

# Viscosity of Marine Lipids in Equilibrium with High Pressure Carbon Dioxide

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Viscosity affects a wide variety of processes through its impact on mass, heat and momentum transfer. Optimal design of processing equipment involving high pressure carbon dioxide (CO<sub>2</sub>) requires viscosity data. Therefore, the objective of this study was to determine the viscosity of fish oil (FO) in the form of fatty acid ethyl esters (FAEE) in equilibrium with CO<sub>2</sub>. The viscosity of FAEE in equilibrium with CO<sub>2</sub> was determined in triplicate at 40, 55, and 70°C and pressures ranging from 0.1 to about 12 MPa using a rotational rheometer equipped with a high pressure cell at shear rates of 300 to 600 s<sup>-1</sup>. Viscosity of FAEE determined at a shear rate of 300 s<sup>-1</sup> and 500 s<sup>-1</sup> decreased with pressure due to dissolution of CO<sub>2</sub>. However, at higher temperatures the solubility of CO<sub>2</sub> in FAEE is lowered leading to a less pronounced viscosity decrease with pressure. At a shear rate of 300 s<sup>-1</sup> the highest viscosity drop of 67% (3.21 to 1.06 mPa.s) with a pressure increase from 0.1 to 11.8 MPa was observed at 40°C, whereas it was only 35% (2.03 to 1.33 mPa.s) at 70°C. Rheological measurements showed dilatant behavior for the FAEE saturated with CO<sub>2</sub> at elevated pressures, with a flow behavior index increasing with pressure from about 1.1 to 1.6 at 40°C. Additionally, at 40°C a new experimental approach using a Quartz Crystal Microbalance (QCM) was used to determine the relative change in viscosity of FAEE in equilibrium with CO<sub>2</sub> under high pressure compared to atmospheric conditions and the results were in general agreement with those obtained with the rotational viscometer. Novel process development and design of equipment, such as spray nozzles, filters and packed columns will benefit from a better understanding of the rheological behavior.

## INTRODUCTION

Processing of lipids involving high pressure CO<sub>2</sub> has increasingly gained interest in the past two decades. The trend towards environmentally friendly technologies taking advantage of high pressure and supercritical CO<sub>2</sub> (SC-CO<sub>2</sub>) as well as gas-expanded liquids is fuelled by stricter environmental and safety regulations in many countries. Furthermore, the increased consumer demand for bioactive lipid components has stimulated a rapidly growing market for functional foods and nutraceuticals, including heart-healthy polyunsaturated fatty acids (PUFAs). Fractionation of fish oil components using SC-CO<sub>2</sub> has been investigated extensively, with a focus on continuous processing in packed columns [1, 2]. In order to understand flooding behavior and optimize the performance of such packed columns the knowledge of viscosity data is fundamental. Lipids when saturated and expanded with CO<sub>2</sub> under pressure undergo significant changes in their physical properties, including density, interfacial tension and viscosity. However, there is lack of viscosity data for gas-expanded lipids in the literature. Therefore, the objective of this study was to determine the viscosity and rheological behavior of fish oil FAEE in equilibrium with CO<sub>2</sub> at 40, 55 and 70°C and pressures of up to about 12 MPa.

## MATERIALS AND METHODS

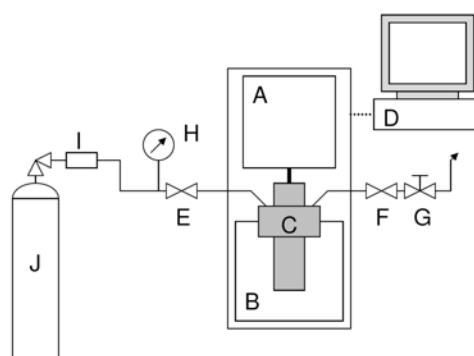
### Materials

Refined fish oil extracted from anchovy and sardine was purchased from Ocean Nutrition (Halifax, NS, Canada) in the form of FAEE for the viscosity measurements. The fatty acid profile as provided by the manufacturer stated a level of 40% and 20% for EPA (C20:5) and DHA (C22:6), respectively. 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 calibration purposes of the QCM. Bone dry carbon dioxide with a purity of 99.9% (Praxair, Edmonton, AB, Canada) was used for the viscosity 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 FAEE into the apparatus.

### Viscosity measurements using the rotational rheometer

Viscosity and rheological properties of FAEE in equilibrium with CO<sub>2</sub> were determined in triplicates for every isotherm at 40, 55 and 70°C and pressures of up to 12.4 MPa using a computer controlled rotational rheometer (UDS200, Anton Paar, Graz, Austria) equipped with a bob and cup setup in a thermostated pressure cell (Fig. 1). The pressure cell was equipped with a magnetic coupling pressure head. The system was pressurized using a CO<sub>2</sub> cylinder with a helium headspace at 12.4 MPa and a pressure reducing regulator. The high pressure cell was flushed with CO<sub>2</sub> and preheated to the desired temperature prior to experiments. In order to fill the high pressure cell, the outlet tubing was disconnected and a known amount of FAEE (11.5 mL) was filled into the cell. The pressure level was increased by adjusting the pressure reducing regulator. Since there was no mechanical mixing, equilibration and saturation of FAEE with CO<sub>2</sub> at elevated pressures inside the pressure cell took up to about 10 hr for each pressure setting as indicated by a constant viscosity value. In a previous study, we found that FAEE expanded up to about 60% by volume when equilibrated with CO<sub>2</sub> at pressures of up to 15 MPa at 40°C. The expansion of FAEE may lead to errors if the ball bearings of the magnetic coupling inside the top of the pressure head of the rheometer are submersed in expanded FAEE. Therefore, CO<sub>2</sub> was carefully bubbled through the headspace of the high pressure cell every 3 hr and, prior to measurements, to purge out expanded excess FAEE. For the first set of measurements, FAEE viscosity was determined at a constant shear rate of 300 s<sup>-1</sup> and 500 s<sup>-1</sup> at all pressure and temperature levels. Then, the rheological properties were evaluated at each pressure level by measuring shear stress ( $\tau$ ) at shear rates ( $\dot{\gamma}$ ) ranging from 300 to 600 s<sup>-1</sup>. The flow consistency index (K) and the flow behaviour index (n) were determined according to the power law:

$$\tau = K * \dot{\gamma}^n \quad (1)$$

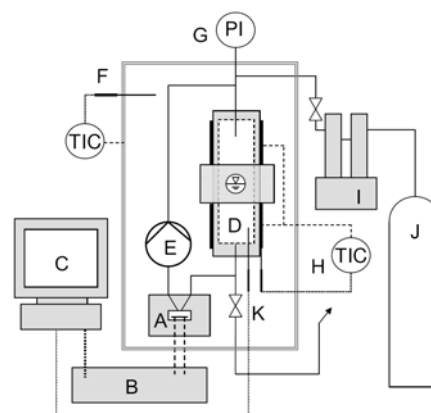


**Figure 1:** Rheometer setup: A) rheometer, B) thermostat, C) high pressure cell, D) computer, E) inlet valve, F) outlet valve, G) metering valve, H) pressure indicator, I) pressure reducing regulator, J) CO<sub>2</sub> cylinder

### Viscosity measurements using the quartz crystal microbalance

The apparatus consisted of a 200 mL high-pressure view cell with an internal diameter of 40 mm (Nova-Swiss, Effretikon, Switzerland) equipped with electric heaters and placed in a temperature controlled insulated air bath (Fig. 2). The temperatures of both the air bath and electric heaters were individually controlled by means of J-type thermocouples connected to controllers (Chromalox 1601, Pittsburgh, PA). The temperatures of both the liquid inside the view cell as well as the circulating air bath were continuously recorded to a computer by means of thermistors connected to a custom made data acquisition (DAQ) system. The DAQ system consisted of a signal amplifier and conditioner designed to linearize and transform the nonlinear response of the thermistors into voltage, which was measured by a 10 bit DAQ card (DI-194RS, DATAQ Instruments, Inc., Akron, OH) and visualized by means of a chart recorder software (WinDaq/Lite, DATAQ Instruments, Inc., Akron, OH). The thermistor DAQ system was calibrated against a precision digital thermometer (Model 4000, Control Company, Friendswood, TX) with a resolution of 0.001°C using an insulated heating waterbath (Haake H3, Karlsruhe, Germany). With a sampling rate of 50 Hz the DAQ system allowed continuous monitoring and recording of the air bath temperature as well as liquid temperature inside the view cell with a resolution of 0.01°C. The air bath and the electric heaters were usually capable of maintaining the liquid circulated inside the view cell at a constant temperature to within +/- 0.1°C. However, in case of fish oil FAEE following a stepwise increase in CO<sub>2</sub> pressure the oil temperature fluctuated by about +/- 0.5°C due to exothermic and endothermic mixing effects caused by dissolution of CO<sub>2</sub> into the lipids. The view cell was pressurized with CO<sub>2</sub> by means of a piston pump (ISCO Model 250D, Isco Inc., Lincoln, NE). The pressure was monitored using a digital pressure indicator with 0.05% full scale accuracy (Druck Model DPI 104, General Electrics Sensing, Billerica, MA). The equilibration process was accelerated by circulating the liquid from the bottom of the view cell by means of a custom made magnetically-driven piston pump and spraying it into the upper CO<sub>2</sub>-rich phase.

The change in viscosity was determined by means of a Quartz Crystal Microbalance (QCM) placed in a custom made high pressure cell, which was connected in line between the view cell and the circulation pump by means of PEEK tubing to electrically insulate the QCM from the rest of the system to avoid interference and noise in the frequency signal. The sensor crystal was a 9 MHz AT-cut quartz crystal with polished wrap-around gold plated electrodes (ICM, Oklahoma City, OK). The crystal had a diameter of 14 mm, a stiffness of  $29.01 \times 10^9$  N/m<sup>2</sup> and a density of 2649 kg/m<sup>3</sup>. The resonant frequency of the QCM was determined by means of an integrated Research Quartz Crystal Microbalance (RQCM, Maxtek, Santa Fe Springs, CA) hooked up to a computer with RQCM software allowing to record frequency shifts of the crystal. To determine the frequency response of the QCM in pure ethanol at elevated pressures the system was completely filled with ethanol and pressurized using an HPLC pump (Beckman Model 110A, Fullerton, CA).



**Figure 2:** Quartz Crystal Microbalance (QCM) setup: A) QCM cell, B) resonator/frequency counter (RQCM), C) computer, D) high pressure view cell, E) circulation pump, F) thermostated circulating airbath, G) pressure indicator, H) temperature controlled electric heaters, I) syringe pump, J) CO<sub>2</sub> cylinder, K) thermistor

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## Calibration of the Quartz Crystal Microbalance

The resonant frequency of an AT-cut quartz crystal submersed in a fluid depends on numerous factors, including the thickness of the crystal, mass loading on the crystal, pressure, temperature, surface roughness as well as viscosity and density of the surrounding fluid [3, 4]. By keeping the temperature constant during all experiments, avoiding mass loading on the electrode surface and using a crystal with an optically polished surface the main factors affecting the resonant frequency of the QCM submersed in a fluid are pressure  $P$ , density  $\rho$  and viscosity  $\eta$ . In that case, the total frequency shift due to changes in pressure, density and viscosity are described by:

$$\Delta f = \Delta f_p + \Delta f_{\rho\eta} \quad (2)$$

The first term  $\Delta f_p$  describes the frequency shift due to hydrostatic pressure [5]:

$$\Delta f_p = f_0 \cdot \alpha \cdot (P - P_0) \quad (3)$$

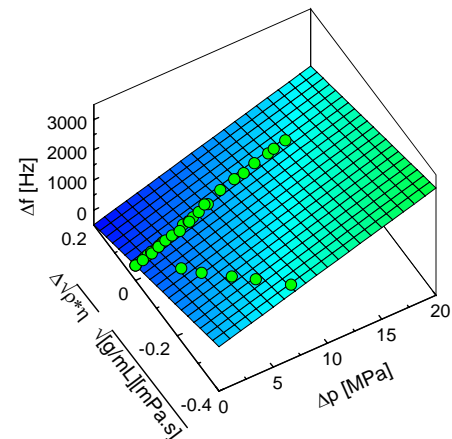
where  $f_0$  is the fundamental crystal frequency at a reference pressure  $P_0$  and  $\alpha$  is a constant. The second term  $\Delta f_{\rho\eta}$  represents the relationship relating the change in density and viscosity to the crystal frequency. A simple relationship was proposed by Kanazawa and Gordon [3]:

$$\Delta f_{\rho\eta} = -c \cdot n \cdot f_0^{3/2} \cdot (\sqrt{\rho \cdot \eta} - \sqrt{\rho_0 \cdot \eta_0}) \quad (4)$$

where  $c$  is a coefficient dependant on the quartz crystal's shear modulus and density, and  $n$  is the number of faces that are immersed in the liquid. The above equation (2) can therefore be written as a combination of the two linear relationships as follows:

$$\Delta f = C_1 \cdot \Delta P + C_2 \cdot \Delta \sqrt{\rho \cdot \eta} \quad (5)$$

In order to determine the constants  $C_1$  and  $C_2$  for the crystal used in this study, the resonant frequency of the crystal while submersed in liquid was recorded at atmospheric pressure up to about 15 MPa. To separate the effect of pressure from the combined effect of density and viscosity on crystal frequency two liquids are required where the density and viscosity change differently with respect to pressure. Thereby, it is possible to generate two different frequency response curves, which are then used to derive the plane surface described by Eq. (5) as illustrated in Figure 3. Ideally the two liquids should be chosen such that the change in the viscosity and density with pressure is little for one liquid while the second liquid exhibits a great change in viscosity and density. In that case the response curves are spread over the plane surface, which improves accuracy of the calibration. The liquid systems used in this study for calibration at 40°C were pure ethanol at hydrostatic pressures ranging from 0.1 to 15 MPa and CO<sub>2</sub>-expanded ethanol at pressures of up to about 10 MPa. For both liquid systems the density and viscosity data are available in literature [6-9]. While density and viscosity of pure ethanol exposed to hydrostatic pressure do not change substantially, the density and viscosity of CO<sub>2</sub>-expanded ethanol change considerably with increasing CO<sub>2</sub> pressure. By measuring the frequency response of the QCM at various pressures in both fluid systems the constants  $C_1$  and  $C_2$  could be determined. The values calculated for the QCM system used in this study are  $C_1 = 82.35$  Hz/MPa and  $C_2 = 2180.9$  Hz/(mPa.s\*g/mL)<sup>1/2</sup>. The value for the constant  $C_1$  translates into an  $\alpha$  value of  $0.93 \cdot 10^{-5}$  MPa<sup>-1</sup> for the 9 MHz QCM, which agrees well with the  $\alpha$  values of



**Figure 3:** Calibration surface plot of the QCM using pure ethanol and CO<sub>2</sub> expanded ethanol.

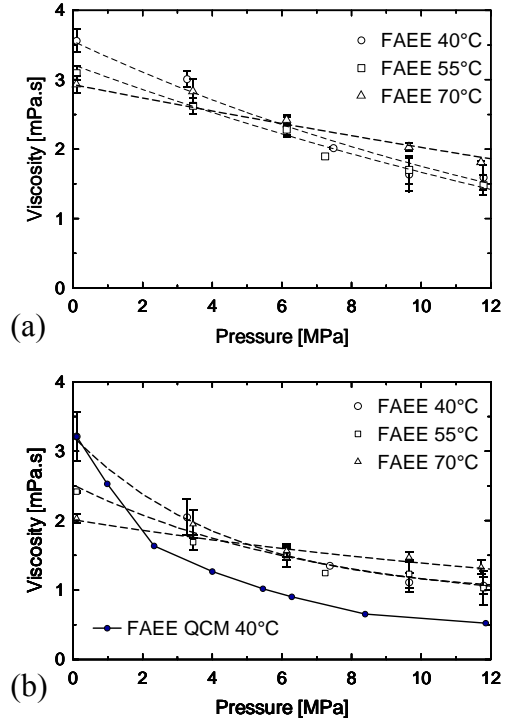
$1.045 \cdot 10^{-5} \text{ MPa}^{-1}$  and  $1.02 \cdot 10^{-5} \text{ MPa}^{-1}$  reported by Stockbridge [5] and obtained using a correlation given in [4], respectively, for 5 MHz AT-cut crystals. Besides Eq. (5) and its constants, the density of the surrounding fluid is required in order to calculate the viscosity from the measured frequency shift of the quartz crystal submersed in the fluid. In a previous study, we determined the density of CO<sub>2</sub>-expanded FAEE [10], which facilitated to ascertain the change in viscosity of CO<sub>2</sub>-expanded FAEE at 40°C and elevated pressures using the frequency response of the QCM.

## RESULTS AND DISCUSSION

Viscosity of fish oil FAEE saturated with CO<sub>2</sub> was determined at shear rates of  $300 \text{ s}^{-1}$  and  $500 \text{ s}^{-1}$  at 40, 55, and 70°C and pressures of 0.1 to 12.4 MPa. The effects of both temperature and pressure on viscosity of FAEE expanded with CO<sub>2</sub> are substantial (Fig. 4). At atmospheric pressure, the viscosity decreased exponentially with temperature. Moreover, at 40°C the viscosity decreased with increasing CO<sub>2</sub> pressure by about 67% from 3.21 to 1.06 mPa.s at 0.1 and 12.4 MPa, respectively. The effect of pressure on viscosity was more pronounced at lower temperatures due to a higher solubility of CO<sub>2</sub> in oil. At elevated pressures of up to 12 MPa the isotherms approached viscosity levels of about 1 mPa.s with a slightly higher value at 70°C.

The model given in Eq. (6) correlates the measured viscosity data obtained with the rotational rheometer to pressure and temperature. The parameters (Table 1) are valid for the temperature and pressure range investigated, with pressure  $P$  in MPa, temperature  $T$  in K and viscosity  $\eta$  in mPa.s.

The viscosity of CO<sub>2</sub>-expanded FAEE at 40°C measured with the QCM is shown in Figure 3(b). In order to determine the relative change of viscosity with the QCM an initial viscosity value is needed. The viscosity of FAEE determined at atmospheric pressure and 40°C with the rotational rheometer served as the reference point. The viscosity values obtained by using the QCM decreased more substantially than those obtained by using the rheometer. The AT cut quartz crystal is a thickness shear mode resonator which transmits an acoustic wave into the surrounding fluid perpendicular to the crystal surface. Thus, defining a specific shear rate for this QCM sensor is difficult.



**Figure 4:** Viscosity of fish oil FAEE in equilibrium with CO<sub>2</sub> as a function of pressure at various temperatures and shear rates of  $500 \text{ s}^{-1}$  (a) and  $300 \text{ s}^{-1}$  (b). QCM results are illustrated by the solid dots and line (b). Dashed lines were calculated using Eq. (6). Error bars represent standard deviation of triplicate measurements.

**Table 1.** Parameters for viscosity model

Shear rate	$300 \text{ s}^{-1}$	$500 \text{ s}^{-1}$
$A_1$	5.144	25.24
$A_2$	-1.347E-02	-8.252E-02
$A_3$	1.714 E-02	4.018E-01
$A_4$	1634	680.7
$A_5$	2.030	4.802E-01
$A_6$	-5.758E-03	-1.352E-03

$$\eta(P, T) = (A_1 + A_2 \cdot T) + \left[ A_3 \cdot e^{\left(\frac{A_4}{T}\right)} - (A_1 + A_2 \cdot T) \right] \cdot e^{[-(A_5 + A_6 \cdot T) \cdot (P - 0.1)]} \quad (6)$$

Furthermore, the effect of non-Newtonian behavior of the surrounding liquid on the response of the QCM is not fully understood. Nevertheless, the information obtained with the QCM allows detection of the relative change in viscosity at elevated pressures with a sensor a fraction of the size of a conventional rheometer. Further research is needed to assess the accuracy of the QCM measurements.

The rheological behavior of CO<sub>2</sub>-expanded FAEE was assessed over the entire pressure range by means of the rotational rheometer. FAEE expanded with CO<sub>2</sub> showed shear thickening behavior, with the flow behaviour index at 40°C increasing from  $n=1.1$  to  $n=1.6$  at 0.1 and 12.4 MPa, respectively (Fig. 5a). However, at elevated temperatures a decrease of the flow behavior index, possibly due to decreased solubility of CO<sub>2</sub> in the liquid phase, was observed. The flow consistency index ( $K$ ) decreased at 40°C with pressure (Fig. 5b), while at higher temperatures it increased.

## CONCLUSIONS

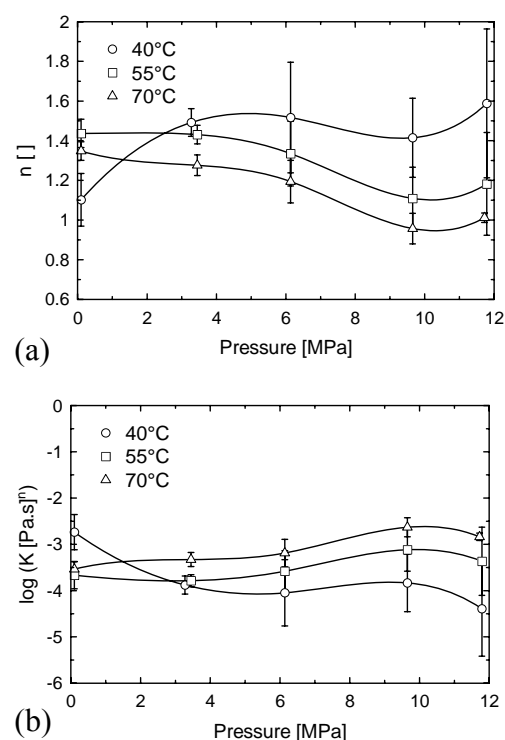
Viscosity of fish oil FAEE in equilibrium with CO<sub>2</sub> was determined at 40, 55 and 70°C and pressures of up to 12 MPa. The viscosity decreased substantially with increasing pressure. At 40°C the viscosity decreased to about one third of the initial value due to the high solubility of CO<sub>2</sub> in FAEE. At elevated pressures, CO<sub>2</sub> expanded FAEE showed pronounced shear thickening behavior. A new approach using a Quartz Crystal Microbalance was used to detect changes in viscosity of gas-expanded FAEE. The preliminary results obtained with the QCM are promising and indicate that this method could be used for continuous monitoring of liquid viscosity under high pressure conditions. Fundamental viscosity data will help to better understand flooding behavior of packed columns operated under high pressure or to optimize spray nozzle design. New process development as well as optimization and modeling of existing processes will benefit from the viscosity data.

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**Figure 5:** Flow behavior index  $n$  (a) and flow consistency index  $K$  (b) of CO<sub>2</sub>-expanded FAEE versus pressure. Error bars represent standard deviation of triplicate measurements.