THE KINETIC STUDY OF THE ENZYMATIC ACIDOLYSIS OF TRIACYLGLYCEROLS FROM SUNFLOWER OIL IN ENZYMATIC REACTORS

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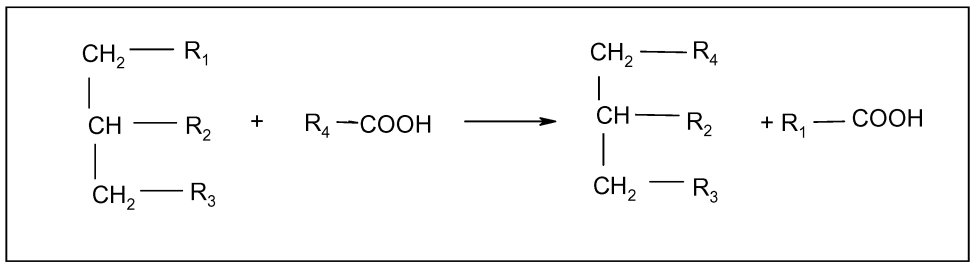
Improvement of the sunflower oils (SO) quality is an important issue related with the area of research for "Food quality and safety". The upgrading of SO is a subject of great interest from economical and social point of view. Long chain fatty esters (LCFE) are useful functional molecules responding to the requirements of numerous applications in food, cosmetics, pharmaceutical and lubricant industry. In the present work a kinetic study of enzymatic acidolysis reactions of triacylglycerols (TAG) from SO with erucic acid (EA) was performed at atmospheric pressure operation (APO) and also in supercritical carbon dioxide (SC CO_2). The fatty acid specificity of 1,3 position specific *Rhizomuchor miehei* lipase in transesterification reaction was investigated. All reactions were carried out using a specific quantity of enzyme and modifying all other parameters of operation. The optimal conditions found in SC CO_2, when using lipase Lipozyme RM IM from *R. miehei*, where: a temperature of 333 K, an agitation rate of 600 rpm and a pressure of 20 MPa. The thermodynamic parameters showed that in SC CO_2 the activation energy of 3.50 kJ/mol and deactivation enthalpy of 27.93 kJ/mol are in good agreement with the thermodynamic model. The results showed that SC CO_2 is a good alternative as reaction medium for biotransformation processes such as enzymatic acidolysis by incorporation of EA into TAG from SO.

1. INTRODUCTION

Enzymes are considered as nature's catalysts. Lipases differ greatly as regards both their origin (which can be bacterial, fungal, mammalian, etc.) and their properties. They can catalyse hydrolysis or synthesis of a wide range of different carboxylic esters, liberate organic acids and glycerol. Additionally, they show highly specific activity towards glyceridic substrates. They constitute the most important group of biocatalysts for biotechnological applications and have potential applications in the detergent, food, leather, textile, oil and fat, cosmetics, paper, and pharmaceutical industries (Hasan et al., 2006; Kojima et al., 2006).
Lipases are versatile biocatalysts. Most regioselective lipases act preferentially on ester bonds at the sn-1 and sn-3 position of the triglyceride structure, whereas few lipases are active at the sn-2 position. They can be found with optimum activities over a wide range of temperatures. Several three-dimensional structures of these enzymes have been resolved allowing the design of rational engineering strategies of the triglyceride structure. In additional to their hydrolytic activity on triglycerides, they can catalyse other reaction such as esterification, interesterification, acidolysis, alcoholysis and aminolysis (Houde et al., 2004).
Research about the ability of immobilized lipases to catalyse acidolysis reactions have been previously reported: the Novozym 435, from *Candida antarctica*, can modify the fatty acid composition of borage oil (*Borago officinalis L.*) in hexane, by incorporation of docosahexaenoic acid (DHA) (Namal Senanayake and Shahidi, 2002; Lai et al., 2005); lipase catalysed acidolysis of TAG of soybean oil with oleic acid in organic solvent was

studied by Cossignania et al. (2004); enzymatic acidolysis of refined, bleached and deodorized palm olein with caprylic acid was presented by Zhao et al. (2007). Lipase-catalysed acidolysis of SO with a mixture of palmitic-stearic acids was performed in a batch bioreactor to produce structured lipids (Carrin and Crapiste, 2008).
The sunflower crops are the 3rd most common oleaginous culture in the world. The sunflower seeds are a very good source of vegetable oil. From the economical value point of view, SO is a very good source for valorisation, due to the variety of fatty esters contained. Sunflower is also a good source of vegetative enzymes. The fatty esters play an important role in different applications: food (human and animal feeds), non-food applications (pharmaceutical field, cosmetics, bio-detergents, biodiesel etc.) (Pereyra-Irujo and Aguirrezabal, 2007).
Very long chain fatty acids (VLCFA) are fatty acids characterized by an acyl chain with more than 20 carbon atoms, which found important applications in industry as well as in agronomy. VLCFA like EA (C22:1) have many uses (more than 1000 patented or potential industrial applications have been described for EA alone). EA is a monounsaturated omega-9 fatty acid. The oil with high EA content could be used in the production of nylon, plasticizers and lubricants. An alternative method for incorporating VLCFA in TAG from SO is the acidolysis reaction using immobilized enzymes as catalysts in supercritical fluids (SCFs). The application of SCFs enables also design of integrated reaction and separation processes. The reaction rate can be increased if SCFs are applied due to higher diffusivity and to reduce viscosity of reaction system especially when the processes are mass transfer limited. SCFs also display unique substrate specificity at relative mild reaction conditions. Carbon dioxide is the preferred choice, due to its non-toxicity, non-flammability and low cost. Dense CO_2 could be used as »green« processing medium, especially when organic solvents should be avoided.
Factors affecting the enzyme stability in SCFs (water activity, pressure and temperature, number of pressurisation–depressurisation steps) and inhibition of enzymes are well documented for hydrolases (Knez and Habulin, 2002; Knez et al., 2005; Habulin et al., 2007a). Studies on kinetics and thermodynamics of lipase catalysed LCFE synthesis in SCFs have been previously reported (Laudani et al., 2007a; Laudani et al., 2007b, Habulin et al., 2008a). The potential of using SC CO_2 as a safe reaction medium for the lipase-catalysed synthesis of sugar fatty acid esters and enzymatic synthesis of citronellol laurate in organic media were reported in recent studies (Šabeder et al., 2006; Habulin et al., 2008b).
The aim of this study was to perform enzymatic acidolysis reaction and kinetic studies through incorporation of VLCFA like EA (C22:1) into TAG from SO. Lipozyme IM from *R. miehei* was used as a catalyst. The study of the reaction thermodynamics was performed as well, evaluating the reaction entropy, enthalpy, activation energy and Gibb's free energy of deactivation. The acidolysis reaction, catalysed by immobilized lipase was performed at atmospheric pressure and also in SC CO_2. Lipase-catalysed acidolysis of TAG is shown in Scheme 1.
At APO, the temperature and the EA concentration were optimized and the thermodynamic properties of the enzymatic catalysed acidolysis of TAG from SO with EA were determined. In SC CO_2 medium temperature, pressure and stirring rate were optimized and the thermodynamic properties of the reaction were determined, too.



Scheme 1: General equation of acidolysis reaction of TAG with fatty acid.

2. MATERIALS

Enzyme preparation. Lipase (Lipozyme RM IM) from *R. miehei* immobilized on a macroporous anion exchange resin was obtained from NOVO Nordisk A/S, Bagsvaerd, Denmark.
TAG from sunflower oil. The sunflower oil from Tovarna olja GEA d.d., Slovenia, was used in acidolysis reactions.
Chemicals. All free fatty acid standards and other chemicals, as well as EA (for transeterification-acidolysis) were purchased from Sigma-Aldrich (Germany). Sodium hydroxide solution (0.025 M) was purchased from Merck (Darmstadt, Germany). Phenolphthalein was provided by Kemika (Zagreb, Croatia). All reagents and solvent were of analytical or chromatographic grade.
Gas. Carbon dioxide 2.5 (99.5 %) was supplied by Messer MG Ruše, Slovenia.

3. METHODS

3.1 Reactions at APO

The acidolysis reactions of TAG with lipase were carried out under different conditions. Substrates (40 mL of SO + necessary quantity of EA and *n*-hexane) were mixed and heated at different temperatures (313 K, 333 K, 353 K, 363 K and 373 K) at atmospheric pressure. The reaction was considered to begin when the enzyme was added. The reactions were performed in the three-neck round-bottom flask heated on an oil bath and using a magnetic stirrer with a rate of 400 rpm. The kinetic of the reaction was followed by sampling at different time intervals fallowing by analysis of samples. The total reaction time was 6 h. The samples were collected for quantitative analysis. The first method was the detection of the percentage of free fatty acid (FFA) by titration and the second method was gas chromatographic (GC) analysis.

3.2 Reactions in SC CO_2

The acidolysis reactions were performed in SC CO_2 using a high pressure batch reactor, considering the effect of temperature, pressure and stirring rate. The reaction mixture consisted of 40 mL of SO + necessary amount of EA and *n*-hexane as a solvent. The reactions were performed at pressure of 8 MPa, 10 MPa, 20 MPa and 30 MPa and different temperatures (313 K, 333 K, 353 K, 363 K and 373 K), for a total period of reaction of 5 hours. The reaction mixture was continuously stirred at 0 rpm, 200 rpm, 400 rpm and 600 rpm to ensure a homogeneous dispersion of the enzyme particles in the reaction medium. The detailed description of the batch reactor system can be found in literature (Habulin et al., 2008a). Initially, the reaction mixture was put into the reactor and then, when the temperature was stabilized, enzyme preparation was added and CO_2 was pumped into the reactor by a high-pressure pump (PM-101, NWA GmbH, Lorrach, Germany), up to the desired pressure. Pressure was measured by using a Digibar manometer (mod. PE 200, HBM GmbH, Darmstadt, Germany). The reaction temperature was obtained by immersion of the autoclave in an oil bath, and monitored with an overall accuracy of ± 0.5 K by a digital thermometer (mod. GTH 1150, Greisinger Electronic GmbH, Regenstauf, Germany), equipped with a thermocouple (WAT-LOW, Missouri, USA). Samples from the reaction mixture were collected by means of a high-pressure valve (mod 100VM-4073, Autoclave Engineering, Pennsylvania, USA), at fixed times in order to monitor the product evolution. If pressure drop occurred after the sample collection, the pressure of the system was returned to the operational conditions by pumping fresh CO_2: the changes of the CO_2 amount were established not to affect significantly the composition of the reaction mixture. The reaction temperature was first optimized, and then the effect of pressure and stirring rate were studied.

3.3 Analytical methods

At different time intervals during the reaction, samples (1000 μL) were collected from the reaction mixture. The FFA content was quantified from the amount of residual FFA in the reaction mixture by two analytical methods. The first method was the detection of the percentage of FFA by titration with 0.025 M NaOH, using phenolphthalein as indicator and ethanol as quenching agent (25 mL of 0.1 wt.% phenolphthalein solution in ethanol). The second method was GC analysis (Bratfalean et al., 2008).

4. RESULTS AND DISCUSSIONS

4.1 Enzymatic acidolysis reactions at APO

The acidolysis reaction was catalyzed by lipase (Lipozyme RM IM) from *R. miehei* and performed at atmospheric pressure. Reaction parameters such as EA concentration and temperature were optimized and the thermodynamic properties of the enzymatic acidolysis of TAG from SO with EA were determined.

4.1.1 Influence of temperature

The most important factor influencing enzyme activity is temperature, which may affect enzyme stability and affinity of enzyme for substrate. In the industrial use of enzymes a major factor considered is their thermostability, mostly because of the potential for minimizing their thermal degradation. The study of the temperature effect on acidolysis reaction was performed at APO, by varying the temperatures from 313 K to 373 K with a step of 10 K, at a constant stirring rate of 400 rpm. The reaction mixture consisted of: SO/organic solvent (*n*-hexane) in a ratio of 1:5 (vol./vol.), 0.25 g of enzyme/mL of SO. The experiments were performed during 6 hours of reaction run. Temperature and time have positive effect on acyl migration being temperature effect the greatest (Kim and Akoh, 2005). The results are presented in Fig. 1, and the values show that the highest conversion of FFA (76.52 %) was obtained at the temperature of 353 K after 240 min of the reaction run. This shows that the lipase was fully active after incubation at 353 K and that *n*-hexane may be used as an organic solvent medium. A decrease of EA incorporation was observed at temperatures of 333K and 313K. Similar results were obtained by Kim et al. (2001) and Carrin and Crapiste (2008). The lowest conversion of FFA should be attributed to much higher temperature, which was 373 K. In the first 20 min of the reaction incorporation of EA was the fastest and reached between 52 % and 90 % of maximal incorporation, obtained at 360 min of reaction run. The effect of temperature on the total conversion at temperatures higher than 353 K during the acidolysis process showed deactivation of the lipase at such reaction conditions. The curve of conversion function vs. time in Fig. 1 shows that at 363 K the maximal value of 52 % is obtained after 180 min, followed by a decrease in conversion, while at 373 K the decrease in conversion happened much sooner, namely after 60 min of the reaction run and maximal conversion was much lower (40 %).

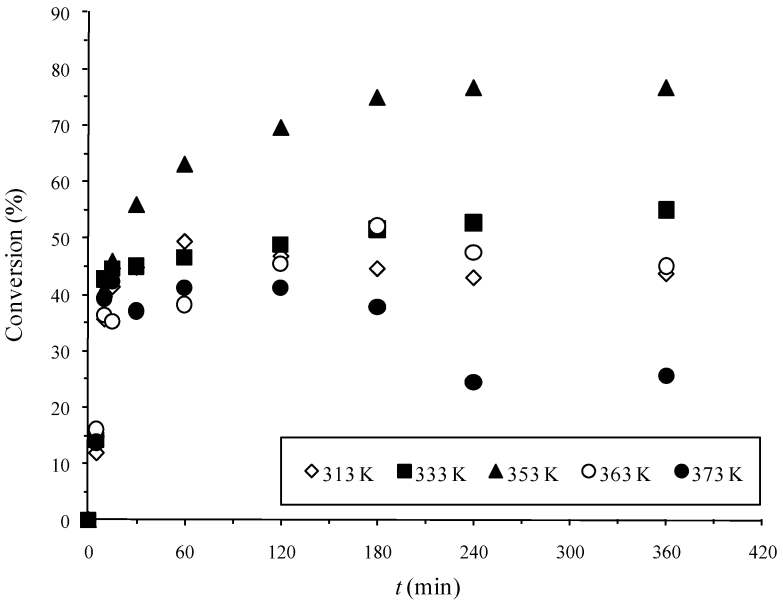


Fig. 1: The effect of temperature on the total conversion during the acidolysis reactions at APO.

For the incorporation of selected long-chain fatty acid (LCFA) into TAG a study on the thermal stability of the immobilized lipase from *R. miehei* compared with the thermal stability of the immobilized lipase from *Candida antarctica* (Novozym 435) was reported by Hamama and Shahidi (2008). It was shown that the lipase from *R. miehei* was fully active after being incubated at temperatures higher than 333 K. Therefore, the results of the present work are in good agreement with other studies on thermal stability of the immobilized lipase from *R. miehei.*

4.1.2 Influence of EA concentration

The effect of EA concentration on enzymatic acidolysis was studied at APO, at temperature of 353 K and at stirring rate of 400 rpm. The reaction mixture consisted of: SO/organic solvent (*n*-hexane) in a ratio of 1:5 (vol./vol.), 0.25 g of enzyme/mL of SO, and different EA concentrations (0.1 mg/mL of SO and 0.2 mg/mL of SO). The reaction rate was influenced by EA concentration. The results are presented in Fig. 2, and they show that the conversion is higher (80 % FFA) when higher concentration of EA (0.2 mg/mL) was applied. This may be explained by a good mass transfer between EA into and FFA from TAG. The driving force for mass transfer is a difference in concentration. The concentration of EA in the reaction mixture had an impact on the reaction rate and consequently on the conversion at incorporation of EA into TAG. Of course, substrate inhibition, which appears at high substrate concentrations, must be taken into account and therefore the reaction should be carried out at not too high EA concentration.

The major products of an acidolysis are FFA and TAG. Separation of these products is usually carried out by neutralization of FFA with hydroalcoholic solution of KOH or NaOH and extraction of TAG by hexane. Using this method, low TAG recovery yields could appear and high amounts of FFA lead in formation of much soap, in which a considerable proportion of TAG could be trapped. Therefore, SC CO_2 was used as reaction medium to combine reaction and separation in one step and to avoid formation of by-products.

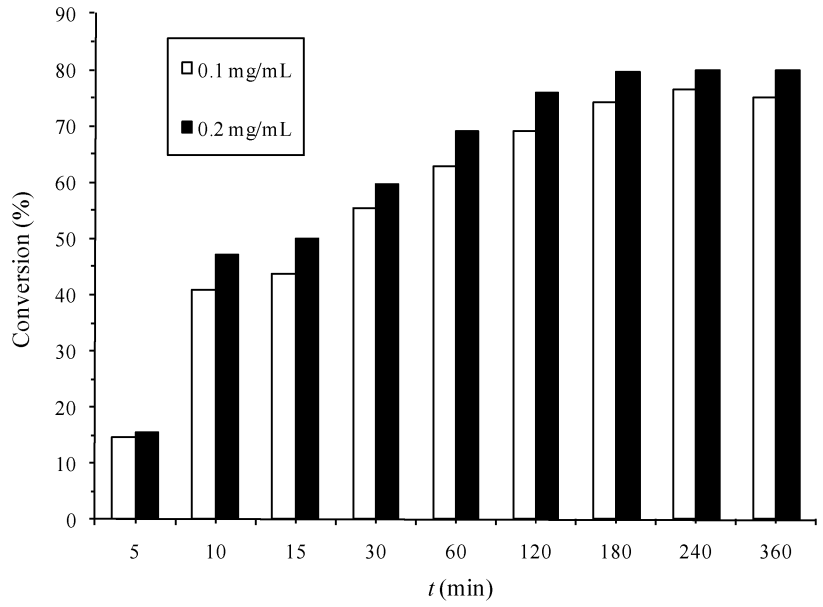


Fig. 2: The effect of EA concentrations on conversion at acidolysis at APO.

4.2 Enzymatic acidolysis in SC CO_2

Recently SCFs were used for developing biotransformation processes and enzymatic catalysis reactions. They are receiving increased attention as reaction media because they permit higher reaction rates compared with conventional solvents. Therefore, the effect of pressure, temperature and the properties of SCFs on enzymatic reactions were extensively studied (Rezaei et al., 2007). SC CO_2 may provide an attractive medium for transesterification (acidolysis) reactions of TAG due to its high diffusivity and low viscosity which increase mass transfer of the substrates into the catalyst particle.

4.2.1 Influence of temperature

The critical temperature of the majority of the gases that are commonly used in their dense supercritical fluid state as enzymatic reaction media are in the same temperature range where enzymes retain their highest stability and activity. Till now, no standard rule for determination of optimal temperature for the enzymatic reactions in SCFs was set. Many parameters could have an influence on optimal temperature for enzymatic reactions. One of this parameters is a decrease in enzyme activity, which could appear due to thermal deactivation by increase in temperature. Physical properties of the solvent could also have an influence on determination of optimal temperature. Rising the temperature, lipase molecule firstly unfolds reversibly but then formation of incorrect structures, destruction of disulfide bonds and/or hydrolysis of peptide bonds may occur (Chulalaksananukul et al., 1993). The first investigation in SC CO_2 studied the influence of temperature on the FFA conversion. The effect of temperature was studied at 10 MPa and stirring rate of 400 rpm. This effect is related to the enzyme activity and stability and the CO_2 solvating power. The reactions were performed using a high-pressure batch reactor, which has been previously described by Knez and Habulin (2002). The influence of temperature on the enzymatic activity and stability was studied in the range between 313 K and 373 K, during 5 hours of reaction performance in the supercritical medium using lipase from *R. miehei* as catalyst, dispersed in the reaction mixture. The reaction mixture consisted of SO/organic solvent (*n*-hexane) in a ratio of 1:5 (vol./vol.), 0.25 g of enzyme/mL of SO, and 0.2 mg of EA /mL of SO.

The results are shown in Fig. 3. These suggest that the maximum conversion of 80.15 % which was obtained at 333 K, was higher than at APO, where it was reached at much higher temperature, namely at 353 K.

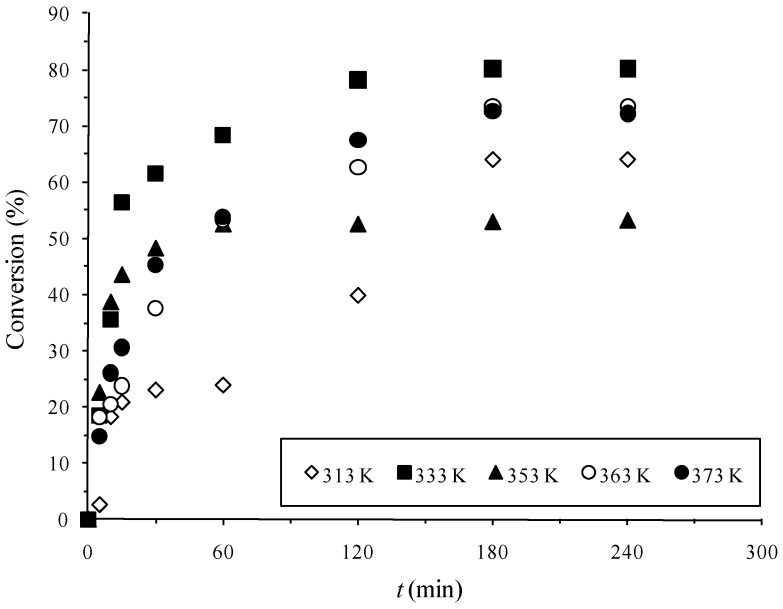


Fig.3: The effect of temperature on the total conversion during the acidolysis reaction in SC CO_2.

The immobilized lipase was found to increase its thermostability in this medium. This could be attributed to the rigidity of enzyme molecules in SC CO_2. The increase of reaction temperature usually results in an acceleration effect, according to the Arrhenius law, during reactions catalyzed by enzymes, with increase in temperature from 313 K to 333 K.

Higher temperature favours higher yields for endothermic reactions due to the shift of thermodynamic equilibrium since higher temperature increases the solubility within substrate (such as solubility of EA) and decreases the viscosity of reaction medium. A compromis between incorporation of EA and lipase deactivation has to be done to choose the proper reaction temperature. Based on reaction kinetics, it was shown that the reaction rate is higher at higher temperature. In the case of temperatures 353 K, 363 K and 373 K the reaction rates were slowly, which demonstrated a slight inactivation of the enzyme at these reaction conditions, and an insignificant growth rate of conversion at higher temperatures. Comparing these results with the results obtained at APO, the conversions at the mentioned high temperatures were still higher in the supercritical medium, which confirms the advantages of this medium for enzymatic reactions. At 373 K and APO a rapid enzyme inactivation occurred, while at the same temperature in the supercritical medium only a slight decrease in the conversion was observed. In the study of Kim et al. (2004) the optimal temperature for the production of structured lipids by lipase-catalyzed acidolysis in SC CO_2 was determined at 328 K, whereas the incorporation of caprylic acid decreased with further increase in temperature. When reaching a too high temperature the enzyme may become denatured. Lin and Chen (2008) demonstrated that the optimum temperature for enriching n-3 PUFA into TAG of borage oil via lipase-catalyzed reactions under supercritical condition was 333.15 K which is in good agreement with present study of acidolysis reaction in SC CO_2.

4.2.2 Influence of the pressure

The enzyme behaviour in sub and SCFs is very important when enzymatic reactions would be performed in these media. At low pressures the density of a SCF is gas-like, but with increase in pressure the density approaches that of a liquid. The solubility of the substrates tends to increase with increase in pressure. The pressure of CO_2 is likely to affect the reaction performance by changing the acidolysis reaction rate or the reactants solubility. Solubility of the substrates in SC CO_2 is strongly associated to the density which can be varied by changes in temperature and pressure. In general a pressure increase acts positive for enzymatic reactions or there are no changes in the reaction rates. However, it is necessary to investigate if the changes in the physical proprieties of the substrate due to the presence of SC CO_2 affect the rate of the lipase catalyzed acidolysis. On the other hand, the reaction can be influenced by the changed enzyme activity or selectivity because of the presence of SC CO_2.

The effect of the pressure on enzyme activity and stability was investigated varying the pressure between 8 MPa and 30 MPa and using the substrate concentration described above. The investigation was performed at a constant temperature of 333 K and a stirring rate of 400 rpm. The reaction mixture consisted of**:** SO/organic solvent (*n*-hexane) in a ratio of 1:5 (vol./vol.), 0.25 g of enzyme/mL of SO, and 0.2 mg of EA /mL of SO. The results, illustrated in Fig. 4, show that the FFA conversion after 4 hours of incubation has similar values for all studied pressures except for the pressure of 8 MPa.

However, the reaction rate is influenced by the pressure, especially in the first 60 min. Thus, lower conversions were recorded at 8 MPa and 30 MPa while at 10 MPa and 20 MPa the conversion reached was much higher, being the highest at 20 MPa. The same pressure optimum was found for enzymatic hydrolysis of sunflower oil in SC CO_2, while with higher (30 MPa) and lower pressures (10 MPa), the concentration of linoleic and oleic acid slightly decreased (Habulin et al., 2007b). The highest incorporation of caprylic acid into corn oil in SC CO_2 was obtained at 24.13 MPa, while with further increase in pressure a slight decrease in caprylic acid incorporation was detected (Kim et al., 2004).

It is possible that a pressure of 8 MPa may not be high enough for reducing the viscosity of the reaction mixture, and the reaction rate may be determined by a slower mass transfer of the substrates. At the same time, a high pressure (30 MPa) may affect the resin used as the enzyme carrier, and this may be reflected in the reaction rate.

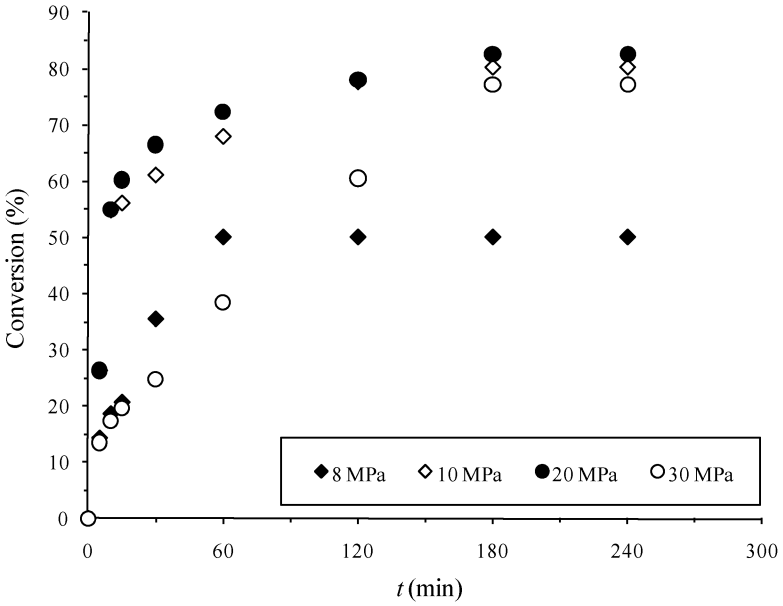


Fig. 4: The effect of the pressure on the total conversion during the acidolysis reaction in SC CO_2.

The solubility of the substrates tends to increase with increase in pressure. Furthermore, highly pressurized SC CO_2 could affect the lipase, changing its specific activity. Not because of the pressure itself, but because of the pH changes in the reaction mixture. Catalytic activity of the enzyme preparation could also be reduced or destroyed by formation of carbamates between CO_2 and lysine residues on the surface of enzyme.
The results reported by Lin and Chen (2008) and Lin et al. (2006) about the pressure effect of SC CO_2 on acidolysis reactions suggest that the solubility of substrates was optimal at pressures between 10 MPa and 20 MPa. Therefore, the results of the present work are in good agreement with their study.

4.2.3 Influence of stirring rate

It is well known fact that for heterogeneous catalysis in SCFs external mass transfer limitations can be minimized or even no external mass transfer limitations occur in these media. Therefore the effect of stirring rate was studied in the range between 0 rpm and 600 rpm at a temperature of 333 K and substrate concentration described above. Lipase catalyzed acidolysis was performed in SC CO_2, for 5 hours reaction time. The reaction mixture consisted of**:** SO/organic solvent (*n*-hexane) in a ratio of 1:5 (vol./vol.), 0.25 g of enzyme/mL of SO, and 0.2 mg of EA /mL of SO. The influence of the stirring rate on the acidolysis reactions is illustrated in Fig. 5. This shows that the time necessary for reaching maximum conversion and the rate of reaction slightly depend on the stirring rate.
In the recent studies about influence of stirring rate on lipase catalyzed synthesis in SC CO_2 by Habulin et al. (2008a) it was reported that at the stirring rate higher than 500 rpm a decrease in reaction rate was observed.
Thus, lower reaction rates were recorded at 0 rpm. Experimental results of this study show that between 200 rpm and 600 rpm only in the first 60 min a minimal influence on reaction rate could be observed. The best results occurred at 600 rpm. These show that the time of reaching maximal conversion and the rate of reaction do not depend on the stirring rate, since maximal conversion was obtained after 120 min at each of the examined stirring rate. This indicates that in the SC CO_2 external mass transfer limitations were set to a minimal value, which is another proof for advantages of this reaction medium.

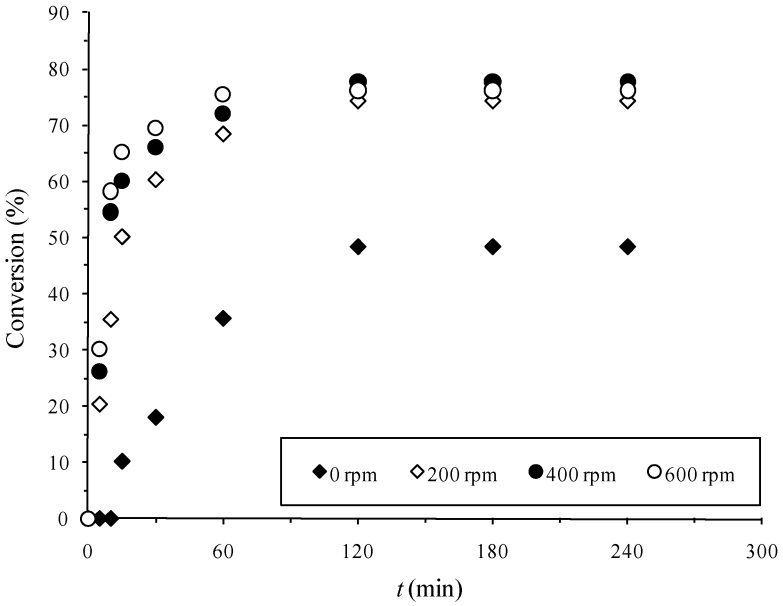


Fig. 5: The effect of the stirring rate on the conversion during the acidolysis reaction in SC CO_2.

4.3 Thermodynamic parameters of acidolysis reaction at APO and in SC CO_2

Thermodynamic parameters (Marangoni, 2003) such as: free energy of deactivation (ΔG_d), enthalpy (ΔH_d), and entropy (ΔS_d) of acidolysis reactions at APO and in SC CO_2 were determined.

The rate constants have been calculated between 313 K and 373 K, and the activation energy (*E*a) was derived using the Arrhenius plot. The thermodynamic quantities for the process of acidolysis have been estimated by plotting ln v_i versus $1/T$, at APO and also in SC CO_2. The results are shown in Fig. 6 and in supporting information. The slope on the fitting lines was determined using linear regression. The effect of temperature on the equilibrium constant of the acidolysis reaction was determined by the temperature dependence of the standard Gibbs free energy and by using the relationship between this energy and the equilibrium constant under constant pressure of 10 MPa with the following equation:

$$K_d = e^{-\frac{\Delta G_d}{RT}} \rightarrow \Delta G_d \quad (1)$$

Where: K_d is equilibrium constant between inactivated and activated enzyme; ΔG_d (kJ/mol) is Gibb's free energy of deactivation; T (K) is temperature and R (J/mol K) is the universal gas constant. The thermodynamic parameters values are presented in Table 1.

From Arrhenius plot (Fig. 6) it is obvious that the activity of the lipase between 313 K and 353 K increased and with the further temperature rise thermal deactivation occurred, regardless of the medium in which reaction was performed.

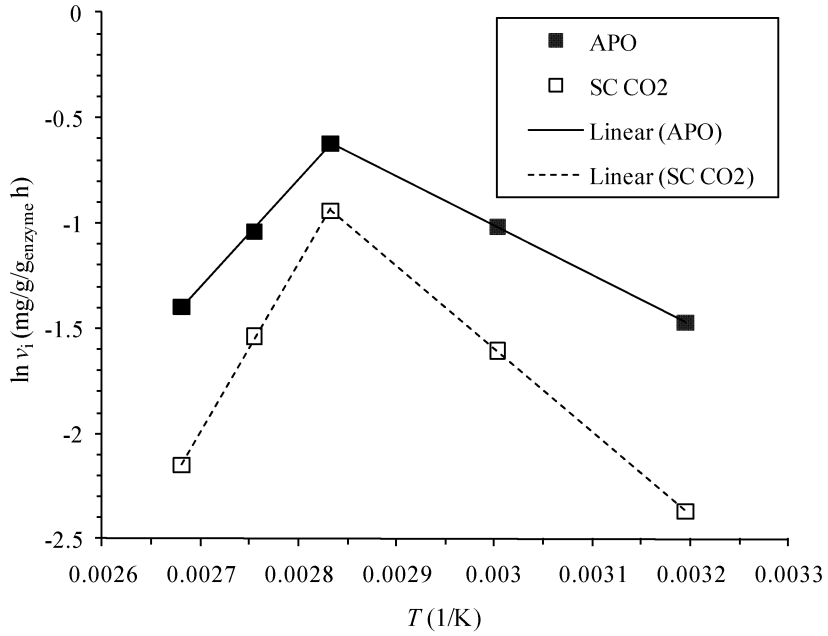


Fig 6: Arrhenius plot for the acidolysis reaction of TAG from SO at APO and in SC CO_2.

The energy of activation in the case of acidolysis reaction in SC CO_2 of 3.50 kJ/mol show a thermodynamic characterization of enzyme stability compared to acidolysis reaction at APO, where the energy of activation was 4.97 kJ/mol. Enthalpy and entropy of chemical reaction provide valuable information about the nature of the transition state, and hence about the reaction mechanism. A large amount of stretching, squeezing or even breaking of chemical bonds is necessary for the formation of the transition state, when value for enthalpy is high. The entropy of activation gives a measure of the inherent probability of the transition state, apart from energetic considerations. Equilibrium constant K_d has at both condition (reaction performed at APO and also in SC CO_2.) value about one, which indicate that enzyme preparation contains inactivated and activated enzyme in equal proportions. Thermodynamic properties suggest that SC CO_2 is a better environment for the enzymatic incorporation of EA into TAG from SO.

Table 1: Thermodynamic parameters for deactivation of lipase during of acidolysis reactions at APO and SC CO_2.

Magnitude	**Value (APO)**	**Value (SC CO_2)**
	Lipase *R. miehei*	**Lipase** *R. miehei*
*E*a (kJ/mol)	4.97	3.50
ΔH_d (kJ/mol)	54.56	27.93
K_d	1.018	1.010
ΔG_d (kJ/mol)	54.98	51.86
ΔS_d (J/mol K)	138.98	147.33

5. CONCLUSIONS

It has been demonstrated that the biotransformation process in SC CO_2 is a good alternative reaction medium for enzyme-catalyzed acidolysis reactions by enzymatic incorporation of EA into TAG of SO. These studies illustrate a preliminary research on improving SO quality by acidolysis reaction.
The obtained results are proving the possibility of introducing the monounsaturated fatty acid with long chain into TAG; introducing EA may offer potential nonfood applications.
The results illustrate an experimental model for the improvement of SO quality, which could be used for improvement of different types of oil by enrichment in polyunsaturated acids, which can protect the organism against hyper cholesterolemia.
Advantages of using SC CO_2, as a reaction medium, are not shown only in obtained better thermodynamic properties, higher conversion (80.15 %) at lower temperature (333 K) and shorter reaction time (180 min) in comparison with reaction performed in *n*-hexane (C = 76.52 %; T = 353 K; t = 240 min), but also in possibility of easier separation of products from unreacted reactants by changing the temperature and/or pressure after reaction.

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