

## Oils & Fats

# Production of concentrated fish oils with Novozymes Lipozyme® 435

## Application sheet

Lipases can be used for ester and triglyceride synthesis, as well as the more common reactions of hydrolysis and Interesterification. Lipozyme 435 is a nonspecific lipase with activity against a broad range of fatty acids and it is particularly useful for ester production, acidolysis and generation of omega-3-boosted lipid products.

### Benefits

- Broad specificity accepts a wide range of alcohols and fatty acids as substrates for modification
- Specific enzyme reaction results in a very low level of by-products
- Reactions can be carried out at mild temperatures limiting destruction of heat-labile components
- Immobilized product resulting in no enzyme in the final product

### Products

Lipozyme 435 is an immobilized enzyme composed of a *Candida B* lipase absorbed onto a spherical bead-form carrier based on cross-linked methacrylic esters.

Lipozyme 435 has an average particle size of 450–650  $\mu$  and a bulk density of ~0.4. A typical particle size distribution is shown in figure 1.

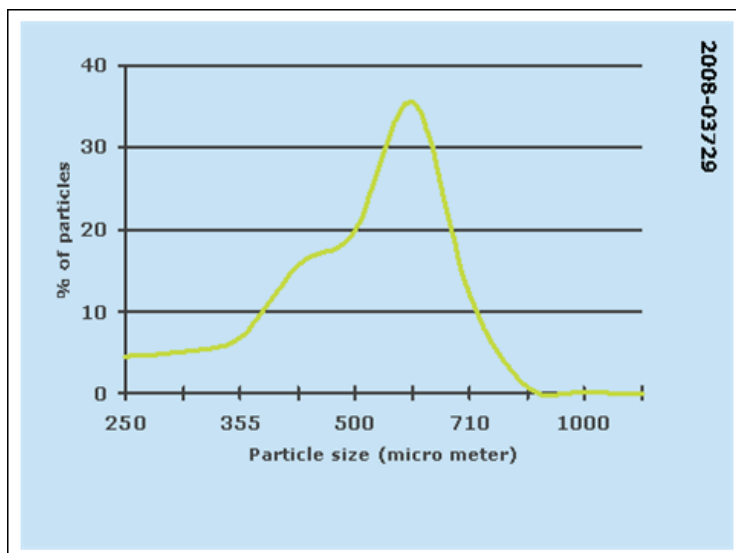


Fig. 1. Particle size distribution of Novozymes Lipozyme® 435.

More information about the abovementioned product is available at the Customer Center.

## Performance

Lipozyme 435 is a very heat-tolerant product with a maximum activity in the range of 70–80 °C (see figure 2). Due to thermal inactivation when running the reaction at elevated temperatures it is recommended to operate in the range of 40–60 °C for optimal productivity.

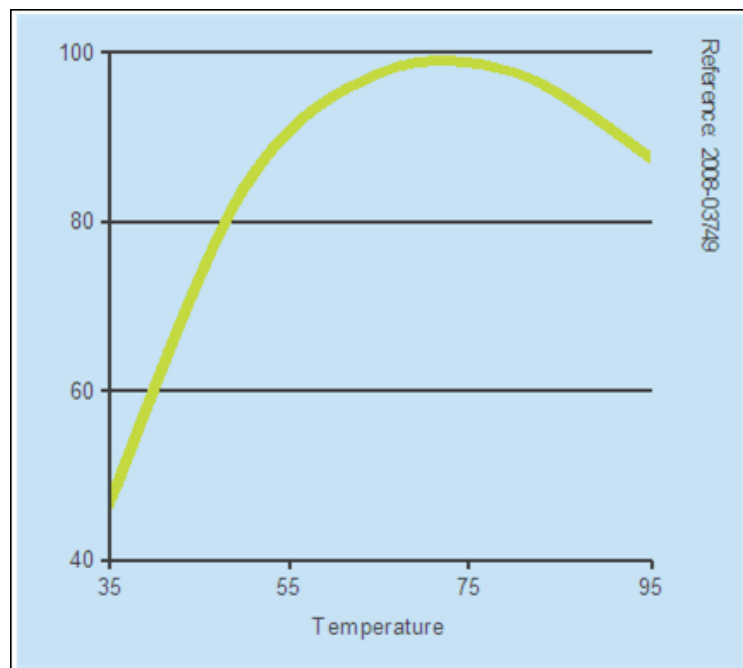


Fig. 2. Temperature and activity of Novozymes Lipozyme<sup>®</sup> 435.

While the material presented above is intended to provide useful information about the features of our enzyme products, in many cases we cannot fully represent the conditions specific to an actual production plant.

Please contact your Novozymes representative for further guidance relevant to your own operational needs.

## Usage

### Production of triglycerides with high levels of EPA and DHA

A triglyceride containing high levels of EPA and DHA can be synthesized by reacting a mixture of glycerol and these long-chain fatty acids in the presence of Lipozyme 435. A mixture of glycerol (14.9 mmol) was combined with an 87% PUFA concentrate (55% EPA and 32% DHA, 45.3 mmol) and was stirred at 65 °C with 10% by weight Lipozyme 435 under a vacuum of 0.1 mm mercury. After 24 hours samples were taken and the FFA level determined by titration. A level of 3% FFA was found equivalent to 97% incorporation of FFA into the glycerol backbone (Ref.: Patent no. US 5,604,119).

Alternatively, using the ethyl ester of the fatty acid enables a reduction of the vacuum required during the reaction.

## Reactor systems for resynthesis of triglycerides

Lipozyme 435 can be applied in a batch reactor in which the reactants are introduced and maintained in a heated, stirred suspension until the reaction is complete. The reactor can be fitted with a slit screen (150 micrometer slit) to allow for easy removal of the triglycerides after the reaction and addition of extra enzyme. As the reaction should run under vacuum to drive the synthesis of the triglycerides, sufficient head space and surface area are needed to allow for the removal of the ethanol or water produced in the reaction.

Alternatively, the conversion can also be conducted in a fixed-bed reactor where an external vacuum flashing is used to remove the water or ethanol. The reactor should also be fitted with a temperature alarm to control the operating temperature.

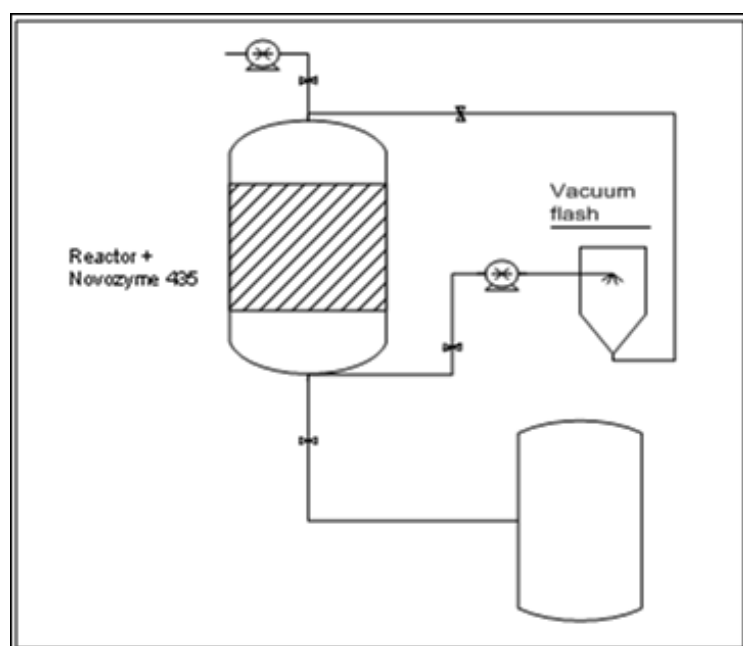


Fig. 3. Semicontinuous conversion of esters.

As an alternative, a reactor configuration using an Iso-Mix<sup>®</sup> nitrogen sparging system coupled to a water absorption column can be used.

## Storage in application

When stored as recommended, the product is best used within six months from date of delivery. Storage at customer's warehouse 0–25 °C (32–77 °F).

In unbroken packaging – dry and protected from the sun. The product has been formulated for optimal stability. Extended storage or adverse conditions such as higher temperature or higher humidity may lead to a higher dosage requirement.

## Safety, handling, and storage

Enzymes are proteins. Inhalation of dust or aerosols may induce sensitization and may cause allergic reactions in sensitized individuals. Some enzymes may irritate the skin, eyes, and mucous membranes upon prolonged contact. Powdered enzymes are readily inhaled and should be handled only with specific precautions to prevent inhalation of dust. All equipment and handling procedures must be designed to control airborne dust. Personal respiratory protection is recommended in all cases where full dust control is not secured.

All spills, however minor, should be removed immediately. Use respiratory protection. Major spills should be carefully shoveled into plastic-lined containers. Minor spills and the remains of major spills should be removed by vacuum cleaning or flushing with water (avoid splashing). Vacuum cleaners and central vacuum systems should be equipped with HEPA filters. Wear suitable protective clothing, gloves and eye/face protection as prescribed on the warning label. Wash contaminated clothes. A Material Safety Data Sheet is supplied with all products. See the Safety Manual for further information regarding how to handle the product safely.

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**Novozymes A/S**

Tel: +45 4446 0000

Fax: +45 4446 9999

Krogshøjvej 36

2880 Bagsvaerd

Denmark

food@novozymes.com

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