Polymer-associated Particles Formation of Fucoxanthin Derived from Brown Seaweed (*Saccharina japonica*) by Gas Saturated Solution Process

[1]A. S. M. Tanbirul Haque, [2]Hee-Chul Woo and [1]Byung-Soo Chun*

[1]Department of Food Science and Technology, Pukyong National University, 45 Youngso-ro, Nam-Gu, Busan 608-737, Republic of Korea,

[2]Department of Chemical Engineering, Pukyong National University, Busan 608-739,

Republic of Korea

*E-mail: bschun@pknu.ac.kr, Fax: +82-51-629-5824

ABSTRACT

Seaweed provides for an excellent source of bioactive compounds, such as a carotenoid, dietary fibre, protein, vitamins, essential fatty acids and minerals. Interest in seaweed lipid has been on the rise owing to the recognition of important bioactive molecules like conjugated fatty acids, pigments that have profound physiological effects in treatment of tumours and other cancer related problems. So seaweed has been used as human foods, cosmetics, fertilizers and source of chemicals for medicine and industries. In this study, extract was collect from *Saccharina japonica* using supercritical CO_2 with ethanol, methanol and ethanol solvent. Highest fucoxanthin was found in supercritical CO_2 with ethanol extracted oil, it was 0.196 ± 0.09 mg/g dry-weight. This extract was used for micronization using gas saturated solution process (PGSS). The particle formation of functional material with biodegradable polymer was performed by supercritical carbon dioxide (SC-CO_2) in thermo stated stirred vessel. PGSS process was carried out at temperatures, ranging from 45 to 55°C and pressures, ranging from 15 to 25 MPa to measure the optimum condition for the fucoxanthin content particles. 400 μm nozzle size was used and reaction time was 1 hr. Fucoxanthin content in particle was measured by HPLC. In particle highest inclusion was found 58.16% at 50˚C/15MPa. The produced particles were characterized by particle size analyser (PSA) to determine their shape and distribution size.

INTRODUCTION

Seaweeds are popular in Japan, China and Korea as one of major components in their daily diet [1]. Brown seaweed pigments especially carotenoids are of interest as antioxidant and anticancer as reported by Mori et al. [2]. Fucoxanthin as major part of carotenoids in brown seaweed had a specific alenic bonds, where 5, 6-monoepoxida play an important role within fucoxanthin structure [3]. Some workers had reported that fucoxanthin had a variety of effects on human health such as anticarcinogen, anti-inflammation, antioxidant and antiobesity [4 - 9]. Interest in seaweed lipids has been on the rise, owing to the recognition of important bioactive

molecules like conjugated fatty acids and pigments (especially fucoxanthin), that have profound physiological effects in the treatment of tumors and other cancer related problems [10-12]. Fucoxanthin occurs in great abundance in brown seaweed, but is absent in higher plants [13]. Sachindra et al. [14] found that fucoxanthin isolated from wakame brown seaweed had a various radical scavenging activities, and it was reported an increase utilization of this seaweed as food due to its beneficial for human health.

Supercritical carbon dioxide (SC-CO_2) is an attractive supercritical solvent, low critical temperature and the fact that it is non-flammable, non-toxic and inert. In recent years, the use of supercritical fluid extraction (SFE) for the removal of organic compounds from different liquid and solid matrices has attracted much attention. This technique has some advantages over more conventional separation techniques, largely due to the unique physical properties of SFs. SFE using CO_2 is promising process for the extraction and fraction of edible oils containing labile PUFAs and lipid soluble bioactive compounds.

The formulation of natural substances together with a biocompatible or biodegradable carrier material to form composites or encapsulates has great potential for the pharmaceutical, cosmetic and food industries [15]. In addition several clinically approved pharmaceutical products use biodegradable polymers to regulate the rate of drug release within the body [16, 17].

Different techniques have been applied for this purpose, including spray-drying, freeze-drying, liquid antisolvent crystallization or milling processes. These technologies present several disadvantages, such as the production of coarse particles with broad particle size distribution, the degradation of the product due to mechanical or thermal stresses, or the contamination of the particles with organic solvents or other toxic substances. For this reason, different alternative precipitation methods are being investigated.

Particle formations techniques SC-CO_2 such as the rapid expansion of supercritical solutions (RESS), particles from gas saturated solutions (PGSS), and supercritical anti-solvent (SAS) precipitation have received much attention as precipitation methods alternative to those using organic solvents [18]. These methods are important for drug delivery systems to successfully obtain composites or encapsulates that comprise an active compound loaded into a matrix of a carrier material, thus improving product preservation as well as controlling the dissolution rate of the active compound [15].

Small particles of pharmaceuticals with a narrow particle size distribution plays a vital role in the design of conventional drug delivery systems like tablet, capsule, injection; biphasic drug delivery system like suspension and emulsion and the controlled drug delivery systems of implants, transdermal, micro emulsions and nanoparticulate [19-25]. PGSS can be used to produce micro particles with a narrow size distribution; therefore, it is a key technique used in the food and pharmaceutical industries, because it results in solvent-free products [26]. Therefore, the aim of this study was to extract oil using SC-CO_2 and measurement the fucoxanthin in oil. Making particle with oil and biodegradable polymer, fucoxanthin content particle size and particle morphology also analysis.

MATERIALS AND METHODS

Materials

Brown seaweed *S. Japonica* was collected from Guemil-eup, Wando-gun, Jeollanam-do, South korea. Pure carbon dioxide (99.99%) was supplied by KOSEM (Sangbuk-myeon Yangsan, Korea). All other chemicals used in this study were of analytical or HPLC grade.

Sample preparation

Fresh *S. Japonica* samples were washed with fresh water to dispose unused materials and then cut into small pieces. The small pieces of seaweeds were dried in freeze drier (Eyela FDU-2100, Tokyo Rikakikai Co., LTD, Japan) equipped by square-type drying chamber (Eyela DRC-1000, Tokyo Rikakikai Co., LTD, Japan) at temperature -80˚C for 3 days. After then, the dried samples were finely ground by mechanical blender (PN SMKA-4000 mixer) and sieved by 710 μm stainless steel sieving mesh. These dried sieved samples were stored at -20^{o}C.

Solvent Extraction

For solvent extraction ethanol and methanol were used as a solvent. 200 g of freeze dried crash sample was placed in the beaker and stirred for 24 h by stirrer at 40°C at 250 rpm. After extraction, the solvent was evaporated in a rotary vacuum evaporator at 40°C. The extracted crude oil was collected in a vial and stored at -20°C until further analysis.

SC-CO_2 extraction

The set-up of a laboratory scale of supercritical fluid extraction (SFE) process was used. 200 g of freeze dried sample was loaded into the stainless steel extraction vessel. A thin layer of cotton was placed at the bottom of the extraction vessel. Before plugging with cap another layer of cotton was used at the top of the sample. CO_2 was pumped at constant pressure into the extraction vessel by high pressure pump (Milroyal, Milton Roy, USA) up to the desired pressure. A back pressure regulator was used to control the pressure of CO_2.The extraction temperature was maintained by connecting the extraction vessel with water bath. Flow rates and accumulated gas volume passing through the apparatus were measured using a gas flow meter (Shinagawa, Tokyo, Japan). As a co-solvent ethanol was used. After SC-CO_2 extraction, the oil was stored at -20°C until further use and analysis. *S. Japonica* was extracted at temperature 50°C and pressure was 25 MPa for 2 h. The flow rates of CO_2 were kept constant at 27 g/min for all extraction time.

Particle formation using PGSS process

The experiments were carried out using PEG 8000 (g/mol) and SC-CO_2 extracted oil with different pressures, temperatures. Sematic diagram of PGSS process was shown in Figure 1. PGSS experiment began by delivering SC-CO_2 to the precipitation chamber until the desired pressure was reached. PEG and oil (20:1) in reactor were melted by SC-CO_2 and mixed by stirred wheel. These experiments were carried out at temperatures, ranging from 45 to 55°C and pressures, ranging from 15 to 25 MPa. The mixture was stirred at 300 rpm and the nozzle size was 400 μm. The duration for reactions was 1 h. After reaction, material with PEG were delivered through the nozzle and collected from a separator.

Analysis of Particle Size by PSA (Particle size analyzer)

The size distribution of the PEG and oil with PEG powder were measured by particle size analyzer (LS 13320, Beckman Coulier, USA). The result from the analysis is the relative distribution of volume of particles in the range of size classes. From this basic result, the data on particle size distributions are calculated. The frequency curve is useful for displaying the results to show the peaks in the graph. The peak of the frequency curve gives the modal diameter, the most commonly occurring particle diameter.

Analysis of fucoxanthin content by HPLC

The methods of Terasaki [27] and Noviendri [28] were adopted for fucoxanthin content determination by HPLC. All HPLC analyses were carried out using a Waters 600 E HPLC system (Water, Milford, USA) equipped with a Tunable absorbance detector (water) Fucoxanthin content in seaweed extract was determined by reversed-phase HPLC (RP-HPLC) with methanol-acetonitrile (7:3, v/v) as the mobile phase at a flow rate of 1.0 mL/min. All RP-HPLC analyses were carried out at ambient temperature using a RP column (XTerra® MS C_{18}, 5.0 µm particle size, 250 mm× 4.6 mm i.d; Waters, Milford, USA) protected with a guard column (10× 4.6 mm i.d.) having the same stationary phase. Briefly, an aliquot of seaweed extract was dissolved in mobile phase, filtered with a 0.22 µm membrane filter and a detection wavelength was set at 450 nm for detecting fucoxanthin. A standard curve prepared using authentic standard was used for quantification of fucoxanthin content in seaweed samples. Fucoxanthin content in seaweed samples were expressed as mg/g dried weight of seaweed sample. The amount of fucoxanthin was quantified from peak area using a standard curve with commercial fucoxanthin (Sigma-Aldrich, St. Louis, USA).

RESULTS AND DISCUSSION

Fucoxanthin amount

Fucoxanthin of *S. Japonica* was determined using different solvent extraction. The quantitative data on fucoxanthin of *S. Japonica* is presented in Table 1. Results show that *S. Japonica* contained a considerable amount of fucoxanthin. The highest amount of fucoxanthin was found in SC-CO_2 with ethanol extracted oil, it was 0.196 ± 0.09 mg/g dry-weight. Kanazawa et.al also reported same amount of fucoxanthin from S. japonica [29]. Lowest amount was found in methanol extract, it was 0.025±0.05 mg/g dry-weight. Xiao et. al. also reported fucoxanthin in *S. japonica* was 0.03 mg/g dry-weight [30]

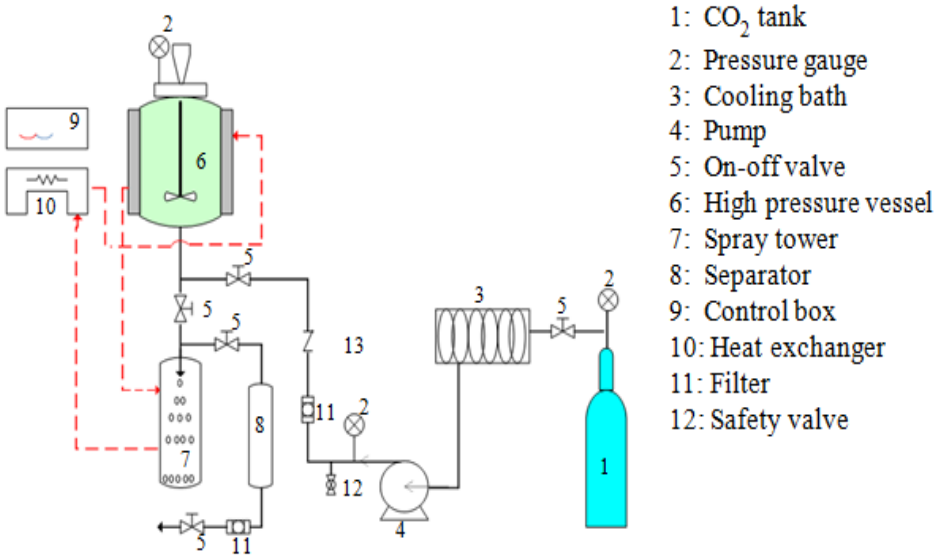

Figure 1: Schematic diagram of PGSS process.

Table 1: Fucoxanthin content in *S. Japonica* using different solvent

Using Solvent	Fucoxanthin mg/g dry weight sample
Ethanol	0.093±0.07
Methanol	0.025±0.05
SC-CO_2 with ethanol	0.196±0.09

Means ± SD (n = 3).

After particle formation with oil and PEG fucoxanthin was found in particle. It was observed that significant amount of fucoxanthin inclusion occurred in particle (Table 2). Highest inclusion was found 58.16% at 50˚C/15MPa. Lowest amount was 33.83% at 55˚C/25MPa. Increasing pressure with fixed temperature inclusion rate was decrease. It may be occurred due to high temperature some fucoxanthin was denatured.

Table 2: Fucoxanthin content in particle

Particle condition (˚C/ MPa)		Fucoxanthin content (mg/g)	Inclusion rate (%)
45	15	0.102±0.03	52.04
	20	0.097±0.09	49.49
	25	0.0787±0.06	40.15
50	15	0.114±0.08	58.16
	20	0.0982±0.05	50.10
	25	0.0678±0.03	34.59
55	15	0.0991±0.02	50.56
	20	0.0754±0.07	38.47
	25	0.0663±0.04	33.83

Means ± SD (n = 3).

Analysis of Particle Size by PSA

The size distributions of original PEG and *S. Japonica* oil particles with PEG 8000 obtained by PGSS using SC-CO_2 under different conditions are shown in Figure 2 (a-d). In this study, the average particle size of PEG before PGSS process was 178.688μm. Average particle size was decrease in all condition. The average size of *S. Japonica* oil particles with PEG was found to be ranged from 100.17 μm to 155.174 μm. Temperature and pressure was moderately affected the size of *S. Japonica* oil particles with PEG. In 55˚C the particle size was smaller than 45˚C. At 55 ˚C and 15 MPa highest smaller particle was found. In a previous work, an analysis of experimental results of micronization of polyethylene glycol by PGSS-drying showed a relationship between the saturation concentration of carbon dioxide in the solution and particle size indicating that if the concentration of carbon dioxide in the gas saturated

solution is increased, the atomization is more effective leading to the production of smaller particle.

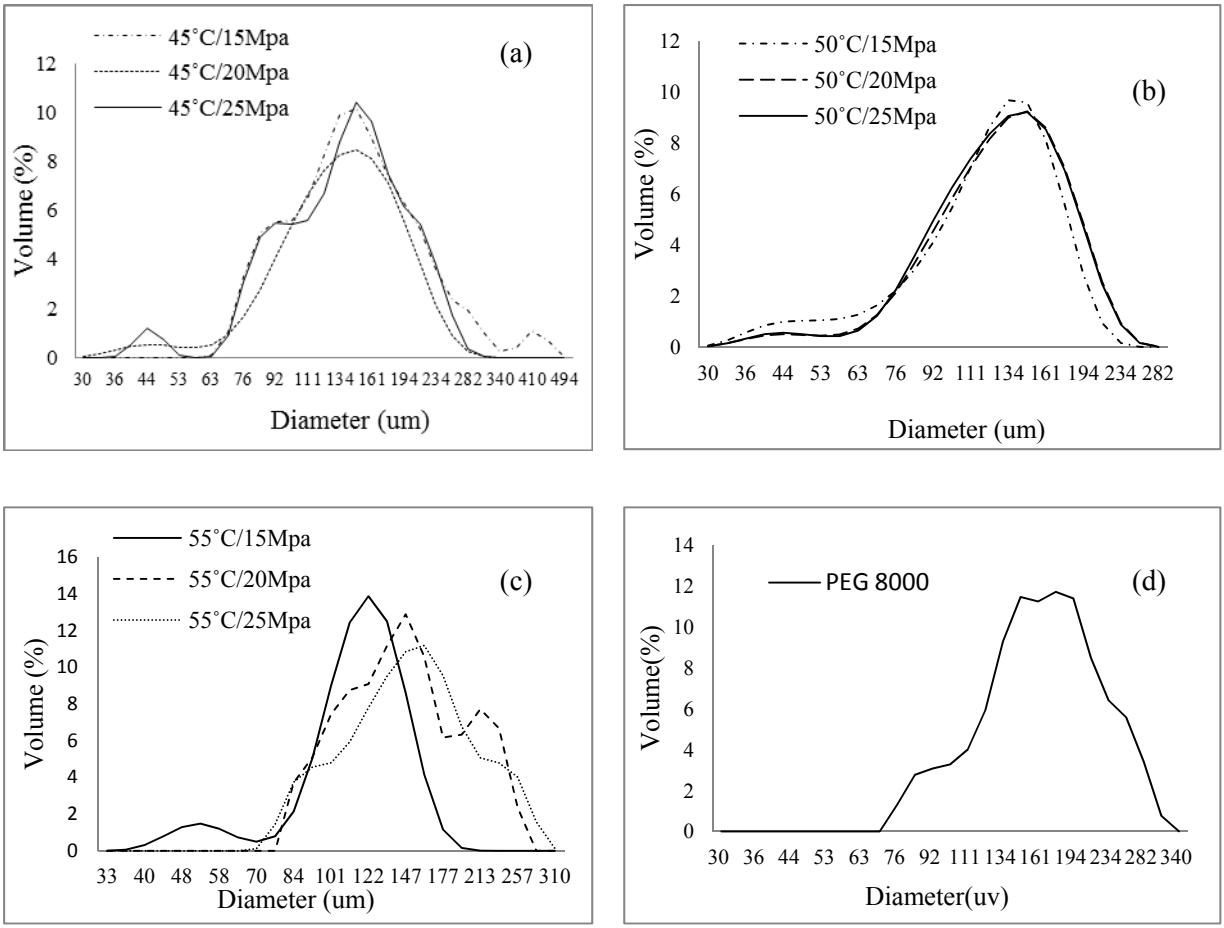

Figure 2: *S. Japonica* oil content particle's size and distribution volume formed by PGSS at fixed temperature and different pressure (a) 45˚C, (b) 50˚C, (c) 55˚C (d) PEG 800 .

CONCLUSION

S. japonica contents significant amount of fucoxanthin. Among different solvent extraction best result found at SC-CO_2 with ethanol where highest amount of fucoxanthin was extracted. So it will be a good solvent for fucoxanthin extraction. On the other hand after particle formation with extract and polymer it also contain fucoxanthin and inclusion rate more than fifty percent. After particle formation it can be easily stored and this particle can be used in various fields like food, cosmetic and pharmaceutical industry.

ACKNOWLEDGEMNT

This research was supported by the Ministry of Oceans and Fisheries (2013-1039449).

REFERENCES

[1] SACHINDRA, N.M., AIRANTHI, M.K.W.A., HOSOKAWA, M., MIYASHITA, K., Journal of Food Science Technology, Vol. 47, **2010**, p. 94.
[2] MORI, K., OOI, T., HIRAOKA, M., OKA, N., HAMADA, H., TAMURA, M., Marine Drugs, Vol. 2, **2004**, p. 63.
[3] MATSUMO, T., Fisheries Science, Vol.67, **2001**, p. 771.
[4] OKOZUMI, J., NISHINO, H., MURAKOSHI, M., IWASHIMA, A., TANAKA, Y., YAMANE, T., Cancer Letters, Vol. 55 **1990**, p. 75.
[5] NOMURA, T., KIKUCHI, M., KUBODERA, A., KAWAKAMI, Y., International Journal of Biochemistry and Molecular Biology, Vol. 42, **1997**, p. 361.
[6] KIM, J.M., ARAKI, S., KIM, D.J., PARK, C.B., TAKASUKA, N., TORIYAMA, H.B., Carcinogenesis, Vol. 19, **1998**, p. 81.
[7] SHIRATORI, K., OHGAMI, K., ILEIVA, I., YIN, X.H., KOYAMA, Y., MIYASHITA, K., Experimental Eye Research, Vol. 81, **2005**, p. 422.
[8] MAEDA, H., HOSOKAWA, M., SASHIMA, T., FUNAYAMA, K., MIYASHITA, K., Biochemical and Biophysical Research Communication, Vol. 332, **2005**, p. 392.
[9] GERASIMENKO, N.I., CHAYKINA, E.L., BUSAROVA, N.G., ARISIMOV, M.M., Applied Biochemistry and Microbiology, Vol. 46, **2010**, p. 426.
[10] HOSOKAWA, M., KUDO, M., MAEDA, H., KOHNO, H., TANAKA, T., MIYASHITA, K., Biochimica et Biophysica Acta, Vol. 1675, **2004**, p. 113.
[11] KOHNO, H., SUZUKI, R., NOGUCHI, R., HOSOKAWA, M., MIYASHITA, K., TANAKA, T., Japanese Journal of Cancer Research, Vol. 93, **2002**, p. 133.
[12] KOHNO, H., SUZUKI, R., YASUI, Y., HOSOKAWA, M., MIYASHITA, K., TANAKA, T., Cancer Science, Vol. 95, **2004**, p. 481.
[13] BALLARD, L.J., GLASGOW, L.A., HOSKINS, L.C., KROHE, T., Spectrochimica Acta.Vol. 45A(12), **1989**. p. 1235.
[14] SACHINDRA, N.M., SATO, E., MAEDA, H., HOSOKAWA, M., NIWANO, Y., KOHNO, M., Journal of Agricultural and Food Chemistry, Vol. 55, **2007**, p. 8515.
[15] COCERO, M.J., MARTIN, A., MATTEA, F., VARONA, S., Journal of Supercritical Fluid, Vol. 47, **2009**, p.546.
[16] TRACY, M.A., Biotechnology Progress, Vol. 14(1), **1998**, p.108.
[17] OKADA, H., Advanced Drug Delivery Reviews, Vol. 28(1), **1997**, p. 43.
[18] MISHIMA, K., Advanced Drug Delivery Reviews, Vol. 60, **2008**, p.411.
[19] Budavari, S., 1989. An Encyclopedia of Chemicals, Drugs and Biological. 11th ed. The Merck Index, Merck and Co. Inc, Rahway, New Jersey, US.
[20] MISHIMA, K., Advanced Drug Delivery Reviews, Vol. 60, **2008**, p. 411.
[21] TURK, M., LIETZOW, R., Journal of Supercritical Fluid, Vol.45, **2008**, p. 346.
[22] YILDIZ, N., TUNA, S., DOKER, O., ALIMLI, A.C., Journal of Supercritical Fluid, Vol.41, **2007**, p. 440.
[23] PARK, S.J., YEO S.D., Korean Journal of Chemical Engineering, Vol. 25, **2008**. p. 575.
[24] TANDYA, A., FOSTE,R N.R., DEHGHANI, F., Journal of Supercritical Fluid, Vol. 37, **2006**, p. 272.

[25] LI, G., CHU, J., SONG, E.S., ROW, K.H., LEE, K.H., LEE, W.J., Korean Journal of Chemical Engineering, Vol. 23, **2006**, p. 482.
[26] PATHAK, P., SUN, Y.P., MEZIANI, M.J., DESAI, T.J., Journal of Supercritical Fluid, Vol. 37, **2006**, p.279.
[27] TERASAKI, M., HIROSE, A., NARAYAN, B., BABA, Y., KAWAGOE, C., YASUI, H., SAGA, N., HOSOKAWA M., MIYASHITA, K., Journal of Phycology, Vol.45, **2009**, p. 974.
[28] NOVIENDRI, D., JASWIR, I., SALLEH, H.M., TAHER, M., MIYASHITA, K., RAMLI, N., Journal of Medicinal Plants Research, Vol. 5, **2011**, p. 2405.
[29] KANAZAWA, K., OZAKI, Y., HASHIMOTO, T., DAS, S.K., MATSUSHITA, S., HIRANO, M., OKADA, T., KOMOTO, A., MORI, N., NAKATSUKA, M., Food Science and Technology Research, Vol. 14, **2008**, p. 573.
[30] XIAO, X., SI, X., YUAN, Z., XU, X., LI, G., Journal of Separation Science, **2012**, Vol. *35*, p. 2313.