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Supercritical Carbon Dioxide Extraction of PUFA Rich Oil from Freeze Dried Tuna Red Meat

R. Yathavamoorthi[1,2], C. T. Nithin[1,3], T. R. Ananthanarayanan[1,4], Suseela Mathew[1*], J. Bindu[1], R. Anandan[1] and T. K. S. Gopal[1]

[1]*Central Institute of Fisheries Technology, P.O. Matsyapuri, Cochin - 682 029, India*

Abstract

Supercritical fluid extraction (SFE) is an emerging technology for extraction and isolation of valuable compounds from natural products. Supercritical carbon dioxide (SCO_2) is one of the most commonly used solvents in SFE and has gained importance as a "green" or environment friendly solvent. In this study, polyunsaturated fatty acid (PUFA) rich oil from freeze dried yellowfin tuna (*Thunnus albacares*) red meat was extracted using supercritical carbon dioxide. Red meat, a by-product obtained from tuna processing forms about 9-11% of the total body weight of tuna. Lipid extraction from freeze dried tuna meat was performed at a temperature of 60°C and pressure of 35 MPa for 3 h. The flow rate of CO_2 was kept constant at 175 l h^{-1}. The extracted oil was collected in two separators both held at 5 MPa pressure and temperature of 50°C and 40°C respectively. The antioxidant tocopherol (0.5%) was added to the extracted oil and stored at 2-4°C for further analysis. The yield of oil obtained was 5% and it was found to be rich in polyunsaturated fatty acids like docosahexaenoic, eicosapentaenoic and arachidonic acid constituting 31, 5 and 4% of the total fatty acids respectively. Palmitic and stearic acid were the most abundant saturated fatty acids present constituting 23 and 15% of total fatty acids respectively. Oleic acid contributed 18% of the total fatty acids. SFE was effective in extraction of fatty acids from tuna red meat with minimal losses.

Keywords: Supercritical carbon dioxide extraction, PUFA, Tuna red meat

Introduction

Fish and fish oils are known to possess many protective properties against cardiovascular diseases (Kris-Etherton et al., 2002), rheumatoid arthritis (Rennie et al., 2003), depression (Nemets et al, 2006), age related cognitive decline (Morris et al., 2005; Obulesu et al., 2015) and neurological disorders such as Alzheimer's disease (Lukiw, & Bazan, 2008). Most commonly used methods for extraction of fish oil are wet reduction, cold extraction or centrifuging, enzymatic extraction, solvent extraction and alkali digestion. However, these methods possess several limitations. Despite its higher yield, extraction of oil by wet reduction requires high amount of refining to make it suitable for edible purposes (Linder et al., 2005). Cold extraction results in lower yield (Nuria et al., 2012) while solvent extraction possesses the drawback of leaving traces of organic solvents in the extracted oil (Ahluwalia et al., 2013).

In recent years, supercritical fluid extraction (SFE) has become an attractive technology for extraction and isolation of valuable compounds from natural products (Linder et al., 2005). It uses moderate temperatures and provides an oxygen free media, to reduce oxidation of the omega-3 during the extraction process of PUFA from fish. Supercritical fluid extraction possesses high tunability, as the physicochemical properties of a given fluid, such as density, diffusivity, dielectric constant and viscosity can be easily controlled by changing the pressure or the temperature. The technology also helps to avoid the co-extraction of polar impurities such as heavy

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* *E-mail: suseela1962@gmail.com*

[2] *Present Address: Export Inspection Agency- Chennai Sub Office, Nellore - 524 003, India*

[3] *Present Address: National Institute of Fisheries Post Harvest Technology and Training, Cochin - 682 016, India*

[4] *Present Address: Foodastha, Cochin - 682 301, India*

metals and hence possesses high selectivity (Kawashima et al., 2006; Catchpole et al., 2000). Supercritical fluid extraction of fish oil and subsequent microencapsulation has been proposed as an alternative to prevent oxidative rancidity as it uses lower temperatures and inert atmospheres (Nuria et al., 2010). Supercritical fluids have liquid like densities and higher diffusion coefficient and low surface tension resulting in easy penetration of the supercritical solvent into the porous structure of the solid matrix to release the solute (Ahluwalia et al., 2013). The most widely used supercritical fluid is carbon dioxide (CO_2), as it is considered to be a green solvent and is generally regarded as safe (GRAS), non-toxic, cheap and non-flammable. Moreover CO_2 can be obtained in its pure form and is gaseous under ambient conditions, therefore easy to separate from the extracted products after processing (Nuria et al., 2010). It has mild critical conditions (31.06°C and 7.386 MPa) that allow to process thermo labile compounds like omega-3 PUFA (Ahluwalia et al., 2013). Extraction and fractionation of oils from plant and animal sources has received widespread interest due to their direct applications in food and pharmaceutical industries for generation of high-value products. Supercritical fluid extraction is gaining its popularity for obtaining high quality fish oil (Letisse et al., 2006; Rubio-Rodríguez et al., 2008).

Tuna red meat is usually utilized for low value applications such as production of fishmeal or animal feeds due to its unappealing flavour and black colour. Mumthaz et al. (2010) have investigated the fatty acid profile of yellowfin tuna red meat and found that it is an excellent source of MUFA and PUFA. SFE of oil from tuna red meat will help in producing high quality fish oil for human consumption without impairing the allied feed industry as the material after extraction of oil will be still suitable for feed production. Hence the present study was aimed to understand the efficacy of supercritical carbon dioxide extraction of oil from freeze dried tuna red meat. Fatty acid profile of the oil obtained by SFE was compared with that of tuna red meat extracted by means of solvent extraction.

Materials and Methods

Frozen yellowfin tuna red meat was obtained from Moon Fishery India Pvt. Ltd., Aroor, Kerala and freeze dried at Accelerated Freeze Drying Company, Ezhupunna, Kerala in a commercial-scale freeze drier (GEA Process Engineering A/S, Soeborg, Denmark). Freeze drying was done at -56.6°C and an operating pressure of 0.5 MPa. Soon after freeze drying, the red meat was packed in moisture proof laminated pouches flushed with nitrogen and subsequently stored in a chill room maintained at 2±2°C until further processing. Supercritical fluid extraction was carried out at Synthite Industrial Chemicals Ltd, Kolenchery, Kerala in a super critical fluid extraction plant (Haian Huada Model No: 220-50-48, Zhejiang, China). Prior to extraction, the freeze dried material was passed through a horizontal drum press to increase the surface area. Lipid extraction was performed at a temperature of 60°C at 35 MPa pressure. Flow rate of CO_2 was kept constant at 175 l h^{-1}. Extracted oil was collected in two separators held at 5 MPa pressure and temperature of 50°C and 40°C respectively. Total extraction time was 3 h. A diagrammatic representation of the extraction setup is given in Fig. 1. Tocopherol (0.5%) was added to the extracted oil and stored in an opaque bottle at 2-4°C for further analysis. Yield of oil (%) obtained was calculated by the following equation

$$\text{Yield (\%)} = \frac{\text{Weight of oil}}{\text{Weight of freeze dried red meat}} \times 100$$

For determining the fatty acid profile, lipids from yellowfin tuna red meat was extracted as per the method of Folch et al., (1957). Oil obtained by supercritical fluid extraction was directly used for analysis.

Fatty acids methyl esters (FAMEs) were prepared by the method of Metcalfe et al. (1966). Briefly, to 100 mg of oil, 5 ml of 0.5 N Methanolic NaOH was added and refluxed for 5 min in a water bath under a stream of nitrogen. Esterification was done by refluxing with 6 ml of BF_3- methanol for 5 min. After cooling, 6 ml saturated NaCl was added. This solution was extracted thrice with petroleum ether and washed with double distilled water and filtered through anhydrous sodium sulphate, evaporated and made up to 1 ml in petroleum ether for further analysis. The corresponding fatty acid methyl esters were analysed in a Perkin Elmer Autosystem XL Gas Chromatograph attached to flame ionization detector. An Elite 225 capillary column measuring 30 m length and 0.25 mm inner diameter with a film thickness of 0.25 µm was used for the analysis. Helium at a flow rate of 0.5 ml min^{-1} was used as the carrier gas. Oven temperature was initially held

at 110°C for 4 min and was programmed to increase to 240°C at a rate of 2.7°C min^{-1}, held at 240°C for 3 min and then programmed to increase to 280°C and held for 5 min. FID was maintained at 275°C. The total run time was about 41.28 min.

Samples were analyzed in triplicates and values are reported as mean±standard deviation.

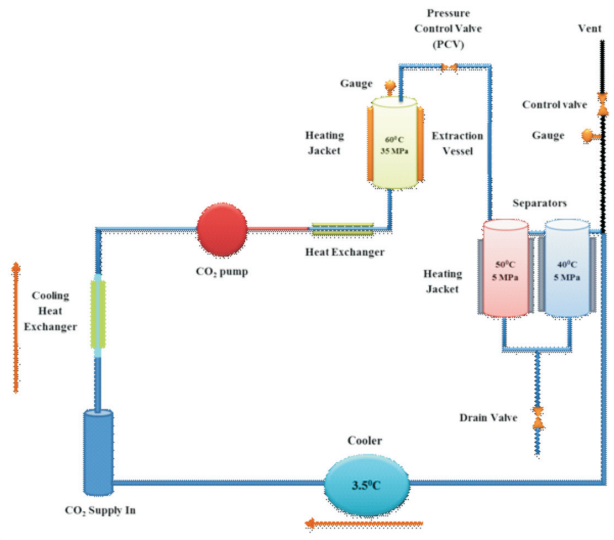

Fig. 1. Diagrammatic representation of the supercritical extraction setup

Results and Discussion

An yield of 5±0.74% oil was obtained by using the SFE. It has been observed that major factors affecting the extractability of oil by SFE are the internal structure (which affects the internal mass resistance and solubility of the fish oil in supercritical fluid) and fatty acid profile (Rubio-Rodríguez et al. 2012).

Fatty acid profile of oil obtained from freeze dried tuna red meat after SFE and that of tuna red meat by conventional method is given in Table 1. Tuna red meat used for the present study had a PUFA content of 43% of the total fatty acids. Saturated and monounsaturated fatty acids contributed 37% and 20%, respectively. PUFA content in the oil extracted by SFE was found to be 40%. Saturated and monounsaturated fatty acid contributed 38 and 22% respectively. Extracted oil was found to be rich in polyunsaturated fatty acids like docosahexaenoic acid (29% of total fatty acids), eicosapentaenoic acid (4.7% of total fatty acids) and arachidonic acid (4% of total fatty acids). Palmitic acid (21% of total fatty acids) and stearic acid (14% of total fatty acids) were the most abundant saturated fatty acids present. Oleic acid contributed 16% of the total fatty acids. The SFE was effective in extracting linolenic acid, Cis 5-Eicosenoic acid and Arachidic acid with minimal losses when compared to the chemical

Table 1. Fatty acid profile (percentage of total fatty acids) of oil from tuna red meat by SFE and solvent extraction method

Fatty acids		Oil obtained by SFE	Solvent extraction method
Saturated fatty acids (SFA)			
C14	Myristic acid	2.53±0.19	2.18±0.22
C16	Palmitic acid	20.94±1.60	22.21±1.54
C18	Stearic acid	13.74±1.05	12.21±1.27
C20	Arachidic acid	0.87±0.07	-
Monounsaturated fatty acids (MUFA)			
C16:1	Palmitoleic acid	3.93±0.30	4.42±0.43
C18:1n9	Oleic acid	16.43±1.26	16.41±0.82
C20:1	Cis 5-Eicosenoic acid	1.84±0.14	-
Polyunsaturated fatty acids (PUFA)			
C20:5n-3	Eicosapentaenoic acid (EPA)	4.70±0.36	4.28±0.21
C22:6n-3	Docosahexaenoic acid (DHA)	28.69±2.19	32.82±2.46
C18:3n-3	Linolenic acid	0.81±.06	-
C18:2n-6	Linoleic acid	1.72±0.14	2.27±0.12
C20:4	Arachidonic acid	3.80±0.29	3.22±0.26

extraction process used. Rubio-Rodríguez et al., 2008 have reported that oil can be extracted from freeze-dried hake offcuts by using supercritical carbon dioxide at a pressure of 25 MPa and a temperature of 313 K. Sahena et al. (2010) have reported highest yield of oil from the skin of Indian mackerel were achieved from the soaking and pressure swing techniques at 35 MPa and 75°C. It is evident from present study that extraction of oil from tuna red meat by SFE is a viable and effective option before utilizing it as feed for animals.

Designing and implementing appropriate measures to dispose the waste generated during processing calls for added cost and provides no significant income to processing companies. Need of the hour is to effectively convert the generated waste to value added products. Such technologies will help in minimising the pollution and creating job opportunities. Red meat is a by-product from tuna processing plants which is generally discarded or utilized for low value applications like production of animal feeds. Supercritical fluid extraction of oil from the red meat can produce high quality PUFA rich oil for human consumption before utilizing the same for production of animal feeds.

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