High-laurate canola oil in production of structured lipids

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Abstract

Structured lipids (SL) containing lauric acid and long-chain polyunsaturated fatty acids (LC PUFA) were produced using high-laurate canola oil (Laurical 35) and eicosapentaenoic acid (EPA, C20:5 n-3), docosapentaenoic acid (DPA, C22:5 n-3), and docosahexaenoic acid (DHA, C22:6 n-3) as substrtaes. Laurical 35, containing 37% lauric acid and 34% oleic acid, was subjected to acidolysis reaction in the presence of enzyme catalysts from *Mucor miehei, Pseudomonas sp*., and *Candida rugosa*. The process variables for optimization of incorporation of LC PUFA into Laurical 35 were the amount of enzyme (2-6%), reaction temperature (35-55°C) and incubation time (12-36 h). The maximum incorporation of EPA, DPA and DHA into Laurical 35 were 62.2, 50.8 and 34.1% under conditions (enzyme concentration 4.36-5.41%, temperature of 38.7-43.7°C and reaction time of 23.9-44.7 h. Both LC PUFA and lauric acid were mainly esterified to the sn-1,3 positions of the modified oils. The modified oil was found to be less stable than the original oils because of its higher degree of unsaturation and removal of active antioxidants from the original oils. The resultant products containing both medium-chain fatty acid (C12:0) and LC PUFA may serve as nutraceutical and functional food ingredients.

Key words: Acidolysis, EPA, DPA, DHA, high-laurate canola oil, lipase, medium-chain fatty acids, ω3 fatty acids, positional distribution, response surface methodology, structured lipids, oxidative stability, conjugated dienes (CD), thiobarbituric acid reactive substances (TBARS).

Introduction

Specialty lipids include a wide range of products amongst which structured lipids are a main class. Structured lipids (SL) are triacylglycerols (TAG) or phospholipids (PL) in which fatty acids are placed in specific locations in the glycerol backbone and are produced using a chemical or enzymatic process. Much attention has been paid to SL due to their potential biological functions and nutritional perspectives. Designing SL with selected fatty acids at specific locations of the TAG for medicinal application has attracted much attention. The position of fatty acids (FA) in the TAG molecules (*sn*-1, *sn*-2, and *sn*-3) would have a significant impact on their metabolism in the body. In general, FA at the terminal positions of TAG (*sn*-1 and *sn*-3) are hydrolyzed by pancreatic lipase and absorbed while those at the middle position of TAG (*sn*-2) remain unchanged and are used in the synthesis of new TAG. For example, it may be desirable to develop a SL containing LC PUFA at the *sn*-2 position with medium-chain fatty acids (MCFA) at the *sn*-1,3 positions for patients with mal-digestion as well as cystic fibrosis.

Recognition of the health benefits associated with consumption of seafoods (n-3 fatty acids) is one of the most promising developments in human nutrition and disease prevention research in the past three decades. Long-chain n-3 fatty acids present in seafoods and algal sources include eicosapentaenoic acid (EPA, C20:5 n-3), docosapentaenoic acid (DPA, C22:5 n-3), and docosahexaenoic acid (DHA, C22:6 n-3). DPA has not been studied in much detail because of availability problems as it is present in a much lower concentration in marine oils as compared with EPA and DHA. It is also difficult to purify it from mixtures containing EPA and DHA which have similar physico-chemical properties (Yazawa, 2001).

High-laurate canola oil was produced by Calgene's Inc. (Davis, CA) in order to provide an alternate to several palm kernel oil fractions (Del Vecchio, 1996). In this study, the ability of different lipases to catalyze the acidolysis of high-laurate canola oil (Laurical 35) with EPA, DPA, and DHA was explored. Effects of enzyme amount, reaction temperature and time on the incorporation of LC PUFA into Laurical 35 using response surface methodology (RSM), were also investigated. The oxidative stability of the resultant structured lipids (SL) was then assessed.

Material and Methods

Methods

Acidolysis Reaction. The reaction mixture contained Laurical 35 (70 mg), fatty acid to Laurical 35 at a mole ratio 3:1, enzyme amount (2-6%), water (2%), and hexane (3.0 mL). The mixture was kept at temperatures ranging from 35 to 55°C for 12 to 36 h.

Preparation of Fatty Acid Methyl Esters (FAMEs). Fatty acid profiles of products were determined following conversion to their corresponding methyl esters (FAMEs) and these were analysed by gas chromatography.

Stereospecific analysis. Positional distribution (sn-2 and sn-1+ sn-3) of fatty acids in modified and unmodified Laurical 35 was carried out with standard methods using gas chromatography and enzymatic reactions.

Experimental Design for Response Surface Analysis. Experimental design for response surface analysis was performed as

described in our previous studies (Hamam and Shahidi, 2006 [a,b]).

Oxidative stability tests. The stability of the products was tested using standards measures of oxidation (conjugated dienes, CD; and thiobarbituric acid reactive substances, TBARS).

Results

The fatty acid profile of high-laurate canola oil (Laurical 35). The original oil contained 37.0% lauric acid as well as oleic (33.9 %) and linoleic (3.35 %) acids.

Enzyme screening. Lipases from *Candida rugosa, Mucor miehei,* and *Pseudomonas sp.* were most effective, for incorporating EPA, DPA, and DHA into Laurical 35, respectively.

Positional distribution. Positional distribution analysis of modified Laurical 35 revealed that both lauric acid and LC PUFA (EPA and DHA) were located at the sn-1 + sn-3 positions of the modified oils. Meanwhile DPA was randomly distributed over the three positions of the modified Laurical 35 with DPA.

Response Surface Methodology. Table 1 shows critical values for the three factors (enzyme load, reaction time and temperature) examined. The maximum incorporation of EPA (62.2%) into Laurical 35 was predicted at 4.36% enzyme load at 43.2°C over 23.9h. The stationary point for the degree of DPA incorporation (%) into Laurical 35 reached a maximum of 50.8% at 5.41% enzyme concentration, and 38.7°C in 33.5 h. Similarly, the maximum incorporation of DHA (34.1%) into Laurical 35 was obtained when enzyme amounts, reaction temperature and time were 5.25%, 43.7°C, and 44.7h, respectively. EPA proved to be more reactive than DPA or DHA. EPA has five double bonds while DHA has six. The more double bonds the chain has in the cis configuration, the more bent it is. Since DHA has six cis double bonds, it becomes quite curved compared to EPA and hence DHA has more steric hindrance than EPA. DPA and DHA have the same chain length and both belong to the n-3 family. However, DPA has one less double bond than DHA and hence less steric hindrance Therefore, the structural differences (chain length, number and location double bonds) between these three molecules had a marked effect on their incorporation into Laurical 35.

Oxidative stability tests. The present results indicate that modification of Laurical 35 LC PUFA resulted in its lower stability as evidenced by both CD and TBARS, due to the incorporation of highly susceptible FA, DHA or DPA or EPA, to oxidation. Another possible explanation could be attributed to the formation of tocopheryl esters from reaction of free carboxylic acids in the medium and tocopherols present in the oil during the process of preparation of SL and these do not render any stability to the resultant modified oils (Hamam and Shahidi, 2006).

Table1. Canonical analysis of response surface for acidolysis of high-laurate canola oil with EPA or DPA or DHA

Factor	Laurical 35, EPA (%)	Laurical 35, DPA (%)	Laurical 35, DHA (%)
Amount of enzyme (w %, X1)	4.36	5.41	5.25
Reaction temperature (C, X2)	43.2	38.7	43.7
Reaction time (h, X3)	23.9	33.5	44.7
Stationary point	maximum	saddle	saddle
Predicted value [a]	62.2	50.8	34.2
Observed value [b]	61.1 ± 0.95	48.7 ± 0.62	38.8 ± 3.11

[a] Predicted using the polynomial model

[b] Mean value of triplicate determinations ± standard deviation

Conclusions

Lipases from *Candida rugosa, Mucor miehei,* and *Pseudomonas sp* were most effective, for incorporating EPA, DPA, and DHA into Laurical 35, respectively. RSM showed that the maximum incorporation of EPA (62.2%) into Laurical 35 was possible using 4.36% enzyme, at 43.2°C and over 23.9h. In Laurical 35-based SL, maximum incorporation of DHA (34.3%) was achieved at a 5.25% enzyme, at 43.7°C, over 44.7 h. The maximum incorporation of DPA into Laurical 35 (50.8%) was obtained when enzyme amount, reaction temperature and time were 5.41%, 38.7°C and 33.5 h, respectively. Incorporation of LC PUFA into Laurical 35 was in the order EPA>DPA>DHA. EPA or DHA as well as lauric acid were mainly esterified to the sn-1,3 positions of the modified oils. Enzymatically modified Laurical 35 with EPA, DPA, and DHA had a higher conjugated dienes (CD) value than the unmodified starting material. Modified Laurical 35 with DPA was less stable than DHA- or EPA- modified Laurical 35 as reflected in both CD and TBARS values.

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