

Concentration of omega-3 fatty acids using enzymes

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ABSTRACT: Omega 3 fatty acids derived from fish oils have been shown to be associated with improved health, cardiac function and several other benefits. Fish oils are a major source of these components but the level is insufficient for use in nutritional supplements. Also, the ethyl ester of the fatty acids is not as bio-available as a tri-glyceride making the latter form preferable. This article examines enzymatic routes for the production of omega 3 concentrates and future possibilities for new production processes.

KEYWORDS: Omega 3 fatty acids, fish oil, lipase.

INTRODUCTION

Omega-3 fatty acids are a family of polyunsaturated fatty acids which have in common a carbon-carbon double bond in the ω -3 position. The most important nutritionally essential ω -3 fatty acid is: ALA (α -linolenic acid). EPA (eicosapentaenoic acid), and DHA (docosahexaenoic acid) are technically not essential but the forms associated with most nutritional benefits. EPA & DHA can be synthesised in the body from ALA but the conversion is very slow and varies from individual to individual. SDA (stearidonic acid) is an intermediate in the synthesis of EPA and DHA from ALA and it has been suggested that as it converts more quickly to EPA and DHA, that it could form a useful dietary additive (Whelan, 2009).

ALA is found in vegetable oils, Stearidonic acid in blackcurrant and echium oils and EPA & DHA from fish oils where the content varies by species and season and site of catch. In order to obtain sufficient quantities then these foods or food components *must* be part of the diet. However, the levels found in food components are too low and addition of sufficient fish oils to raise the levels to that desired may leave a "fishy taint". Also there is a shortage of good quality fish oil to serve as a source of EPA & DHA. Fish oils have to be extracted from fish and due to their chemical structure they are inherently unstable and prone to oxidation. Finally due to pollution not all fish stocks are suitable sources of DHA & EPA. For these reasons omega 3 sources for food supplementation are further processed to boost the content of DHA & EPA and remove undesirable contaminants and flavours.

EXTRACTION OF EPA/DHA FROM FISH OIL

The content of EPA/DHA in fish oils is lower than desired and needs to be increased in fish oil products if taints are to be avoided and application amounts kept to a reasonable level. The ratio between EPA and DHA is different according to fish type and growth conditions but on average the total level is approximately 20 percent in the fish lipids. A number of chemical processes for concentrating EPA and DHA including Urea fractionation, Silver nitrate extraction have been proposed but these are not ideal for producing components for food or pharmaceutical grade products (Breivik, 2007). Other strategies combining enzymes and physico-chemical processes were developed to provide the higher concentration products required by the food and other industries. One process starts with a chemical esterification

of the fish oil to produce ethyl esters of the fatty acids and glycerol. This then allows for the partial separation of the desired EPA and DHA esters from the more saturated esters by short path (molecular) distillation. The ethyl ester of DHA has a boiling point of $\sim 450^{\circ}\text{C}$ compared to $>900^{\circ}\text{C}$ for the triglyceride and short path (vacuum) distillation allows for these labile compounds to be separated. This is the preferred production route because although the difference in boiling point between the ester and free fatty acid is not great, if fatty acids were used they would have to be derived from thermal fat splitting which would affect these labile compounds. The process is limited to producing a threefold concentration of polyunsaturated fatty acid esters (i.e. approximately 65 percent by weight). For higher concentrations, molecular distillation in two stages is applied. The end product in both of these cases is a mixture of ethyl esters of the unsaturated fatty acids together with other saturated esters depending on the degree of concentration applied.

CURRENT PRODUCTION OF HIGH LEVEL EPA AND DHA TRI-GLYCERIDES

The tri-glyceride (TG) form of the poly unsaturated fatty acids (PUFAs) is considered to be nutritionally more favourable due to poor absorption of the ester forms (Anon, 2010). In addition, the content of ethanol that is associated with the esters may have religious and/or nutritional consequences. The solution to these issues is to re-convert the EPA and DHA to a lipid and eliminate both the ethanol and the absorption issues. A condensation reaction of EPA & DHA either as fatty acid or ethyl ester with glycerol offers a route to produce highly concentrated tri-glycerides (Figure 1).

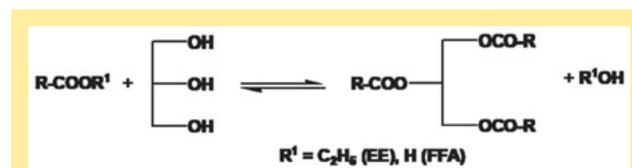


Figure 1. Condensation of fatty acid or ethyl ester with glycerol.

While this can be carried out chemically, an enzymatic condensation offers a number of advantages.

- Lipases are specific in their action and so by-product formation is virtually absent
- Lipases function under mild reaction conditions which improves stability of labile components

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- The condensation reaction mimics the synthesis of tri-glycerides within mammalian systems
- No chemicals are used or need to be discharged to the environment

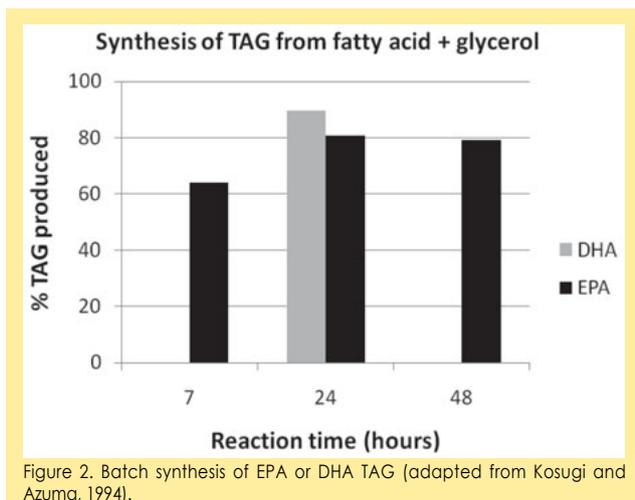


Figure 2. Batch synthesis of EPA or DHA TAG (adapted from Kosugi and Azuma, 1994).

Typically, immobilised esterase, EPA and DHA esters and glycerol will be reacted in a stirred batch reactor until the desired degree of conversion is achieved. Vacuum needs to be applied, as this will remove the generated ethanol which otherwise would inhibit the condensation reaction. Critical factors governing the speed of the condensation reaction will be temperature, enzyme dosage and ethanol removal. Higher temperatures will increase reaction rate but at the same time lead to a faster thermal deactivation of the enzyme. For this reason, processing is normally below 70°C and preferably closer to 60°C. This is however, significantly lower than the chemical condensation and will reduce the deterioration of the PUFA portion. Ethanol (and water) removal is the second critical parameter and reactors should be designed to have a high surface area to volume ratio to enhance evaporation. Ethanol is more volatile than water, so condensation with ethyl esters has been the preferred route. However, ethanol is also toxic to the enzyme catalyst and for this reason the fatty acid may be preferable for long term stability of the enzyme. This stability is required to improve the reaction economics and allow for as many cycles of reaction as possible with a single enzyme batch.

FURTHER APPLICATIONS OF ENZYMES IN THE PROCESSING OF FISH OILS

The first stage of the existing process is the production of ethyl esters by chemical esterification. Although the use of the chemical catalyst is well established, both here and in other food fat esterification reactions, it is being increasingly questioned. Chemical interesterification leads to excessive by-products, resulting in a move away from this towards enzymatic conversion which is essentially free of these compounds. The yields obtained for chemical esterification are also lower than desired due to the non specific nature of the catalyst and the high temperatures required. These higher temperatures are associated in general oil processing with the production of undesired trans isomers. PUFAs are also susceptible to this conversion and processing temperatures of > 180°C will result in their rapid formation (Mjøs and Solvang, 2006) and polymerization of the triglycerides.

An enzymatic esterification process offers the possibility to carry out this reaction at lower temperatures, with less production of by-products and hence overall higher yields. Figure 3 summarises the likely yields with three different lipase formulations. As the formulation giving the highest yield is also the one for the

condensation reaction, a sequential process using the same enzyme should be possible. The excessive toxicity of ethanol to the *C. antarctica* B lipase can be reduced by stepwise addition of the alcohol (Deng et al., 2005, Xu et al., 2007) resulting in an improved enzyme working life.

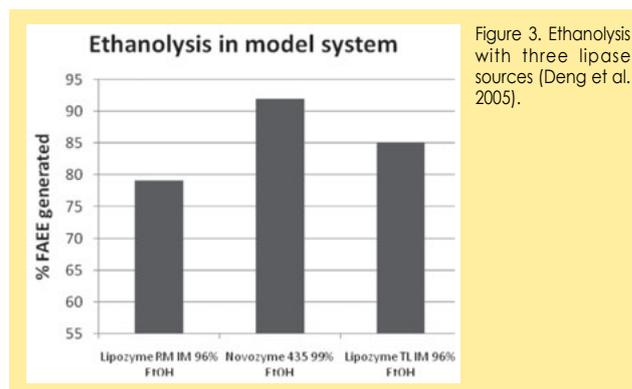


Figure 3. Ethanolysis with three lipase sources (Deng et al. 2005).

To improve overall enzyme reaction rate, the ethyl esters can be converted to free fatty acids prior to the condensation reaction. A hydrolysis reaction with soluble or immobilised *C. antarctica* B lipase will convert the esters to the free fatty acid plus ethanol. The reaction is run at ~60°C and under vacuum to remove the generated ethanol together with some of the added water required for the hydrolysis. A 100 percent hydrolysis yield is not required as the remaining ethyl ester can also be used for the condensation. Applying the predominately fatty acid containing substrate will however, increase enzyme working life and reaction rate, resulting in an anticipated better utilisation of the plant.

Other suggestions as to how enzymes can be applied are based on the selectivity of different lipases for EPA and DHA. For example, as EPA & DHA are found mainly at the Sn2 position, hydrolysis with a 1.3 specific lipase can preferentially remove non omega 3 acids from the tri-glyceride. If the resulting mono-glyceride is then used in the condensation reaction above, utilising another source of EPA or DHA or another fatty acid of interest, then modified lipids can be produced without the need for short path distillation (Cowan, 2009).

Also ethanolysis reactions can use the ability of lipases to differentiate between EPA and DHA to alter the balance of the two PUFA types. In this reaction (Table 1) the immobilised *Rhizomucor miehei* lipase shows a strong preference for the synthesis of the EPA ester, leaving the DHA fatty acid attached to the glycerol backbone. Short path (molecular) distillation could then separate the ester from the higher boiling point partial glyceride.

Time (hours)	Ethyl ester fraction EPA recovery	Residual glycerides DHA recovery
2	1.6%	100%
12	31.5%	97.2%
24	60.2%	90.2%

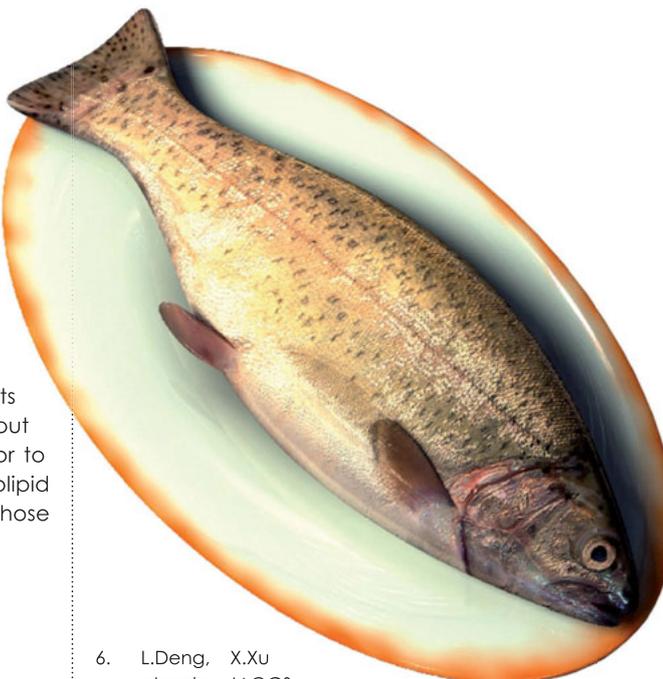
Table 1. Separation of EPA and DHA by ethanolysis of Tuna oil with *Rhizomucor miehei* lipase (Halldórsson et al., 2003).

CONCLUSIONS

Omega 3 containing products are widely used as nutritional supplements and food additives due to their beneficial effects in a number of areas. In order to facilitate their use and to avoid increasing saturated fat consumption, the beneficial PUFAs need to be separated and supplied separately. Increasingly, high Omega 3 tri-glycerides are replacing EPA and DHA ethyl esters as the preferred form for their application due to their improved bioavailability and avoidance of ethanol.

Using a combination of processing methods, extraction and concentration of Omega 3 fatty acids can be readily accomplished using an enzymatic condensation reaction for the critical last stage. However, enzyme technology can be applied in all of the processing steps to increase yields, decrease energy and chemical consumption and improve product purity. This will offer the producer of these products economic advantages and the final consumer, the possibility to reduce chemical processing of food stuffs.

Enzyme technology does not only produce products that are of higher purity and create less by-products but also offers the possibility to alter EPA and DHA ratios or to add alternative lipid functionalities such as phospholipid groups. This will lead to a wider range of products than those currently available with amore adapted function.



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