Selective enzymatic hydrolysis of triglycerides in supercritical carbon dioxide in continuous-flow packed-bed reactor

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The fatty acid composition of dietary fats is connected with human health and infant development. One of the most significant aspects is the content and ratio of omega-3 and omega-6 polyunsaturated fatty acids. Being able to acquire products enriched with omega-3 or omega-6 from industrial vegetable oils would have a potential for the development of dietary supplements and could make us less reliant on marine resources for purpose of their production.

As different fatty acids are not uniformly distributed over glycerol positions in triglycerides of vegetable oils[1], controlled hydrolysis with *sn*-1,3 regiospecific lipase yields products of different fatty acid profiles. Partial regioselective hydrolysis yields a product composed of diglycerides, monoglycerides, and free fatty acids, each of these fractions having a different ratio of omega-3 and omega-6 fatty acids.

Unlike most enzymes, pancreatic *sn*-1,3 regiospecific lipase is well stable in supercritical carbon dioxide (Sc-CO_2), which has many advantages in comparison with water, which is usually the first solvent of choice in case of enzymatic reactions. Sc-CO_2 provides excellent mass transfer properties and allows fine control of processing parameters which is very important for this cause since desired compositional change is a function of hydrolysis conversion. After depressurization, Sc-CO_2 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.

Our objective was to study compositional changes in the product of vegetable oil hydrolysis with regards to the ratio of omega-3 and omega-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. In the first column carbon dioxide was saturated with water which serves as a reagent and aids the enzyme to hold required conformation. The oil was dissolved in the second column. The third column 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 ratio of omega-3 and omega-6 in resulting free fatty acids and partial glycerides was experimentally tested. Enzyme's ability to withstand experimental conditions was also studied. Reaction products were analyzed with HPLC-MS which can provide very detailed information about the composition of particular hydrolysate fractions.

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

References

[1]H. Sovova, M. Zarevúcka, Lipase-catalysed hydrolysis of blackcurrant oil in supercritical carbon dioxide, Chemical Engineering Science 58 (2003) 2339 – 2350.

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