J. of Supercritical Fluids 50 (2009) 97–104

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Density of marine lipids in equilibrium with carbon dioxide

Bernhard Seifried, Feral Temelli*

Department of Agricultural, Food and Nutritional Science, University of Alberta, 2-06C Agriculture/Forestry Building, Edmonton, Alberta, Canada T6G 2P5

ARTICLE INFO

Article history:
Received 12 January 2009
Received in revised form 29 May 2009
Accepted 30 May 2009

Keywords:
Density
Fish oil
Marine lipids
Supercritical CO_2
Volumetric expansion

ABSTRACT

The density of marine lipids in equilibrium with carbon dioxide (CO_2) was determined using a view cell equipped with a novel spring balance based on Archimedes' principle. The densities of fish oil triglycerides (TG) and fish oil fatty acid ethyl esters (FAEE) were measured at pressures ranging from 0.1 to about 25 MPa and temperatures of 40, 55 and 70 °C. In the pressure and temperature ranges investigated, the density increased with pressure and decreased with temperature. The density increase from atmospheric pressure to about 25 MPa at temperatures of 40, 55 and 70 °C was 4.1, 3.2, 2.7% and 5.3, 4.0, 3.6% for TG and FAEE, respectively. Volumetric expansion of fish oil TG and FAEE saturated with CO_2 was determined at 40 °C and pressures ranging from 0.1 to 22 MPa. With increasing pressure a relative volume change of up to 38 and 67% was observed for TG and FAEE, respectively. The density and volumetric expansion of lipids in equilibrium with CO_2 are important for optimal design of high pressure processes involving mass and momentum transfer.

1. Introduction

During the past century, the health benefits attributed to the consumption of marine fish have gained great attention. Research has established the link between long chain polyunsaturated fatty acids (LC-PUFA) found in fish oil and its health benefits [1,2]. The two most important LC-PUFA in fish oil, namely eicosapentaenoic (C20:5 ω-3, EPA) and docosahexaenoic acids (C22:6 ω-3, DHA) belong to the family of omega-3 fatty acids, where the first double bond begins with the third carbon atom from the methyl end of the fatty acid chain. Omega-3 PUFA obtained from marine sources are mainly consumed in the form of either triglycerides (TG) or fatty acid ethyl esters (FAEE). The increased awareness of consumers about the numerous health benefits of omega-3 PUFA has led to a growing demand for food products and supplements containing EPA and DHA. The content of EPA and DHA in marine fish oil depends on numerous factors, such as species, origin, catch season and feed of the fish, with EPA and DHA levels of up to 21.5 and 36%, respectively [3–5]. Alternative sources for EPA and DHA besides fish oil include microbial or single cell oil, where certain microbial strains (*Crypthecodinium cohnii*) are capable of producing oils containing up to 50% DHA of the total fatty acids [6].

In order to be suitable for human consumption crude fish oil needs to be refined and purified. The refining process for fish oils aims mainly at removing undesirable compounds such as free fatty acids, off-aromas and peroxides. On the other hand, high-value compounds, such as squalene, vitamin A and vitamin D obtained from fish oils can be used for nutraceuticals and cosmetics. Fish oils with EPA and DHA concentrations of up to 300 mg/g can be obtained by winterization, blending, solvent crystallization and/or vacuum distillation [7]. For applications requiring higher EPA and DHA levels, the fish oil TG are hydrolyzed to liberate the fatty acids, which can then be converted into FAEE or methyl esters (FAME). The esters are subsequently fractionated by various methods to obtain higher concentrates of DHA and EPA [8]. Conventional methods to fractionate fish oil esters include vacuum or short path distillation [9], solvent crystallization and urea complexation [10,11]. These methods often require the use of flammable and toxic organic solvents or elevated process temperatures, which can lead to polymerization and degradation of thermally labile PUFA [7,8]. Besides the aforementioned methods, selective enzymatic hydrolysis of fish oil TG using fatty acid specific lipases is also used to facilitate separation and concentration of EPA and DHA from other fatty acids present in fish oils [12–14].

Processing of heat sensitive materials, such as marine lipids containing PUFA using supercritical carbon dioxide (SC-CO_2) offers numerous advantages due to the moderate critical temperature and pressure (31 °C, 7.3 MPa) of CO_2. Fractionation of fish oil TG, FAEE and FAME using SC-CO_2 has been investigated by numerous researchers and has been reviewed previously [15]. Over the last decade, progress has been made towards continuous fractionation using countercurrent packed columns. Fractionation of fish oil FAEE in a pilot plant countercurrent packed column was carried out to separate components of low molecular weight (LMC; C14–C18) from high molecular weight components (HMC; C20–C22), obtaining HMC concentrations in the raffinate of greater than 95 wt.% at

* Corresponding author. Tel.: +1 780 492 8189; fax: +1 780 492 8914.
E-mail address: feral.temelli@ualberta.ca (F. Temelli).

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doi:10.1016/j.supflu.2009.05.011

a yield greater than 95% [16,17]. Catchpole et al. [18] studied the continuous fractionation of squalene from shark liver oil in a countercurrent packed column obtaining squalene of up to 99% purity.

Modelling and design of such fractionation processes, in terms of mass transfer and understanding flooding behaviour of countercurrent packed columns, falling film extraction or spray processes, require physical properties such as density and viscosity of both the liquid and gaseous phases as well as interfacial tension [19–23]. Experimental data for the density of fish oil TG and FAEE are limited in the literature. Chang et al. [24] determined the density of highly concentrated DHA and EPA ethyl esters (90 and 96%, respectively) in equilibrium with CO_2 at 40 and 60 °C and pressures of up to about 23 MPa using a U-tube densitometer. Smith et al. [25] assessed the density and volumetric swelling factors of mixtures of CO_2 with fish oil and fish oil ethyl esters at various compositions using a vibrating tube densitometer at 40 °C and pressures ranging from 0.1 to about 20 MPa. A high pressure pycnometer was used by Staby and Mollerup [26] to determine the density of a fish oil ethyl ester mixture at 10, 40 and 70 °C and pressures up to 5.9, 15.64 and 22 MPa, respectively; however, they reported large uncertainty in their density data, presumably caused by flashing of the gas saturated esters into the evacuated pycnometer during the sampling procedure. Tegetmeier et al. [27] determined the density of corn oil in equilibrium with CO_2 using a magnetically coupled balance and sinker based on Archimedes' principle similar to the device used in this study.

Due to the lack of experimental density data for marine lipids in the literature, the objective of this study was to measure the density of fish oil TG and FAEE in equilibrium with CO_2 at 40, 55 and 70 °C and pressures ranging from 0.1 to 25 MPa. For this purpose, a view cell together with a novel device consisting of a spring balance equipped with a sinker was designed and built. The second objective was to determine the volumetric expansion of fish oil TG and FAEE in equilibrium with CO_2 at 40 °C and pressures ranging from 0.1 to about 22 MPa. Furthermore, the results of density and volumetric expansion for both TG and FAEE were analyzed in relation to the solubility data of CO_2 in the liquid phase taken from literature.

2. Experimental

2.1. Materials

Corn oil (Mazola®) was purchased at a local store and used without further treatment for density measurements. Refined fish oil extracted from anchovy and sardine was kindly provided by Ocean Nutrition Canada (ONC, Halifax, NS, Canada) in the form of TG and FAEE for the density measurements. The fatty acid profiles as provided by the manufacturer for the fish oil in the form of TG (ONC product code: XOTDHA-NG) stated a level of 8 and 25%, whereas that for the FAEE (ONC product code: XO4020EE) was 42 and 21% for EPA and DHA, respectively. Further specifications for both the fish oil TG and FAEE are listed in Table 1. Both TG and FAEE were used without further treatment and stored at 4 °C in aluminum bottles with nitrogen filled headspace to minimize any degradation. Food grade anhydrous ethanol (Commercial Alcohol, Winnipeg, MB, Canada) was used for cleaning and density measurements. Bone dry carbon dioxide with a purity of 99.9% (Praxair, Edmonton, AB, Canada) was used for the density measurements. Nitrogen with a purity of 99.998% (Praxair, Edmonton, AB, Canada) was used to fill the headspace of the aluminum bottles containing the fish oil after each opening and to flush the syringe used to inject fish oil into the apparatus.

Table 1
Fatty acid profile and specifications for FAEE and TG as provided by the manufacturer.

	FAEE Expressed as EE	TG Expressed as TG
Fatty acid profile		
EPA, mg/g	390 (42%)	70 (8%)
DHA, mg/g	200 (21%)	230 (25%)
Total omega-3 PUFA, mg/g	670 (72%)	330 (36%)
Specifications		
Free fatty acids, % oleic acid	0.5	0.2
Acid value, mg KOH/g	1.0	0.4
p-Anisidine value	8	12
Peroxide value, mequiv./kg	3	0
Moisture level, %	0	0

2.2. Volumetric expansion measurements

2.2.1. Apparatus to determine volumetric expansion

The apparatus (Phase Monitor II, Supercritical Fluid Technologies Inc., Newark, DE) used to measure the volumetric expansion of fish oil TG and FAEE is shown in Fig. 1. It consists of a variable volume high pressure view cell (3–30 mL) equipped with a CCD camera and temperature controlled electric heaters. The view cell was pressurized with CO_2 by means of a syringe pump (ISCO Model 250D, Isco Inc., Lincoln, NE) instead of the original hand-operated pump of the system. The internal volume of the view cell was adjusted to about 5 mL by movement of the piston. The apparatus equipped with a thermocouple and pressure transducer was connected to a computer, which allowed continuous recording of temperature and pressure along with the images from the CCD camera. Thus, images were recorded continuously facilitating the observation of the volumetric expansion.

2.2.2. Determination of volumetric expansion

The volumetric expansion of fish oil in the form of TG and FAEE in equilibrium with CO_2 was determined in triplicate at 40 °C and pressures ranging from 0.1 to about 22 MPa. Prior to experiments, fish oil (3 mL) was placed at the bottom of the view cell and continuously stirred during experiments. Furthermore, a capped capillary with an inner diameter of 1.5 mm and height of 15 mm was filled with fish oil up to a height of approximately 3 mm under nitrogen using a syringe with a fine needle tip to avoid inclusion of gas bubbles. The filled capillary was then placed in the capillary holder and inserted into the preheated view cell. Fish oil was placed at the bottom of the view cell to minimize the amount of FAEE or TG extracted by SC-CO_2 from within the capillary during expansion studies. Most of the lipids dissolving into the CO_2 to reach phase equilibrium are estimated to come from the oil placed at the bottom part of the view cell, since mass transfer is proportional to the mass trans-

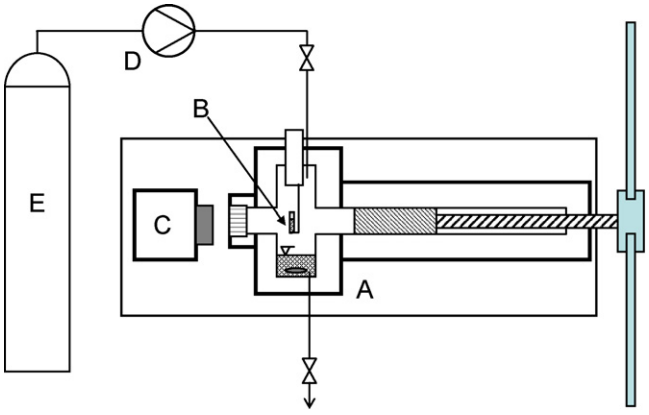

Fig. 1. Apparatus to study volumetric expansion: (A) thermostated view cell with magnetic stir bar, (B) holder with glass capillary, (C) CCD camera, (D) syringe pump, and (E) CO_2 tank.