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in Continuous-Flow Packed-Bed Reactor.**

Pleskač, Ondřej
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Selective Enzymatic Hydrolysis of Triglycerides in Supercritical Carbon Dioxide in Continuous-Flow Packed-Bed Reactor

Student: Ing. Ondřej Pleskač

Supervisor: Ing. Marie Sajfrtová, Ph.D.

Supervising Expert: Ing. Helena Sovová, CSc.

The sufficient content and balanced ratio of ω -3 and ω -6 polyunsaturated fatty acids in dietary fats are essential for human health and infant development.¹ Selected vegetable oils (black currant seed oil, hemp seed oil, chia seed oil) provide a great source of these fatty acids but their ratio is not always optimal. Having a method for refinement of these oils to manipulate the ω -3: ω -6 ratio, would have a potential for the dietary supplements development and could make us less reliant on marine resources for purpose of their production.

Different fatty acids are not uniformly distributed over glycerol positions in triglycerides of vegetable oils.² Hence partial regioselective hydrolysis yields diglycerides, monoglycerides, and free fatty acids with different fatty acid compositions from the original oil. Because of the change in fatty acid composition, the ratio of ω -3: ω -6 should also differ in these product fractions.

The regioselective hydrolysis is catalyzed by pancreatic *sn*-1,3 regiospecific lipase which is unlike other enzymes well stable in supercritical carbon dioxide (sc-CO₂). Usage of sc-CO₂ as a reaction media has many advantages in comparison with water, which is usually the first solvent of choice in terms of enzymatic reactions. Sc-CO₂ provides excellent mass transfer properties and allows fine control of processing parameters which is very important for this cause since desired compositional change is presumed to be affected by hydrolysis conversion. After depressurization, CO₂ leaves products without traces of solvents, suitable for the pharmaceutical industry. It also offers a possibility of downstream separation by precipitation driven by a change of solvent density via pressure.

This research aims to study compositional changes in the product of blackcurrant (*Ribes nigrum*) seed oil hydrolysis in sc-CO₂ concerning the ratio of ω -3 and ω -6 fatty acids. The blackcurrant seed oil was chosen as a model substrate for its balanced fatty acid profile.

The experimental apparatus consisted of three columns connected in series. In the first column, carbon dioxide was saturated with water, which serves as a hydrolysis reagent and aids the enzyme to hold required conformation. Wet CO₂ then continued to the second column, which served as an oil saturator. The prepared reaction mixture was then introduced to the third column, which served as a continuous-flow packed-bed reactor, filled with regioselective lipase (Lipozyme) immobilized on a macroporous resin.

The influence of reaction temperature (30, 40, 50 °C), pressure (15, 20, 25 MPa), and mean residence time on the ω -3: ω -6 ratio in diglycerides was investigated. Enzyme's ability to withstand experimental conditions and to sustain its activity was also studied.

Preliminary results confirmed compositional changes between partial glycerides and original oil. High selectivity towards hydrolysis in *sn*-1 and *sn*-3 was observed. The activity of Lipozyme showed a slightly decreasing trend during course of the reaction.

References

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