Astaxanthin stability and color change of krill during subcritical water treatment

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Abstract Pacific krill (*Euphausia pacifica*) contains high amounts of astaxanthin, a carotenoid pigment with strong antioxidative activities. In this study, the effects of subcritical water temperatures (100–180 °C) and times (0–10 min) on color changes (L^* , a^* , and b^*) and astaxanthin degradation in Pacific krill were investigated. In addition, an aqueous solution of pure astaxanthin and that of crude astaxanthin from Pacific krill, both at a concentration of 5 mg/L, were subjected to treatment under subcritical water conditions using a flow-type reactor to compare the degradation of free astaxanthin and astaxanthin fatty acid esters. To compare the results of the different treatment conditions on the properties of astaxanthin, the severity factor ($\log R_0$) was calculated, which ranged from 0.38 to 3.52. The extractability of astaxanthin enhanced when the subcritical water treatment was carried out at $\log R_0$ values of 2.00–2.44. In contrast, astaxanthin underwent 50% degradation at $\log R_0 > 2.44$. The changes in the a^* values correlated well with the astaxanthin content in the treated krill, while the b^* and L^* values might

relate to the browning components forming owing to Maillard reaction. The results show that free astaxanthin was less stable than crude astaxanthin under subcritical water treatment.

Keywords Astaxanthin · Pigment · Subcritical water · Color · Stability

Introduction

Pacific krill (*Euphausia pacifica*) is a small crustacean found in the North Pacific Ocean. Around 30,000–40,000 ton per year of Pacific krill had been commercially caught in the waters of Japan during 2005–2010 (Ministry of Agriculture, Forestry and Fisheries 2016). Though the harvested krill is majorly used for animal and aquaculture feed production, it is also used for human consumption and in the pharmaceutical industry (Nicol and Endo 1999). The red spots on the translucent pink shell of the fresh krill appear due to the presence of astaxanthin (3,3'-dihydroxy- β,β -carotene-4,4'-dione) and its fatty acid esters (Auerswald et al. 2008). Pacific krill is, therefore, an excellent source of oil with high polyunsaturated fatty acid and astaxanthin contents (Tou et al. 2007). Astaxanthin is also well known for its medicinal properties; for example, it prevents cardiovascular diseases and cataracts and also acts as an immune booster (Higuera-Ciaparara et al. 2007). However, astaxanthin is susceptible to degradation by oxidation and heat, and the pigment color change in *E. pacifica* is the most noticeable change observed during its thermal treatment. Niamnuy et al. (2008) reported a correlation between the decrease in the redness and the loss of astaxanthin in shrimp during drying. The effect of thermal treatment on

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pigment coloration can be quantified using color parameters or by the amount and nutritive value of astaxanthin (Nawar 1996).

Astaxanthin is a xanthophyll carotenoid and it is found in some microorganisms and marine animals such as salmon, shrimp, crab, and krill (Ambati et al. 2014). It is a fat-soluble, red pigment and possesses higher antioxidative activity than β -carotene and vitamin E (Kobayashi et al. 1997). Its stability in different carriers and under different storage conditions has been investigated. Astaxanthin in rice bran, gingelly, and palm oils was found to be stable at 70–90 °C with 84–90% of retention (Ambati et al. 2007), whereas it showed 50% degradation at 120 and 150 °C. Raposo et al. (2012) reported a loss of 90% of astaxanthin when subjected to spray drying at 180/110 °C (inlet/outlet temperatures, respectively) and stored at −21 °C under nitrogen for 9 weeks. The thermal degradation of astaxanthin in shrimp during different processing methods, i.e., boiling, vacuum drying, and hot-air drying, was also investigated, and it was observed that exposure to heat for even a short time accelerated the degradation of astaxanthin (Niamnuy et al. 2008).

Subcritical water extraction is an effective water-based method to extract both water-soluble and water-insoluble bioactive compounds (Hassas-Roudsari et al. 2009). Temperatures between 100 and 374 °C and pressure sufficient to maintain water in the liquid state are generally employed in subcritical water extraction (Ramos et al. 2002). This results in the modification of the properties of water, such as a reduction in its dielectric constant and increase in diffusivity with increasing temperature (Carr et al. 2011).

Previously, we have reported that the extracts and solid residues obtained from subcritical water treatment of Pacific krill can be used as a food ingredient (Koomyart et al. 2015a, b, 2016 Tamiya et al. 2016). However, color changes in the extract and solid residue during the subcritical water treatment have never been studied despite the fact that color is a very important factor influencing the acceptance of a product. Thus, the objective of the present study was to investigate the effects of the treatment temperature and time on the color change and stability of astaxanthin during subcritical water treatment of Pacific krill, using the severity factor ($\log R_0$) as a parameter for comparing the results of different treatment conditions. In addition, the stability of crude astaxanthin extracted from Pacific krill was compared with that of pure astaxanthin under subcritical water conditions. This quantitative analysis should be useful for optimizing the condition for preparing the ingredient from subcritical water treatment of Pacific krill.

Materials and methods

Materials

Frozen, raw Pacific krill (*Euphausia pacifica*) with an average weight of 0.1 ± 0.05 g and length of 18 ± 0.65 mm was purchased from Hamaichi (Wakayama, Japan). The frozen krill was stored at −30 °C until use. Astaxanthin (from algae, 98% purity), methanol, hexane, and acetone were purchased from Wako Pure Chemical Industries (Osaka, Japan). Sodium hydroxide was purchased from Nacalai Tesque (Kyoto, Japan).

Batch-type subcritical water treatment of krill

Subcritical water treatment of krill was conducted in a batch-type, pressure-resistant vessel (117 mL, Taiatsu Techno, Osaka, Japan). Thawed krill (30 g) and water (30 mL) were heated together in a tightly closed vessel using a 200 W mantle heater (Heater Engineer, Tokyo, Japan) connected to a TXN 700B thermo-controller (As One, Osaka, Japan) to maintain the temperature in the range of 100–180 °C. The temperature was measured by using a type K thermocouple inserted in the vessel. The time required to attain the desired temperature was 17–31 min (Table 1). The vessel was maintained at a constant temperature for 0–10 min before it was removed and cooled in an iced water bath. The liquid and solid fractions were separated and collected as described in our previous work (Koomyart et al. 2016). To assess the color and content of astaxanthin, the solid residue was frozen at −30 °C for 12 h, lyophilized at −50 °C and 90 Pa for 48 h, and then ground into a powder form using a mortar and pestle. All subcritical water treatments were conducted in duplicate.

Flow-type subcritical water treatment of crude and pure astaxanthin

The crude astaxanthin solution obtained from raw krill at a concentration of 5 mg/L (as free astaxanthin) was prepared in the same way as described in the “Astaxanthin analysis” section (without the saponification step). A sample of pure astaxanthin (2.5 mg) was dissolved in 2.5 mL of 95% ethanol and then diluted to 500 mL with distilled water to obtain a 5 mg/L solution. The solution was kept in a screw-capped bottle after flushing the head space with nitrogen gas. A stability test of astaxanthin was conducted under subcritical conditions in a flow-type reactor in a temperature range of 100–140 °C. The pure astaxanthin solution and the crude astaxanthin extract were released using an LC-10AT VP high-pressure pump (Shimadzu, Kyoto, Japan) through a tubular reactor (SUS316, 0.8 mm

Table 1 Severity factors ($\log R_0$) of subcritical water treatment at various temperatures and times

Reactor	T (°C)	Heat-up time (min)	Log R_0 at isothermal reaction time (min)					
			0	2	4	6	8	10
Batch-type	100	17	0.38	0.69	0.91	1.08	1.22	1.32
	120	21	1.04	1.31	1.51	1.65	1.76	1.85
	140	26	1.62	1.88	2.08	2.23	2.34	2.44
	160	28	2.37	2.55	2.69	2.80	2.88	2.95
	180	31	2.94	3.15	3.3	3.4	3.45	3.52
Flow-type	120		0.27	0.88				
	140		0.85	1.47				
	160		1.37	1.97				

i.d. $\times$ 2 m) immersed in an oil bath (BO400/410, Yamato Scientific, Tokyo, Japan) at 100, 120, or 140 °C, and flowed directly to the cooling coil immersed in an iced water bath to terminate the reaction immediately (Fig. 1). The pressure inside the reactor was maintained at 10 MPa by a back-pressure valve (Upchurch Scientific, Oak Harbor, WA, USA). The treatment time (30, 60, 90, and 120 s) was set by adjusting the flow rate in accordance with the densities of water at ambient and treatment temperatures. The concentrations of astaxanthin in the feed (C_0) and reactor effluent (C) were measured using high-performance liquid chromatography (HPLC).

Severity factor

The severity factor ($\log R_0$) is used to express the combined effect of temperature and time as a single parameter (Ruiz et al. 2013). It is calculated using Eq. 1 as follows:

$$\log R_0 = \log \left(\int_0^t \exp \left[\frac{T(t) - 100}{14.75} \right] dt \right) \quad (1)$$

where t is the reaction time (min), T is the temperature (°C), 100 is the reference temperature, and 14.75 is an empirical parameter related to activation energy, assuming pseudo-first order kinetics. Table 1 shows $\log R_0$ values for each subcritical water treatment condition in both batch- and flow-type reactors.

Color measurement

The color of the raw krill, krill liquid extract, and solid residue obtained from subcritical treatment were measured with an SA4000 color-difference meter (Nippon Denshoku Industries, Tokyo, Japan) using a D65 light source and at an observed angle of 10° using the dried sample (1.5 g) or liquid solution (3 mL) in a sample glass cup (16 mm i.d. $\times$ 28 mm height). The sample glass cup was placed on a sample port and covered with black plastic before the color parameters (L^* : lightness, a^* : redness, b^* : yellowness) were recorded. The total color differences (ΔE^*) were calculated using the following Eq. (2):

Fig. 1 Schematic diagram of flow-type apparatus for subcritical water treatment

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

where L_0^* (61.5, 81.0), a_0^* (12.0, 0), and b_0^* (9.4, 0) are the reference values obtained from untreated krill and pure water, respectively.

Astaxanthin analysis

Astaxanthin is the most abundant carotenoid in krill and is typically present as fatty acid esters (Takaichi et al. 2003). Before quantification with conventional HPLC, astaxanthin was hydrolyzed to remove the fatty acids. A solution of sodium hydroxide in methanol is commonly used for the base-catalyzed saponification to hydrolyze astaxanthin esters to obtain free astaxanthin (Yuan and Chen 1999).

The solid residue or liquid extract from the krill treatment was extracted and saponified according to the methodology adopted by Yuan and Chen (2000) with some modifications. Freeze-dried residue powder (1.0 g) or the liquid extract (5 mL) was mixed with 10 mL of a solution of dichloromethane and methanol (1:3 v/v) in a screw-capped vial. The headspace was flushed with nitrogen and the mixture was stirred in the dark for 3 h. The mixture was then centrifuged at $9600 \times g$ for 5 min at 4 °C. The precipitated residue or the upper layer was removed from the solution containing astaxanthin. The volumes of astaxanthin-containing solutions obtained from the residue and the extract were adjusted to 10 and 5 mL, respectively, with the extraction solvent. Then, the astaxanthin solution (5 mL) was saponified by mixing with 0.1 mol/L solution of sodium hydroxide in methanol (1 mL). The vial was flushed with nitrogen and maintained at 4 °C in the dark for 12 h. The solvent was then removed by evaporation under a stream of nitrogen. The dry and free astaxanthin was dissolved in chloroform for HPLC analysis.

The HPLC system comprised an LC-10AD VP pump, an ultraviolet–visible light detector (SPD-10A) (Shimadzu, Kyoto, Japan), and a Luna Silica(2) column (3 μ m, 100 Å, 150×4.6 mm, Phenomenex, Torrance, CA, USA). The mobile phase comprised a mixture of hexane and acetone (82:18 v/v), and the flow rate was 1.2 mL/min. 20 μ L of the sample was injected at ambient temperature and astaxanthin was monitored at 474 nm. Standard solutions of astaxanthin (5–25 mg/L) were used to plot a calibration curve. The amounts of astaxanthin in the raw krill (A_{Raw}), liquid extract (A_{Ext}), and solid residue (A_{Res}) were used to calculate the percentage loss of astaxanthin during the subcritical water treatment as follows:

$$\text{Astaxanthin loss, (\%)} = \frac{A_{Raw} - (A_{Ext} + A_{Res})}{A_{Raw}} \times 100 \quad (3)$$

Results and discussion

Subcritical water treatment of krill was carried out under various conditions to evaluate the degradation of astaxanthin and to observe changes in the color of krill. The effect of different temperatures and times employed in the treatment was expressed as the severity factor ($\log R_0$), as shown in Table 1. The values of $\log R_0$ ranged from 0.38 to 3.51. Other researchers have also used the severity factor to describe the changes in several responses during the subcritical water treatment or similar thermal treatments of biomaterials (Kim et al. 2013, 2014; Khuwijitjaru et al. 2014).

Color change during subcritical water treatment

We have proposed that the liquid extract and solid residue obtained from subcritical water treatment of krill can be used to give shrimp-like flavor to food (Koomyart et al. 2015a, b, 2016). Color is an important factor that can affect the acceptance of such ingredients.

Figure 2 depicts the images of the solid residues and liquid extracts obtained at different $\log R_0$ values. The color changes were observed in both the solid residue and the liquid extract. At low values of $\log R_0$ (0.38–1.00), the solid residue slightly changed color from light pink as in the raw krill to reddish-pink, while the liquid extract turned a brighter orange with the increase in $\log R_0$. At $\log R_0 > 1$, the solid residues and liquid extracts turned yellowish-brown and reddish-brown, respectively. The reddish-pink and orange colors of the residue and the extract, respectively, could be attributed to the release of astaxanthin from the carotene-protein complex when the protein was denatured by heat (Maoka 2011) at low $\log R_0$, while the brown color of the products can be explained by the Maillard reaction occurring during the treatments at high $\log R_0$ values.

Figure 3 depicts the plots of L^* , a^* , b^* , and ΔE^* against $\log R_0$ for liquid extracts and solid residues. Figure 3a shows changes in the a^* value, manifested as difference in the shades of red coloration, during the subcritical water treatment at different values of $\log R_0$. At $\log R_0 < 1$, the solid residue exhibited an increase in the a^* value, corresponding to the development of red color. This reflects the release of astaxanthin from the carotene-protein complex, as discussed previously. However, a^* values of the solid residue exhibited a decreasing trend at $\log R_0 > 1$, probably owing to the migration of astaxanthin into the solution, which earlier increased the redness of the liquid solution. Furthermore, the rapid decrease in the a^* values of the liquid extracts at $\log R_0 > 2$ can be attributed to the degradation of astaxanthin; the details are provided later.

Fig. 2 Appearances of the solid residue and liquid extract obtained by subcritical water treatment of raw Pacific krill at various $\log R_0$ values

Liquid
Solid
$\log R_0$	0	0.38	1.65	1.88	2.95	3.15	3.52
	Raw krill	100 °C 0 min	120 °C 6 min	140 °C 0 min	160 °C 10 min	180 °C 2 min	180 °C 10 min

Fig. 3 Changes in the **a** a^* , **b** b^* , **c** L^* and **d** ΔE^* values of the solid residue (closed symbols) and liquid extract (open symbols) during subcritical water treatment of Pacific krill as a function of severity factor ($\log R_0$)

Figure 3b depicts the plots between b^* , whose positive value represents yellowness, and $\log R_0$ values. The b^* values of the solid residues gradually increased with $\log R_0$ and reached a plateau at $\log R_0 > 2.5$, while those of the liquid extract continually increased with $\log R_0$. As the $\log R_0$ value increased, both the light-orange liquid extract and the light-pink solid residue became reddish-brown (Fig. 2). This is equivalent to a shift to yellowness, which can be explained by a higher rate of Maillard reaction at higher $\log R_0$ values during the treatment. The increased amount of the brown product formed by the Maillard reaction was reflected by the lightness value, L^* (Fig. 3c). An increase in the $\log R_0$ values caused the solid residue and liquid extract to turn darker. The color change was reflected by a gradual decrease in the L^* value. The value of the color difference (ΔE^*), which represents an overall difference of color between a sample and a reference, significantly

increased with the increase in the treatment severity (Fig. 3d). At $\log R_0 = 3.52$, the maximum ΔE^* values attained by the solid residue and the liquid extract were 22 and 10, respectively.

Astaxanthin content during the subcritical treatment

Extensive Maillard reaction occurs during the subcritical water treatment of biomaterials and influences the color change in the treated product. However, the relationship between the amount of natural pigments such as astaxanthin and the associated color change of the product during subcritical water treatment has not been reported.

Figure 4 shows the HPLC chromatograms of astaxanthin both in the liquid extract and the solid residue, obtained by the subcritical water treatment of krill. The

Fig. 4 HPLC chromatograms of astaxanthin in liquid extract and solid residue obtained by subcritical water treatment of Pacific krill. **a** Standard astaxanthin, **b** unsaponified astaxanthin of krill extract at $\log R_0$ 0.38, **c** unsaponified astaxanthin of krill residue at $\log R_0$ 0.38, **d** unsaponified astaxanthin of krill residue at $\log R_0$ 3.52, and **e** saponified astaxanthin of krill residue at $\log R_0$ 0.38

HPLC analysis enables classification of free astaxanthin and astaxanthin fatty acid esters. Pure astaxanthin appeared as a large peak of the *all-trans* astaxanthin isomer (Grynbaum et al. 2005) at a retention time of 5.6 min. A small peak was observed at 5–6 min (Fig. 4a), which should correspond to the *cis*-isomers because astaxanthin is readily isomerized. The chromatogram of the unsaponified sample obtained from the treatment at $\log R_0 = 0.38$ showed four peaks of astaxanthin esters at retention times of 1.8–4.0 min and two peaks of free astaxanthin (Fig. 4b, c). However, the peaks corresponding to free astaxanthin disappeared for the treatment at $\log R_0 = 3.52$ (Fig. 4d). The saponified sample showed two large and two small peaks of free astaxanthin (Fig. 4e). This is because astaxanthin obtained from the krill consisted of many isomers, particularly 9-*cis*- and 13-*cis*-astaxanthins (Grynbaum et al. 2005). The content of free astaxanthin in the raw krill, as calculated from the mass difference between the saponified and unsaponified samples, was 3.4 ± 0.9 mg/kg-raw krill, which was equal to 18% of the total astaxanthin content, whereas that in Antarctic krill (*Euphausia superba*) is within 7–22% of the total astaxanthin content (Takaichi et al. 2003; Grynbaum et al. 2005; Maoka et al. 1985; Yamaguchi et al. 1983).

The astaxanthin contents in the raw krill, solid residue, and liquid extract, and the total astaxanthin content

calculated as the sum of the astaxanthin contents of the extracts and residues are presented in Fig. 5. The total astaxanthin content in the raw krill was found to be 18.41 ± 1.56 mg/kg-raw krill, while the krill subjected to subcritical water treatment at $\log R_0 = 0.38$ –3.52 contained astaxanthin in the range of 5.17–15.21 mg/kg-raw krill or 28–83% of the initial value. The gradual loss in the total astaxanthin content with $\log R_0$ indicated a significant degradation of astaxanthin during the subcritical water treatment. Other researchers also reported the loss of astaxanthin in other samples. For example, Yang et al. (2015) reported total astaxanthin losses of 2.35, 35.73, and 64% after thermal processing with microwave, boiling, and frying, respectively, for 3 min.

The astaxanthin content in the liquid extract gradually increased on increasing the $\log R_0$ values from 0.38 to 2.44. The highest astaxanthin content of 4.32 ± 0.60 mg/kg-raw krill was achieved at $\log R_0 = 2.44$. This gradual increase in the astaxanthin content can be explained by the fact that the increasing extraction temperature decreased the polarity or dielectric constant of water, which facilitated the extractability of astaxanthin, a nonpolar compound, in water (Khuwjitjaru et al. 2002). However, a decrease in the astaxanthin content of both the solid residue and liquid extract was observed at higher $\log R_0$ values. It should be noted that changes in the astaxanthin content in both the liquid extracts and solid residues showed a similar pattern as that observed for the changes in the a^* values, discussed previously.

Fig. 5 Total astaxanthin contents (dotted-line symbols), and astaxanthin contents in the solid residues (open symbols) and liquid extract (closed symbols) obtained by subcritical water treatment at various $\log R_0$ values. Inset shows the crude extract of astaxanthin of solid krill residue (upper row) and liquid krill extract (lower row)

Stability of astaxanthin

Figure 6 shows the astaxanthin losses during the batch-and flow-type treatment under subcritical water conditions at various $\log R_0$ values. The loss of astaxanthin in any system showed an approximate linear relationship with $\log R_0$. This suggests that the $\log R_0$ value is useful for predicting astaxanthin degradation in subcritical water treatment. The results also indicate that in the batch-type treatment, the natural astaxanthin in krill, which is mostly present as fatty acid esters and forms a complex with protein, was more resistant to degradation than the astaxanthin esters and free astaxanthin in the crude extract and pure solution, respectively. The complexation of astaxanthin with protein prevents its degradation, and the degradation might occur after the protein complex is broken down. When the $\log R_0$ value was increased from 0.27 to 1.97, pure astaxanthin degraded by 24–86%, while crude astaxanthin degraded by 18–78%. Schweiggert et al. (2007) also reported that the esterified carotenoid was more stable to thermal processing than the nonesterified one.

Conclusion

The effects of subcritical water treatment conditions as represented by the severity factor ($\log R_0$) on the color change and astaxanthin content in the solid residue and liquid extract of Pacific krill were investigated. Higher $\log R_0$ values enhanced the extractability of astaxanthin. However, more than 70% of astaxanthin in the krill degraded during treatment at $\log R_0 > 3.00$. It was also observed that the degradation of astaxanthin in its natural fatty acid ester form was slower than that in its free form.

Fig. 6 Astaxanthin loss of natural astaxanthin in krill, crude extract, and pure astaxanthin solution during the treatment under subcritical water conditions at various $\log R_0$ values

This information is useful for industry to develop a stable form of astaxanthin ingredient.

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References

- Ambati RR, Ravi S, Aswathanarayana RG (2007) Stabilization of astaxanthin in edible oils and its use as an antioxidant. *J Sci Food Agric* 87(6):957–965
- Ambati RR, Moi PS, Ravi S, Aswathanarayana RG (2014) Astaxanthin: sources, extraction, stability, biological activities and its commercial applications—a review. *Mar Drugs* 12(1):128–152
- Auerswald L, Freier U, Lopata A, Meyer B (2008) Physiological and morphological colour change in Antarctic krill. *Euphausia superba*: a field study in the Lazarev Sea. *J Exp Biol* 211:3850–3858
- Carr AG, Mammucari R, Foster NR (2011) A review of subcritical water as a solvent and its utilisation for the processing of hydrophobic organic compounds. *Chem Eng J* 172(1):1–17
- Grynbaum MD, Hentschel P, Putzbach K, Rehbein J, Krucker M, Nicholson G, Albert K (2005) Unambiguous detection of astaxanthin and astaxanthin fatty acid esters in krill (*Euphausia superba* Dana). *J Sep Sci* 28(14):1685–1693
- Hassas-Roudsari M, Chang PR, Pegg RB, Tyler RT (2009) Antioxidant capacity of bioactives extracted from canola meal by subcritical water, ethanolic and hot water extraction. *Food Chem* 114:717–726
- Higuera-Ciaparara I, Félix-Valenzuela L, Goycoolea FM (2007) Astaxanthin: a review of its chemistry and applications. *Crit Rev Food Sci Nutr* 46(2):185–196
- Khuwijitjaru P, Adachi S, Matsuno R (2002) Solubility of saturated fatty acids in water at elevated temperatures. *Biosci Biotechnol Biochem* 66(8):1723–1726
- Khuwijitjaru P, Pokpong A, Klinchongkon K, Adachi S (2014) Production of oligosaccharides from coconut meal by subcritical water treatment. *Int J Food Sci Technol* 49(8):1946–1952
- Kim DS, Myint AA, Lee HW, Yoon J, Lee YW (2013) Evaluation of hot compressed water pretreatment and enzymatic saccharification of tulip tree sawdust using severity factors. *Bioresour Technol* 144:460–466
- Kim Y, Kreke T, Mosier NS, Ladisch MR (2014) Severity factor coefficients for subcritical liquid hot water pretreatment of hardwood chips. *Biotechnol Bioeng* 111:254–263
- Kobayashi M, Kakizono T, Nishio N, Nagai S, Kurimura Y, Tsuji Y (1997) Antioxidant role of astaxanthin in the green alga *Haematococcus pluvialis*. *Appl Microbiol Biotechnol* 48(3):351–356
- Koomyart I, Nagamizu H, Khuwijitjaru P, Kobayashi T, Shiga H, Yoshii H, Adachi S (2015a) Subcritical water treatment for producing seasoning from semidried Isada krill. *J Food Process Eng* 37(6):567–574
- Koomyart I, Nagamizu H, Khuwijitjaru P, Kobayashi T, Shiga H, Yoshii H, Adachi S (2015b) Compositions, flavor and antiradical properties of products from subcritical water treatment of raw Isada krill. *Int J Food Sci Technol* 50(7):1632–1639
- Koomyart I, Nagamizu H, Khuwijitjaru P, Kobayashi T, Shiga H, Yoshii H, Adachi S (2016) Direct treatment of isada krill under subcritical water conditions to produce seasoning with shrimp-like flavor. *Food Technol Biotechnol* 54(3):335–341

- Maoka T (2011) Carotenoids in marine animals—review. *Mar Drugs* 9(2):278–293
- Maoka T, Katsuyama M, Kaneko N, Matsuno T (1985) Stereochemical investigation of carotenoids in the Antarctic krill *Euphausia superba*. *Bull Jpn Soc Sci Fish* 51:1671–1673
- Ministry of Agriculture, Forestry and Fisheries (2016) Statistic Report of Fisheries and Aquaculture. Ministry of Agriculture, Forestry and Fisheries, Tokyo
- Nawar WW (1996) Lipids. In: Fennema OR (ed) *Food Chem*. Marcel Dekker Inc, New York, pp 225–314
- Niamnuy C, Devahastin S, Soponronnarit S, Vijaya Raghavan GS (2008) Kinetics of astaxanthin degradation and color changes of dried shrimp during storage. *J Food Eng* 87(4):591–600
- Nicol S, Endo Y (1999) Krill fisheries: development, management and ecosystem implications—review. *Aquat Living Resour* 12:105–120
- Ramos L, Kristenson EM, Brinkman UAT (2002) Current use of pressurized liquid extraction and subcritical water extraction in environmental analysis. *J Chromatogr A* 975(1):3–29
- Raposo MFJ, Morais AMMB, Morais RSC (2012) Effects of spray drying and storage on astaxanthin content of *Haematococcus pluvialis* biomass. *World J Microbiol Biotechnol* 28(3):1253–1257
- Ruiz HA, Rodríguez-Jasso RM, Fernandes BD, Vicente AA, Teixeira JA (2013) Hydrothermal processing, as an alternative for upgrading agriculture residues and marine biomass according to the biorefinery concept: a review. *Renew Sust Energy Rev* 21:35–51
- Schweiggert U, Kurz C, Schieber A, Carle R (2007) Effects of processing and storage on the stability of free and esterified carotenoids of red peppers (*Capsicum annuum* L.) and hot chilli peppers (*Capsicum frutescens* L.). *Eur Food Res Technol* 225(2):261–270
- Takaichi S, Matsui K, Nakamura M, Muramatsu M, Hanada S (2003) Fatty acids of astaxanthin esters in krill determined by mild mass spectrometry. *Comp Biochem Physiol B* 136(2):317–322
- Tamiya S, Koomyart I, Kobayashi T, Shiga H, Yoshii H, Khuwijitjaru P, Adachi S (2016) Preparation of liquid and solid seasonings with shrimp-like flavor from Isada krill under subcritical water conditions by steam injection. *Food Sci Technol Res* 22(3):317–323
- Tou JC, Jaczynski J, Chen Y (2007) Krill for human consumption: nutritional value and potential health benefits. *Nutr Rev* 65(2):63–77
- Yamaguchi K, Miki W, Toriu N, Kondo Y, Murakami M, Konosu S, Satake M, Fujita T (1983) The Composition of carotenoid pigments in the Antarctic krill *Euphausia superba*. *Bull Jpn Soc Sci Fish* 49:1411–1415
- Yang S, Zhou Q, Yang L, Xue Y, Xu J, Xue C (2015) Effect of thermal processing on astaxanthin and astaxanthin esters in Pacific white shrimp *Litopenaeus vannamei*. *J Oleo Sci* 64(3):243–253
- Yuan JP, Chen F (1999) Hydrolysis kinetics of astaxanthin esters and stability of astaxanthin of *Haematococcus pluvialis* during saponification. *J Agric Food Chem* 47(1):31–35
- Yuan JP, Chen F (2000) Purification of *trans*-astaxanthin from a high-yielding astaxanthin ester-producing strain of the microalga *Haematococcus pluvialis*. *Food Chem* 68(4):443–448