Preparation of Seasoning with Shrimp–like Flavor from the Aqueous Residue of Isada Krill under Subcritical Water Conditions

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Conditions were examined to prepare a seasoning with shrimp–like flavor from the aqueous residues formed after the recovery of oil-soluble functional components in Isada krill caught from the Sanriku coast in 2017 and 2018 upon treatment under subcritical water conditions. Treatment at ≥160°C increased the amount of insoluble material and decreased the concentration of soluble matter. The color of the aqueous residue depended on the properties of the treated residue obtained from the 2017 and 2018 catches showed a decrease in the fishy odor and an increase in fragrant and burnt aromas. The preference score obtained for the residue obtained from the 2017 catch increased after the treatment process. On the other hand, the aqueous residue obtained from the 2018 catch, which did not show any improvement in the preference score upon treatment, was improved when diluted 2 or 4 times after the treatment process. GC–MS analysis revealed that pyridines and pyrazines related to shrimp–like flavor were produced in both aqueous residues obtained from the 2017 and 2018 catches upon treatment under subcritical water conditions.

Keywords: Isada krill, seasoning, sensory evaluation, subcritical water

1. Introduction

Isada krill (Euphausia pacifica) is a small euphausid caught on the Sanriku coast around February to April, which is an abundant resource [1,2]. However, due to the rapid deterioration in quality by endogenous enzymes [1,3], its use in food is limited [3], and most Isada krill is used as fishing bait and feed for cultured fish. On the other hand, Isada krill contains many functional lipids such as eicosapentaenoic and docosahexaenoic acids [4], as well as 8-hydroxyeicosapentaenoic acid, which can decrease plasm and hepatic triacylglycerols [5]. These lipid components are expected to be used as supplements. In the process of recovering useful oily ingredients from Isada krill, a large amount of aqueous residue is formed, but it is, at present, discarded.

Water that maintains a liquid state in the range of 100°C, which is its boiling point at normal pressure, to 374°C, which is its critical temperature. Subcritical water has a lower dielectric constant and higher ion product than water at normal temperature [6,7]. Because of these characteristics, subcritical water has attracted a significant amount of attention as a reaction solvent and extractant. We have previously reported that the treatment of Isada krill [8–12] or its residual waste solution [13] under subcritical water conditions at 140 to 200°C reduced the fishy odor and enhanced shrimp–like flavor.

In this context, the effect of the conditions under which the aqueous residue was treated on the properties of the treated residue was examined. The characteristics of the treated residues obtained from the different catches were compared. Since we intended to use the treated residue as a liquid or powdered seasoning with shrimp–like flavor, the odor of the treated residue was evaluated by a panel of volunteers.

2. Materials and Methods

2.1 Materials

The aqueous residues obtained after the recovery of useful oil–soluble ingredients from Isada krill caught in 2017 and 2018 were supplied by Kokuyo (Ofunato, Japan) and designated residue–2017 and residue–2018, respectively. The residues were stored in a freezer at −80°C prior to use. The frozen residues were thawed at room temperature and centrifuged at 1500×g for 10 min to remove the insoluble matter. The supernatant was used
for treatment under subcritical water conditions.

2.2 Treatment of the aqueous residue under subcritical water conditions

The aqueous residue was treated under subcritical water conditions using a pressure–resistant batch-type SUS-316 vessel with a maximum volume of 117 mL (Taitsu Glass, Osaka, Japan). The aqueous residue (ca. 70 mL) was poured into the vessel and the vessel was tightly sealed and heated at 120–200°C using a heating mantle. The temperature inside the vessel was measured using a K-type thermocouple. After reaching the desired temperature, the vessel was maintained for an additional 5 min at that temperature to perform the treatment process. The vessel was then cooled using running tap water to terminate the treatment process.

In batch operation, the temperature changed with time. The combined effect of temperature and time was expressed using a single parameter called the severity factor \( R_0 \) [14], which was calculated using Eq. (1).

\[
R_0 = \int_0^T \exp \left( \frac{T(t) - 100}{14.75} \right) dt
\]

where \( t \) is time [min], \( T \) is temperature [°C], 100 is the reference temperature [°C], and 14.75 is an empirical parameter [°C].

The treated residue was centrifuged (H-3G a, Kokusan, Saitama, Japan) at 1500×g for 10 min to remove insoluble matter and the supernatant was used for analysis and sensory evaluation. The precipitate was dried at 50°C for ca. 2 d and at 105°C for a further 3 h to estimate the insoluble matter concentration from the weight of the dried precipitate.

2.3 Properties of the treated residue

The concentration of soluble matter was measured using a PAL-S pocket refractometer (Atago, Tokyo, Japan) as the Brix concentration. The pH value of the treated residue was also measured using a B-71X compact pH meter (Horiba, Kyoto, Japan).

The color parameters \( L^* \): lightness, \( a^* \): redness, and \( b^* \): yellowness) of the original and treated residues were measured using a CM-5 color–difference meter (Konica-Minolta, Tokyo). The total color difference (\( \Delta E^* \)), was calculated using Eq. (2).

\[
\Delta E^* = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}
\]

where the subscript 0 indicates the original (untreated) residue. The \( L_0^* \), \( a_0^* \), and \( b_0^* \) values were 22.54, 4.21, and 7.41 for residue–2017, and 3.63, 2.01, and 4.53 for residue–2018, respectively.

2.4 Sensory evaluation of the odor of the residues

The preference scores (dislike to like) and smell properties (shrimp–like, fragrant, burnt, rotten, and fishy odor) were evaluated by 13 and 43 well-informed panelists (men and women aged 18 to 24) for residue–2017 and residue–2018, respectively. A five-point hedonic scale (−2: Dislike extremely, −1: Dislike, 0: Neither like nor dislike, +1: Like and +2: Like extremely) was used for preference scoring. A five-point intensity scale (1: Slight to 4: Strong) was used for grading the odor. A volume of 1 to 1.5 mL of the original or treated residue was placed in an amber glass vial with a cap, and the vial was served to the panelists at room temperature.

Residue–2018 treated at 140°C was diluted 2\( \times \) times (\( n \) =0 to 5) and subjected to the same sensory evaluation as describe above.

The sensory evaluation was conducted with the approval of the ethics committee of Kyoto Gakuen University, which is the former name of Kyoto University of Advanced Science.

2.5 Gas chromatography–mass spectrometry (GC–MS) analysis of the odor

Gas chromatography mass spectrometry analysis of the odor was performed in the same manner as in our previous study [13]. Briefly, a GC–MS QP–2010 (Shimadzu, Kyoto) with electron ionization (ED) and a DB–WAX Ultra Inert column (0.25 mm×30 m, Agilent, Santa Clara, CA, USA) was used. The original or treated residue (2 mL) was placed in a 20 mL screw-capped vial. A fiber for solid-phase microextraction (SPME, Sigma–Aldrich, St. Louis, MO, USA) was inserted into the head space of the vial and stored at 40°C for 5 min to adsorb the volatile compounds.

3. Results and Discussion

3.1 Properties of the treated residues

Figure 1 shows examples of the temperature change observed during the treatment of the aqueous residue (residue–2018) under subcritical water conditions using a stainless steel pressure–resistant vessel. Using Eq. (1), the \( R_0 \) values were calculated for all the samples treated under various conditions (Table 1).

The concentration of soluble and insoluble matter, and the pH of the residue–2017 and -2018 samples treated
under various conditions were plotted against the severity factor $R_s$, as shown in Fig. 2. The concentration of soluble matter in the original residue–2017 and residue–2018 samples were almost 15% (Brix concentration). Treatment of the residues under subcritical water conditions produced insoluble matter. Under the treatment conditions with an $R_s$ value of $\geq 500$ (corresponding to treatment at $\geq 160^\circ$C), the amount of insoluble matter increased and the concentration of soluble matter decreased. Similarly, the pH of the residue treated under subcritical water conditions increased.

The color characteristics of the aqueous residue varied greatly depending on the year it was caught (Fig. 3). The original residue–2017 sample had a lighter color and larger $L^*$ value than the original residue–2018 sample. Treatment of residue–2017 decreased the $L^*$ value, indicating that the residue became darker. On the other hand, residue–2018 had a dark color and the lightness increased slightly when treated under the conditions with a high $R_s$ value. However, the color of original and treated residue–2018 samples was still darker than that of the residue–2017 sample. The $a^*$ values for both residue–2017 and residue–2018 decreased upon the treatment under conditions with a large $R_s$ value. That is, the greenness of the residue became slightly stronger during the treatment process. The $b^*$ value of residue–2017 was almost constant regardless of the treatment conditions used, but that of residue–2018 increased slightly under the treatment conditions with a large $R_s$ value, which increased the yellowness.

### 3.2 Sensory evaluation of the odor

Figure 4 shows the preference scores for the residue–2017 and residue–2018 sample treated under various conditions.
conditions, which are expressed by the severity factor. When raw Isada krill was treated under subcritical water conditions with a severity factor in the range of 400 to 1000, liquid seasoning with a high preference score and high intensity of shrimp-like flavor was obtained [12]. Residue−2017 had a higher preference score than the original residue when treated under subcritical water conditions, except for the conditions with an $R_0$ value of ~1000. On the other hand, the preference score of residue−2018 was almost independent of the treatment conditions used, and it was neither liked nor disliked or somewhat disliked.

The individual odor characteristics (shrimp-like, fragrant, burnt, rotten, and fishy) were assessed (Fig. 5a–d, respectively). Treatment under subcritical water conditions tended to increase the fragrant and burnt odor, and decrease the rotten and fishy odor. On the other hand, the intensity of the shrimp-like flavor was almost independent of the treatment conditions.

The quality and preference of a flavor depend on its concentration [15]. Therefore, the preference score and the smell characteristics of the residues treated at 140°C were assessed after being repeatedly diluted 2-fold. Among the odor characteristics, the intensity of the shrimp-like flavor and fishy odor, which seem to positively and negatively affect the preference score, respectively are shown in Fig. 6, as well as the preference score. The dilution of residue−2017, the preference score of which increased upon treatment under subcritical water conditions, decreased the intensity of the shrimp-like flavor, but there were no significant change in the preference score and the intensity of the fishy odor. On the other hand, the fishy odor of residue−2018, which had a relatively low preference score, decreased upon dilution, but the intensity of the shrimp-like flavor did not decrease until 8-fold dilution. Consequently, the preference score for the residue−2018 sample treated at 140°C increased when diluted 2- or 4-fold.

### 3.3 GC–MS analysis of the odor

Figure 7 shows the GC–MS chromatograms for residue−2018 treated at 140, 160 and 180°C. The chromatogram for the original residue is also shown. It has been reported that pyridines and pyrazines are formed during shrimp roasting and krill boiling, and that they cause the
shrimp–like flavor [16,17]. We previously reported that pyridines and pyrazines were eluted in the retention time range between 9.6 to 20.4 min [13]. The retention times of some pyridines and pyrazines under the analytical conditions were 9.6 min (pyridine), 12.3 min (2-methylpyrazine), 13.2 min (3-methylpyrazine), 14.1 min (2,5-dimethylpyrazine), 14.3 min (2,6-dimethylpyrazine), 16.6 min (2,3,5-trimethylpyrazine), and 16.8 min (3,5-dimethylpyridine) [13]. The original residue exhibited no or few peaks in this retention time range. On the other hand, some peaks were detected in this retention time range for the residues treated at 140, 160 and 180°C, indicating that compounds exhibiting shrimp–like flavor can be formed during the treatment process conducted under subcritical water conditions.

The chromatograms for residue–2017 treated under subcritical water conditions also exhibited peaks in the same retention time range (data not shown).

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イサダからの水溶性残渣液の亜臨界条件下での処理によるエビ風味をもつ調味料の調製

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イサダ（学名ツノナシオキアミ, 別名アミエビ）は早春に三陸沿岸で漁獲される, 資源量が豊富な小型のアミ類である[1,2]. イサダは内在性の強い酵素活性をもつため, 漁獲後短時間で品質が低下する[1,3]. したがって, 食品への利用は限られており, 大半は釣りの撤き餌や養殖魚の餌として利用されている[3]. 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, 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. これら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℃の範囲で液体状態を保った水を亜臨界水という. 著者らは, イサダ[8-12]やその煮汁[13]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.

そこで, 煮汁と同様に, イサダから脂溶性の有効成分を回収する際に排出される水溶性残渣液についても, 亜臨界条件下で処理すると, エビ風味の調味液が調製できると期待される. また, その調味液に賦形剤を添加し, 噴霧乾燥すると, エビ風味をもつ調味粉末が得られる.

そこで, 2017年および2018年に漁獲されたイサダから脂溶性の有用成分を回収したあとの水溶性残渣液を種々の温度（120～200℃）で処理し, 处理液の特性を評価した. さらに, 調味料としての利用を想定しているため, 处理液の嗜好性およびエビ風味, 香ばしさ, 焦げ臭, 廃棄臭および生臭さの強度について官能評価を実施した. なお, 回分操作では時間とともに温度が変化する昇温過程の影響が大きいと思われるため, 温度と時間の効果をseverity factor（式(1)）という1つの指標で評価した.

160℃以上の処理温度では, 不溶物の生成が多くなり, 可溶物の濃度が低下した. また, 处理液のpHも上昇した（Fig. 2）.

イサダの組成は漁獲年, 漁獲時期, 漁場などに依存する. また, 水溶性残渣液の色調も漁獲年などに依存し, 亜臨界条件下での処理後の色調も異なっていた（Fig. 3）. しかしあの漁獲年の水溶性残渣液も亜臨界条件で処理すると, 生臭さが低減し, 香ばしさと焦げ臭が強くなった（Fig. 5）. 2017年度の水溶性残渣液は亜臨界条件下での処理により嗜好性が向上した. 一方, 2018年度の水溶性残渣液については, 处理による嗜好性の向上が認められなかった（Fig. 4）. しかし, 2017年および2018年は水溶性残渣液についての官能評価を行ったところ, 2018年度の試料は, 2倍または4倍に希釈するとエビ風味は低下しないが, 生臭さが軽減されるため, 嗜好性が向上した（Fig. 6）.

エビ風味はピリジン類やピラジン類に由来するといわれる. そこで, GC-MSによるベッドスペース分析を行ったところ, 水溶性残渣液にはこれらに相当するピークは認められなかったが, 2017年度および2018年度の水溶性残渣液を140, 160および180℃で処理した液はいずれもピリジン類やピラジン類に由来するピークが出現した.