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NOTE

Extraction of Docosahexaenoic Acid (DHA) from Lyophilized *Thraustochitrium* sp.

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Solvent extraction, soxhlet method, and supercritical fluid extracion were considered, respectively, as the method of choice for the recovery of DHA from lyophilized *Thraustochitrium* sp., and the results of corresponding extraction were compared. Supercritical fluid extraction seems to be the most appropriate process with respect to time, process simplicity, and extractant intoxicity.

Docosahexaenoic acid (DHA) is one of the representative component of ω-3 polyunsaturated fatty acids, and is purported to have various physiological activities including reduced susceptibility to arthritis and cardiovascular disease (5). Hence, the interest in DHA for food additives as well as possible therapeutic agents has been rapidly increased with an expectation for DHA to act as a highly beneficial dietary additive for human health (7).

At present, a commercially available source of DHA is marine oils, which is obtained through isolation from the body oil of cold water fishes such as anchovy, mackerel, sardine and menhaden. However, such DHA from fish oils is considered unattractive and has several disadvantages because it contains substantial amounts of undesirable fatty acids and cholesterol with an unpleasant odor and color, making it unsuitable for pharmaceutical materials or a food additive (6).

This fact motivates the search for alternative means of producing DHA. Among the conventional sources for DHA are marine algae (2) or fungi. In particular, the filamentous fungus, *Thraustochitrium* sp. has been demonstrated to contain a considerable amount of DHA in its lipid matter (1).

While several reports have been there to extract the lipids from freeze-dried algae (3) or fungi (4), extraction of DHA from lyophilized *Thraustochitrium* sp. has not been attempted. In this work, three different types of extraction were attempted for the development of DHA recovery process from the lyophilized fungi. Solvent extraction, soxhlet method, and supercritical fluid extraction were investigated.

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Gas Chromatographic Analysis of DHA

Lipids extracted from freeze-dried cells were methylated and the methyl esters of the fatty acids were analyzed with Hewlett-Packard (HP) gas-liquid chromatograph with HP-1 (methyl silicone gum) column (5 m × 0.53 mm × 2.65 um). The temperature gradient was programmed to run from 150 to 220°C with the rate of 5°C/min. Helium was the carrier gas and flowed through the column at 40 ml/min. Residence time of DHA was certified using authentic DHA (98%) from Sigma Co. Quantitation of DHA was done using external standard method based on the calibration curve from the signals of authentic DHA samples with concentration range from 1 to 10 μg per 1 ml of chloroform.

Solvent Extraction

Lyophilized cells were homogenized and 10 g of fungi powder (200 mesh/400 mesh) was located in the 250 ml silicone capped flask. 100 ml of solvent was poured into the flask. The flask was put in the rotary shaker at 200 rpm, and the temperature was set to 30°C. Fig. 1 shows the result of the time dependency of the amount of DHA in the extracting solvents. After 24 h, most of the extraction seems to be completed, and no more significant increase was observed. Hence, 24 h was considered to be the appropriate extraction time for the extraction of DHA from freeze-dried cells, and was adopted as extraction time in the following experiments.

From the Fig. 1, the final amount of DHA was different according to the solvent used as an extractant. Two times lager amount of DHA was obtained from the chloroform:methanol (2:1) mixture over hexane. This seems to be due to the difference of polarity beteween two solvents. Table 1 supports the polarity dependence

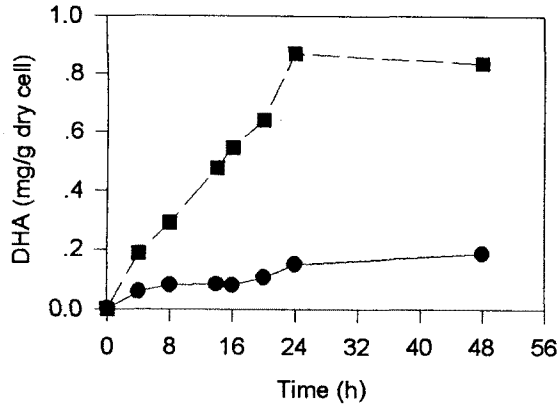

Fig. 1. Time course of solvent extraction.
100 ml of hexane (●) and chloroform: methanol (2:1) (■) was poured, respectively, into 250 ml silicone capped flask, and 10 g of freeze-dried cell powder was charged. The flask was put in a rotary shaker at 200 rpm. The reaction temperature was 30°C.

Table 1. Polarity dependence of solvent on the solvent extraction for the recovery of DHA from lyophilized *Thraustochitrium* sp.

Solvent	Polarity	DHA (mg/g dry cell)
Hexane	0.0	0.147
Cyclohexane	0.2	0.209
Diethel ether	2.8	0.216
Chloroform	4.1	0.773
Methanol	5.1	0.680
Chloroform:methanol (2:1)		0.869

of solvent on the extraction of DHA. As the polarity of extracting solvent went higher, more DHA was extracted.

In case of the solvent extraction, further step is needed to separate biomass solid from liquid solvent. Furthermore, additional treatment is indispensable for the removal of toxic solvent from DHA.

Soxhlet Extraction

10 g of lyophilized *Thraustochitrium* sp. powder (200 mesh/400 mesh) was put in the timble and 200 ml of extracting solvent was filled in the bottom flask. Through the extraction, solvent was evaporated and condensed to flow down across timble, wetting matrix, and recycled to bottom flask. As the biomass matrix was placed on the timble made out of sintered glass, solvent could only penetrate the sintered glass layer. Hence, filtration was achieved, simultaneously.

After 24 h operation, concentrated solvent was obtained in the bottom flask. The removal of solvent was done using rotary evaporator to yield fungal oil. Hexane and chloroform were chosen as extractants and the results were compared as in Table 2. The extracted amount of DHA was comparable with that of solvent extraction.

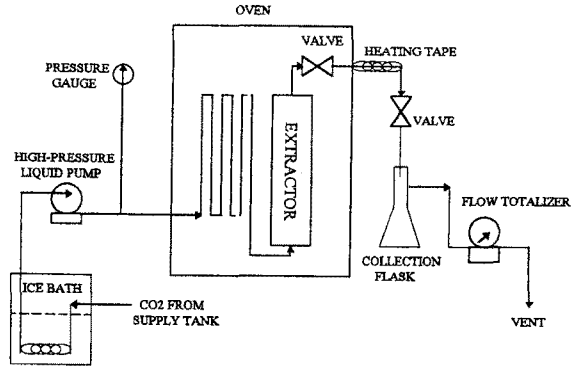

Fig. 2. Experimental apparatus for supercritical fluid extraction.

Table 2. Results of Soxhlet extraction and supercritical fluid extraction for the production of DHA from freeze-dried *Thraustochitrium* sp.

Extraction method	Extractant	DHA (mg/g dry cell)
Soxhlet extraction	Hexane	0.174
Soxhlet extraction	Chloroform	0.852
Supercritical fluid extraction	Carbon dioxide	0.251

Soxhlet method over solvent extraction allows the reduction of solid-liquid separation step like filtration or centrifugation required to remove particles from solvents.

Supercritical Fluid Extraction (SFE)

The apparatus for SFE unit is shown at Fig. 1, and the operation condition was refered to Cygnarowicz-Provose, M. *et al.* (4). The equipment consists of CO_2 supply tank, ice bath, high-pressure liquid pump, pressure gauge, extraction cell, oven, expansion valve, collection flask, and vent. Carbon dioxide was used for supercritical fluid. The extraction was achieved at 5,500 psi and 40°C. The flow rate of carbon dioxide was 3 SLPM (standard liters per minute). 3.475 g of powder (200 mesh/400 mesh) was charged between layers of glass wool to an extraction vessel. The high pressure gas passing downstream of the compressor was heated in a tube preheater to about 40°C and was passed through the extraction vessel. The extraction took just 10 min (data are not shown).

The amount of DHA extracted is shown in Table 2. The amount of extracted DHA was larger than that of solvent extraction with nonpolar solvent. On the other hand, better extraction was achieved with solvent extraction using polar extractant than that of SFE with CO_2. This seems due to the nonpolar property of CO_2. SFE, however, has the absolute merits over solvent or soxhlet methods with respect to the acquisition of pure DHA enliched lipid without any additional step for separation of extractant.

Moreover this inexpensive extractant, CO_2, is a non-

toxic solvent which eliminates the problem derived from remaining toxic solvents.

From the above results of extractions, supercritical fluid extraction using CO_2 seems to be one of the most appropriate choice for the recovery process of DHA from lyophilized *Thraustochitrium* sp.

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