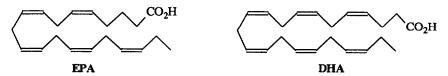
## THE PREPARATION OF TRIGLYCERIDES HIGHLY ENRICHED WITH ω-3 POLYUNSATURATED FATTY ACIDS VIA LIPASE CATALYZED INTERESTERIFICATION

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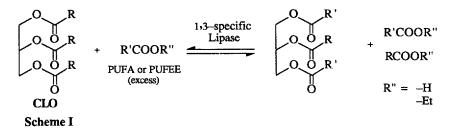
Abstract. The preparation of triglycerides highly enriched with n-3 polyunsaturated fatty acids, EPA and DHA in particular, via interesterification of cod liver oil and free fatty acid or ethyl ester concentrates catalyzed by an immobilized 1,3-specific lipase from Mucor miehei, is described.

In recent years the health aspects of marine fat have been studied extensively<sup>1-4</sup>. This interest was initiated upon epidemiological studies of Danish scientists on the Greenland Eskimos regarding coronary heart diseases<sup>5</sup>. The beneficial effect has been attributed to the n-3 polyunsaturated fatty acids, which are characteristic of marine fat, *cis*-5,8,11,14,17- eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA), in particular<sup>6,7</sup>. These desirable fatty acids are bound into triglycerides, which is the natural form of fatty acids in fish oils<sup>8</sup>. Cod liver oil, a well-known food supplement for generations, for instance, is a complicated mixture of more than fifty different fatty acids bound into the natural triglycerides of which there is usually 8-9 % each of EPA and DHA and 22-24 % the total of n-3 polyunsaturated fatty acids<sup>9</sup>.

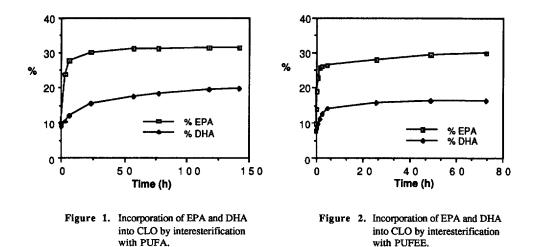


Within the pharmaceutical industry there has been increasing demand for these fatty acids and then almost exclusively in the natural triglyceride form<sup>6,7</sup>. It is possible to prepare triglycerides containing up to 30 % EPA and DHA directly from fish oils, without splitting the fat, for which several methods are available<sup>8,10</sup>, such as winterization, molecular distillation and solvent crystallization. To achieve an oil concentration of EPA and DHA totalling above 30 % is on the other hand often difficult mainly due to the great variety of the combinations of the fatty acids in triglyceride oils. However, once set free as individual fatty acids or esters fractionation is feasible up to the 65 -80 % EPA + DHA level by a number of methods<sup>8,10</sup>, such as supercritical fluid extraction, urea complexation, chromatography, molecular distillation and above the 90 % level each of EPA and DHA by HPLC<sup>11</sup> and Corey's chemical modification<sup>12</sup>. Resynthesis of triglycerides from such highly enriched EPA and DHA concentrates has not been described. The reaction of glycerol with either free fatty acids or monoesters generally affords triglycerides contaminated with large quantities of monoand diglycerides, both chemically<sup>13</sup> and enzymatically<sup>14</sup>. However, interesterifications, which are based on exchanging the fatty acid components of a triglyceride or a mixture triglycerides for either free fatty acids (acidolysis) or the fatty acids of other triglycerides or monoesters, have been used successfully on the much simpler vegetable oils. These have been carried out either chemically<sup>15,16</sup> by using e.q. sodium or sodium alkoxide, or enzymatically<sup>17-19</sup> by lipases.

In this communication we report a facile method for the preparation of triglycerides containing very high ratios of n-3 polyunsaturated fatty acids, particularly EPA and DHA. This method is based on lipase catalyzed interesterification of cod liver oil (CLO) with either polyunsaturated free fatty acid (PUFA) or ethyl ester (PUFEE) concentrates (Scheme I), either in the absence of any solvent or in a non-polar organic solvent such as hexane. These concentrates were prepared from appropriate fish oils by urea complexation<sup>20</sup>, molecular distillation<sup>21</sup> or a combination of both. The lipase that was used is Lipozyme, an immobilized lipase from the fungus *Mucor miehei*, provided by Novo industri A/S in Denmark<sup>19,22</sup>. The use of enzymes as catalysts in organic synthesis has increased markedly in the last several years<sup>23,24</sup>. The use of lipases has been well established as chiral catalysts for asymmetric hydrolysis in an aqueous media<sup>23</sup>, but more recently the use of lipases in organic media has been increasing<sup>25</sup>.



In a typical procedure a mixture of refined  $CLO^{26}$  and PUFA or PUFEE in the ratio of 1:3 (wt:wt) was stirred at 60-65 °C with 10 % Lipozyme under nitrogen for 48 hours. Samples were systematically collected during the process. In the PUFA case they were extracted under alkaline condition to separate the free fatty acids and the triglyceride product, whereas in the PUFEE case the monoester separation was carried out either by the aid of preparative TLC on silica gel or molecular distillation. In both cases the fatty acid analysis of the triglyceride product was obtained by capillary GLC. Figures 1. and 2. show typical EPA/DHA incorporation curves for the PUFA and the corresponding results for the PUFEE cases, respectively. In the former case a PUFA concentrate of 39 % EPA and 24 % DHA was used and when an equilibrium had been reached a triglyceride product of 31 % EPA and 20 % DHA was afforded. In the latter case a PUFEE concentrate of 40 % EPA and 19 % DHA was used, which at an equilibrium afforded a triglyceride product of 30 % EPA and 16 % DHA.



As can be noticed from these figures the exchange process is considerably faster for the ethyl esters. Also, there is a marked difference in the incorporation rate between EPA and DHA, the latter being considerably slower, especially in the overall slower PUFA case, which takes more than twice as long to reach an equilibrium. Under the condition described above the interesterification applying ethyl esters has the advantage of a considerably faster process. On the other hand the application of PUFA affords triglycerides free of any mono- and diglycerides, whereas the ethyl ester case affords a product which is contaminated with 5-6 % diglycerides as a consequence of minor partial hydrolysis due to the essential water content<sup>19</sup> of the immobilized lipase. The procedure described represents a viable practical method which conveniently has been scaled up for both the PUFA and PUFEE cases to the kg scale as a batch process.

Obviously the fatty acid composition of the triglyceride products reflects the initial PUFA or PUFEE concentrate composition. Thus, we have succeeded in preparing EPA-enriched triglycerides of 40 % EPA and 25 % DHA with well over 70 % total n-3 polyunsaturated fatty acid content as well as DHA-enriched triglycerides of 48 % DHA and 12 % EPA, by using appropriate EPA or DHA enriched concentrates, respectively. There is no doubt that a considerable part of the triglycerides present contains three long-chain n-3 polyunsaturated fatty acid components<sup>13</sup> within the same triglyceride molecule and in fact, our results indicate that there does not seem to be any limit on the EPA/DHA content of triglycerides. Our results also appear to indicate that the 1,3-specific lipase does not affect the composition of the mid-position of the triglycerides very much, although acyl migration is known to occur at the mid-position during an interesterification process<sup>17,19</sup>. The mid-position of the triglycerides in CLO is reported to be enriched with the polyunsaturated fatty acids<sup>27</sup>, DHA in particular. We are currently investigating the possible participation of the mid-position of the original triglyceride substrate during the course of the interesterification process.

More details will be reported soon regarding optimal conditions such as the effect of temperature, time, substrate composition, triglyceride/concentrate ratio, lipase dosage, water content of the Lipozyme and the consequential partial hydrolysis during the process, both for the PUFA<sup>28</sup> and the PUFEE<sup>29</sup> lipase catalyzed CLO interesterifications, as well as the enzyme productivity<sup>30</sup>.

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