方、イサダはエイコサペンタエン酸やドコサヘキサエン酸などの機能性脂質に加え、脂肪燃焼効果をもつ8-ヒドロキシエイコサペンタエン酸を多く含有する[5]ため、その脂溶性成分はサプリメントなどへの利用が期待されている。イサダから脂溶性の有効成分を回収する工程では大量の水溶性残渣液が排出されるが、現状では用途がなく、廃棄されている。

常圧での沸点である100℃から臨界温度の374℃の範囲で液体を保った水を亜臨界水という。著者らは、イサダを亜臨界水の条件下で処理すると生臭さが大きく低減し、エビ風味を発現することを見出した。また、イサダの煮汁についても同様の結果を得ている[8]。

そこで、イサダから脂溶性の有効成分を回収する際に出される水溶性残渣液を亜臨界水条件下で処理し、エビ風味の調味液または調味粉末を製造する研究を進めている。しかし、水溶性残渣液の固形物濃度は、イサダの漁獲期や脂溶性成分の回収方法などにより、Brix値で5～15%と大きく変動する。また、亜臨界水条件下での処理を効率的に行うには、固形物濃度を高める必要がある。

そこで、濃縮過程での品質低下の少ない凍結濃縮法の中でも、特に装置が必要なく、また操作も簡単な凍結融解法による水溶性残渣液の濃縮について検討した。

水溶性残渣液を−20℃または−80℃で大きさの異なる球形または直方体状に完全に凍結したのち、室温（26±2℃）で融解し、融解過程における融解率と融解液の固形分濃度の変化を測定した。凍結融解法による水溶性残渣液の濃縮について検討した。

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