Kinetics and specificity of Lipozyme-catalysed oil hydrolysis in supercritical CO_2
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Kinetics and specificity of Lipozyme-catalysed oil hydrolysis in supercritical CO_2

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Abstract

Blackcurrant seed oil is rich in linoleic and linolenic acids. The aim of the study was to obtain by partial hydrolysis a mixture of free fatty acids of different composition than that of the hydrolyzed oil. The oil was dissolved in supercritical carbon dioxide flowing through a packed bed reactor. The catalyst was Lipozyme®, a 1,3-specific lipase from *Mucor miehei* immobilised on a macroporous ion-exchange resin. The operating conditions were temperature 40 °C, pressure 15-28 MPa, and superficial velocity 0.1-0.7 mm s^{-1}. The composition of the hydrolyzate was determined using colorimetric method and chromatographic methods PTLC, GC, HPLC, and LC-NMR. The maximum reaction rate per unit amount of enzyme 2.6 10^{-3} mol s^{-1} kg^{-1} was achieved at maximum flow velocity and at maximum pressure. The mixture of liberated fatty acids contained higher percentage of saturated fatty acids than the hydrolyzed oil, in accordance with the fact that saturated acids occupy in triacylglycerols of vegetable oils the *sn*-1 and *sn*-3 positions. According to analytical methods PTLC+GC and HPLC (but not according to LC-NMR) the concentration of γ-linolenic acid in the mixture of free fatty acids in the hydrolyzate was by order of magnitude lower than its concentration in the oil.

Keywords: blackcurrant oil, lipase, hydrolysis, supercritical carbon dioxide, mass transfer

1. Introduction

Enzymatic reactions of vegetable oils dissolved in supercritical carbon dioxide in a continuous-flow tubular fixed-bed reactor were investigated e.g. by Miller *et al.* (1991), Hampson and Foglia (1999), Temelli and co-workers (Rezaei and Temelli 2001, Martinez *et al*. 2002), and Turner *et al.* (2004).

Our study of enzymatic hydrolysis of blackcurrant seed oil aims to partially separate linolenic acids, which are important in human nutrition, from other fatty acids in the oil, utilizing the enzyme specificity. It means to find an enzyme that would preferentially liberate either linolenic acids or other acids from triacylglycerols forming the oil, and learn to hydrolyse the oil to the required degree of conversion corresponding to favourable substrate-to-product ratio. Supercritical CO_2 (SC-CO_2) was chosen as a solvent because of its good transport properties, protection of polyunsaturated fatty acids against degradation, easy separation from the solutes after the reaction, and easy integration of oil extraction from seeds with the reaction into one technological step. The scheme of extraction and reaction with SC-CO_2 solvent is shown in Figure 1.

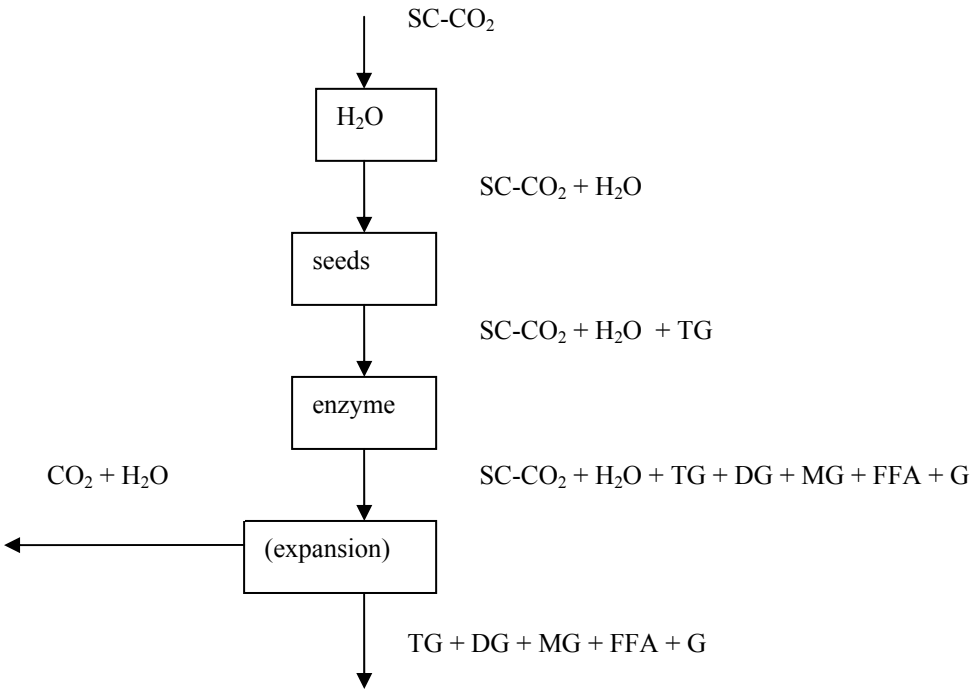

Fig. 1. Scheme of oil (TG, triacylglycerols) extraction and reaction.

Enzyme Lipozyme was tested previously (Sovová and Zarevúcka 2003). Its activity showed no decreasing trend during several weeks of exposure to dense carbon dioxide, and the composition of liberated free fatty acids determined by preparative thin layer chromatography followed by methyl esterification and gas chromatography was different from that of the oil: the saturated fatty acids and α-linolenic acid were liberated faster and γ-linolenic acid slower than other fatty acids. However, large fluctuations of apparent enzyme activity were observed. Irregular accumulation of low-soluble glycerol near active sites, connected with an increased mass transfer resistance, was hypothesised to cause the fluctuation.

In this work, extensive experimental study of the kinetics of blackcurrant seed oil hydrolysis was carried out. The effects of flow rate and repeated partial depressurization of reactor on enzyme activity were studied at 40 ºC with different amounts of enzyme. Measures for maintaining the enzyme activity were introduced and proved to be efficient, and the effect of pressure on the reaction rate was measured. The decrease of Lipozyme activity during its storage in the refrigerator was evaluated, too.

2. Theoretical

2.1. Concentration of substrate near enzyme

Oil hydrolysis takes place in three stages:

$$\mathrm{TG + H_2O \rightarrow DG + FFA}$$
$$\mathrm{DG + H_2O \rightarrow MG + FFA}$$
$$\mathrm{MG + H_2O \rightarrow G + FFA}$$

where TG, DG, FFA, MG and G represent triacylglycerols, diacylglycerols, free fatty acids, monoacylglycerols and glycerol, respectively. As the amount of water in the mixture was much higher than the reaction stoichiometry requires, the reaction was assumed to be independent of water concentration and the only substrate affecting the kinetics is oil.

The rate of enzymatic liberation of fatty acids is simulated by Michaelis-Menten kinetics

$$v = v_{max}\, c_e/(K_m + c_e). \qquad (1)$$

The rate of substrate transport to enzyme is equal to the rate of reaction:

$$v = k_f\, a(c - c_e). \qquad (2)$$

Combining equations *(1)* and *(2)* we get quadratic equation in c_e, substrate concentration near enzyme, which has only one positive solution

$$c_e = \left(-b + \sqrt{b^2 + 4K_m c}\right)\Big/2 \quad where \quad b = K_m - c + v_{\max}/\left(k_f\, a\right). \qquad (3)$$

For $v_{max} >> k_f a$, c_e approaches zero and the reaction is controlled solely by mass transfer according to equation

$$v = k_f a c. \qquad (2a)$$

When $v_{max} << k_f a$, the substrate concentration near enzyme approaches its concentration in bulk fluid, c, and the reaction is controlled solely by enzyme kinetics,

$$v = v_{max}\, c/(K_m + c). \qquad (1a)$$

As the mass transfer coefficient k_f increases with increasing flow velocity, an increase in the measured reaction rate v with flow velocity indicates the dependence of reaction rate on mass transfer.

2.2. Relationship between conversion and average rate of reaction

As the substrate is consumed in the course of the reaction its rate decreases. When the initial substrate concentrations c_0 and c_{e0} are used in experimental data evaluation, the average reaction rate v must be converted to the initial reaction rate at the reactor inlet. Retardation factor f_δ dependent on degree of conversion δ is introduced for this purpose:

$$f_\delta = \frac{v(\delta)}{v(\delta=0)} = \frac{\delta c_0}{v(\delta=0)\tau}. \qquad (4)$$

Conversion δ is in the range 0-1, it is the measure of the substrate depletion, and τ is the residence time of the reactor where plug flow is assumed. For example, the relationship between the reaction rate parameters, residence time, conversion and initial substrate concentration for enzyme kinetics according to *Eq. (1a)* is (Miller *et al.*, 1991)

$$\delta c_{e0} = v_{max}\tau + K_m \ln(1-\delta). \qquad (5)$$

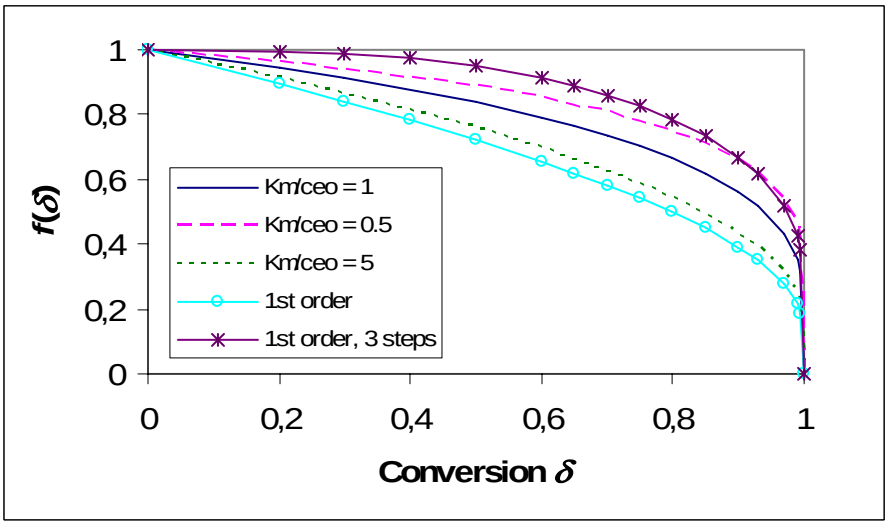

Fig. 2. Retardation factor according to Eq. (6) for Michaelis-Menten kinetics with three values of parameter K_m/c_{e0}, according to Eq. (6a) for a single first order reaction, and for three-step hydrolysis calculated with the ratio of rate constants r_1, r_2, r_3 1:0.9:1.7.

After substitution $v(\delta=0)\tau = [\delta c_0 - K_m \ln(1-\delta)]c_0/(K_m+c_0)$ from *Eqs (1a), (5)* to *Eq. (4)* and its rearrangement we obtain relationship

$$f_\delta = \frac{1+K_m/c_{e0}}{1-(K_m/c_{e0})\ln(1-\delta)/\delta} \qquad (6)$$

with parameter K_m/c_{e0}. The course of the retardation factor is shown in Figure 2 for three values of the parameter. Similarly, for the first order kinetics with reaction rate

$$v = r\,c \qquad (2b)$$

the retardation factor is derived as

$$f_\delta = -\frac{\delta}{\ln(1-\delta)}. \qquad (6a)$$

It is a limiting case of the Michaelis-Menten kinetics for $K_m/c_0 \to \infty$ (see Fig. 2). When the conversion in the three-step hydrolysis of oil is related to the liberated fatty acids and c_{FFA} is the fatty acid concentration at reactor outlet, the retardation factor is

$$f_\delta = \frac{c_0\delta}{v(\delta=0)\tau} = \frac{c_{FFA}}{3v(\delta=0)\tau}. \qquad (4a)$$

The factor for the three-step hydrolysis decreases with increasing conversion slower than in the previous case because new substrates are produced in the first and second steps. Fig. 2 shows the course of the retardation factor calculated for the three-step hydrolysis using first order reaction model with plug flow (Sovová and Zarevúcka, 2003) with the ratio of rate constants in the first, second and third step fixed as explained in section 4.5. Up to about 40% conversion the value of f_δ is close to one and thus the factor may be omitted in the calculations. When the reaction is controlled by Michaelis-Menten kinetics, the range of conversion where f_δ is close to one is even broader.

3. Experimental

3.1. Materials

Blackcurrant seed oil was used in two forms, either as the TG fraction of oil used already in the previous work (Sovová and Zarevúcka, 2003) with fatty acid composition 45.8 % linoleic, 14.3 % γ-linolenic, 13.9 % α-linolenic, 10.8 % oleic, 5.7 % palmitic, 2.9 % stearidonic, and 1.4 % stearic acid and 5.4 % other acids (w/w)), or directly extracted from the seeds harvested in 2006. This oil contained a negligible amount of free fatty acids and thus it could be used in the experiments without refining. Its fatty acid composition was 45.0 % linoleic, 14.1 % α-linolenic, 13.1 % oleic, 11.1 % γ-linolenic, 6.2 % palmitic, 2.8 % stearidonic, 1.5 % stearic acid, and 6.1 % other acids (w/w). 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.

3.2. 3.2 Extraction and reaction

The scheme of the solvent flow is in Figure 1. Pressurized carbon dioxide was pumped by ISCO 260D syringe pump consecutively to the first saturator containing water, the second saturator containing either oil or crushed seed, and the reactor filled

with immobilized enzyme and glass beads. Inner diameter of the saturators and reactor was d_r = 0.008 m, 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 colorimetric analysis. 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 other experimental conditions are listed in Table 1. The applied pressure was 15, 20, 25 and 28 MPa.

Table 1. Reaction conditions

Series	Enzyme storage, months	$M_e \times 10^6$, kg	P, MPa	Extractor contents	$u \times 10^3$ m s^{-1}	No. of runs
A	9	56	15	Oil	0.12-0.68	8
B	10	55	15	Oil	0.15-0.65	7
C	10	25	15	Oil	0.14-0.69	7
D	11	15	15	Oil	0.15-0.70	3
E	13	75	15	Oil	0.17-0.61	3
F	14	16	15	Oil	0.16-0.62	1
G	21	31	15	Oil	0.13-0.47	8
H	26	42	15-28	Seed	0.18-0.42	3
I	28	35	15-28	Seed	0.16-0.42	7
J	31	72	15-28	Seed	0.27-0.48	4

The hydrolysate precipitating from the first 10-15 ml CO_2 (measured in the pump) was collected in each experimental run separately. As preliminary experiments showed, this volume was sufficient to rinse the equipment and to stabilise the reaction rate. Then, five to six samples of hydrolysate were taken at different flow rates. The valves at reactor inlet and outlet were then closed and the equipment was left overnight at room temperature.

Physical properties of the solution in the reactor were assumed to be equal to the properties of pure CO_2. Its density was calculated according to Altunin and Gadetski (Angus *et al*., 1976) and viscosity according to Ulybin and Makarushkin (1976). The solubility of oil and water in SC-CO_2 was estimated using the correlations published by del Valle and Aguilera (1986) and Sovová and Zarevúcka (2003), respectively. Comparison of the calculated oil solubility with the measured concentration of hydrolysate in CO_2 shows that the solvent was saturated with oil both in the experiments with refined oil in the saturator and in the experiments with the saturator filled with crushed seeds.

Table 2. Properties of CO_2, oil and water solubility in CO_2 and pressure factor at 40 ºC.

P, MPa	ρ, kg/m3	$\mu \times 10^6$, Pa s	S_o, mol m^{-3}	S_w, mol m^{-3}	f_P	f_{Pexp}
15	781.2	67.0	1.5	92	1.000	1.0
20	840.6	78.4	3.3	104	0.863	0.9
25	880.2	87.4	5.4	111	0.777	0.76
28	899.3	92.2	6.8	115	0.738	0.67

3.3. Analytical methods

The content of free fatty acids N_{FFA} (mol) in hydrolysate samples (10-20 mg) was determined by the colorimetric method according to Kwon and Rhee (1986) using blue cupric acetate-pyridine reagent; the absorbance was measured at 714 nm. The mass fractions of TG, DG+MG, and FFA in selected hydrolysate samples were determined using preparative TLC and the fatty acid composition was analysed by gas chromatography as described by Sovová and Zarevúcka (2003). The LC-NMR experiments were conducted to analyse the composition of free fatty acids in the hydrolysate as described by Sýkora et al. (2007).

4. Results and discussion

4.1. Calculation of reaction characteristics

To compare the experiments with different enzyme loadings, specific reaction rate $V = vV_r/M_e$ was introduced and calculated as

$$V = N_{FFA}/(M_e t_s). \qquad (7)$$

Conversion to free fatty acids, expressed as mass ratio, is

$$\delta = N_{FFA}\, M_{wFFA}/M_s = 0.2784\, N_{FFA}/M_s. \qquad (8)$$

In this calculation we assume that the mass of hydrolysate is equal to the initial mass of substrate. Molecular weight of hydrolysate fractions (TG, DG, MG, FFA) is taken equal to the molecular weight of major component, linoleic acid, and its compounds. Thus, $M_{wFFA} = 0.2784$ kg/mol and $M_{wTG} = 0.8734$ kg/mol. The substrate concentration in CO_2 at the reactor inlet is then

$$c_0 = M_s\rho/(M_{CO2}M_{wTG}) = 1.145\, M_s\rho/M_{CO2}.. \qquad (9)$$

Superficial velocity of carbon dioxide in the reactor is calculated according to equation

$$u = 4\, M_{CO2}/(t_s\rho\pi d_r^2) = 19\,894\, M_{CO2}/(t_s\rho). \qquad (10)$$

4.2. Effect of flow velocity

The specific reaction rate was plotted in logarithmic scale against superficial velocity (Fig. 3). The straight lines for individual series are shifted vertically because the enzyme activity of different enzyme fillings was not identical. The slope of the straight curves, 0.83, was adjusted according to the correlation between Sherwood number, Reynolds number and Schmidt number published by Tan *et al*. (1988)

$$Sh = 0.38\, Re^{0.83}\, Sc^{1/3} \qquad (11)$$

from which follows the dependence of mass transfer coefficient on superficial velocity and physico-chemical properties (where diffusivity D is assumed to be inversely proportional to viscosity, according to Wilke-Chang correlation):

$$k_f = const.\, D^{2/3} (\rho/\mu)^{0.5}\, u^{0.83} = const.\, \rho^{0.5}\, \mu^{-1.17}\, u^{0.83} \qquad (12)$$

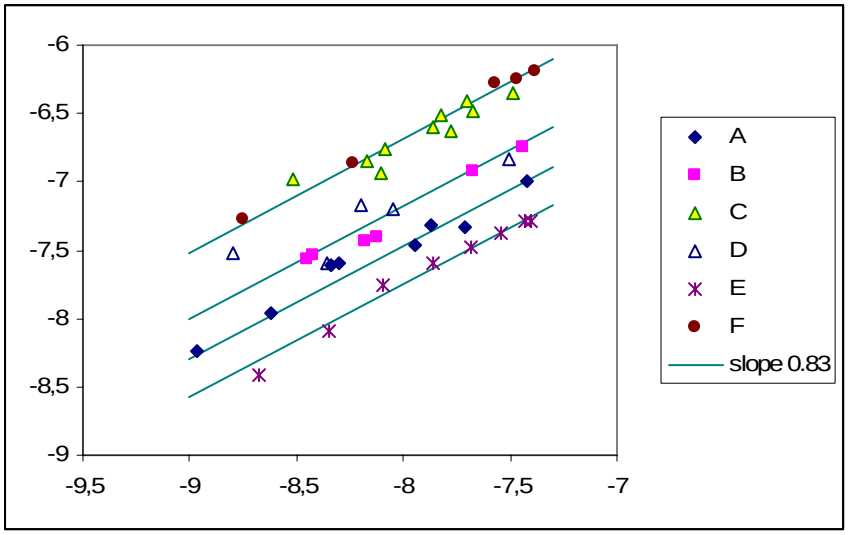

Fig. 3. Effect of superficial velocity u (m/s) on specific reaction rate V (mol s^{-1} kg^{-1}). Experimental series A-F, pressure 15 MPa, substrate concentration 1.0-1.6 mol m^{-3}.

Thus, the reaction was controlled by mass transfer, contrary to our expectations. The reason for it may be that the decisive part of transport is not to the surface of particles but through the macropores of enzyme carrier where the diffusion is hindered. In comparison with the work of Miller *et al.* (1991) where in the range of superficial velocities 0.7-2.4 mm/s the reaction was independent of mass transfer, lower superficial velocities (0.1-0.7 mm/s) were applied in the present study.

4.3. Effect of pressure

The reaction rate increased with increasing pressure (Figure 4). The pressure affects both oil solubility and solvent properties. The positive pressure effect is due to the large increase in oil solubility (see Table 2) and thus substrate concentration c_0. The negative effect of increasing fluid phase viscosity, on the other hand, is less pronounced.

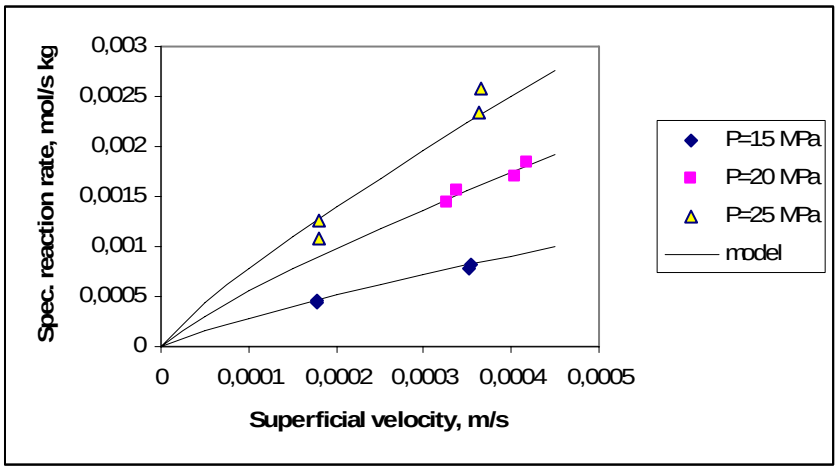

Fig. 4. Specific extraction rates at different pressures in series H. Model: V = const. $u^{0.83}$ with the constant of proportionality equal to 0.6 for 15 MPa, 1.15 for 20 MPa and 1.65 for 25 MPa.

4.4. Water and effect of partial depressurization

A decrease in enzyme activity was observed after several runs in series A-E. Special attention was then paid to the effect of water concentration on the enzyme activity and stability. As the first saturator was at ambient temperature, water concentration in CO_2 in the reactor at 40 ºC was between 50 and 70 % of saturation. Between experimental runs, however, the closed reactor was cooled to room temperature, the pressure dropped and the solution was oversaturated with water, which could partially precipitate. When the pressure in the reactor was kept constant by supplying CO_2 in the course of cooling as in series H or when dry CO_2 was passed through the reactor et the end of the run as in series I and J, the activity of enzyme that was not exposed to the oversaturated water solution was stable.

4.5. Mass fractions and reaction rates in three steps

Eight samples from series I were separated using preparative thin layer chromatography into three fractions: triglycerides TG, combined di- and monoglycerides DMG, and free fatty acids FFA, and the mass of the fractions was determined. Figure 5 shows the experimental data DMG versus TG together with a

simulation of this curve using a plug flow model of hydrolysis with rate constants r_1, r_2 and r_3 for the three steps of reaction to DG, MG and G, respectively (Sovová and Zarevúcka, 2003). The ratio of the rate constants was adjusted to fit the experimental data. As shown in 2.2, the corresponding retardation factor was close to 1 for conversion to free fatty acids lower than 40-50 % and thus in most of our experimental data the retardation could be neglected and the measured reaction rate was taken identical with the initial reaction rate at substrate concentration c_0.

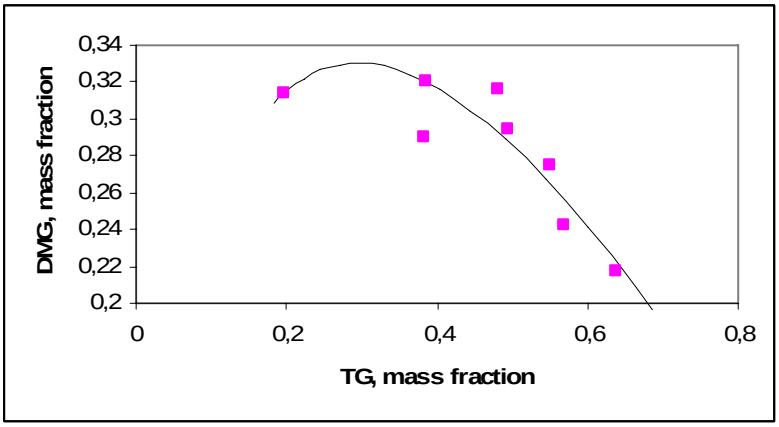

Fig. 5. Experimental composition of hydrolysate fractions and their simulation with a model containing the rate constants ratio 1:0.9:1.7.

4.6. Kinetic parameters in the model for hydrolysis

Relationship for the specific reaction rate was obtained from *Eq. (2)* using *Eqs. (4), (12)*:

$$V = \nu V_r/M_e = const.\ \rho^{0.5}\mu^{-1.17}\, u^{0.83} a_e (c_0 - c_{e0})\, f_\delta = K_u\, f_P\, u^{0.83}\, (c_0 - c_{e0})\, f_\delta \qquad (13)$$

where K_u includes the specific enzyme surface, a_e, and CO_2 properties at 15 MPa μ_{15}, ρ_{15}, and f_P is the pressure effect on mass transfer coefficient. Thus, *Eqs (1)-(3)* were transformed to

$$V = V_{max}\, c_{e0}/(K_m + c_{e0}) f_\delta = K_u\, f_P\, u^{0.83}\, (c_0 - c_{e0})\, f_\delta, \quad f_P = (\rho/\rho_{15})^{0.5}\, (\mu_{15}/\mu)^{1.17},$$

$$c_{e0} = \left(-b + \sqrt{b^2 + 4K_m c_0}\right)\Big/ 2 \quad where \quad b = K_m - c_0 + V_{\max}\Big/\left(K_u\, f_P u^{0.83}\right). \qquad (14)$$

The f_p values are listed in Table 2. Using kinetic parameters V_{max}, K_e, K_m and experimental c_0 and u, the specific reaction rate and substrate concentration c_{e0} were calculated for each sample. The kinetic parameters were adjusted, keeping constant K_u in each series and constant K_m and V_{max}/K_u ratio throughout the calculation, to minimise the sum of squares of differences between the calculated and experimental specific reaction rates. The result is shown in Table 3. The pressure factor was

adjusted in the optimisation, too, giving the values f_{Pexp} not much different from theoretical values f_p (see Table 2).

Despite the mass transfer resistance, the experimental specific reaction rate was comparable with the enzyme activity declared by Fluka (>40 U/g, where 1 U corresponds to the amount of enzyme which liberates 1 μmol oleic acid/min at pH 8.0 and 40 ºC, it is $V = 40/60\,000 = 0.67 \times 10^{-3}$ mol s^{-1} kg^{-1}).

Table 3. Model parameters (K_m = 0.070 mol m^{-3}), range of experimental specific reaction rate, conversion to FFA, experimental bulk concentration of oil and calculated oil concentration near enzyme.

Series	K_u, $m^{2.17}s^{-0.17}kg^{-1}$	$V_{max} \times 10^3$, mol s^{-1} kg^{-1}	$V \times 10^3$, mol s^{-1} kg^{-1}	δ	c_0, mol m^{-3}	c_{e0}, mol m^{-3}
A	0.39	2.6	0.1-0.9	0.11-0.59	0.7-1.3	0.009-0.039
B	0.42	2.8	0.1-1.2	0.12-0.61	1.0-1.6	0.016-0.042
C	0.70	4.7	0.2-1.8	0.10-0.54	1.0-1.7	0.015-0.044
D	0.49	3.4	0.5-1.1	0.12-0.22	0.8-1.3	0.010-0.039
E	0.25	1.7	0.0-0.7	0.04-0.51	1.2-1.4	0.012-0.049
F	0.72	4.9	0.7-2.0	0.23-0.35	1.0-1.5	0.011-0.053
G	0.49	3.4	0.2-1.4	0.18-0.61	0.8-1.9	0.007-0.037
H	0.38	2.6	0.4-2.6	0.24-0.60	1.1-7.4	0.011-1.35
I	0.31	2.1	0.3-2.3	0.14-0.47	0.9-8.7	0.013-1.77
J	0.23	1.6	0.2-1.7	0.23-0.48	0.6-8.5	0.005-1.43

4.7. Changes in fatty acid composition

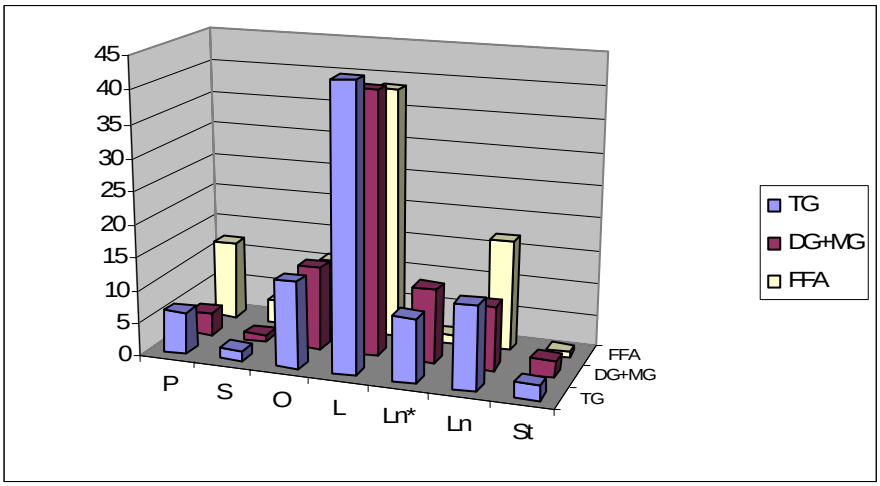

Fig. 6. Percentage of individual fatty acids (P palmitic, S stearic, O oleic, L linoleic, Ln* γ-linolenic, Ln α-linoleic, St stearidonic acid) in triacylglycerols, di- and monoacylglycerols, and free fatty acids at TG:DG+MG:FFA 57:24:19 (w/w).

Figure 6 shows a typical result of the analysis of hydrolysate composition using preparative TLC followed by GC of fatty acid methyl esters. As Lipozyme is a 1,3-specific enzyme, the composition of fatty acids liberated from triacylglycerols depends on the fatty acid distributions at *sn*-1,3 positions in the molecules of blackcurrant seed oil. The composition of TG fraction is within the error of measurement equal to the composition of initial oil. As expected, the percentage of saturated fatty acids (palmitic and stearic acid) in FFA fraction is higher than in the oil because saturated fatty acids occur in most vegetable oils almost entirely at *sn*-1,3 positions and therefore they are liberated preferentially. The percentage of α-linolenic acid is also increased and the percentage of γ-linolenic acid in FFA fraction is very low. Thus, it could be concluded that α-linolenic acid is in TG molecules of blackcurrant seed oil also preferentially at *sn*-1,3 positions while γ-linolenic acid is almost completely at *sn*-2 position. The results of LC-NMR analysis of free fatty acids in hydrolysates confirm the increase in saturated fatty acid concentration, however, they do not show any change in the percentage of free Ln and *Ln acids, compared to their percentage in oil. The difference must be explained by further experiments.

5. Conclusions

The maximum apparent enzyme activity measured in supercritical carbon dioxide was three times higher than that declared by the supplier. Nevertheless, the reaction was mass transfer controlled and the substrate concentration at the enzyme was lower than K_m in most cases. The maximum reaction rates were achieved at 28 MPa with the highest substrate concentrations. An increase in the velocity in the reactor is recommended to suppress the mass transfer resistance. The enzyme activity was stable when the reactor was dried before depressurization.
The mixture of liberated fatty acids contained higher percentage of saturated fatty acids than the hydrolyzed oil, in accordance with the fact that saturated acids occupy in triacylglycerols of vegetable oils the *sn*-1 and *sn*-3 positions. According to analytical methods PTLC+GC and HPLC (but not according to LC-NMR) the concentration of γ-linolenic acid in the mixture of free fatty acids in the hydrolyzate was by order of magnitude lower than its concentration in the oil.

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Nomenclature

a specific interfacial area in reactor, $m^2\ m^{-3}$
a_e specific surface of immobilised enzyme, $m^2\ kg^{-1}$
c substrate concentration in bulk fluid, $mol\ m^{-3}$
c_0 substrate concentration in bulk fluid at reactor inlet, $mol\ m^{-3}$

c_e substrate concentration near enzyme, mol m^{-3}
c_{e0} substrate concentration near enzyme at reactor inlet, mol m^{-3}
d_r reactor inner diameter, m
f_P pressure factor for mass transfer coefficient, defined by Eq. (14), m s^{-1}
f_{Pexp} adjusted pressure factor for mass transfer coefficient, m s^{-1}
f_δ retardation factor defined by Eqs (4) and (4a), m s^{-1}
k_f mass transfer coefficient, m s^{-1}
K_m Michaelis constant (substrate concentration when $v = v_{max}/2$), mol m^{-3}
K_u mass transfer constant defined by Eq. (13), $m^{2.17}s^{-0.17}kg^{-1}$
M_{CO2} carbon dioxide passed through the reactor during sampling, kg
M_e enzyme in the reactor, kg
M_s hydrolysate sample, kg
M_{wTG} molecular weight of trilinolein, kg mol^{-1}
M_{wFFA} molecular weight of linoleic acid kg mol^{-1}
N_{FFA} free fatty acids in the sample of hydrolysate, mol
P pressure, MPa
r reaction rate constant for the first order kinetics, s^{-1}
S_o solubility of oil in carbon dioxide, mol m^{-3}
S_w solubility of water in carbon dioxide, mol m^{-3}
t_s sampling time, s
u superficial velocity in reactor, m s^{-1}
v reaction rate, mol m^{-3} s^{-1}
v_{max} maximum reaction rate, mol m^{-3} s^{-1}
V $(= vV_r/M_e)$ specific reaction rate, mol s^{-1} (kg enzyme) $^{-1}$
V_{max} maximum specific reaction rate, mol s^{-1} (kg enzyme) $^{-1}$
V_r reactor volume, m^3
δ conversion to free fatty acids, -
μ fluid viscosity, Pa s
ρ fluid density, kg m^{-3}
τ residence time, s

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