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Direct Treatment of Isada Krill under Subcritical Water Conditions to Produce Seasoning with Shrimp-Like Flavour

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Summary

Characterization, sensory evaluation, and astaxanthin stability of isada krill under various subcritical water conditions were investigated to optimize the quality of krill extract and residue for producing food seasoning. Raw krill (82 % wet basis moisture content) without additional water was treated in a pressure-resistant vessel for 10 min at a temperature range of 100–240 °C. The yield of water-soluble protein was maximized by treatment at 200 °C and decreased with treatment at higher temperatures. The degradation of large molecules and the concomitant production of small molecules depended on the treatment temperature. Astaxanthin in the krill was unstable at temperatures higher than 140 °C. The odour intensities of krill extract and residue increased with higher treatment temperature; however, the highest intensity of pleasant shrimp-like flavour was obtained by treatment at 140 °C. Subjective preference scores were the highest for extract and residue obtained at 140 °C. Thus, treatment at 140 °C is the most promising method for production of seasoning with shrimp-like flavour from isada krill.

Key words: subcritical water, astaxanthin, shrimp-like flavour, Isada krill, odour intensity

Introduction

Isada krill (Euphausia pacifica), also known as North Pacific krill, is a small, shrimp-like crustacean (euphausi-aid) that is found mainly around the Sanriku coast in Japan. Krill readily deteriorates after harvesting, owing to the action of endogenous proteases in the digestive tract, with the production of unpleasant colour, odour and taste (1), restricting its usage. The krill is mainly used in low-value-added products, such as animal and aquaculture feeds, and fishing bait, and only a small amount is directly consumed by humans. High content of fluoride in exo-
skeleton reported for Antarctic krill (Euphausia superba) and some other species of euphausiids raised concerns of toxicity of using krill for a direct human diet (2). However, krill is an abundant resource with high nutritional value, rich in protein, polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), vitamin E and astaxanthin (2). Astaxanthin has a role in determining the colour of crustacean products (3). Its stability during drying and storing processes has been studied (4); however, its stability during a hydrothermal processing, as in subcritical water treatment, has not been reported yet.

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Subcritical or superheated water is water in the liquid state in the temperature range of 100–374 °C, at pressures of 1–22 MPa. Under subcritical conditions, the relative dielectric constant and polarity of water decrease with the increase in temperature. The reduced polarity of subcritical water gives it properties of organic solvents; thus, it can be used for extraction of organic compounds (5). Another feature of subcritical water is a high ion production, which indicates that the water acts as a stronger acid or base catalyst than water at lower temperatures and pressures. Therefore, subcritical water has been widely used to extract and hydrolyse the natural materials for production of valuable compounds, such as proteins, essential oils and bioactive compounds (including phenolic compounds and antioxidants). Furthermore, the production of hydrolysates from fish skin by using subcritical water improved or modified their functionalities (6). Extraction under subcritical water conditions in a closed vessel also promoted the flavour development of tea (7).

Subcritical water has been used in the production of protein hydrolysates and amino acids for use as food additives, for example, from defatted krill (8) and squid viscera (9). We have also applied the treatment to semi-dried or raw krill by mixing the krill with water at a mass ratio of 1:1, to produce extracts and residues having pleasant, shrimp-like flavour (10,11). Using the products as seasoning should be safe regarding fluoride toxicity because only a small amount will be applied to food products. However, mixing of the krill with water resulted in the dilution of the extract, which decreased the flavour intensity. Therefore, in this study, we described the direct treatment of raw krill, without the addition of water, under subcritical conditions, to produce extracts and residues, which is possible because the water content of raw krill is around 80 % of its fresh mass. The effects of treatment temperature on the properties of the extracts and residues were examined to determine the optimal treatment conditions. Sensory evaluations of the extracts and residues were also carried out.

Materials and Methods

Materials

The average mass and length of krill, which were purchased from Hamachi (Wakayama, Japan) in a frozen state and thawed before use, were (0.07±0.01) g and (18.0±0.65) mm, respectively. The krill contained on wet mass basis 0.82 % of water and 0.05 % of lipid. Astaxanthin (from algae, 98 % purity), diethanolamine, copper(II) sulfate pentahydrate, bovine serum albumin (BSA) and fluoride were purchased from Wako Pure Chemical Industries (Osaka, Japan). Other reagents were used as purchased.

Treatment under subcritical water conditions

The plastic wrapped frozen krill pack was thawed by immersion in water, and 60 g of the thawed krill was put into a 117-mL pressure-resistant batch-type vessel (Taitsu Techno, Osaka, Japan). The closed vessel was then heated with a 200 W mantle heater (Heat Eng. Japan) equipped with a TXN 700B thermo-controller (As One, Osaka, Japan) to temperatures of 100–240 °C, at intervals of 20 °C. Extractions at these eight temperature points were performed in triplicate. After reaching the desired temperature, it was maintained for 10 min, and then the vessel was immediately immersed in ice water to cool it down to room temperature.

The treated krill was separated into liquid and solid residues by filtration under reduced pressure, followed by pressing as described previously (10). The liquid obtained from the solid residue by pressing was combined with the liquid extract from filtration.

Properties of the extracts

The total solid content of the extracts was determined by hot-air drying of 1 g of extract at 135 °C for 2 h according to ISO method 6496:1999 (12), and is reported as mass fraction in kg per kg of extract.

The protein content of the extracts was determined by the Lowry-Ciocalteu method (13), after centrifugation of the extracts at 4270×g for 20 min at 4 °C. A standard curve was prepared using BSA at concentrations of 100–500 mg/L. Diluted supernatant (0.4 mL) or BSA (0.4 mL) solution was mixed with 2 mL of Lowry reagent and 0.2 mL of 50 % (by mass per volume) Folin-Ciocalteu reagent. The absorbance of the mixture was measured at 750 nm using a UV-1600 spectrophotometer (Shimadzu, Kyoto, Japan).

The lipid content of residues and extracts was determined using the solvent extraction method (14) after hot air drying of residues at 105 °C for 4 h and crushing into powder, and dehydration of extracts with sodium sulfate. A mass of 1 g of dried krill residue powder or dehydrated extract was placed in an extraction thimble (Advantec, Toyo Roshi, Tokyo, Japan) and set in a Soxhlet extractor for over 10 h at 47.5 °C to extract lipids using diethyl ether. The remaining solvent in the extracted oil was removed by evaporation. The residual lipid was weighed and the lipid content was expressed as mass fraction in kg per kg of raw krill.

The pH of the extract was measured using a pH meter (D-51; Horiba, Kyoto, Japan).

Molecular mass distribution analysis

Molecular mass distribution was determined by size exclusion chromatography, using an HPLC system consisting of an LC-10AD pump (Shimadzu) and an YMC-Pack Diol-120 column (500 mm length, 8.0 mm internal diameter; YMC, Kyoto, Japan). The filtered extract (20 μL) was loaded onto the column and eluted with distilled water at a flow rate of 1.0 mL/min. Elution was monitored with an UV-Vis light detector (SPD-10A; Shimadzu) at 280 nm. The mass distribution in the extract was determined by comparison with molecular mass standards comprising glucose oxidase (153 kDa), BSA (66 kDa), myoglobin (16.9 kDa), Tyr-Tyr-Tyr (507 Da), Phe-Phe (312 Da), Phe-Ala (236 Da), and uracil (112 Da), dissolved in distilled water. The elution volume of the extract was converted to the molecular mass using the calibration curve.
of the standards. The curve was expressed by the following equation:

\[ \log M = 7.31 - 0.334V_s \]

where \( M \) and \( V_s \) are molecular mass (Da) and elution volume (mL), respectively.

The freezing point depression, \( \Delta T_f \) (°C), was also measured, using an osmometer (OM802; Vogel, Kevelaer, Germany) to estimate the average molecular masses of solutes (\( M_{\text{soln}} \)) in the extracts, using the following equation:

\[ M_{\text{soln}} = \frac{w_{\text{soln}}K_f}{\Delta T_f} \]

where \( w_{\text{soln}} \) is the fraction of total solid content (g/kg) and \( K_f \) is the molal freezing point depression constant of water (1.86 (°C·kg)/mol).

To quantify peak areas, the printed chromatogram was carefully cut along a peak shape, and the paper was divided into regions of high, medium and low molecular mass by division at 10° and 10² Da. The divided chromatograms were calculated to the relative peak areas of the regions of high, medium and low molecular mass to the total area.

Astaxanthin analysis

Astaxanthin in the liquid extracts or solid residues was extracted and saponified to convert it into its free form. Krill residues were first dried in a hot air oven at 70 °C for 2 h and ground using a mortar and pestle to produce a powder. Extraction and saponification were performed using a modified method of Yuan and Chen (15). Residual powder (0.5 g) or extract solution (5 mL) was mixed with 10 mL of an extraction solvent, which was a mixture of dichloromethane and methanol (1:3, by volume), and the mixture was stirred in the dark for 3 h. The vial was then centrifuged at 9600×g for 5 min at 4 °C. The precipitated residue or the upper layer of the extract was separated from the solution containing astaxanthin. The volumes of astaxanthin-containing solutions obtained from residues and extracts were adjusted to 10 and 5 mL, respectively, with the extraction solvent. Astaxanthin in the solution was de-esterified by mixing with 0.1 mol/L of sodium hydroxide in methanol at the ratio 5:1. Headspace was flushed and kept at 4 °C in the dark for 12 h. The solvent was then removed by evaporation under a stream of nitrogen. Dry, free astaxanthin was redisolved in chloroform for HPLC analysis. Standard solutions of astaxanthin containing 5–25 μg/mL were prepared from a stock solution of 25 μg/mL.

The astaxanthin content was determined using HPLC (Shimadzu), with a Luna® 3 μm Silica (2) 100 Å column (150 mm×4.6 mm; Phenomenex, Torrance, CA, USA) at ambient temperature. The mobile phase was a mixture of hexane and acetone (82:18, by volume), and the flow rate was 1.2 mL/min. The sample injection volume was 20 μL. Astaxanthin loss during the subcritical water treatment was calculated in percentage using the following equation:

\[ \text{Astaxanthin loss} = \left( \frac{A_{\text{raw}} - (A_{\text{ext}} + A_{\text{res}}))}{A_{\text{raw}}} \right) \times 100 \]

where \( A_{\text{raw}} \), \( A_{\text{ext}} \) and \( A_{\text{res}} \) are the absorbances that correspond to astaxanthin concentrations in raw krill, liquid extract and solid residue, respectively.

Odour intensity

Odour intensity was measured at 25 °C using an odour concentration meter (XP-329III; Cosmos, Tokyo, Japan) as an overall strength of perceived odour of the krill extract (1 mL) or krill residue (1 g) in a 5-mL amber glass bottle. The emitted odour compounds were detected by an integrated sensor and were reported as numerical values in an odour unit. The screw-capped bottle containing the sample was incubated in a water bath at 25 °C to reach that temperature; then, the bottle was opened, and the detection probe was immediately placed inside. Measurement was conducted until the monitor displayed a constant value. Odour intensity was expressed in odour units (OU) per headspace volume of the bottle.

Sensory evaluation

The preference scores and shrimp-like flavour intensities of the extract solutions and solid residues were evaluated by 13 panellists. 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, and a five-point intensity scale (1=slight to 5=strong) was used for grading shrimp-like flavour intensity. A volume of 1 mL of the extract or 1 g of the wet residue was placed in an amber glass bottle with a cap, and the bottle was served to the panellists at room temperature. The bottle was shaken well before being opened and sniffed. The scores obtained from the panellists were subjected to analysis of variance (ANOVA) to determine the significance of differences. The least significant difference (LSD) test for randomized complete block design (RCBD) was used to compare the scores for different treatments with statistical significance level of 0.05, using SPSS Statistics v. 17 (IBM, New York, NY, USA).

Results and Discussion

Appearance of extracts

The appearance of extract solutions and solid residues obtained by treatment of krill under subcritical water conditions varied depending on the treatment temperature (Fig. 1). The brown colour of extracts was more intense at higher treatment temperatures (for colour images see: www.ftb.com.hr). Extracts obtained at 100 and 120 °C were light yellow, whereas extracts prepared at 140 and 160 °C were reddish-yellow. Extracts prepared at higher temperatures gradually became brownish-yellow, and the extract prepared at 240 °C was dark brown. The residues treated at low temperatures were reddish-pink. The residues of the treatment at 140–180 °C were light brown, and residue colour became lighter with treatments at temperatures above 200 °C.

Yields of extract solutions and solid residues

Raw krill was treated at various temperatures and separated to give extract solutions and solid residues. Yields of extracts and residues varied depending on treatment temperature (Fig. 2). Inset of the figure shows the temperature profiles during the treatment of the krill using the batch-type vessel. The yields of extracts and
residues showed little variation with treatment temperatures from 100 to 140 °C, but extract yields greatly increased, and residue yields greatly decreased at the temperatures from 140 to 180 °C. The yields of residues on wet mass basis below 140 °C were around 0.3–0.4 kg per kg of raw krill, decreasing significantly above 140 °C. The yields of extracts increased with increasing treatment temperature. At temperatures above 180 °C, the yields of extracts were ≥ 90 % of the raw krill. The respective increases and decreases in the yields of extracts and residues with increasing temperature indicate that subcritical treatment at higher temperature decomposed the krill more efficiently by increasing the ability of the krill water content to act as a solvent.

Total solid and protein contents and pH of extracts

Total solid material and protein contents, and pH of extracts varied according to treatment temperature (Fig. 3). The total solid content in the extracts increased gradually from 0.06 to 0.13 kg per kg of extract with increasing treatment temperature up to 220 °C, levelling off to 0.12 kg per kg at 240 °C. Protein content also increased from 0.03 to 0.11 kg per kg of extract at treatment temperatures up to 200 °C, and slightly decreased to 0.10 kg per kg at higher temperatures. The pH of the extracts showed similar variation and might result from the formation of amines, which is a basic product obtained by cooking materials containing protein at high temperature (16). These observations suggest that proteinaceous substances be the main components in the extracts obtained at higher temperatures (16). However, the decrease of protein content at 220 and 240 °C suggested that at these temperatures the degradation of proteins and the generation of small components, such as organic acids, occur (17,18).

Molecular mass distribution profiles

Molecular mass distribution profiles of liquid extracts varied depending on treatment temperature. The molecular masses ranged from 10^2 to 10^6 Da (Fig. 4a). Chromatograms of extracts obtained at 100–140 °C showed similar patterns, although the signal intensity of the extract at 140 °C was greater than those of the extracts at 100 and 120 °C. Similarly, the extracts obtained at 220 and 240 °C showed similar distribution profiles.

The chromatograms were divided into regions of high, medium and low molecular mass, and peak areas of the regions were measured (Fig. 4b). The peak areas of the regions of high and medium molecular mass increased with increasing treatment temperature up to 180 °C, and decreased at higher temperatures. The total solid content of the extracts showed little variation from 180 to 240 °C (Fig. 3). Therefore, the decline could be ascribed to hydrolysis of high-molecular-mass substances such as protein. The peak area of the low-molecular-mass region sharply
Lipid content

Lipid contents of extracts and residues varied depending on treatment temperature (Fig. 5). Lipids moved more easily from the krill to the liquid extract at higher treatment temperatures. With increasing treatment temperature, the lipid contents of liquid extracts increased from 0.006 to 0.031 kg per kg of raw krill (from 12 to 58 % of total lipid content), whereas those of solid residues decreased from 0.045 to 0.022 kg per kg (from 88 to 42 % of total lipid). Because subcritical water has a low relative dielectric constant (like an organic solvent), which decreases with increasing temperature, more lipids should be extracted and solubilized in the liquid phase at high temperatures (20). For instance, the use of subcritical water to pretreat the oil seed of Datura stramonium increased the yield of extractable lipids by 50 % (21). In addition, collapse of krill shell material could become increasingly important at elevated temperatures, facilitating lipid extraction. Chitosan and protein, which are the shell components of the crustacean organism (22), could be decomposed by subcritical water hydrolysis (23).

Astaxanthin content and stability

Astaxanthin is the main carotenoid that has a role as a pigment in the krill and it exhibits excellent antioxidant capacity. The overall astaxanthin contents in krill extracts and residues obtained by subcritical water treatment, as well as astaxanthin loss during treatment (determined by comparison with total astaxanthin composition of raw krill) vary depending on temperature (Fig. 6). The overall astaxanthin content in the extracts prepared at 100–140 °C increased with increasing treatment temperature, and the highest yield was achieved at 140 °C, with a corresponding reduction in residual astaxanthin content in the residues. This increase in the extraction of astaxanthin from the krill might be due to the reduced viscosity and polarity of subcritical water at higher temperatures, increasing the ability to solubilize the hydrophobic astaxanthin (5). In addition, heating of the krill also helped releasing astaxanthin from protein complex (3). Nevertheless, it was found that the overall astaxanthin content under subcritical conditions at 100–140 °C remained constant even though some astaxanthin was extracted into the liquid solution.
Astaxanthin loss was determined as the difference between the astaxanthin content of raw krill and the sum of the contents of the extracts and residues. In the treatment temperature range of 160–240 °C, the levels of astaxanthin in both the extracts and residues decreased with increasing temperature, resulting in a sharp increase in astaxanthin loss, presumably due to degradation. Almost all the astaxanthin was lost at 220–240 °C (Fig. 6). Astaxanthin has previously been shown to be thermally labile, with Becerra et al. (24) reporting that 14.2 % of astaxanthin was degraded by boiling shrimp in brine (5 % NaCl, by mass per volume) for 15 min.

Odour intensity and sensory evaluation

Treatment temperature had a remarkable influence on the odour intensities of krill extracts and residues (Fig. 7). The odour intensity became stronger as the treatment temperature increased of both the extracts and residues. Degradation of proteins and other substances, which are precursors of odour compounds (25), can increase at higher treatment temperatures.

![Graph showing odour intensity and temperature](image)

Fig. 7. Odour intensity of the liquid extracts (O) and solid residues (Δ) obtained from subcritical water treatment of raw krill at various temperatures

Shrimp-like flavour intensity and preference scores of the krill extracts and residues at various treatment temperatures were measured (Fig. 8). The preference score expressed an indication of the overall quality of the extract or residue as a seasoning. Shrimp-like flavour intensity was evaluated as the magnitude of cooked shrimp aroma. Both the extract solutions and solid residues exhibited a shrimp-like flavour, but its subjective intensity, measured by panellists, did not correlate with the objective odour intensity, measured electronically (Fig. 7). The strongest shrimp-like flavour was detected in the extracts and residues prepared at 140–160 °C. These results are similar to those for the treatment of raw krill mixed with water (10). The temperature dependence of preference scores was similar to that of the shrimp-like-flavour intensity (Fig. 8). The extract and residue obtained at 140 °C had the highest values of both preference score and shrimp-like flavour intensity. Burnt odour, which is ascribed to very dark roast and ash-like substances, was recognised in extracts and residues prepared at temperatures of 180–240 °C, and the preference scores and shrimp-like flavour intensities for these treatment temperatures were low, especially for the extract and residue obtained at 240 °C. Consequently, treatment of the krill at 140 °C was the most promising for producing a seasoning with the desired shrimp-like flavour.

Fluoride mass fraction in krill, which has been reported to be as high as 1.37 and 2.40 mg per g of dry mass of Euphausia crystallorophias and Euphausia superba, respectively (26), raised concerns of utilizing of krill for direct human consumption because the Recommended Daily Allowance (RDA) values of fluoride are only 4 and 3 mg for male and female adults, respectively. However, using the extract and residue as seasoning ingredient should be possible because the amount that needs to be added to food products is usually low.

Conclusions

Raw isada krill, without any addition of water, was treated under subcritical water conditions in the tempera-
ture range of 100–240 °C. The yields of the liquid extract and solid residue increased and decreased, respectively, as the treatment temperature increased. The total contents of solid material and protein in the extracts were higher at higher treatment temperatures. Treatment at high temperatures (220–240 °C) caused degradation of high-molecular-mass substances. Degradation of astaxanthin in both the extracts and residues was significant at treatment temperatures above 160 °C. The highest preference score and shrimp-like flavour intensity were recorded for both the extract and residue prepared at 140 °C. Although suboptimal for overall yields, treatment at 140 °C would seem to be the most promising method for avoiding degradation of krill components, and for producing a shrimp-like flavour seasoning from the krill.

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