Blackcurrant Oil Hydrolysis in SC-CO_2: Experiment and Model

P. Bernasek[a*], M. Zarevucka[b], H. Sovova[a], M. Bartlova[a], K. Stransky[b], J. Cvacka[b]
[a]Institute of Chemical Process Fundamentals AS CR, Rozvojová 135, 16502 Prague, Czech Republic, e-mail: bernasek@icpf.cas.cz, fax: +420 220 920 661
[b]Institute of Organic Chemistry and Biochemistry AS CR, Flemingovo nam. 2, 16610 Prague, Czech Republic

The studied reaction is an enzyme catalysed hydrolysis of highly unsaturated blackcurrant seed oil (*Ribes nigrum*). Biocatalysis under supercritical conditions embodies high enzymatic performance and advanced transport properties of supercritical fluids, in this case carbon dioxide (SC-CO_2). The aim of the study is to collect experimental data concerning the reaction, product composition and to simulate the complex process via mathematical modelling. Several analytical procedures were developed and applied and these are also further described. This contribution contains blueprint of process-affecting parameters and basic mathematical correlation for measured outputs.

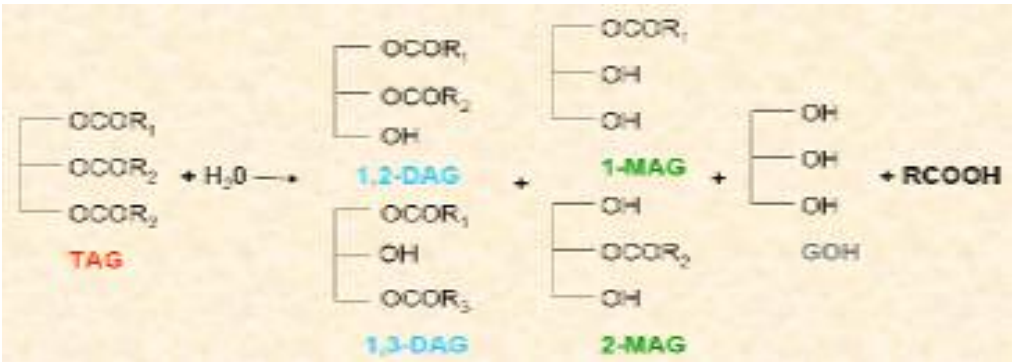
INTRODUCTION

The substrate, blackcurrant seed oil (BCO), contains significant amount of polyunsaturated fatty acids (PUFA) including α- and γ- linolenic acids. Their importance is in human nutrition where are considered as essentials factors. Their content in common food is relatively low and effective separation process would be useful mainly for specials diets and pharmaceuticals.
These acids can be released from several raw materials including mostly vegetable oils. In such oils, we can assume only basic oil compound, triacylglycerol (TAG) is present. The acids are bound in a TAG molecule often with other fatty acids. In case of BCO, the other acids are mostly palmitic, stearic, oleic and linoleic acids. As all these compounds are very similar in polarity and common chemical reactivity any conventional hydrolytic reaction cannot be used to separate them but enzymatic reactions are promising. The oil composition is shown below.

Table 1. Blackcurant seed oil composition

Fatty acid	% w/w
γ-Linolenic	15,11
α-Linolenic	14,66
Linoleic	48,41
Oleic	11,36
Palmitic	5,98
Stearic	1,46
Stearidonic	3,02

The hydrolytic reaction of TAGs is complex one. It can be generally written as



where TAG, DAG, MAG means Tri-, Di-, Mono-acylgycerol and GOH is for glycerol.

EXPERIMENTAL

Refined blackcurrant seed oil composed of triacylglycerols was dissolved in wet supercritical carbon dioxide and led into continuous-flow reactor (16 cm x 8 mm I.D.) containing immobilized enzyme Lipozyme mixed with glass beads where its hydrolysis to di- and monoacylglycerols, free fatty acids and glycerol took place (**Figure 1**). The reaction mixture was separated from the solvent by expansion to atmospheric pressure and analyzed off-line. The reaction was carried out at 15 MPa and 40 ºC with 15-75 mg enzyme and with 0.2-0.9 l/min CO_2 (measured at ambient conditions).

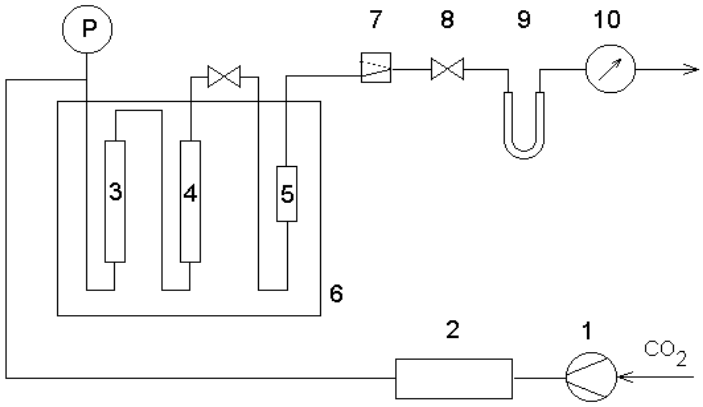


Figure 1. Scheme of the equipment
(1) compressor, (2) pressure control unit, (3) water saturator, (4) oil saturator, (5) reactor, (6) water bath, (7) rinsing valve, (8) expansion valve, (9) trap, (10) gas meter.

ANALYTICAL

Three analytical methods were used. The content of free fatty acids in the reaction mixture was determined using colorimetric method. The fatty acid composition was determined using the standard method of GC analysis and a newly developed LC-NMR method.
Principle of this method is separation of hydrolyzed fatty acids by reveresed-phase HPLC, where the eluence sequence is 1) linolenic a. izomers 2) linoleic a. 3) palmitic and oleic a. 4)

stearic acid 5) MAG, DAG, unreacted TAG – all together - no importance for this analysis. HPLC column is directly connected with NMR-measuring cell, where each acid has its own signal and by calibration we can determine its amount, even in case in synchronous elution of linolenic acid isomers. The differences among NMR signals are shown in **Figure 2** and the whole experiment expressed in time scale in **Figure 3**, where the most important area for linolenic isomer resolution is circled.

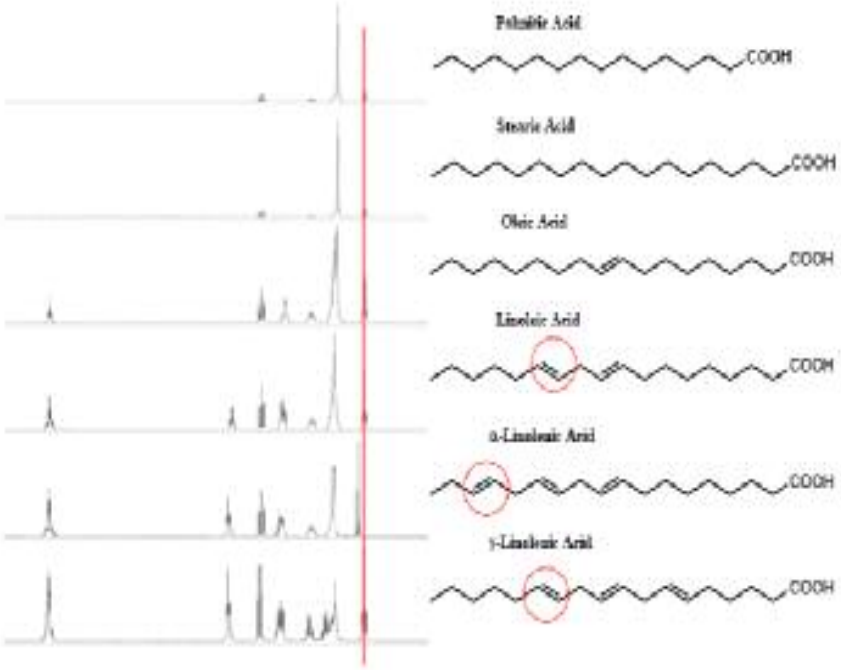


Figure 2. NMR signals

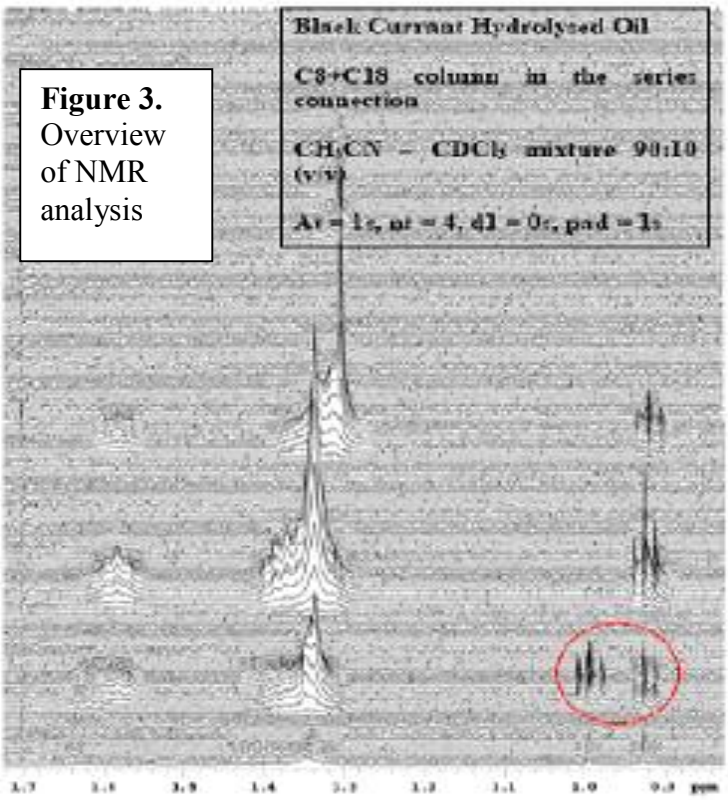


Figure 3. Overview of NMR analysis

REACTION RATE MODELLING

The residence time of the solution in the reactor is $t_r = M/Q$, where M is the mass of solution filling the space between the particles in the reactor and Q is the mass flow of the solution through the reactor, and the initial oil concentration in the solution is c_{10}. As the amount of water in the reaction mixture was much higher than reaction stoichiometry requires, the enzymatic hydrolysis is assumed to be first order reaction with respect to fatty compounds. Let the concentrations of TAG, DAG, MAG, FFA, and GOH in the solution be c_1, c_2, c_3, c_4, and c_5, respectively, and the reaction rates of TAG, DAG, and MAG hydrolysis r_1, r_2, and r_3, respectively. For plug flow, the reaction mixture composition was calculated integrating equations

$$dc_1/dt = -r_1c_1$$
$$dc_2/dt = r_1c_1 - r_2c_2$$
$$dc_3/dt = r_2c_2 - r_3c_3$$
$$dc_4/dt = r_1c_1 + r_2c_2 + r_3c_3$$
$$dc_5/dt = r_3c_3 \quad (1)$$

from $t = 0$ to $t = t_r$ with initial condition $c_1 = c_{10}$, $c_2 = c_3 = c_4 = c_5 = 0$ for $t = 0$.
Alternatively, the mixture composition was calculated for reactor as ideal mixer according to equations

$$c_1 = c_{10}/(1 + r_1t_r)$$
$$c_2 = r_1c_1t_r/(1 + r_2t_r)$$
$$c_3 = r_2c_2t_r/(1 + r_3t_r)$$
$$c_4 = (r_1c_1 + r_2c_2 + r_3c_3)t_r$$
$$c_5 = r_3c_3t_r. \quad (2)$$

The reaction rate is assumed to be proportional to enzyme concentration in the reactor and to the amount of fatty acids in the hydrolyzed compound. Thus, for a constant reactor volume, pressure and temperature, $r_1t_r = 3kN_e/Q$, $r_2t_r = 2kN_e/Q$, and $r_3t_r = kN_e/Q$, where N_e is the mass of immobilized enzyme in the reactor and k is a factor to be determined comparing the calculations with experimental results.

RESULTS

The degree of oil conversion to free fatty acids was examined in dependence on the enzyme amount, age, water amount and solvent flow rate.

Flow rate

The observed non-monotonous conversion dependence on the flow rate was explained by existence of natural convection at lower flow rates and its suppression at higher flow rates. Simulating the reaction, the model for ideal mixer was switched to the model for plug flow when CO_2 flow rate exceeded 0.6 l/min. In long-term experiment with a series of runs where the enzyme in reactor was not exchanged for a fresh one, we observed gradual decrease in the degree of conversion.

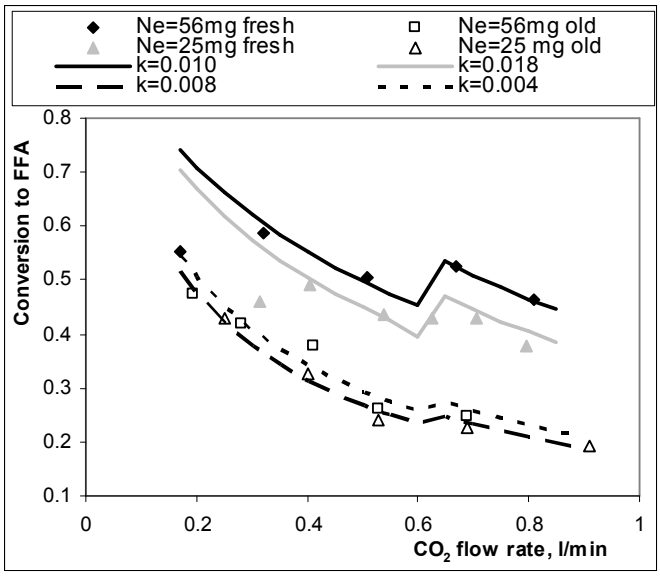


Figure 4. Conversion: experiment and model

The decrease is most probably caused by low soluble glycerol accumulation on enzyme carrier, hindering the substrate approach to the enzyme. It was simulated by decreasing factor k like in **Figure 4**, where $k = 0.018$ min^{-1} for 25 mg of fresh enzyme in the reactor

decreased to 0.008 min^{-1} after several days, and $k = 0.010$ min^{-1} for 56 mg of fresh enzyme similarly decreased to 0.004 min^{-1}. (The difference in k for different amounts of fresh enzyme may be connected with different enzyme deposition in the reactor.)

Dependence on water amount

was measured by change in water solubility in SC-CO_2 achieved by increasing temperature of water saturator from 24°C to 50°C. **Table 2** shows the water solubilities in SC-CO_2 at 15 MPa. During the experiment, the reactor temperature was maintained at 40 °C. No significant dependence was observed (**Figure 5**).

Table 2. Water solubility in CO_2

Temperature, °C	mg water / g CO2
20	1.27
24	1.42
30	1.67
40	2.12
50	2.62
60	3.11

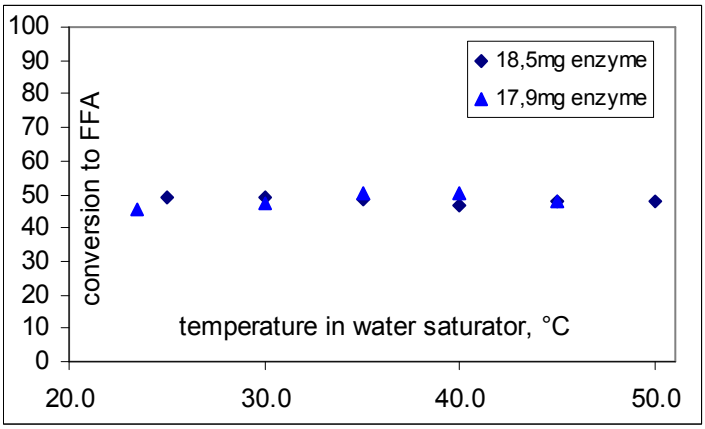


Figure 5. Conversion and water amount in the solvent.

Enzyme aging

Repetitive enzyme use affects its catalytic properties. No accurate statement can be yet made but the tendency is to decrease activity with time (**Figure 6**). The suspected reasons are low-soluble glycerol layer forming on the enzyme surface and pressure stresses. Experiment were made with enzyme load 56mg, 15 MPa, water content 1.42 mg /g CO_2 and flow rate from 0.2-1 l CO_2/min.

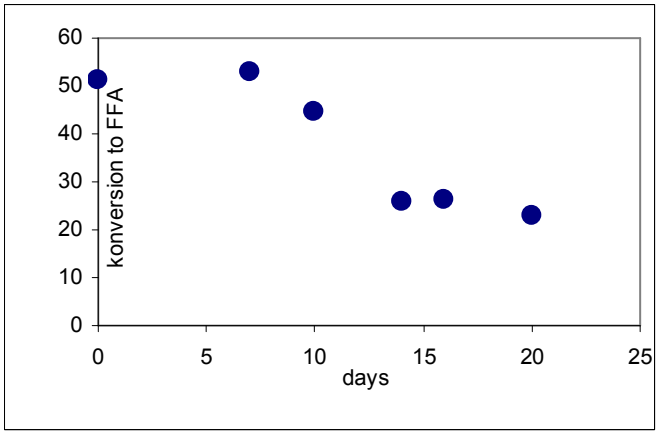


Figure 6. Decrease in conversion for a batch of enzyme used for 20 days.

Enzyme amount

For both fresh and used enzyme the hyperbolic dependence on conversion is approximately fulfilled as suggested in **Figure 7**. The limit in conversion is caused by enzyme catalytic properties to DAG and MAG and the transport properties of fluid.

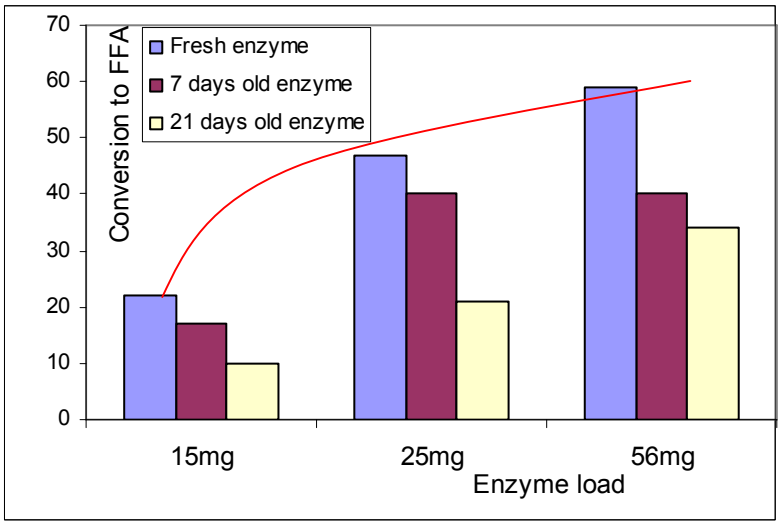


Figure 7. Enzyme load and conversion.

CONCLUSION

The conversion to FFA depends on enzyme amount, enzyme age, and flow rate, written in the order of decreasing importance. The amount of water in the used range had no effect on enzyme properties. The experiments with advanced analysis are in progress.

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