Enzymatic transesterification of *Brassica juncea* seed oil for production of neutraceuticals

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Abstract

The importance of polyunsaturated fatty acids (PUFA) in human nutrition and disease prevention has long been recognized. Both omega-3 and omega-6 PUFA are precursors of eicosanoids, which are involved in many important biological processes in the human body. GLA (gamma linolenic acid) is important for the prevention and treatment of skin disease, premenstrual syndrome, diabetes, inflammatory and autoimmune disorders and cancer. Mustard (*Brassica juncea*) oil could produce neutraceuticals under enzymatic interesterification with short chain fatty acids (C_8-C_{10}) and other important fatty acids. An immobilized lipase from *Thermomyces lanuginosus* (TL IM) could be employed to mediate the continuous transesterification of mustard oil with other unsaturated oils in a packed bed reactor operating at 60-65^{0}C. Introduction of EPA (Eicosapantanoic acid), DHA (Docosahexaenoic acid) and GLA (Gamma linolenic acid) in mustard oil could contribute to the removal of erucic acid and also could be utilized for DAG (Di-acyl glycerol) by taking glycerol and 2-position fatty acids esterification in presence of ethyl alcohol or isopropanol. These products could be complex mixtures of diacylglycerol (DAG) species with good amount of unsaturation and essential fatty acids in appropriate ratio, whose composition depend on reaction conditions.

Key words: Lipase, interesterification, PUFA, DAG, GLA, DHA, EPA, neutraceutical, structured lipids

Introduction

Structured lipids(SLs) are tailor-made fats and oils with improved nutritional or physical properties because of modifications to incorporate new fatty acids or to change the position of existing fatty acids on the glycerol backbone. More recently, SLs were designed to provide simultaneous delivery of beneficial long chain fatty acids (LCFAs) at a slower rate and medium chain fatty acids (MCFAs) at a quicker rate (Babayan 1987; Akoh 1998). SL synthesis yields novel triacylglycerol (TAG) molecules (Akoh 1998). SLs may provide the most effective means of delivering desired fatty acids for nutritive or therapeutic purposes, and for targeting specific diseases and metabolic conditions (Lee and Akoh, 1998). Improvements or changes in the physical and/or chemical characteristics of a TAG can also be achieved when SLs are synthesized

The component fatty acids and their position in the triacylglycerol (TAG) molecule determine the functional and physical properties, the metabolic fate, and the health benefits of an SL. MCFAs are preferentially transported via the portal vein to the liver, because of their smaller size and greater solubility compared to LCFAs (Bell and others 1991; Straarup and Hoy 2000). Therefore, it appears that MCFAs are most useful in a structured lipid that combines their inherent mobility, solubility, and ease of metabolism with more healthful polyunsaturated fatty acids (Cater and others 1997; Akoh 1998). LCFAs are absorbed and metabolized more slowly than either medium or short chain fatty acids; much of the LCFAs may be lost as calcium-fatty acid soap in the feces (Broun and others 1999). Eicosapentaenoic acid, 20:5n-3 (EPA), and docosahexaenoic acid, 22:6n-3 (DHA), found in fish oil, are other n-3 polyunsaturated fatty acids (PUFAs) of interest in SL production.

Therefore, it is evident from the above statements that development of structured lipids is very important for their application in functional food, neutrceuticals, low calorie fats etc. In the present study mustard oil was selected because it contains good amount of n-3 and n-6 fatty acids and was interesterified with EPA and DHA. This process makes mustard oil as a neutraceutical oil for various functional purposes.

Materials and methods

Mustard oil used in experimentation was purchased from local market, Lipase enzyme (Lipozyme TL 1M) was obtained from Novozyme, EPA and DHA was purchased from Merck in the form of Maxepa capsules.

EPA and DHA was first esterified with methanolic potassium hydroxide in organic solvent medium, ester layer was separated and added to mustard oil. The mixture was added with 10% lipase enzyme and was incubated at 60°C for different time periods viz 4, 6, 8 and 12 hours with constant shaking on shaker water bath system. Fatty acid composition of normal mustard oil and the Maxepa from Merck was also undertaken

After the interesterification process for different time periods was completed the mixture was then centrifuged at 10000 RPM for 10 minutes. After centrifugation the enzyme layer oil oil-ester layer was separated. The oil-ester layer was then spotted on silica gel plate with 10 replicates for each treatment. The spotted silica gel plate was then allowed to run in hexane-diethylether (70:30) mobile phase. These plates were allowed to run upto 10cm from the spot. After taking out the plates the lower 0.5cm from spotted point was scratched and dissolved in 10ml heptane and esterified with methanolic

potassium hydroxide for detailed GLC analysis.

Results

The fatty acid composition of mustard oil and Maxepa capsule was analyzed after converting them into methanolic esters. In Table1 the detailed fatty acid composition shows that mustard oil employed in this study contains 48% erucic acid and Maxepa contains 18% EPA and 12% DHA in addition to other fatty acids.

Table 1: GLC profile of major fatty acids of mustard oil and Maxepa

S.No.	Fatty acid composition	Mustard Oil	Maxepa
1.	Oleic	10.39%	16.27%
2.	Linoleic	15.79%	2.66%
3.	Linolenic	12.38%	1.16%
4.	Erucic	48.01%	0.00%
5.	EPA	0.00%	18.05%
6.	DHA	0.00%	11.82%

In our study we have observed that as the time period for enzymatic hydrolysis increases the interesterification reaction decreases after a certain time, which was 6 hours. In Table 2 we have observed that among the four treatments of different time periods the maximum incorporation of EPA and DHA is in

Table 2: GLC profile of major fatty acids of interesterified oil at different reaction periods

S.No.	Fatty acid composition	4hr reaction	6hr reaction	8hr reaction	12hr reaction
1.	Oleic acid	30.35%	34.00%	33.94%	28.00%
2.	Linoleic acid	13.48%	15.45%	18.10%	15.92%
3.	Linolenic acid	6.58%	8.31%	15.23%	8.73%
4.	Eruic acid	0.40%	0.26%	0.10%	0.00%
5.	EPA	27.00%	18.83%	16.00%	27.31%
6.	DHA	7.57%	4.76%	3.30%	4.30%

All fatty acid composition is the average of ten hydrolysis replicates

4 hours reaction but at the same time the erucic acid left in the oil is 0.4% as compared to 6 hours reaction in which the erucic acid left in the interesterified oil is 0.26% only and also the maximum interesterification percentage was observed in this time period.

As the time for reaction increased from 6 to 12 hours the reaction reached to equilibrium and then showed a downtrend. Lipase-catalyzed reactions were a combination of esterification and hydrolysis (reverse reaction) reactions. After reaching equilibrium these reactions showed stagnation and then a downtrend. Structured lipids produced with lipases in organic solvent, where substrates are soluble and hydrolysis can be minimized depending upon the objective for structured lipids. It was understood during the different reactions performed that the type of organic solvent employed dramatically affected the reaction kinetics and catalytic efficiency of an enzyme. The extent to which the solvent affected the activity or stability of the enzyme and the effect of the solvent on the equilibrium position of the desired reaction must both be considered when choosing a solvent for biocatalysis.

Discussion

In our study it is evident that mustard oil can be designed to a 'Neutraceutical oil' with the incorporation of EPA and DHA with lipase catalyzed interesterification. The removal of erucic acid and incorporation of essential fatty acids for the purpose of making it fit for various functional foods. From the study it is also evident that enzymatic interesterification is time and temperature sensitive therefore a proper time period analysis is very important. Also, lipases occur widely in nature and are active at the oil-water interface in heterogeneous reaction systems. Lipase catalyzed interesterification reactions offer the advantage of greater control over the positional distribution of fatty acids in the final product, due to lipases' fatty acid selectivity and regiospecificity. Lipases hydrolyze triacylglycerol (TAGs) to monoacylglycerols, diacylglycerols (DAGs), free fatty acids (FFA), and glycerol. In addition to the ester-interchange reaction, lipases can also catalyze direct esterification, acidolysis, and alcoholysis reactions (Lee and Akoh, 1998). As is evident from the 6 hours reaction onwards where it is clear that the reaction time period increases the interesterification rate and then shows a downtrend. In present study at 60°C the interesterification process was found optimum for a period of 6 hours. Most lipases are optimally active between 30 and 40°C (Shahani 1975). As the temperature increases, enzyme molecules unfold by destruction of bonds, such as sulfide bridges, and may lead to hydrolysis of peptide bonds and deamidation of asparagines and glutamine residues. However, these processes can be avoided in a water-free environment. Immobilization of enzymes also results in greater thermo stability. Additionally, genetically engineered lipases are now available for the synthesis of SLs. It is hoped that the use of biotechnology will reduce the cost of lipases, making the enzymatic route to SLs economically viable. Other factors affecting enzymatic activity and product yield include pH, substrate molar ratio, enzyme activity and load, incubation time, specificity of enzyme to substrate type and chain length, and regiospecificity (Akoh, 1998). Two of the most attractive reasons for choosing enzymatic over chemically catalyzed reactions for SL production are the energy saved and minimization of thermal degradation.

Conclusion

The study shows that mustard oil although contains essential fatty acids in well balance but still it can further be modified into Neutraceutical oil and for particular functional food purposes. The incorporation of EPA and DHA enhances its nutritional and functional value manifold. Although much remains unstudied in the field of SLs, but still the properties of a SLs for reduction in calorific value of foods that normally contain high amounts of fat, or the medicinal properties of rapidly absorbing TAGs composed of medium chain fatty acids (MCFAs) and poly-unstaurated fatty acids (PUFAs), structured lipids definitely provide attributes that consumers will find valuable. Therefore, it is important that further research be conducted that will allow for better understanding and more control over the various esterification processes and reduction in costs associated with large-scale production of SLs.

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