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Characterization of oil including astaxanthin extracted from krill (*Euphausia superba*) using supercritical carbon dioxide and organic solvent as comparative method

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Abstract–Krill oil including astaxanthin was extracted using supercritical CO_2 and hexane. The effects of different parameters such as pressure (15 to 25 MPa), temperature (35 to 45 °C), and extraction time, were investigated. The flow rate of CO_2 (22 $gmin^{-1}$) was constant for the entire extraction period of 2.5 h. The maximum oil yield was found at higher extraction temperature and pressure. The oil obtained by SC-CO_2 extraction contained a high percentage of polyunsaturated fatty acids, especially EPA and DHA. The acidity and peroxide value of krill oil obtained by SC-CO_2 extraction were lower than that of the oil obtained by hexane. The SC-CO_2 extracted oil showed more stability than the oil obtained by hexane extraction. The amount of astaxanthin in krill oil was determined by HPLC and compared at different extraction conditions. The maximum yield of astaxanthin was found in krill oil extracted at 25 MPa and 45 °C.

Key words: Krill, Oil, Astaxanthin, Supercritical Carbon Dioxide Extraction, Hexane

INTRODUCTION

Krill is considered by many scientists to be the largest biomass in the world, with the vast majority harvested for feed for fish farms and a small percentage for human consumption. Lipids of the Antarctic krill, *Euphausia superba* as in aquatic organisms are generally rich in highly unsaturated fatty acids (UFAs) and phospholipids, which have attracted much attention for health benefits [1]. There is a commercial interest in obtaining polyunsaturated fatty acids (PUFAs). Recently, attention has focused on n-3 PUFAs, especially eicopentaenoic acid (EPA, C 20:5 n-3) and docosahexaenoic acid (DHA, C 22:6 n-3), due to their association with the prevention and treatment of several diseases [2]. Studies show that a diet rich in omega-3 fatty acids may help lower triglycerides and increase HDL cholesterol (the good cholesterol). Omega-3 fatty acids may also act as an anticoagulant to prevent blood from clotting. Several other studies also suggest that these fatty acids may help lower high blood pressure [3].

Carotenoid is a generic name used to designate the most common groups of naturally occurring pigments found in the animal and plant kingdoms. Carotenoids are considered suitable as components of various types of products due to their high antioxidant activity, e.g., cancer prevention agents, potential life extenders, inhibiting agents for heart attack and coronary artery disease [4-6]. In addition to Omega-3 fatty acids, Krill oil consists of three components: omega-3 fatty acids similar to those of fish oil, omega-3 fatty acids attached to phospholipids, mainly phosphatidylcholine and natural fat soluble antioxidant, astaxanthin, which is one of the most effective carotenoids whose antioxidant activity is 10 times stronger than those of any other carotenoids such as zeaxanthin, lutein, canthaxanthin and β-carotene and was up to 500 times stronger than vitamin E [7].

Conventional methods based on solvent extraction from natural matrices are time consuming as they involve multiple extraction steps and require large amounts of organic solvents that are often expensive and potentially hazardous [8]. The problems associated with traditional solvent extraction techniques have aroused growing interest in developing simpler, faster, more efficient methods for the extraction of carotenoids from foods and natural products [9,10]. Degradation and decomposition of thermolabile compounds cannot be avoided in a conventional extraction method, since relatively high temperatures are required for these processes. Organic solvents are also harmful to human health as well as the environment.

In recent years, supercritical fluid extraction (SFE) has proved one of the most appealing techniques for solid sample treatment. In fact, supercritical fluids diffuse more readily into matrices than to ordinary liquids, thereby improving the extraction yields of analytes from complex matrices. The SFE technique is a desirable alternative to the solvent extraction of some classes of natural substances from foods [6,11]. Supercritical fluids possess excellent extractive properties such as high compressibility, liquid-like density, low viscosity, high diffusivity [12]. One advantage of supercritical CO_2 (SC-CO_2) relative to traditional organic solvents is that it can be used at a moderate temperature; this allows carotenoid losses through heat-induced degradation to be reduced. In addition, because it avoids the use of organic solvents, the extracted compounds can be employed as nutritional additives and in pharmacological applications [5]. The application of extraction with SC-CO_2 has been widely used in many industrial applications: decaffeination of coffee, extraction of hops and carotenoids, and synthesis of polymers, purification

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and formation of nano particles [12,13]. Yamaguchi et al. [1] reported that when applying SC-CO_2 extraction to krill samples, the extracted oils were composed solely of nonpolar lipids without contamination by phospholipids and their deteriorated lipids. Previous studies also investigated SC-CO_2 extraction of oils rich in PUFAs introducing this technology, as a clean technology, with negligible environmental effect [14,15]. On the other hand, a limitation of SC-CO_2 is that it often fails in quantitative extraction of polar analytes from solid matrices, because of its poor solvating power and the insufficient interaction between SC-CO_2 and the matrix [16]. Polar cosolvent such as ethanol is often used to enhance the solute solubility in SC-CO_2 by interacting with the solute, and thus improving the extraction efficiency. SC-CO_2 can extract majority of astaxanthin without ethanol from matrices containing high lipid, since astaxanthin molecule is lipid soluble and considered containing no strong polar moieties [5]. In our work, we have avoided the use of the cosolvent to prevent products quality; since heat is required to separate the organic solvent and, also, for the measurement of oil stability at different extraction conditions.

Thus, the purpose of this study was to obtain extraction data of krill using SC-CO_2 determined at various conditions (from 35 to 45 °C and from 15 to 25 MPa). At the optimal condition, analyses of extracts were conducted. Extraction yield of extracts (fatty acids, astaxanthin) at different conditions was compared with traditional organic solvents. Also, the stability of oil obtained by SC-CO_2 extraction was also compared to the oil obtained by soxhlet extraction with hexane.

MATERIALS AND METHODS

1. Materials

The krill (*Euphausia superba*) were collected from Dongwon F & B Co., S. Korea. The krill blocks were stored at −80 °C for no longer than 1 year before being used experimentally. The carbon dioxide (99.99% pure) was supplied by KOSEM, Korea. All other chemicals used in different analysis were of analytical or HPLC grade.

2. Sample Preparation

The krill samples (mean body length, 5.15 cm; mean body weight, 0.65 g) were dried in a freeze-drier for about 72 h. The dried samples were crushed and sieved (700 μm) by mesh. The dried samples, called freeze dried raw krill, were then stored at −80 °C until used for SC-CO_2 and organic solvent extraction.

3. SC-CO_2 Extraction

The extraction scale of SFE process used in this work, shown in Fig. 1, consisted of a pump (ILSHIN Metering, Korea) with a maximum capacity of about 30 MPa, a water bath (DW-15 S, Dongwon Scientific system), a chiller (DW-N30L, Dongwon Scientific system, Korea), an extraction cell and a wet gas meter (WNK-1A, Sinagawa Corp., Tokyo). Thirty five grams of freeze dried krill sample was applied in 200 mL stainless steel extraction vessel containing a thin layer of cotton at the bottom. Before plugging with cap another layer of cotton was used at the top of the sample. CO_2 was pumped into the vessel by high pressure pump up to the desired pressure, which was regulated by a back pressure regulator. The vessel temperature was maintained by a heater. Flow rates and accumulated gas volume passing through the apparatus were measured with a gas flow meter. The flow rates of CO_2 were kept constant

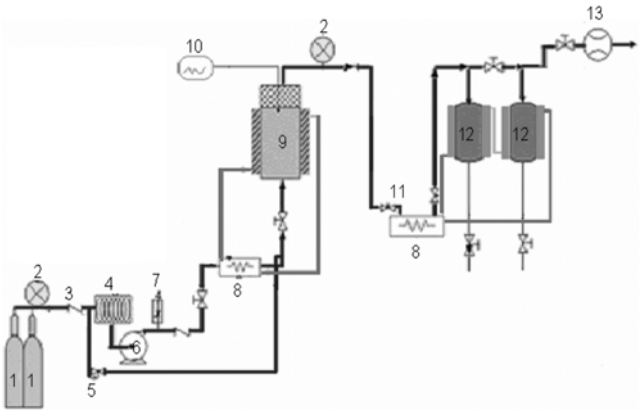


Fig. 1. The schematic diagram of the process of supercritical carbon dioxide extraction.

1. CO_2 tank	8. Heat exchanger
2. Pressure gauge	9. Extractor
3. Check valve	10. Temperature indicator
4. Cooling bath	11. Metering valve
5. Back pressure regulator	12. Separator
6. Pump	13. Gas flow meter
7. Safety valve	

at 22 g/min for all extraction conditions. A cyclone separating vessel was used for the collection of the oil extracted by SC-CO_2. The amount of extract obtained at regular intervals of time was established by weight (g) using a balance with a precision of ±0.001 g. The extracted oil and krill residues were then stored at −80 °C until further analysis and used.

The effects of temperature and pressure on oil extraction from krill were determined at 35-45 °C and 15-25 MPa at a constant extraction time of 2.5 h. To prevent the activity of bioactive compounds in both the extracts and extracted residues, the extractions were performed at low temperature.

4. Soxhlet Extraction by Hexane

To compare the extraction strength of SC-CO_2 with conventional organic solvent extraction, soxhlet extraction was selected. The extraction was carried out in a soxhlet apparatus using hexane as solvent. Three grams of freeze dried raw krill sample was placed into the extraction thimble (28×100 mm, Advantec, Tokyo, Japan) and the extraction was run for 12 h until the color of the condensed solvent at the top of the apparatus was clear. The sample was then dried in the oven at 80±1 °C for 2 h, after which it was cooled in desiccators before reweighing. After that, the extracted oil was stored at −20 °C until further analysis.

5. Determination of Extraction Yield

The weight of the initial freeze-dried krill prior to the extraction with different extraction processes was recorded. Following extraction, the weight of extracted oil was also recorded. The extraction yield was determined according to the following equation:

$$\text{Oil yield} = \frac{\text{weight of extracted krill oil (g)}}{\text{weight of freeze-dried krill subjected to extraction (g)}} \times 100$$

6. Gas Chromatography Analysis for Fatty Acid Compositions

The fatty acid profiles of both krill oil obtained by SC-CO_2 and organic solvent, hexane extraction were analyzed by gas chroma-

tography using a Hewlett Packard gas chromatograph (5890 Series II GC system). The fatty acid methyl esters were prepared according to AOCS official method Ce 2-66 [17]. An Agilent equipped with a flame ionization detector (FID) and DB-Wax capillary column (30 m length×0.250 mm internal diameter, 0.25 μm of film). Carrier gas of fatty acid methyl esters was nitrogen at a flow rate 1.0 mL/min. The column temperature was raised from a constant temperature of 130 °C for 3 min, and then increased to 240 °C at a rate of 4 °C/min and hold at 240 °C for 10 min. The split ratio was fixed at 50 : 1. Both injector and detector temperatures were 250 °C. Fatty acid methyl esters were identified by comparison of retention time with standard fatty acid methyl esters mixture (Supleco, USA).

7. Measurement of Oil Stability

Fats and oils are prone to oxidation. The rapidity of oxidation depends on the degree of unsaturation, the presence of antioxidants, and prior storage conditions [18]. Several methods are used to determine the stability of oil. In our study, oil stability was measured by evaluating free fatty acid content and peroxide value.

7-1. Free Fatty Acid Content of Extracted Oils

Free fatty acids (FFA) of krill oils were determined twice for each sample, and the average values are reported. As described by Bernardez et al. [19], precisely 50 mg of oil was placed into Pyrex tubes with the addition of 3 mL of cyclohexane. Then, 1 mL of cupric acetate-pyridine reagent was added, and tubes were vortexed for 30 sec. After centrifugation at 2,000 g for 10 min, the upper layer was read at 710 nm. The measurement of FFA content of oils was based on a calibration curve obtained from oleic acid standard.

Copper reagent was prepared according to Lowry and Tinsley [20]. Briefly, 5% (w/v) aqueous solution of cupric acetate was prepared and, after filtration, the pH of cupric acetate solution was adjusted to 6.1 using pyridine.

7-2. Peroxide Value

Peroxide value was determined according to the AOCS (American Oil Chemists' Society) method Cd 8-53 [17] with modified amount of sample taken. 1.0 g of krill oil was dissolved in 6 mL of acetic acid-chloroform (3 : 2) solution. Then 0.1 mL of saturated potassium iodine (KI) solution was added to the mixtures and the solution was allowed to stand with occasional shaking for 1 min. Distilled water (6 mL) was immediately added to the solution to allow the mixture to stand. The solution was titrated with 0.1 N of sodium thiosulfate until the yellow iodine color had almost disappeared. Then 0.4 mL of starch indicator solution was added with shaking to extract iodine from chloroform layer, and again titrated until the blue color disappeared. A blank determination was performed with the same procedure. Peroxide value was expressed as milliequivalents peroxide/1,000 g sample.

7-3. Color

The color of the extracted oils was measured in triplicate by means of reflectance spectra in a spectrophotometer (Lovibond, USA). For measurements, samples were placed in a white cup and covered with optical glass. CIE L*a*b* color coordinates (considering standard illuminant D65 and observer 10°) were then calculated. Color changes were measured by the lightness (L*) and the coordinates greenness-redness (a*) and blueness-yellowness (b*).

8. Astaxanthin Analysis by High Pressure Liquid Chromatography

A Waters model 600E system controller (Milford, USA) high pressure liquid chromatograph (HPLC) equipped with a model 484 UV/VIS detector and an Eclipse Plus C18 column (5 μm, 4.6×250 mm, Agilent, USA) were used for astaxanthin analysis. Elution was carried out using a mobile isocratic phase prepared with 10% dichloromethane, 85% ethanol and 5% acetonitrile. Flow rate was 1 mL per minute [21]. Astaxanthin was detected at the wavelength of 470 nm. The amount of astaxanthin in the extract was measured based on the peak area of the standard astaxanthin. For astaxanthin content determination, an external standard (Sigma Chemical Co., St. Louis, MO, USA) was used. The standard was dissolved in a mixture to prepare further dilutions to build the calibration curve (Fig. 2). The extraction efficiency was investigated by the determination of the total amount of astaxanthin in samples using soxhlet extraction with dichloromethane as solvent. The ratio between the amount of astaxanthin in the extracts and the total amount in the samples was defined as the extraction efficiency.

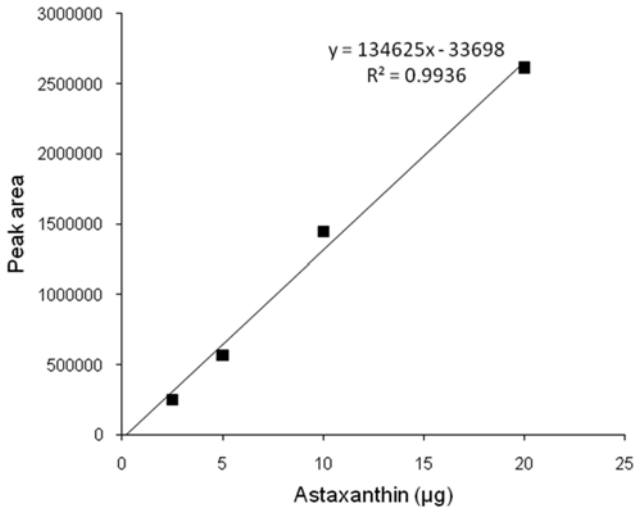


Fig. 2. Calibration curve of standard astaxanthin for estimation of astaxanthin content.

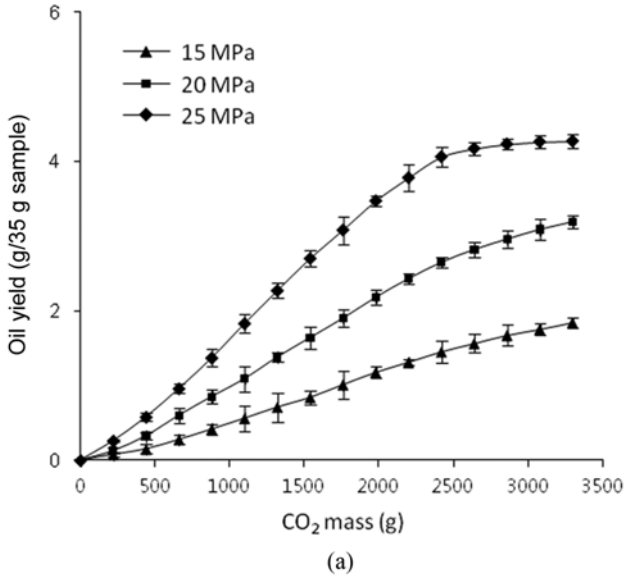
9. Statistical Analysis

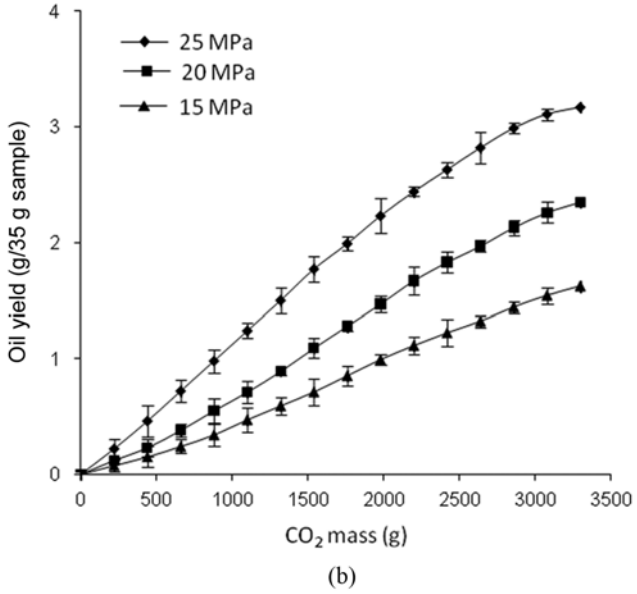
All extractions and analysis of samples from each extraction were done in duplicate in randomized order and means were reported. Data were evaluated by Duncan's multiple range test using SAS 9.1 (SAS Institute Inc., Cary, NC, USA) to evaluate differences in mean values. The least significant difference at the 95% confident level was calculated for each parameter.

RESULTS AND DISCUSSION

1. SC-CO_2 Extraction

The extracted oil from krill was fluid and bright red due to the presence of astaxanthin in it [1]. Fig. 3(a), (b) and (c) present the SC-CO_2 extraction curves of krill oils at different temperatures (45, 40 and 35 °C) and pressure (25, 20 and 15 MPa). The extraction yields ranged from 4.1 to 12.2% depending on the experimental conditions. The highest yield obtained was 4.27±0.08 g/35 g of krill at temperature, 45 °C and pressure, 25 MPa. Ooi et al. [22] mentioned that SC-CO_2 pressure affects both yield and solubility of palm oil. They showed that at higher pressures, the yield increased with temperature from 323.2 to 338.2 K and they attributed this to the





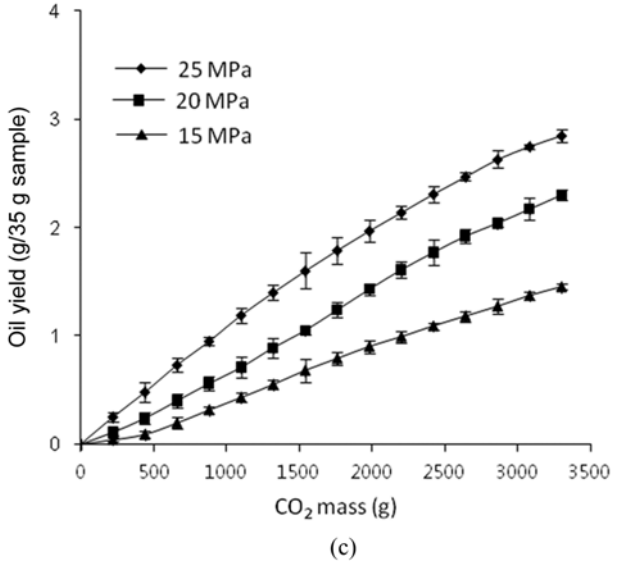


Fig. 3. (a)-(c) SC-CO_2 of oil from krill at different temperatures. (a) 45 °C, (b) 40 °C and (c) 35 °C.

solubility of palm oil increasing with increasing temperature, even though increasing temperature causes the density of SC-CO_2 to decrease. In this work, an increase in temperature led to an increase in the total yield of SC-CO_2 extracted krill oil at a given mass flow rate and pressure. The applied pressure and temperature variation greatly affected the oil solvating power of SC-CO_2 and hence the amount of yield. The yield of extracted oil was increased with the increasing of CO_2 mass, depending on the pressure and temperature. The extracted oil per solvent (CO_2) mass used was increased constantly over the entire extraction time, until nearly all oils was extracted. The change in the slope of the extraction curve (45 °C and 25 MPa) shows that SC-CO_2 extracted almost all extractable oil. The lower yields at low pressure and low temperature are attributable to the fact that krill residues still contained oil. At constant temperature, the density of the SC-CO_2 was increased with the pressure and hence the solvating power, that is, the amount of oil extracted was increased. The effect of pressure can be attributed to the increase in solvent power and by the rise of intermolecular physical interactions [23]. Similar trends have been reported by De Azevedo et al. [24] and Park et al. [25] in the extraction of oil from green coffee and boiled anchovy, respectively.

Compared to other experimental conditions the amount of oil extracted was highest at 45 °C. Regardless of the decreasing of density of the solvent, the oil extraction yield increased with the temperature, which is probably attributed to the increase of the oil components vapor pressure. Yamaguchi et al. [1] reported that up to 45 °C the amounts of extracted oils were almost constant in spite of temperature and pressure examined. Thus, the effect of the increase of solute vapour pressure may have dominated over the solvent density.

2. Comparison of Oil Yield Obtained by SC-CO_2 and Hexane Extraction

The comparison of the yield of the oil obtained by SC-CO_2 and by Soxhlet extraction with hexane is reported in Fig. 4. The highest yield obtained in SC-CO_2 extraction was 12.2% from the run conducted at 25 MPa and 45 °C. The oil yield obtained by hexane

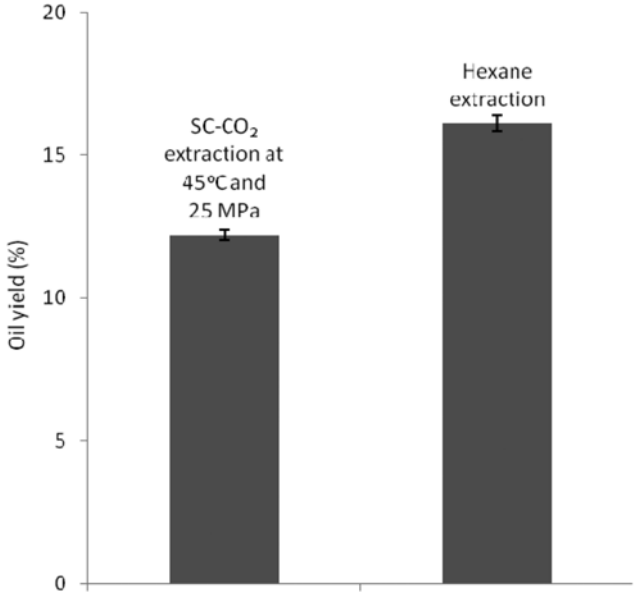


Fig. 4. Krill oil yield extracted by SC-CO_2 and hexane extraction.

extraction was 16.12% (w/w in freeze dried sample). By considering that the extraction of oil using hexane as organic solvent was nearly complete, the highest yield obtained by SC-CO_2 extraction (4.27±0.08 g) represents almost 75.6% of the yield obtained by hexane (5.64±0.13 g). Several researchers have compared SFE with conventional extraction methods. Sanchez-Vicente et al. [26] reported that the maximum yield of peach seed oil obtained by SC-CO_2 was 70%. However, our findings concur with that reported by Yamaguchi et al. [1]. These differences in maximum yield may be occurring due to variation of processing unit used, experimental conditions, sample size, percentage lipid in sample, moisture contain, etc.

The results mentioned above show that SFE could replace solvent extraction methods in a large diversity of samples. In fact, SFE has recently been included in the recommended methods from the Association of Official Analytical Chemists (AOAC) to extract fat from oilseed [18]. On the other hand, for materials with strong interactions between lipids and matrix, methods involving acid or alkaline pretreatment could be necessary. Despite the main advantage of SFE, which is the excellent quality of the resulting product, the main limitations are the cost of SFE equipment, the extraction of nonfat material and incomplete lipid extractions under some conditions. Therefore, further development is needed since each biological material is distinctive. Conditions must be investigated and improved in order to optimize yields for each sample type.

3. Fatty Acid Compositions

The comparison of the fatty acid profile of the oil obtained by SC-CO_2 in different conditions and by Soxhlet extraction with hexane is reported in Table 1. A total of 23 fatty acids were identified in the different extracts analyzed. It is observed that under certain extraction conditions there were slight changes in the fatty acid compositions of the extracted oil. The run at 20 MPa and 40 °C showed the highest percentage (95%) of the total fatty acid. Among saturated fatty acids, palmitic acid (C16:0) was present in the highest concentration ranging from 18.57 to 22.75% of total identified fatty acids. Oleic acid (C18:1) was also found in significant amounts ranging from 18.16 to 20.90%, among the UFAs. EPA (C20:5) in extracts analyzed was present in higher amounts compared to other PUFAs. The percentage of EPA (C20:5) and DHA (C22:6) in total identified fatty acids ranged from 9.41 to 11.27 and 3.11 to 4.91, respectively. The composition of PUFAs obtained in krill oil was indistinguishable from that reported by Yamaguchi et al. [1]. Also, it has been shown that marine fish oils such as cod liver oil and anchovy oil contained about 14-31% of EPA and DHA [18].

On the other hand, results showed that the oil extracted by SC-CO_2 presents a higher value of total fatty acids, particularly PUFAs, than the oil extracted with hexane. This difference can be explained bearing in mind that the soxhlet extraction with hexane occurs at a higher temperature than SC-CO_2, thus possibly leading to thermal degradation of fatty acids, mainly UFAs [27].

4. Oil Stability

Fats and oils are prone to oxidation. The degree of oxidation depends strongly on the level of unsaturation, the presence of antioxidants and prior storage condition [28]. High level of PUFAs is found in marine fish oil. Peroxide value and FFA analyses give an idea of how good or bad oil is at a particular time. FFAs are responsible for the acidity of oil. Changes of FFA content are mainly correlated to hydrolytic reactions in the oil. Peroxide value and FFA content of the oil extracts are shown in Table 2. Comparing the FFA and peroxide value in several oil extracts, it has been observed that the amount of FFA and peroxide value were significantly high in hexane extracted oil than SC-CO_2 extracted. It was also found that among oils obtained by SC-CO_2 in different condition, oil extracted at higher extraction temperature contained high amount of FFA and peroxide value. This result arranged with the high FFA content and peroxide value in hexane extracted oil due to higher temperature. Rubio-Rodriguez et al. [27] reported that higher temperature and storage time caused an important increase of the FFA content in the hake by-products oil. Also, peroxide value is used as a measurement of rancidity of oils which occurs by auto oxidation. Low exposure of oxygen in SC-CO_2 extraction process causes minimal oxidation, especially if CO_2 used is free of O_2 (in this study CO_2 used is 99.99% pure). Thus, the oil obtained by SC-CO_2 extraction showed

Table 1. Fatty acid profile of krill oil extracted with SC-CO_2 and with hexane as determined by the AOAC method (Fatty acids showed only that were found more than 1% of total fatty acids)

Fatty acids[a] (%)	SC-CO_2 (Run for 2.5 h)									Hexane (Run for 24 h)
	25 MPa			20 MPa			15 MPa			
	45 °C	40 °C	35 °C	45 °C	40 °C	35 °C	45 °C	40 °C	35 °C	
C14:0	14.80	15.04	12.86	14.95	15.29	14.89	13.06	15.33	15.42	14.20
C16:0	21.50	22.07	19.78	21.96	22.75	22.13	18.57	21.78	22.24	23.20
C16:1	9.28	9.11	8.23	9.91	10.12	9.33	8.26	9.45	9.98	7.99
C17:1	1.03	1.57	0.92	1.76	2.14	1.06	0.87	1.16	1.97	1.67
C18:0	1.49	1.39	1.22	1.96	2.03	1.95	1.39	1.76	1.66	1.39
C18:1	19.63	19.44	19.18	20.90	20.68	19.84	18.56	19.69	20.64	18.16
C18:2	1.93	1.87	1.14	1.99	1.87	1.95	1.74	1.97	1.88	1.91
C20:0	1.66	1.78	1.57	1.61	1.89	1.67	1.44	1.48	1.66	1.33
C20:1	0.63	0.51	0.48	1.02	0.87	0.59	0.43	0.61	0.74	0.58
C20:2	2.97	2.86	2.56	3.08	2.89	2.48	2.41	2.62	2.81	2.66
C20:5 (EPA)	11.03	10.87	10.13	11.27	10.93	10.27	10.34	10.27	10.76	9.41
C22:6 (DHA)	4.91	3.68	3.11	4.69	3.71	3.59	4.63	3.45	3.52	3.74

[a]Data are the mean value of three replicates. Standard error of the fatty acid constituents was on the order of about ±2%

Table 2. FFA, peroxide value and color of krill oil obtained by SC-CO_2 and hexane extraction

		SC-CO_2 extraction (2.5 h)									Hexane extraction (24 h)
		15 MPa			20 MPa			25 MPa			
		35 °C	40 °C	45 °C	35 °C	40 °C	45 °C	35 °C	40 °C	45 °C	
FFA[a] (g/100 g oil)		2.21± 0.08	2.61± 0.15	3.02± 0.06	2.75± 0.12	3.13± 0.09	3.21± 0.14	3.11± 0.13	3.27± 0.07	3.94± 0.15	6.47± 0.17
Peroxide value[a] (milliequivalent/kg)		3.14± 0.08	4.03± 0.13	4.63± 0.14	4.12± 0.07	4.64± 0.12	5.25± 0.15	5.02± 0.13	5.41± 0.17	6.11± 0.21	8.12± 0.28
Colour[b]	L*	24.38	24.41	24.25	24.04	25.51	24.21	24.13	24.79	24.38	23.54
	a*	+9.21	+9.35	+9.42	+9.32	+10.02	+9.17	+9.26	+9.33	+9.12	+0.34
	b*	+3.75	+4.18	+4.21	+4.15	+4.11	+4.27	+4.25	+4.29	+4.35	+1.42

[a]Mean value of three replicates± Standard Error (S.E.). Lightness (L*), redness (a*) and yellowness (b*)
[b]Values in each column are not significantly different ($p<0.05$)

more stability compared to the oil extracted by hexane.

5. Color

The changes in the lightness (L*), redness (a* value) and yellowness (b* value) over different krill oils extracted are shown in Table 2. Lightness does not seem to be affected by extraction conditions; slight difference of L* value was recorded between SC-CO_2 extracted oil and oil extracted by hexane. The most significant changes are observed in a* values, which are related to the tonality of color, changing from redness (a*) to greenness in hexane extracted oil. The characteristic red color of krill oil is denoted by a high value of b* that shows a slight increase at 45 °C and 20 MPa related to other SC-CO_2 conditions.

6. Extraction Yield of Astaxanthin

Previous literature stated that the solubility of organic compounds in SC-CO_2 depended basically on the balance between fluid density and solute vapour pressure, which are controlled by fluid temperature [22]. In other words, SC-CO_2 is suitable for the extraction of thermally labile compounds due to its low critical temperature. However, previous papers indicate that some substances like astaxanthin could be unstable under high pressure of SC-CO_2 at a temperature, for example, 80 °C that never provokes the decomposition of astaxanthin under atmospheric pressure [29]. Thus, attention should be paid to this fact during the extraction of natural products using SC-CO_2. In this study, the SC-CO_2 extraction of astaxanthin yield obtained at different pressures and temperatures was compared with that obtained with hexane. As shown in Fig. 5, the astaxanthin yield increased with pressure at 35 and 45 °C. The highest yield of astaxanthin was 8.62±0.31 mg/100 g at 25 MPa and 45 °C. But, at 40 °C the maximum amount was found at the pressure 20 MPa. This result can be explained by the increase in the fluid density and the decrease in diffusion coefficient. Careri et al. [10] reported that raising the pressure increases the solvent density, which has a double effect: an increase in the solvating power of the SCF, which enhances the extraction process and reduces the interaction between the solvent and the matrix resulting from the decrease in diffusion coefficient, which makes the yield of extraction process decrease. At 35 and 45 °C the increase in solvating power of the fluid was dominant over the decrease in diffusion coefficient between the fluid and matrix. However, at 20 MPa and 40 °C, the leading effect was the decrease in diffusion coefficient.

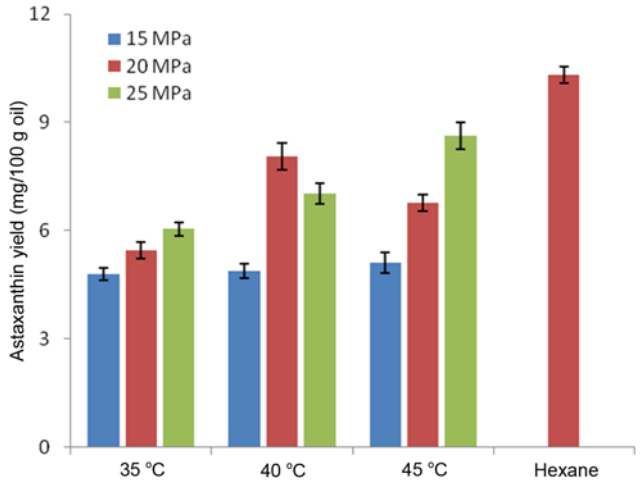


Fig. 5. Astaxanthin yield from krill at different SC-CO_2 extraction conditions and hexane extraction. Data are the mean value ±S.E. (n≤3).

On the other hand, at the pressure 15 and 25 MPa the astaxanthin yield was the maximum at 45 °C. Rising the temperature decreases the solvent density, but can increase the solute vapor pressure, which enhances the extraction yield. The same effect of temperature was reported by Lopez et al. [5] for the extraction of astaxanthin from crustaceans.

For hexane extraction, the amount of astaxanthin obtained was 10.32±0.13 mg/100 g. The highest extraction efficiency of astaxanthin obtained by SC-CO_2 was almost 86% of the total amount of astaxanthin estimated by dichloromethane extraction. This result agreed with that reported by Thana et al. [30] (83.05%) from *Haematococcus pluvialis*.

7. Correlation of Astaxanthin Solubility Using the Chrastil Model

To correlate the solubility of astaxanthin in the SC-CO_2, the Chrastil [31] model was used. The model relates the solubility directly to the density of the gas solvent, avoiding the complexity of the equation of state. It is easy to use and SFE studies have proven its usefulness. The correlations based on experimental density are very useful to determine the solubility of solids and liquids in com-

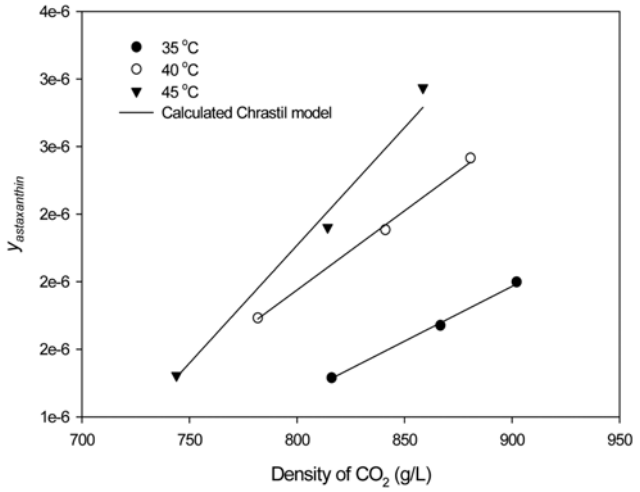


Fig. 6. Correlation of astaxanthin solubility experimental data using Chrastil model.

pressed fluids, as they are both simple and do not require physicochemical properties of the solute. Fig. 6 shows the experimental solubility data. It shows the isotherms and the effects of temperature and solvent density. Wahyu et al. [32] reported that solubility of lipids in SC-CO_2 might be correlated directly to the pressure and temperature by the equation of state. The mole fractions of astaxanthin in solvent were calculated by Eq. (1) and the density relationship in Chrastil model is based on Eq. (2) which is derived from Antoine [31]:

$$\alpha_{asta} = \frac{n_{asta}}{n_{asta} + n_{CO_2}} \quad (1)$$

$$y_{asta} = \rho_{CO_2}^{k} \exp\left(\frac{a}{T} + b\right) \quad (2)$$

where: is the mole fraction of astaxanthin, is moles of astaxanthin and is moles of carbon dioxide. is the solubility of astaxanthin (mol/mol), is the SC-CO_2 density, T is experimental temperature (K) and a, b and k are empirical fitting parameters. Chrastil parameters obtained are shown in Table 3. At a given temperature, nearly a linear relation between solubility of astaxanthin and SC-CO_2 density was obtained. Thus, the solubility data of astaxanthin show its correlation with the Chrastil model. The solubility of fucoxanthin extracted by SC-CO_2 from brown sea weed was also fitted in the Chrastil model [33].

CONCLUSION

The extraction of krill oil using SC-CO_2 and soxhlet extraction with hexane was investigated. The optimal extraction conditions chosen, regarding extraction rate, were 25 MPa and 45 °C. Under those conditions, the extraction yield obtained after 2.5 h was 75.6% compared with the oil obtained by organic solvent extraction. The lipid compositions of oil showed a high amount of ω-3 PUFA, especially EPA and DHA. The main fatty acids were C14:0, C16:0, C16:1, C18:1 and C20:5 in oils of both obtained by SC-CO_2 extraction and organic solvent extraction. The result showed that the highest amount of astaxanthin was extracted at 25 MPa and 45 °C which represent 86% as extraction efficiency. The solubility of astaxanthin was examined and correlated with the Chrastil model satisfactorily. In addition, SC-CO_2 extracted oil showed more stability and better quality than organic solvent extracted oil. Thus, krill oil obtained by SC-CO_2 extraction would be a good source of PUFAs and astaxanthin.

Table 3. Chrastil equation parameters of astaxanthin mole fraction

Parameters of Chrastil equation	Temperature (°C)		
	35	40	45
k	2.74	2.85	2.92
a	1.02	1.04	1.09
b	−21.64	−21.38	−21.73

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