Oil Processing with SC-CO_2: from Seed to Reaction Products

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INTRODUCTION

Combination of supercritical carbon dioxide (sc-CO_2) as environmentally benign and safe medium with enzymatic catalysis is extremely attractive to the food industry. Use of sc-CO_2 as a solvent for vegetable oils enables us to integrate several processes: extraction of oil from seeds, chemical or enzymatic reaction of the dissolved oil in a continuous-flow packed-bed reactor, and, if necessary, a partial fractionation of the obtained mixture of oil and reaction products by their stepwise separation from CO_2 at different pressures and temperatures in a series of separators. For example, fatty acid composition of vegetable oils is determined by gas chromatography after transmethylation of oil triacylglycerols. The sc-CO_2 extraction of oil from seed flakes integrated with the lipase-catalyzed reaction [1, 2] has the advantage of reduced sample handling and shorter analysis time in comparison with conventional extraction followed by transmethylation. Integration of the extraction of canola oil from seeds with enzymatic hydrolysis [3] or ethanolysis [4] on laboratory scale was also studied and proposed as a new process.

In our laboratory, the extraction and enzymatic hydrolysis of blackcurrant seed oil was studied with respect to the rates of extraction and reaction and to the fatty acid composition of oil, free fatty acids and intermediate products. With the help of regiospecific lipase Lipozyme, different rates of liberation of different fatty acids from the oil triacylglycerols were achieved. The blackcurrant seed oil is rich in both α- and γ-linolenic acids and the final purpose of the research is to obtain two fractions of oil hydrolysate differing in the ratio of linolenic acids, both products being suitable for dietary purposes. Ideally, sc-CO_2 would serve as a solvent to extract the oil from seeds, to conduct the reaction, and to fractionate the hydrolysate on the basis of different solubilities of lipid classes.

The reaction rate and the conversion to free fatty acids were measured in a continuous-flow reactor in dependence on the pressure, flow rate and amount of enzyme [5]. The extraction of oil from milled seeds was found to be fast enough to saturate the solvent fed to the reactor with oil. The average reaction rate (**Fig. 1**) increases with increasing flow velocity (due to the flow-dependent mass transfer

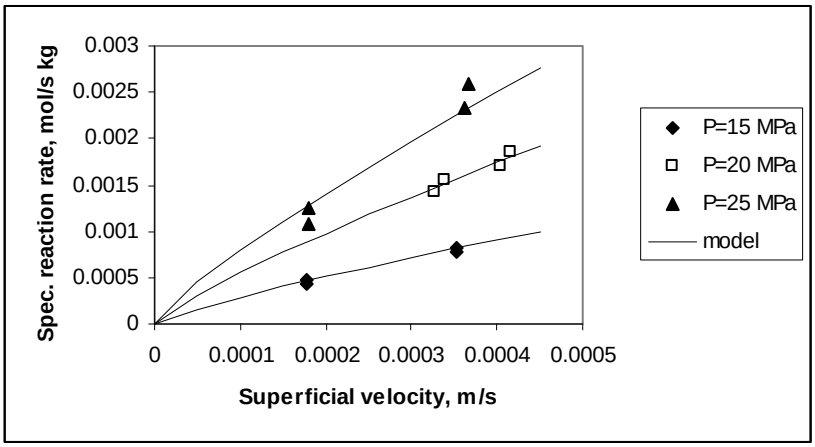

Figure 1: Average rate of liberation of fatty acids in the continuous-flow reactor at 40 ºC per unit mass of enzyme.

solubility in sc-CO_2). A model for the reaction rate was derived, taking into account both one-substrate Michaelis-Menten reaction kinetics (the effect of the concentration of the second substrate, water, could be neglected due to its large surplus in the reaction mixture) and mass transfer of oil substrate to the immobilised enzyme.
In this contribution, the attention is focused on different fatty acid distributions of individual lipid classes in the hydrolysate, and partially also to the conditions for the fractionation of the hydrolysate.

REGIOSPECIFIC HYDROLYSIS OF OIL

The oil hydrolysis is described as an irreversible reaction taking place in three steps:

$$\begin{aligned} &TG + H_2O \rightarrow DG + FFA \\ &DG + H_2O \rightarrow MG + FFA \\ &MG + H_2O \rightarrow G + FFA \end{aligned} \qquad (1)$$

where TG, DG and MG are abbreviations for tri-, di- and monoacylglycerols, respectively, FFA stands for free fatty acids, and G for glycerol.
TG contain in their molecules three acyls of fatty acids at stereospecific positions *sn*-1, *sn*-2 and *sn*-3 (**Fig. 2**). A regiospecific enzyme can distinguish only between the primary positions *sn*-1, *sn*-3 and the secondary position *sn*-2. Thus, Lipozyme liberates preferentially the acids from the primary positions, forming DG with one fatty acid esterified at *sn*-2 position and the other one at either *sn*-1 or *sn*-3 position.

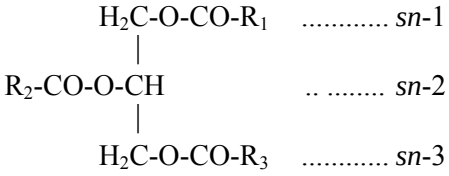

Figure 2: Stereochemical positions in a molecule of triacylglycerol, ester of R-CO-OH fatty acids (Fischer projection).

Let us denote the overall fatty acid composition at the primary stereochemical positions P_i,

$$\sum_{i=1}^{n} P_i = 1 \qquad (2)$$

where n is the number of fatty acids in the oil. Analogously, S_i will be the fatty acid composition at the secondary stereochemical position,

$$\sum_{i=1}^{n} S_i = 1 \qquad (3)$$

The fatty acid composition of the oil is then

$$f_{TGi} = (2P_i + S_i)/3 \qquad (4)$$

and the fatty acid composition of diacylglycerols formed by the regiospecific hydrolysis is

$$f_{DGi} = (P_i + S_i)/2 \quad (5)$$

The fatty acid composition of MG is equal to that of DG because the hydrolysis of diacylglycerols is not regiospecific.

The lipid class of free fatty acids consists of the acids liberated from TG, whose composition is equal to the composition at the primary stereochemical positions P_i, and the acids liberated from DG and MG, whose composition is equal to f_{DGi}. As a result, the FFA composition is

$$f_{FFAi} = \alpha P_i + (1-\alpha)(P_i + S_i)/2 \quad (6)$$

where α is the fraction of the acids liberated from TG and $1-\alpha$ is the fraction of the acids liberated from DG and MG.

Balance equations for the individual lipid classes obtained by the hydrolysis of m_{TG0} moles of triacylglycerols are

$$\begin{aligned} m_{TG} &= m_{TG0} - h_{TG} \\ m_{DG} &= h_{TG} - h_{DG} \\ m_{MG} &= h_{DG} - h_{MG} \\ m_{FFA} &= h_{TG} + h_{DG} + h_{MG} \\ m_{G} &= h_{MG} \end{aligned} \quad (7)$$

where m with respective subscript denotes the moles of the lipid class in the mixture and h_{TG}, h_{DG}, and h_{MG} represent the moles of the lipid classes that have been hydrolyzed. The fraction of fatty acids liberated from triacylglycerols is then

$$\alpha = h_{TG}/(h_{TG} + h_{DG} + h_{MG}) = (m_{TG0} - m_{TG})/m_{FFA}. \quad (8)$$

MATERIALS AND METHODS

Blackcurrant seed obtained from Zemcheba (Chelčice, CR) was harvested in 2006. It contained oil consisting almost solely of triacylglycerols, with a negligible amount of free fatty acids. Lipozyme®, a 1,3-specific lipase from *Mucor miehei* immobilised on a macroporous ion-exchange resin, was supplied by Fluka Chemie AG, Buchs. Carbon dioxide (> 99.9 %) was purchased from Linde Technoplyn, CR.

The scheme of the laboratory high-pressure equipment for the oil extraction and reaction is shown in **Fig. 3**. Pressurized carbon dioxide was pumped by ISCO 260D syringe pump consecutively to the first saturator containing water on glass beads, the second saturator containing milled seed, and the reactor filled with immobilized enzyme and glass beads. Inner diameter of the saturators and reactor was 8 mm and their volumes were 12 ml (saturators) and 4 ml (reactor). The mixture was expanded to ambient pressure in a heated micrometer valve behind the reactor and the precipitating hydrolysate was collected in a vial, weighed, and stored in a freezer before analysis or measurement of separation factors. The sampling time was measured and the mass of CO_2 passed through the equipment was calculated from its volume and density in the pump. The saturator with water was at room temperature and the temperature in the second saturator and in the reactor was maintained at 40 ºC. The applied pressure was 15, 20, 25 and 28 MPa, the solvent flow rate was 0.3-0.6 l/min (expanded).

To measure the separation factors of free fatty acids to other lipid classes in the hydrolysate, one saturator was filled with glass beads and an accurately weighed amount of hydrolysate with a known content of free fatty acids and was connected between the pump and the micrometer valve. After the required temperature and pressure were established in the saturator, CO_2 flow rate lower than 0.1 l/min was applied to ensure equilibrium between the liquid and vapour phases in the saturator. The extract precipitating from the solution flowing out of the micrometer valve was sampled and analysed for the

content of free fatty acids. The equilibrium content of FFA in the liquid phase inside the saturator was calculated from mass balance.

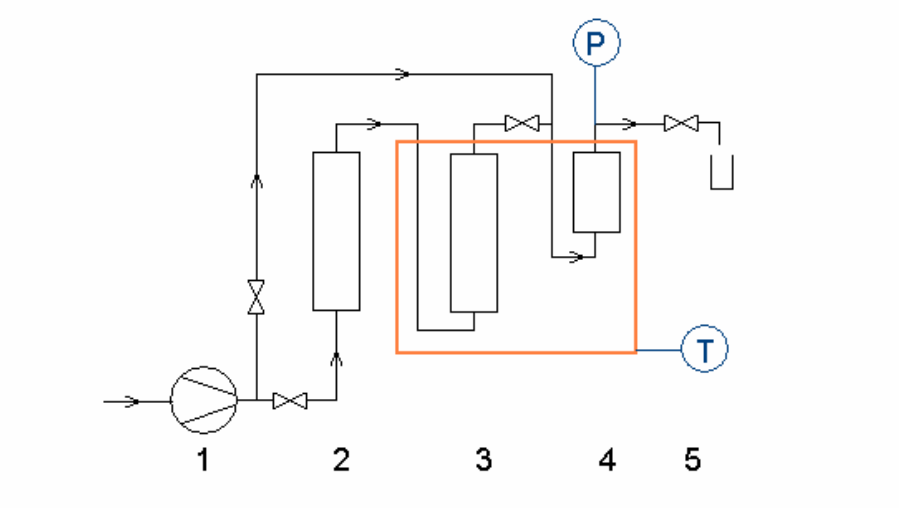

Figure 3: Scheme of extraction/reaction equipment. (1) high pressure pump for CO_2, (2) saturator cotaining water, (3) saturator containing oil, (4) reactor with immobilised enzyme, (5) heated micrometer valve; parts (3) and (4) were heated to 40 ºC.

The content of free fatty acids N_{FFA} (mol) in hydrolysate samples (10-20 mg) was determined by a fast *colorimetric method* according to Kwon and Rhee [6] using blue cupric acetate-pyridine reagent; the absorbance was measured at 714 nm.

The hydrolysate samples were separated into lipid classes (TG, FFA, DG+MG) by *preparative TLC*, developed in a solvent mixture containing light petroleum/diethyl ether/acetic acid (80/20/1.6). Lipid spots were identified using a 10% solution of phosphomolybdic acid in methanol. Fractions corresponding to each lipid class were extracted from the plates with diethyl ether, evaporated and weighted to calculate chemical yields.

Each lipid class was converted to fatty acid methyl esters according to an earlier described method [7] and the products were analysed on the *gas chromatograph* (Hewlet Packard, USA) equipped with a FID detector, a split-splitless injector and a DB-WAX column (30 m × 0.25 mm × 0.25 µm), with hydrogen as carrier gas at flow rate of 40 cm/s. The injection port temperature was 240 ºC, the FID temperature was 250 ºC, the oven temperature started at 200 ºC (20 min) and rose up to 230 ºC (15 min) with a rate of 5ºC/min. The peaks of fatty acid methyl esters were identified and calibrated using standards and equivalent chain length values [8, 9].

The experimental mass fractions of TG, DG+MG and FFA in hydrolysate samples and their fatty acid compositions were compared with the results of the *mathematical model* converted from mole to mass fractions. The adjusted model parameters were the fatty acid compositions at the primary and secondary positions P_i,, S_i in the oil and the fractions of lipid classes m_{TG}/m_{TG0}, m_{DG}/m_{TG0}, and m_{MG}/m_{TG0} in the hydrolysate.

RESULTS

As expected, the composition of TG, DG and MG in the hydrolysates obtained by regiospecific hydrolysis was independent of both reaction conditions and degree of conversion, and the composition of free fatty acids was dependent only on the degree of conversion.

An example of the composition of the product of reaction carried out at 25 MPa and CO2 flow rate of 0.6 l/min (expanded), with 35 mg of Lipozyme and at the solution residence time in the reactor 2 min

is given in **Fig. 4** and in **Table 1.** The figure shows the difference between the fatty acid compositions of lipid fractions: di- and monoacylglycerols are enriched in γ-linolenic and stearidonic acids, while the fraction of free fatty acids is enriched in a-linolenic, palmitic and stearic acids. The content of linoleic acid, the major component, and also the content of oleic acid remain practically unchanged.

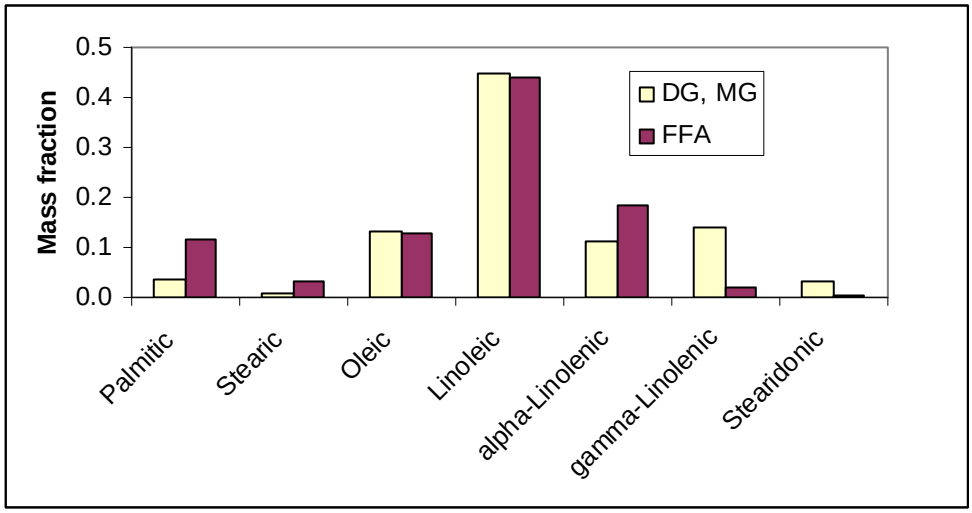

Figure 4: Fatty acid composition of partial glycerols DG, MG and free fatty acids FFA in the hydrolysate consisting of 48.8 wt.% TG, 31.4 wt.% DG+MG, and 19.8 wt.% FFA.

The table lists also the calculated fatty acid composition at the primary and secondary regiochemical positions in blackcurrant seed oil, P_i and S_i. Palmitic and stearic acids are concentrated at primary positions, which is in accordance with the rule that saturated acids in vegetable oils occupy preferably these positions. There was, however, no data on the position of linolenic and stearidonic acids in vegetable oils, and thus the information about the prevailing primary position of α-linolenic acid and prevailing secondary position of γ-linolenic and stearidonic acids in blackcurrant seed oil is new.

Table 1: Fatty acid composition in lipid classes and at regiochemical positions of TG. Lipid classes in the blackcurrant seed oil hydrolysate: TG 48.8 wt.%, DG+MG 31.4 wt.%, FFA 19.8 wt.%

Fatty acid	Experiment, mass fraction			Model, mole fraction	
	TG	DG, MG	FFA	P_i	S_i
Palmitic	0.064	0.036	0.116	0.100	0
Stearic	0.014	0.010	0.031	0.028	0
Oleic	0.126	0.133	0.127	0.128	0.125
Linoleic	0.457	0.450	0.440	0.443	0.481
α-Linolenic	0.148	0.112	0.184	0.188	0.075
γ-Linolenic	0.119	0.141	0.020	0.029	0.263
Stearidonic	0.030	0.034	0.006	0.012	0.056

An example of the results of measurement of the separation factor of FFA is shown in **Fig. 5**. The measurement will continue, looking for the optimum conditions. If the achieved separation of free fatty acids from the rest of hydrolysate is not sufficient, ethyl esters of free fatty acids should be

prepared by enzymatic reaction instead of FFA fatty acids, because their separation factors will be higher than those of FFA due to their better solubility in sc-CO_2. The effects of regiospecificity are expected to remain unchanged.

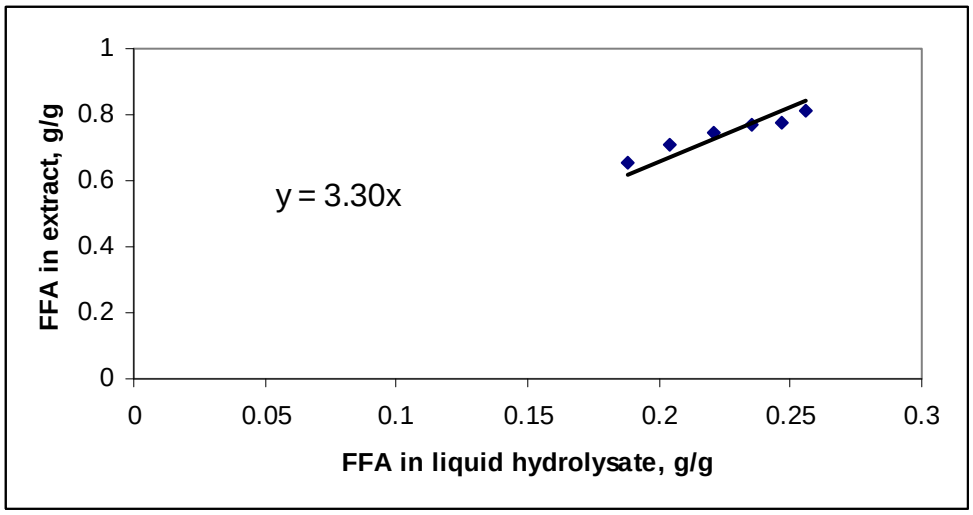

Figure 5: Measurement of separation factor of free fatty acids to the rest of hydrolysate extracted with sc-CO_2 at 11 MPa and 40 °C: the factor is 3.3.

CONCLUSION

The hydrolysis of blackcurrant seed oil dissolved in supercritical carbon dioxide and catalysed by regiospecific enzyme Lipozyme yields partial glycerols whose γ-linolenic-to-α-linolenic acid ratio is higher than 1 and free fatty acids rich in α-linolenic and palmitic acid, containing very low amounts of γ-linolenic and stearidonic acid. Further experiments will be necessary to optimise the conditions for free fatty acid separation from acylglycerols in the hydrolysate.

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REFERENCES:

[1] JACKSON, M.A., KING, J.W., J. Am. Oil Chem. Soc., Vol. 73, **1996**, p. 353
[2] TURNER, C., WHITEHAND, L.C., NGUYEN, T., MCKEON, T., J. Agric. Food Chem., Vol. 52, **2004**, p. 26
[3] REZAEI, K., TEMELLI, F., J. Supercrit. Fluids, Vol. 19, **2001**, p. 263
[4] KONDO, M., REZAEI, K., TEMELLI, F., GOTO, M., KING, J.W., Ind. Eng. Chem. Res., Vol. 41, **2002**, p. 5770
[5] SOVOVÁ, H., ZAREVÚCKA, M., BERNÁŠEK, P., 5th Int. Symp. High Pressure Proc. Tech. Chem. Eng. Proceedings, Segovia, Spain **2007**, p. 75
[6] KWON, D.Z., RHEE, J.S., J. Am. Oil Chem. Soc., Vol. 27, **1988**, p. 1551
[7] STRÁNSKÝ, K., JURSÍK, T., Fett/Lipid, Vol. 98, **1996**, p. 65
[8] STRÁNSKÝ, K., JURSÍK, T., VÍTEK, A., SKOŘEPA J., J. High Resolut. Chromatogr., Vol. 15, **1992**, p. 730
[9] STRÁNSKÝ, K., JURSÍK, T., VÍTEK, A., J. High Resolut. Chromatogr., Vol. 20, **1997**, p. 143