EXTRACTION OF OIL FROM KRILL USING SUPERCRITICAL CARBON DIOXIDE AND ORGANIC SOLVENT AS A COMPARATIVE METHOD.

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Abstract

This study was to investigate the efficient method for the extraction of astaxanthin from krill. To apply supercritical fluid extraction technique in order to recover astaxanthin from krill, it is essential to know the solubility of astaxanthin in supercritical carbon dioxide(S-CO_2) with varying pressure and temperature. In this work, pure and modified S-CO_2 was used as solvent. Experiments were carried out under different conditions. The effects of different parameters, such as pressure (80 to 280 bar), temperature (35 to 45°C), modifier volume and extraction time (1- 4 h), on the SCO_2 extraction for oil including astaxanthin from krill were investigated in order to evaluate their influence on the yield and composition of the krill oil. Organic solvent extraction with the same sample was performed and used as a comparative method. The chemical composition of each supercritical fluid extraction sample has been analyzed by HPLC and GC and the global composition were compared with that obtained by organic solvent extraction. The main fatty acids from krill were Tridecanoic acid ,heptadecanoic acid, palmitic acid, stearic acid, palmitoleic acid, EPA (eicosapentaenoic acid), and DHA (docosahexaenoic acid).

INTRODUCTION

Krill represents a very large biomass, little contaminated by organic pollutants and heavy metals and harvested since 1975 for animal feed and aquaculture [1]. Its expanded use in human health is possible through enzymes, various krill extracts and oils. The fat content of krill is low, but it is rich in EPA and DHA of high bioavailability given their presence in phospholipids. These fatty acids have health benefits and have been reported to reduce the risk of coronary heart diseases [2], and lower blood pressure and plasma triacylglycerol levels [3]. In addition they can improve inflammatory conditions, reduce the symptoms of diabetes [5] as well as a range of other disorders.

Supercritical fluid extraction (SFE) has been used for the determination of organic pollutants in environmental solids with variable success [7]. Additionally, SFE has been shown to produce equivalent or more effective results compared to other extraction techniques such as Soxhlet, sonication and accelerated solvent extraction [8].

In the supercritical state, the difference between the liquid and gas phase has disappeared and the fluid can no longer be liquefied by raising the pressure nor can gas be formed on increasing the temperature. Thus, the physicochemical properties of this fluid, such as density, diffusivity, dielectric constant and viscosity can be easily managed by changing the pressure or the temperature without ever crossing phase boundaries [9]. The main profits of SFE instead of conventional organic solvents are the minimal consumption of organic solvents, the exclusion of oxygen, and the reduction of heat. Modern SFE offers shorter extraction times, potentially higher selectivity and increased sample throughput (due to available automated instruments) compared to conventional solvent extraction techniques [10]. SCO_2 extraction and fractionation of fish oils has been the subject of ongoing research, where a lot of information has been published on fundamental measurements of solubility and phase equilibria of polyunsaturated ω-3 fatty acid fish oil compounds in supercritical fluids [10].

Thus, the purpose of this work was to obtain extraction data of krill using SCO_2, determined at various conditions (from 35 to 45°C and from 80 to 280 bar). At the optimal condition, we were identified the fatty acids in the oil. Also we were compared the astaxanthin obtained from krill by SCO_2 and organic solvent extraction with astaxanthin standard solution.

MATERIALS AND METHODS

Materials

The raw material employed in the experiments was krill and this was obtained from F & F Co., Busan, Korea. The krill was washed and brought to the laboratory in iced condition. And used after crushed (Philips, HR1727) and sieved (700 ㎛, Chung gye sang gong SA). The samples were stored at -80°C in deep freezer (Samwon Freezing Engineering Co., SW-UF-200) and used for lipid extraction by SCO_2 and also for organic solvent extraction. Carbon dioxide with a purity of 99.99% was supplied by local company. Also all other reagents are analytical grade and HPLC grade supplied by Sigma Co.

Supercritical Fluid Extraction Method

Twenty five grams of freeze dried krill sample were applied in 200 mL stainless steel extraction vessel containing 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 heater. Flow rates and accumulated gas volume passing through the apparatus were measured using a gas flow meter. The extracted oil were collected by a cyclone separating vessel. Extraction trials were performed at temperatures between 35 and 45°C and pressure between 80 and 280 bar. SC-Co_2 was introduced into the vessel at a predetermined temperature and pressure. The extracted lipid was collected every 60 minutes .

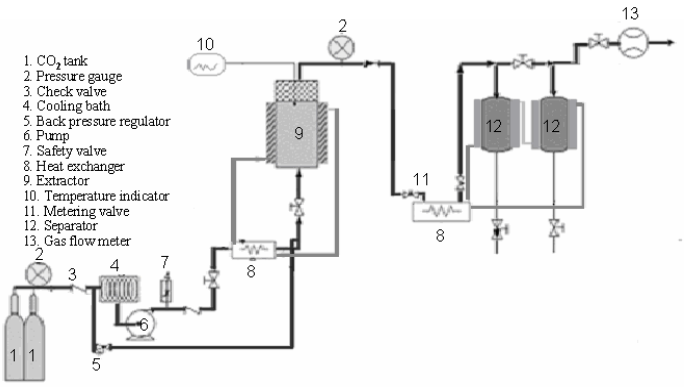


Figure 1: A schematic diagram of SFE process.

Organic solvent Extraction Method

Lipid extraction from krill samples were performed by n-hexane using soxhlet apparatus. The extraction time was 24 hours. After extraction the krill residues remaining in soxhlet apparatus were stored at -80°C.

Analysis of Fatty Acids

The samples were used not only SCO_2 treated powder and oil but also organic solvent powder and oil. For organic solvent extraction, the method described by Bligh *et al.* [14] was used. The samples (0.3 g) were methylated by the AOAC method (996.06 standard) [15], and the methyl esters of fatty acid compounds in the krill were determined by gas chromatography-flame ionization detector (GC-FID) (HP5890II, USA). The column used was a DB-wax column (Agilent, 30 m × 0.25 mm i.d., 0.25 µm film thickness). The GC conditions were: the initial temperature of oven was 40^{o}C, and programmed from 40 to 180^{o}C at 10^{o}C/min, and then to 260^{o}C at 5^{o}C/min, finally at 260^{o}C for 5 min, injector temperature 250^{o}C, injection volume was 1 µL, the split ratio was 100:1, total carrier gas (nitrogen) flow rate was 1.52 mL/sec. A lipid standard (fatty acid methyl ester mixture, Supelco 37 Component FAME Mix) was used to identify the fatty acids.

Analysis of Astaxanthin

HPLC Analysis of extracts Supercritical carbon dioxide extraction method using organic solvent extraction device and compare the extracted ataxanthin was conducted to verify the HPLC analysis. Waters600E (USA) devices using YMC-C30 (250mm X 4.6mm) column was used, the temperature was set to room temperature. Solvents ACN: CH2Cl2: MeOH: H2O: Propionic acid (71: 22: 4: 2: 1 (v / v / v / v / v)) to the 1ml/min yongri use of the flow rate of a single solvent. The percentage of identified astaxanthin was presented by peak area %.

RESULTS AND DISCUSSION

Supercritical Fluid Extraction

The amount of extracted oil was increasing with the increasing of CO_2 mass depending on the pressure and temperature. The amount of extracted krill oil per solvent mass used was increased constantly over the entire extraction period, until almost all the oil was extracted. The change in the slope of the extraction curve (45°C and 250 bar) indicated that SCO_2 extracted almost all krill oil. After SCO_2 extraction at low pressure and low temperature, krill residues contained still oil. At constant temperature, the amount of oil extracted from krill was increased with the pressure. Due to the increase in pressure, the density of the SCO_2 was increased and hence the solvating power. The effect of pressure can be attributed to the increase in solvent power and by the strengthening of intermolecular physical interactions [11]. Similar results were found in the extraction of oil from boiled anchovy [7]. The amount of oil extracted was highest at 45°C as compared to other conditions. Despite of the decreasing of solvent's density, the oil extraction yield was increased with the temperature which can be attributed to the increase of the oil components vapour pressure.

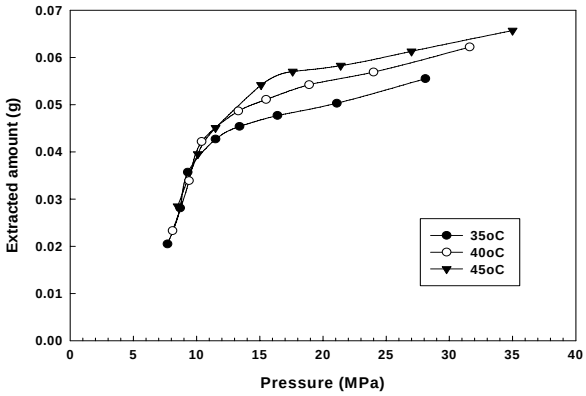


Figure 2: Effect of CO_2 density on the amount of extracted oil

Analysis of Fatty Acids

The main fatty acid composition of krill was presented in **Table 1**. This experiment was performed at 45°C and 250 bar as the optimal condition from the extraction data. The major fatty acids classes were PUFAs, monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs).

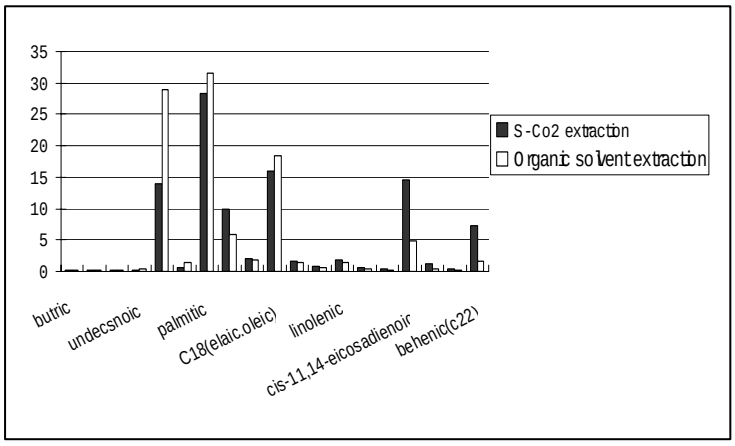


Figure 3: Comparison of fatty acid composition between SCO_2 extraction and organic solvent extraction from krill

In this work, the compositions of the S-CO_2 extraction component and the organic solvent extraction oils were almost similar. Tridecanoic acid, palmitic acid, Stearic acid, heptadecanoic , EPA, DHA were the main compounds in essential oils obtained by organic solvent extraction and S-CO_2 extraction.

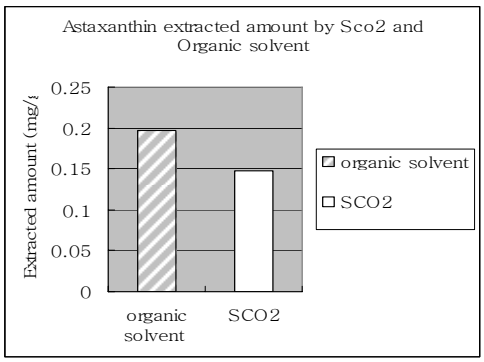
Table 1: Main fatty acids composition in krill.

F. acid	R. Time	Area (%)	
		SCO_2 Extraction	Organic solvent Extraction
14:0	11.164	28.96	13.93
14:1	12.079	1.36	0.61
16:0	15.385	31.64	28.34
17:1	18.390	5.95	9.88
18:0	22.197	1.90	1.99
18:1	23.142	18.48	15.92
18:2 n-6	25.365	1.33	1.60
18:3	32.427	1.32	1.81
EPA (20:5 n-3)	44.633	4.78	14.49
22:0	45.889	0.33	1.14
22:1 n-9	48.638	0.12	0.43
DHA (22:6 n-3)	55.857	1.70	7.36

Analysis of Astaxanthin

The S-CO_2 extracts from krill showed identical chromatographic profiles than that of astaxanthin standard solution. This suggests that the SFE process did not alter the pigment profile in the sample. Astaxanthin is unstable at high pressures and temperatures around 80°C [6]. Even with the use of very high pressures (Above 300 bar), the temperatures of all the treatments were below 80°C, therefore, it is very unlikely that the S-CO_2 process caused any degradation of the pigment present in the raw material**.**

Figure 4:



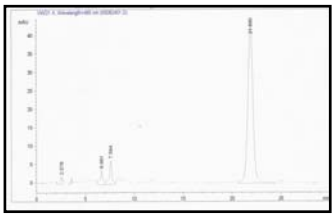


Figure 5: HPLC result of astaxanthin standard solution.

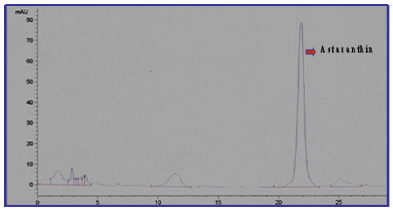


Figure 6: HPLC result of krill extracts by S-CO_2(45□, 250bar)

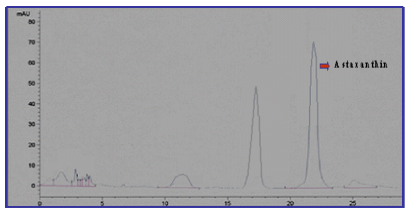


Figure 7: HPLC result of krill extracts by organic solvent

CONCLUSION

According to this study, the supercritical carbon dioxide removed almost all lipids from krill at pressure, 250 bar and temperature, 45°C. Astaxanthin can be completely removed by SCO_2 extraction within the range of conditions used. And the extraction efficiency of functional components was competitive compared to those of organic solvent extraction. Also, it is important to understand the properties between the target materials to be separated and supercritical fluids process as an alternative process to the conventional extraction process.

REFERENCES

[1] Nicol s, Endo Y., Aquat Living Resour ,Vol. 12, p.105-20

[2] Lee, E.-H., Kim, S.-K., Cho, G.-D., Youil publishing Co., Busan, Korea, **1977**, p. 43-46.

[3] Lemaitre, R. N., I. B. King, D., Mozaffarian, L.H. Kuller, R. P., Tracy, D. S. Siscovick The cardiovascular health study. Am. J. Clin. Nutr, Vol.77, **2003**, p .319-325.

[4] Chan, D. C., G. F. Watts,. Mori, T. A,. Barrett, P. H, T. G. Redgrave, and L. J. Beilin Am. J. Clin. Nutr. Vol.77, **2003**, p. 300-307.

[5] Dallongeville, J., Yarnell ,J., Ducimetiere, P., Arveiler ,D., Ferrieres J., Montaye, M., Luc G., Evans,A., Bingham,A..,Hass,B., Ruidavets,J.B.,Amouyel, P.,Circulation, vol.108 ,**2003**, p. 820-825.

[6] YAMAGUCHI, K et al.. J. Agric. Food Chern, **1986**, p.34, 904-907.

[7] Park, J. Y., M. K. Lee, M. S. Uddin, B. S. Chun, Biotechnology and Bioprocess Engineering, **2008**, p. 298-303.

[8] Dean, J. R. John Wiley & Sons, Inc., NY, USA, **1998**, p. 123.

[9] Hawthorne, S. B., C. B. Grabanski, E. Martin, D. J. Miller, Chromatogr J.. *A,* vol.892, **2000**, p.421-433.

[10] Assis, L. M., J. S. S. Pinto, and F. M. Lancas , *J. Microcol. Sep,* vol.12, **2000**, p. 292-301.

[11] Morita, A., and O. Kajimoto J. Phys. Chem. Vol.94, **1990**, p.6420–16420.

[12] Sihvonen, M., E. Järvenpää, V. Hietaniemi, R. Huopalahti, *Trends Food Sci. Technol.* Vol.10,**1999**, p. 217-222.

[13] Turner, C., J. W. King, and L. Mathiasson , *J. Chromatogr. A,* vol.936, **2001**, p. 215–237.

[14] Chun, B.-S., Kim, Y.-W., Song, S.-K., Kim, S.-K., Wilkinson, G. T. *Proceedings of 5th ISASF Meeting.* March 23-25. Nice, France, **1998**

[15] Chun, B.-S., K.-Y. Kang, D.-H. Ahn, G. T. Wilkilnson, *Korean J. Chem. Eng,* Vol.22, **2005**, p. 399-405.