Seasoning Production from the Residual Waste Solution of Isada Krill Processing by Its Treatment under Subcritical Water Conditions

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The residual waste solution obtained during the recovery of functional components from Isada krill was treated under subcritical water conditions. Properties of the treated solution, such as solid, protein, and carbohydrate contents, were measured. The characteristic smell of the solution was evaluated. The smell intensity increased with a rise in the treatment temperature by gasification of the soluble components. In particular, the treatment at 160–180 ℃ strengthened the shrimp-like fragrant smell and retained the unpleasant fishy and rotten smell at lower level. The treated solution, therefore, may serve as a promising seasoning ingredient. The gas chromatography mass spectrometry analysis of the smell revealed the formation of pyridine- and pyrazine-related compounds after the treatment that contributed to the improvement in shrimp–like flavor.

Keywords: flavor improvement, Isada krill, seasoning production, sensory evaluation, subcritical water

1. Introduction

Krill is a small crustacean that inhabits in oceans and serves as a prey in the marine ecological system [1]. Isada krill (Euphausia pacifica) mainly inhabits the Sanriku offing of Japan [2]. The catch of Isada krill is more than 70,000 tons a year [3]. However, Isada krill is susceptible to autolysis, which accelerates the process of quality deterioration after fishery [4] and results in the development of unpleasant amine–like odor. As a consequence, Isada krill has limited use as a food ingredient.

Isada krill is a rich source of valuable nutrients such as eicosapentaenoic acid, docosahexaenoic acid, and astaxanthin [5]. Recovery of these substances from Isada krill has been industrially performed [6]. Isada is known to contain a promising component, 8-hydroxy-eicosapentaenoic acid, that may serve as a functional food ingredient [7]. The useful components contained in Isada krill are recovered as follows: The krill is enzymatically treated to be hydrolyzed, and then heated to inactivate the enzyme. The hydrophobic components are recovered by adsorption and desorption using an adsorptive resin. The recovery process generates large quantities of residual waste solution with fishy smell, and additional costs are incurred for the disposal of this waste solution. Therefore, effective utilization of the residual solution is desirable.

Subcritical water, water maintained in its liquid state under pressurized condition between 100℃ and 374℃, exhibits two characteristic properties—low dielectric constant and high ion product. These properties of subcritical water facilitate the catalytic activity and extraction ability of hydrophobic substances [8,9]. Due to these properties much attention has been paid to utilization of subcritical water to food processing residues in last two decades [10–12].

We have previously reported that the subcritical water treatment of raw Isada krill results in the decrease in the fishy smell and increases the shrimp–like fragrant smell [13–15]. As the residual solution may retain some of the original components from Isada krill, its treatment with subcritical water would be favorable to improve the smell of the solution. In this study, the residual waste solution was treated with subcritical water, and sensory evaluation of smell was performed to investigate the application of the treated solution for flavor seasoning. Compounds formed during the subcritical water treatment were also identified.
2. Materials and Methods

2.1 Materials

The residual solution obtained after the processing of Isada krill was supplied from Koyo Chemical (Osaka, Japan). The solution was stored in a freezer at −25°C until use. The reagents used were purchased from Wako Pure Chemical Industries (Osaka, Japan) or Nacalai Tesque (Kyoto, Japan).

2.2 Treatment of the Residual Solution under Subcritical Water Conditions

The treatment of the residual solution under subcritical water conditions was performed using a small pressure-resistant batch-type SUS-316 vessel with a maximum volume of 10 mL (Taiatsu Glass, Osaka, Japan). The residual solution (6.5 mL) was poured into the vessel. The vessel was tightly sealed and dipped in an oil bath thermostated at 120–200°C. The temperature inside the vessel was measured using a K-type thermocouple. Once the desired temperature was attained, the vessel was maintained for an additional 5 min to perform the treatment. The vessel was then moved to an ice bath to terminate the treatment process.

2.3 Properties of the Treated Solution

The total soluble solid content of the solution was estimated by using a hot air oven method. The oven was preheated to 135°C, and the sample solution (3 mL) in a crucible was placed in the oven and heated for 3 h. After the crucible reached a constant weight, its weight was recorded to estimate the solid content. The solid content was expressed as mass fraction in gram solid content per gram of the solution. Ash content was estimated by the same method but the sample solution (3 mL) was ashed at 550°C for 4 h. The content was expressed as gram of ash per gram of solution.

Protein content of the solution was determined by Lowry–Folin’s method [16]. One milliliter of the solution (500 times diluted) was mixed with Lowry’s reagent (0.5 mL) and the mixture was stored for 15 min at room temperature. Folin–Ciocalteu reagent (0.05 mL) was added to the above mixture and the mixture was further stored for 30 min at room temperature. The absorbance of the resultant mixture was measured at 750 nm using a U-5100 spectrophotometer (Hitachi High-Tech Science, Tokyo, Japan). A standard curve was prepared using bovine serum albumin.

Freezing point depression of the solution was measured using an osmometer (OM802, Vogel, Kevelaer, Germany). Salinity, pH, and UV/VIS spectra of the solutions were measured using PAL-ES1 pocket salt meter (Atago, Tokyo, Japan), D–51 pH meter (Horiba, Kyoto, Japan), and U–5100 spectrophotometer, respectively.

The smell intensity of the solution was measured after placing the solution (1 mL) into a test tube (14 mm I.D × 100 mm). The smell intensity in the head space was measured using an odor sensor (XP–329IIIR, NEW COSMOS ELECTRIC, Osaka, Japan) at room temperature for 1 min with batch and level modes.

2.4 Sensory Evaluation of the Smell of the Solutions

Properties of smell (shrimp-like, fragrant, fishy, burnt, and rotten smell) were scored on a five-level intensity scale (0: slight to 4: strong). The treated or untreated solutions (1.5 mL) were kept in 5-mL screw-capped vials before being served to 20 well-informed panelists.

2.5 Gas Chromatography Mass Spectrometry (GC–MS) Analysis of the Smell

Gas chromatography mass spectrometry analysis of the smell was performed using GC–MS QP–2010 (Shimadzu, Kyoto, Japan) with electron ionization (EI). The solution (2 mL) or 20-min roasted shrimp shell (ca. 2–cm height in a vial) 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 volatiles. A DB–WAX Ultra Inert column (0.25 mm × 30 m, Agilent, Santa Clara, CA, USA) was used. The column temperature was programed as follows: 40°C for 5 min, 40°C to 200°C at 5°C/min, and 200°C for 10 min. The injector, interface, and ion source temperatures were 220°C, 250°C, and 250°C, respectively.

3. Results and Discussion

3.1 Properties of the Treated Solution

The soluble solid content, salinity, and ash content of the untreated solution were 2.8% (w/w), 0.36% (w/w), and 0.32% (w/w), respectively. As salinity and ash content values were almost the same, most of the ash contained sodium chloride. The content of organic matter in the solution was ca. 2.5% (w/w), and 0.84% (w/w) and 0.03% (w/w) of it were proteins and carbohydrates, respectively. This solution was treated in the temperature range of 120–200°C.
Figure 1 shows the effect of the treatment temperature on the appearance of the solutions. The untreated solution was light yellow, while the treatment at 120°C did not induce any remarkable change in the color tone. On the other hand, the color of the solution changed to brown and became darker with an increase in treatment temperature. This trend was remarkable upon treatment of samples at 160°C or higher temperatures. Figure 2 shows the effects of the treatment temperature on the properties of the solutions. The solid content of the treated solutions ranged from 2.1% to 2.8% (w/w) and was lower than that of the untreated samples. As the ash content was approximately 0.3% (w/w), the content of organic matter was ca. 1.8%–2.5% (w/w). On the other hand, the freezing point depression was 0.48±0.01 K regardless of the treatment temperature (molality of the solutes was equivalent to ca. 0.26 mol/kg) and was unaffected by the treating conditions. These results indicate that the small decrease in the solid content was associated with the gasification of a part of the components and that the soluble matter decomposed into small molecules.

The untreated residual solution was slightly alkaline (pH 8.08), probably due to the presence of trimethylamine as described later. The pH value increased with an increase in the treatment temperature and reached 9.11 after treatment at 200°C. These results indicate the formation of basic compounds.

The formation of bases may be related to some factors. Chitin is a major constituent of the shell of crustaceans such as krill, lobster, crab, and insects and comprises N-acetylglucosamine (GlcNAc) monomers. Based on the composition of the residual solution, some of the carbohydrates in the solution were suggested to be glucosamine–related compounds. Carbohydrates such as GlcNAc become reactive at temperature of 170°C or higher [17]. Subcritical water may hydrolyze a part of GlcNAc to yield glucosamine, thereby contributing to the increase in pH. In addition, products of Maillard reaction and caramelization would contribute to the change in the pH value. Significant pH change was observed following treatment at 160°C or higher temperatures. This temperature was slightly lower than that of GlcNAc, indicating that the treatment at 160°C may also promote the reaction.

The antioxidative ability of the solution increased with an increase in the treatment temperature. As the changes in the color tone may be related to the Maillard reaction and caramelization effects, the reaction products such as melanoidin and 5-hydroxymethyl furfural improved the antioxidative activity.

### 3.2 Sensory Evaluation of the Smell

The intensity of smell was enhanced with an increase in the treatment temperature, and the solutions treated at 180–200°C showed stronger smell as compared to others (Fig. 2). This observation may be attributed to the gasification of the solid content. Figure 3 shows the effects of treatment temperatures on the properties of
smell (shrimp-like, fragrant, fishy, rotten, and burnt smell). For untreated residual solution, the smell intensity was low but the fishy and shrimp-like smell was relatively prominent. On the other hand, each score increased with an increase in the treatment temperature. Although the intensity of unpleasant smells (fishy, rotten, and burnt) increased after treatment at 140–180°C, the enhancement of the favorable fragrant smell (shrimp-like and fragrant smell) was more obvious. The treatment at 200°C resulted in an increase in the unpleasant smell, which may be a side-effect of the high temperature treatment. These results suggest that the treatment at 160–180°C would favorably influence the smell properties and particularly enhance the favorable shrimp-like fragrant smell.

The overall preference would be improved by the treatment under subcritical water conditions. It is, therefore, suggested that the treatment is effective for producing seasonings with shrimp-like fragrant smell from the residual solution.

3.3 Identification of the Smell Components

The treatment of the residual solution under subcritical water conditions yielded a shrimp-like fragrant smell. To identify the compounds related to this smell, headspace analysis of the treated solutions was performed by GC–MS (Fig. 4). Smells of the roasted shrimp shell and untreated residual solution were analyzed as positive and negative controls, respectively.

Every solution, including the untreated sample, contained trimethylamine (Table 1). However, other conspicuous peaks were absent in the untreated solution. As trimethylamine may act as a major unpleasant smelling component, the untreated solution particularly showed fishy smell. No significant change in the GC–MS chromatogram was observed between control sample and those treated at 120°C.

The treatment of samples at 140°C or higher temperatures resulted in some distinct peaks that corresponded to the reaction products. These peaks became intensive, especially when the treatment temperature was 160°C or higher. The products obtained after treatment at 140–200°C were estimated. Some peaks were tentatively identified and pyridine– and pyrazine–related compounds (pyridines and pyrazines) were detected (Table 1). Major compounds such as pyridine, 3-methylpyrazine, and 3,5-dimethylpyrazine were detected both in the treated solution and roasted shrimp shell. However, a difference in their smell compounds was observed. The treated solutions showed relatively stronger peaks of pyrazines such as 2–methylpyrazine, 2,5–dimethylpyrazine, 2,6–dimethylpyrazine, and 2,3,5–trimethylpyrazine that were weak for the roasted shrimp shell.

Table 1  Identified compounds with smell from the residual solution of Isada krill treated under subcritical water conditions or the roasted shrimp shell.

| Peak No. | Retention time (min) | Compounds with smell |
|----------|----------------------|----------------------|
| 1        | 1.15                 | Trimethylamine        |
| 2        | 9.59                 | Pyridine             |
| 3        | 12.33                | 2-Methylpyrazine      |
| 4        | 13.19                | 3-Methylpyrazine      |
| 5        | 14.11                | 2,5-Dimethylpyrazine  |
| 6        | 14.31                | 2,6-Dimethylpyrazine  |
| 7        | 14.82                | 2,3-Dimethylpyrazine  |
| 8        | 15.82                | 3-Ethylpyridine       |
| 9        | 16.20                | 2-Ethyl–5–methylpyrazine |
| 10       | 16.56                | 2,3,5–trimethylpyrazine |
| 11       | 16.84                | 3,5–Dimethylpyridine  |
| 12       | 17.21                | 2-Ethyl–5–methylpyridine |
| 13       | 17.72                | 2,5–Dimethyl–3–ethylpyrazine |
| 14       | 18.27                | 2,6–Diethylpyrazine   |
| 15       | 18.50                | 2,3,5,6–Tetramethylpyrazine |
| 16       | 19.11                | 3,5–Diethyl–2–methylpyrazine |
| 17       | 20.39                | 2,6–Dimethyl–3–ethylpyridine |
These basic fragrant compounds would contribute to the pH increase and favorable smell. As described above, protein and glucosamine were detected in the residual solution. It was reported that pyridines and pyrazines are among the Maillard reaction products and may be formed during shrimp roasting and krill boiling [18,19]. Therefore, it is reasonable that the treated solutions exhibited shrimp-like fragrant smell in this study.

To confirm the formation of pyrazines, UV/VIS spectra of the treated solutions were measured. Spectra of the visible light region showed an increase in the absorbance associated with the Maillard reaction with an increase in the treatment temperature (Fig. 5). In addition, the treatment at higher temperatures increased the absorbance in the UV region. Absorption peaks between 250 and 280 nm shifted to shorter wavelength following treatment at 160°C or higher temperatures, and the solution treated at 200°C showed the maximal absorption peak at 257 nm. Absorption peaks around 260 nm supported the formation of pyrazine. These results corresponded with the fact that the smell intensity significantly enhanced at 160°C or higher temperatures. The results of GC-MS and UV/VIS spectra suggest that various types of pyridines and pyrazines were easily formed during treatment under subcritical water conditions.

In conclusion, the treatment under subcritical water conditions would be effective for the efficient utilization of the residual waste solution obtained during the recovery of valuable compounds from Isada krill. The treated solution exhibited shrimp-like fragrant smell and would be promising for flavor seasoning.

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亜臨界水条件下での処理によるイサダ処理残渣液からの風味原料の調製

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イサダ（学名ツノナシオキアミ、別名アミエビ）は、三陸沿岸で早春に漁獲される小型のアミで、資源量は豊富である[2,3]。水揚げ直後のイサダは鮮やかな桜色であるが、強い酵素活性により、数時間で黒色し、異臭を生じる[4]。地元では、水揚げ後すぐに蒸煮し、乾燥した半乾燥イサダが食用として販売されているが、大半は養殖魚の餌やつり餌（撒き餌）としての利用に留まっている。しかし、イサダはドコサヘキサエン酸（DHA）、エイコサペンタエン酸（EPA）、アスタキサンチンなどの機能性脂質を含有する[5]。さらに、EPAの8位が水酸化された8-ヒドロキシエイコサペンタエン酸（8-HEPE）という強力な抗肥満成分を含有することが見出され[7]。イサダからこれらの脂質を取り出し、機能性食品素材として利用する試みが行われている。

その工程では、廃棄物として水溶性残渣が生成する。常圧では水は100℃で沸騰し気体（水蒸気）になるが、高山ではそれより低い温度で気化する。一方、圧力鍋の中や深海の底では、沸点は100℃より高くなる。すなわち、沸点は圧力に依存し、圧力が高いと水は374℃（臨界温度）まで液体状態を保つ。このように、常圧での沸点である100℃から臨界温度の範囲で、加圧することにより液体状態を保つ水を亜臨界水または加圧熱水という。常温常圧の水に比べて、比誘電率が低く、イオン積が大きいという2つの特徴がある[8,9]。前者の特徴から、亜臨界水は常温の水には溶け難しいものを溶解除することができる。後者の特徴から、亜臨界水は水素イオンと水酸化物イオンの濃度が高くなり、種々の反応を促進する。このような性質を利用し、未利用資源や廃棄物から有用なものをつくろうとする研究が活発に行われている[10–12]。筆者らは水が亜臨界状態となる条件でイサダを処理すると、生臭さが消え、エビ風味が増強されることを見出した[13–15]。

本論文では、イサダから機能性脂質を取り出したあと、水溶性残渣を廃棄することなく、120～200℃の範囲で、水が亜臨界状態を保つ条件下で水溶性残渣を処理し、エビ風味を呈する調味液に効率的に変換する生産する条件について検討した。

水溶性残渣は黄色を呈している。120℃での処理では色調は変化しなかったが、処理温度が高くなると処理液は褐色になり、高温ほど色調が強くなった（Fig. 1）。亜臨界条件下での処理により（Fig. 2）、固形物濃度がわずかに低下し、揮発性物質の生成を示唆した。また、処理温度が高いくほどの処理液のpHが高くなり、塩基性物質の生成を示唆した。さらに、処理温度の上昇に伴い処理液が示す抗酸化活性が上昇した。これはMaillard反応やカaramel化に起因すると思われる。水溶性残渣を処理する温度が処理液の臭気特性（エビ臭、香ばしさ、生臭さ、腐敗臭および焦げ臭）に及ぼす影響を20名のボランティアにより官能評価した（Fig. 3）。140～180℃で処理すると、生臭さ、腐敗臭、焦げ臭という不快な特性が低下し、逆に好ましいエビ風味や香ばしさが強まった。しかし、さらに高温の200℃では不快臭が強くなった。これらのことより、160～180℃での処理がもっとも好ましがと示された。

エビ風味を呈する成分を特定するため香気成分のヘッドスペースGC−MS分析を行った（Fig. 4）。未処理の水溶性残渣を含め、すべての液のヘッドスペースはトリメチルアミンを含んでいた。しかし、亜臨界条件下で処理した液では、それ以外にははっきりした不快な成分のピークは認められなかった。140～200℃で処理した液からはピラジンやピリジン類が認められた（Table 1）。ときに、エビ風味に関連する2-メチルピラジン、2,5-ジメチルピラジン、2,6-ジメチルピラジン、2,3,5-トリメチルピラジンといったピラジン類の強いピークが認められた。