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**Topical issue on:**

**LIPIDES DU FUTUR**  
**LIPIDS OF THE FUTURE**

PROCEEDINGS

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# Lipases industrial applications: focus on food and agroindustries

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**Abstract** – Enzymes developed and produced for industrial applications represent a market estimated at a global value comprised between \$5000 million and \$5500 million in 2016. The major applications for industrial enzymes include food and beverages (dairy, bakery, fruit juices, beer, wine), detergents, biofuel productions, animal feed, and other applications such as textiles, leather, and paper processing. Altogether, food and feed applications account for 55–60% of the global enzymes market, and market is still growing at an estimated 6–8% annual growth. The lipases category represents less than 10% of the global enzymes market, with a broad range of industrial applications: detergents, oil processing, food processing and pharmaceutical end-users. Existing applications and new development in the food and agroindustries sectors are reviewed.

**Keywords:** industrial enzymes / enzyme producers / food / agroindustries / lipases / enzymes

**Résumé – Utilisation des lipases dans les agroindustries et les aliments.** Les enzymes développées et produites pour les applications industrielles représentent un marché mondial dont la valeur est comprise entre \$5000 millions et \$5500 millions en 2016. Les principales applications industrielles des enzymes comprennent les applications agroalimentaires (produits laitiers, boulangerie, jus de fruits, bière, vin) et les applications non alimentaires telles que les détergents, la production de biocarburants, la nutrition animale ou encore les textiles, le cuir et l'industrie papetière. Globalement, les applications en nutrition humaine et animale représentent entre 55 % et 60 % du marché mondial des enzymes, avec une croissance annuelle estimée entre 6 % et 8 %. Les lipases représentent moins de 10 % du marché mondial des enzymes industrielles et sont utilisées dans de nombreux segments industriels tels que les détergents, la production des huiles et corps gras, les produits alimentaires et le secteur pharmaceutique. Cet article propose une revue d'applications actuelles et de développements récents dans les secteurs alimentaires et agroindustriels.

**Mots clés :** industrielles / producteurs d'enzymes / industries agroalimentaires / agroindustries / lipases

## 1 Introduction to industrial enzymes

### 1.1 Industrial enzymes applications

The following are the main application areas for industrial enzymes:

- cleaning agents, including laundry and dishwasher detergents, household care and personal care products;
- food and beverages, including dairy, baking, fruit and vegetable processing, brewing;
- animal feed;
- biofuel, including both first and second generation biofuels;
- others, including fine chemistry, pharmaceutical industry, textile and leather processing, waste treatment, and paper processing to name a few.

The global market for industrial enzymes is estimated at a global value comprised between \$5000 million and

\$5500 million in 2016. Food and beverages is the largest application for industrial enzymes (Fig. 1). The global demand for processed food combined with a tremendous increase in the per capita income of the population in emerging economies are driving the demand for food and beverages, leading the food industry to optimize their production. In many food processing applications, the use of enzymes significantly improves yield and quality.

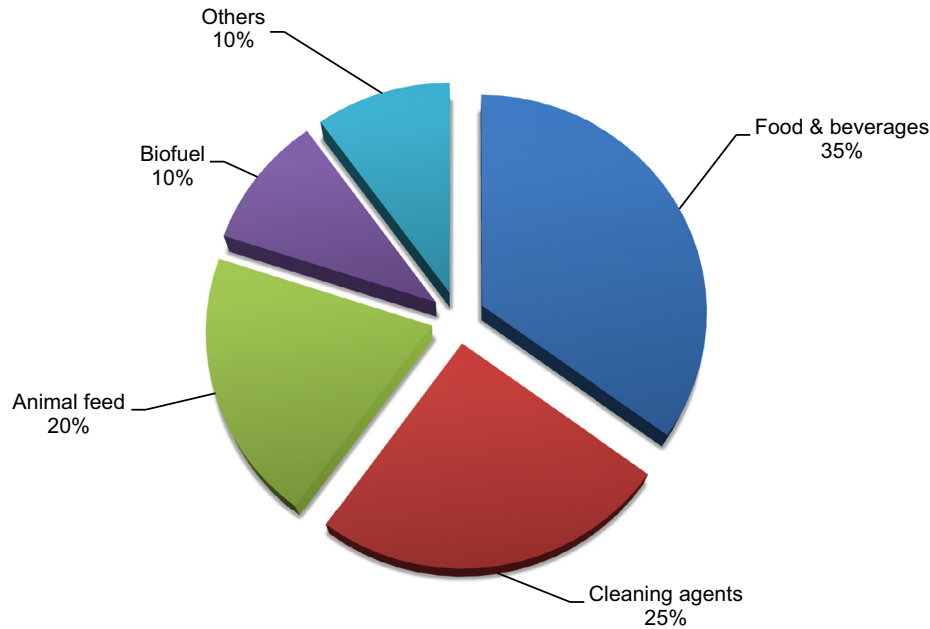
Altogether, food and feed applications account for 55–60% of the global enzymes market, and market is growing at an estimated 6–8% annual growth in the period 2016–2020.

### 1.2 Enzyme functionalities

The global industrial enzymes market is driven by three leading categories: carbohydrases, proteases and lipases:

- the carbohydrases segment accounts for almost 50% of the industrial enzymes market, up to 70% for the food and beverages application;

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**Fig. 1.** Global enzymes market share (m.s.) by application (2015).

- proteases represent between 25% and 30% of the global market;
- lipases account for less than 10% market share.

Other categories include oxydases (*e.g.* glucose oxydase), phytase, transglutaminase and other enzymes, which are most of the time associated with specific applications and markets combinations.

### 1.3 Industrial enzymes production and key players

Industrial enzymes can be obtained from microbial, plant and animal sources. Most of the enzymes currently developed for industrial applications are obtained from microorganisms (bacteria, yeast or fungi). Microbial enzymes account for 90% of the global market and their producers are located mainly in Europe and Asia.

The 3 dominant global enzyme producers (Novozymes, Dupont, DSM) account for more than 75% of the global enzymes business. Novozymes accounts for 45% to 50% (Fig. 2) of the market and is the sole company active in all enzyme markets and being almost fully dedicated to the enzymes business. Other active players based in Europe are AB Enzymes, BASF, Chr. Hansen, Kerry and Soufflet Biotechnologies.

Japan also has long time established enzyme suppliers such as Ajinomoto, Amano, Nagase and Shin Nihon.

Finally, there are numerous enzymes producers also in China and India serving both local and global markets.

## 2 Lipases for industrial applications

Lipases (triacylglycerol acyl hydrolases, E.C. 3.1.1.3) are a class of enzymes, which catalyze the hydrolysis of long chain triglycerides. In humans and monogastric animal species,

lipases enzyme naturally occur in the stomach and pancreas where they function to digest fats and lipids. For industrial applications, animal derived lipases are still used in some specific medical sectors (*e.g.* pig pancreas lipase to supplement lipase-deficient patients).

Microbial lipases are produced by fungal, yeast, and bacterial species. The following micro-organisms account for most of the industrial microbial lipases production: *Candida* sp., *Aspergillus* sp., *Rhizomucor* sp., *Rhizopus* sp., *Humicola* sp., *Yarrowia lipolytica* and *Pseudomonas* sp. They are developed, produced and marketed by the key enzyme players for different applications (Tab. 1).

Industrial lipases application scope covers various industries and applications such as oleo-chemicals, detergents, polymers, food processing, pharmaceutical, waste, cosmetics and biodiesel (Tab. 2).

## 3 Lipases for the food and agro-industrial applications

Lipases have many applications and benefits in the food and agroindustries, where they have quantitative and/or qualitative impacts. For instance, in the vegetal oil processing, lipases allow a significant increase in oil yield and at the same time the end product is of better appearance. In baking and dairy applications, lipases are added to enhance and accelerate the development of aromatic notes. In the food industry, lipases find a great interest in the egg processing sector to enhance the emulsification properties of egg yolk lipids.

Lipases are also being developed to create new functional ingredients and functional foods, such as cocoa butter equivalents or human milk fat equivalents.

The following lipase applications are reviewed: dairy, baking, cocoa butter substitutes, human milk fat substitutes, egg processing and edible oil production.

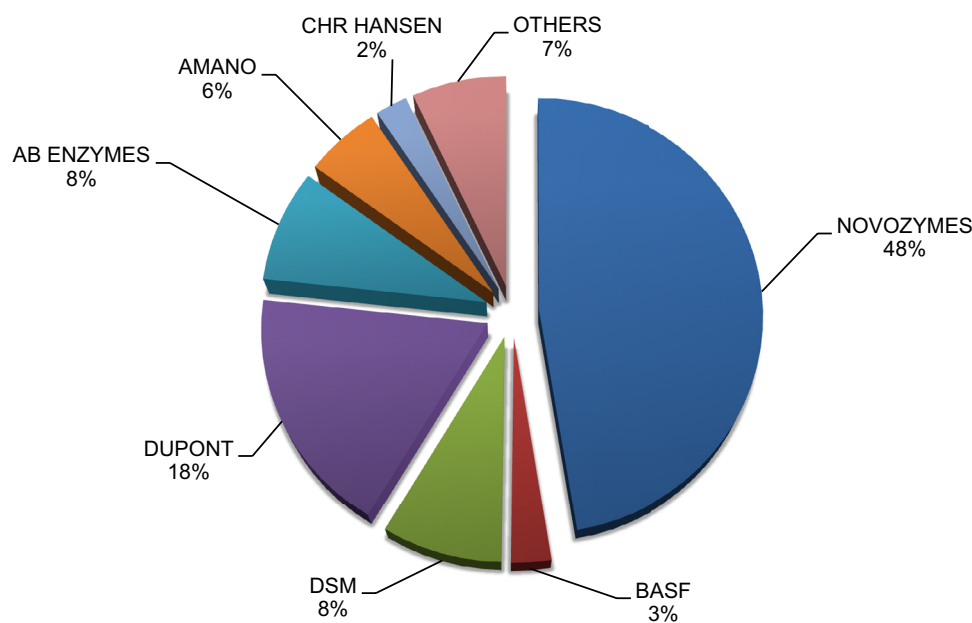


Fig. 2. Industrial enzymes market share, estimation by company (2015).

Table 1. Some examples of commercial lipases from the leading global enzyme suppliers (adapted from suppliers technical literature).

Enzyme producer	Product examples	Application
NOVOZYMES	Lipase 435 Lipolase	Multipurpose lipase Detergents
DSM	Gumzyme Maxapal A2	Oil degumming Egg processing
AB ENZYMES	Rohalase PL, Rohalase F, Veron Hyperbake	Oil degumming Baking
AMANO	Lipase DF	Baking

### 3.1 Dairy

Lipases are used to break down milk fats and give characteristic flavors to cheeses. The flavor comes from the free fatty acids produced when milk fats are hydrolyzed (Jooyandeh *et al.*, 2009). Both animal and microbial enzymes are used in the industry. Animal lipases are obtained from kid, calf and lamb, while microbial lipase is derived by fermentation, mainly with the fungal species *Rhizomucor meihei*. Animal and microbial lipases have different action patterns and the industry uses both according to the expected flavor profile (hydrolysis of the shorter fats is preferred because it results in the desirable taste of many cheeses while the hydrolysis of the longer chain fatty acids can result in “soapy” notes).

### 3.2 Baking

In baking industry, (phospho)lipases can be used to substitute or supplement traditional emulsifiers through the degradation of wheat lipids to produce emulsifying lipids *in situ*. Lipase in baking also enhance the flavor of bakery

products by liberating short-chain fatty acids through esterification. In synergy with other commonly used baking enzymes (amylases, xylanases...), lipases contribute to increase the loaf volume and improve crumb firmness, allow to prolong the shelf-life of baked products and improve their texture and softness (Robert, 2015).

#### 3.2.1 Cocoa butter substitutes

Cocoa butter is a fat mainly formed by triacylglycerides (TAGs) with two saturated fatty acids (palmitic and stearic acids) and one mono-unsaturated fatty acid (oleic acid). Thanks to its unique structure, cocoa butter shows unique properties in the food processing industry (mouthfeel, melting behaviour...). However, cocoa butter supply is uncertain and its price highly fluctuant. Enzymatic processes have been developed to catalyse interesterification reaction of different edible oils (such as sunflower oils) to produce fats having a composition and properties comparable to those of cocoa butter (Bhaggan, 2016). Such products are called cocoa butter equivalents or cocoa butter substitutes (CBS).

### 3.3 Human milk fat substitutes

Human milk fat (HMF) contains different lipids: oleic (30–35%), palmitic (20–30%), linoleic (7–14%) and stearic acids (5.7–8%). Unlike in vegetable oils and in cow’s milk fat, in HMF, palmitic acid, the major saturated fatty acid, is mostly esterified at the *sn*-2 position of the TAGs, while unsaturated fatty acids are at the external positions. The fatty acid profile of HMF has a crucial effect on its digestibility and intestinal absorption in infants. Human milk fat substitutes (HMFS) have been obtained by *sn*-1,3 lipase-catalyzed acidolysis of tripalmitin, butterfat, palm oil, palm stearin or lard (rich in palmitic acid in *sn*-2 position) with free fatty acids (FFA) from different sources.

**Table 2.** Overview of lipases industrial applications (adapted from Choudhury, 2015).

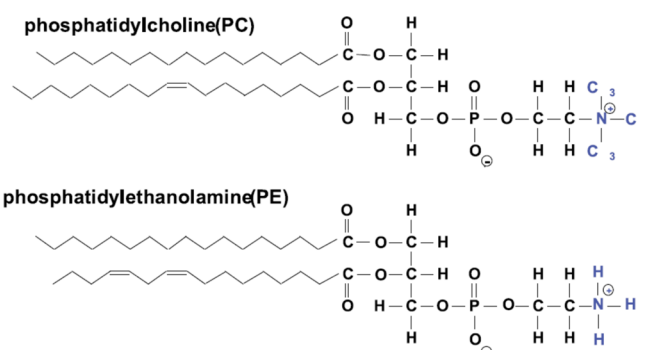
Industry sector	Applications and benefits
Fats and oleo-chemistry	The current trend in the oleo-chemical industry is to involve the use of immobilized lipase to catalyze the hydrolysis, esterification and inter-esterification of oils and fats as an alternative to physico-chemical processes (energy saving, specificity of reactions)
Detergents	Next to proteases as detergent additives, lipases are the second most important group of detergent enzymes to contribute to oil and fats stain and traces removal. Lipases are used in both laundry and dishwashing formulations commercial detergents where they have been optimized to operate under various pH and temperature conditions. Lipex <sup>®</sup> and Lipolase <sup>®</sup> from Novozymes are two examples of lipases sold to the detergent industry
Polymers	Lipases can be used to assist the production of polymers, such as polyester, PLA (polylactide), PCL (polycaprolactone) and many others, in alternative to chemically catalysed reactions (Pollet, 2015) Immobilised lipases are also used in lignin transesterification reactions to produce lignine oleate, bringing new properties to the polymer (Husson, 2015)
Food processing	Lipases are utilized in dairy products for flavor development but also in the processing of other foods such as meat products, baked foods, cocoa butter processing and others
Medical and pharmaceutical sectors	Due to their high level of specificity, lipases can be used to produce active pharmaceutical compounds. For example, enantioselective enzymes are used as an alternative technology to chiral chromatography (Gerard, 2015) Lipases are also applied in the production of lysophospholipids from phospholipids and to extract and produce functional lipids (Lennon, 2015)
Pulp and paper	The presence of hydrophobic components (mainly triglycerides and waxes) in wood are detrimental to many of the processes in the production of paper and pulp, and lipases can be used to remove those undesirable triglycerides
Waste / effluent / sewage treatment	Lipases are added to eliminate the thin layers of fats formed at the surface of waste water reservoirs and therefore recover active oxygen transport conditions which are necessary in maintaining optimum biomass growth
Cosmetics and perfumery	Lipases are used in the production of surfactants (e.g. mono-acylglycerols and diacylglycerols) via the controlled esterification of glycerols In the cosmetic and fragrances industries lipases can be used in the synthesis of citronellyl butyrate and valerate
Biodiesel	Lipases are used to produce biodiesel from various feedstocks such as palm oil or animal fats. Thermostable lipases have been developed to optimize the application of enzymes in biodiesel production (Nielsen, 2016)

The commercial Betapol<sup>®</sup> product is manufactured by biocatalytic processes by IOI Loders Croklaan (Bhaggan, 2016), through the acidolysis between lard and soybean fatty acids, catalyzed by the *sn*-1,3 selective lipase from *Rhizomucor miehei* (Lipozyme<sup>®</sup> RM).

### 3.4 Egg processing

Eggs provide functional ingredients to the food industry with a variety of properties including foaming, gelation, emulsifying in batters and mayonnaise and improved texture of baked goods. Egg lipids are responsible for the emulsifying properties. Lipases can greatly improve egg lipids emulsifying power for better performance and lower egg yolk addition rate in processed food recipes, such as dressings and mayonnaise-like products (Biocatalysts UK technical literature).

Egg yolk emulsification for dressings: the global production of emulsified dressings is estimated at 3 millions of metric tons per year, consuming roughly 150 000 metric tons of egg yolk in their process. One third of the market for emulsified dressings is concentrated in Russia and Eastern Europe countries. The market is highly industrialized, with global



**Fig. 3.** Chemical structure of most important phospholipids in egg yolk.

players such as Nestlé, Kraft and Unilever. Egg yolk is a complex oil-water emulsion composed of 50% water, 32% lipids and 16% protein. Approximately, 1/3 of the lipids are phospholipids, of which approximately 80% is phosphatidylcholine (PC). Egg yolk also contains phosphatidylethanolamine (Fig. 3).

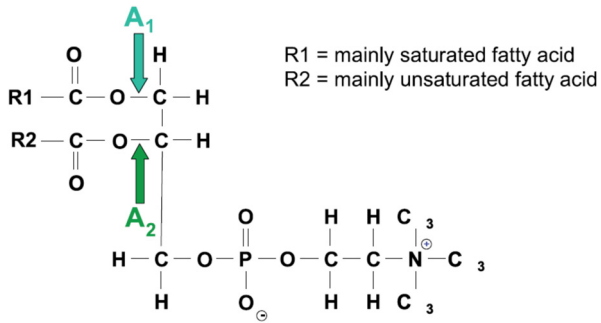


Fig. 4. Action of phospholipases in A1 and A2 positions.

The enzymatic conversion of egg yolk phospholipids into lyso-phospholipids will increase the emulsion stability. Enzyme producers have developed different phospholipases (PL) acting at different positions (Fig. 4).

Egg processing lipases can be extracted from pork pancreas (e.g. Lipomod 699, Biocatalysts, UK) or from micro-organisms (e.g. Maxapal A2, DSM, NL).

Using pork pancreas lipases, the conversion rate from phospholipids to lysophospholipids is typically over 80% after 1 hour reaction. However the application of such enzyme is limited by its animal origin (vegan consumers, Halal/Kosher restrictions). Lipases cloned and expressed in the fungus *Aspergillus niger* (such as DSM Maxapal A2) also reach high

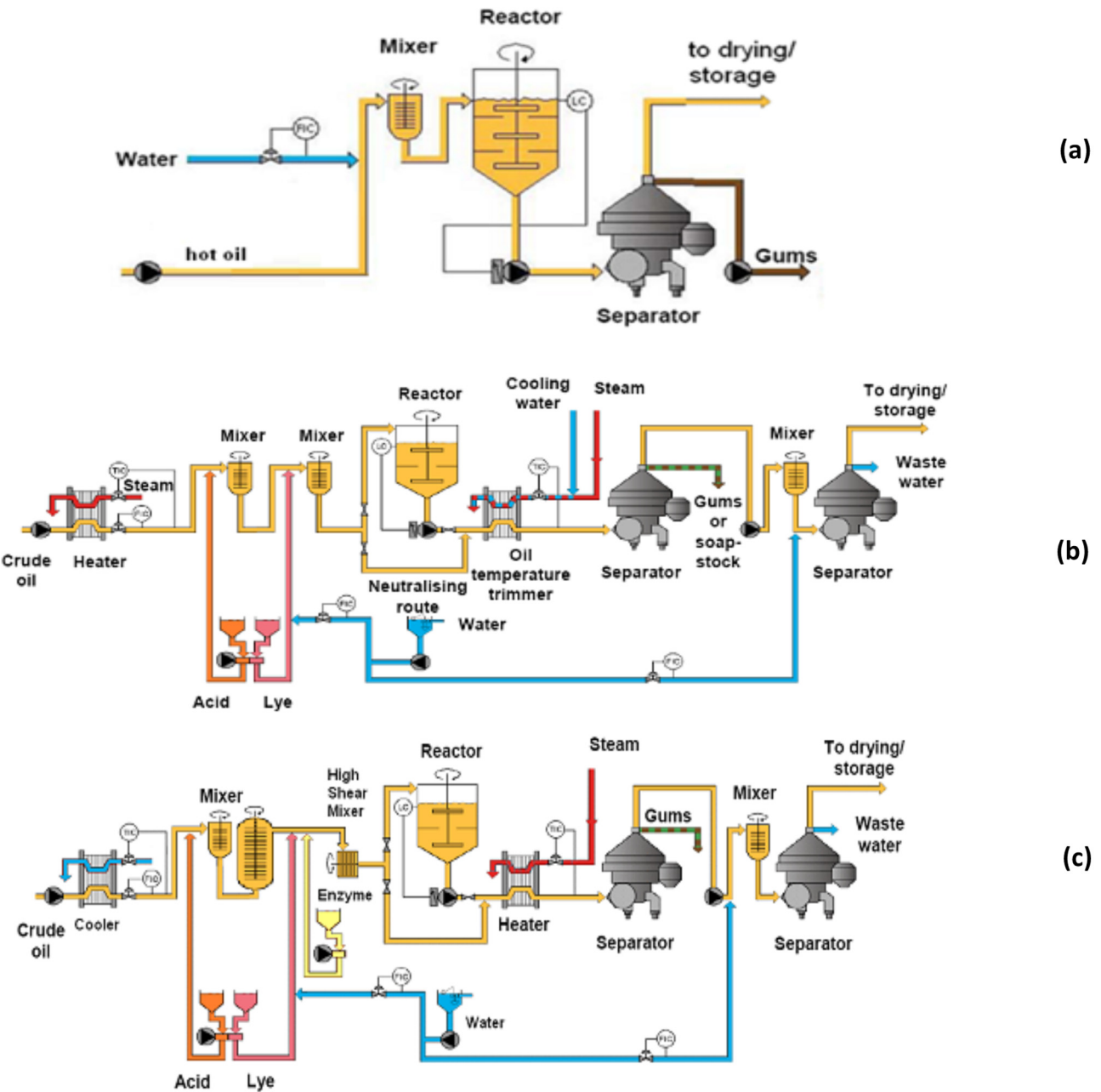


Fig. 5. Oil degumming flowcharts comparing three technologies (a) high temperature water extraction (b) process with acidification and (c) enzyme assisted process (from Alfa-Laval technical documentation).

Topical Issue

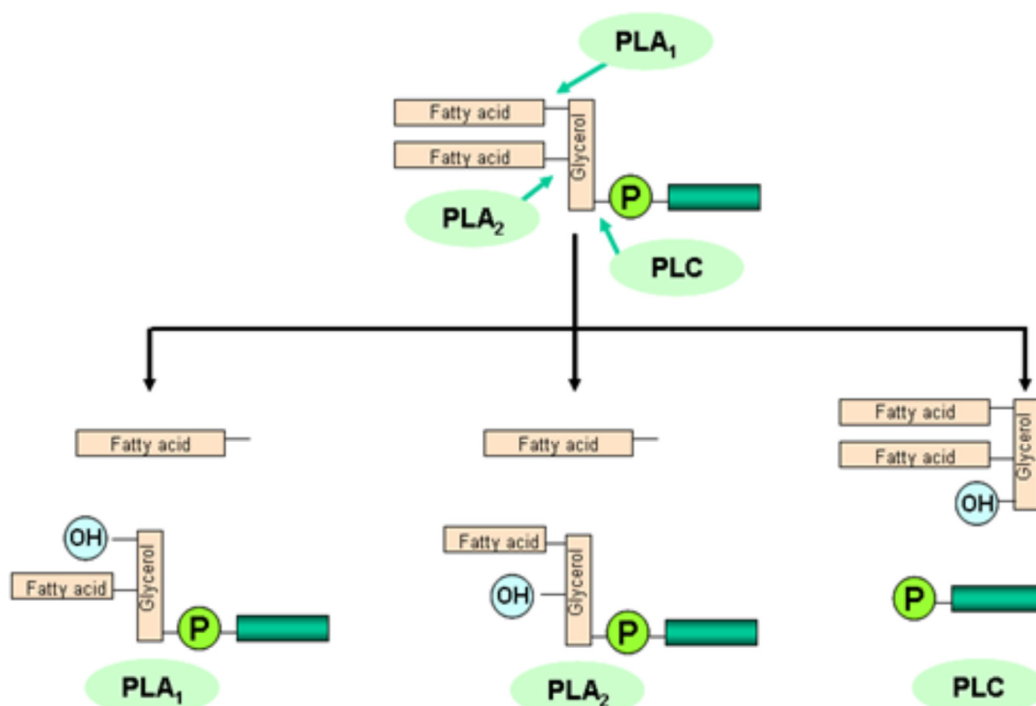


Fig. 6. Mode of action of different phospholipases (PL) used for oil degumming. Adapted from Galhardo (2012).

conversion yield but some industry players are not in favour of using enzymes produced by such modified micro-organisms (MGM).

### 3.5 Edible oil production

Edible oils from plants are used in different industries: food, feed and fuel. Food consumption of vegetal oils is close to 150 million of metric tons per year, of which 1/3 being palm oil. Production of edible vegetal oils is dominated by three leading global players: ADM, Bunge and Cargill.

When refining vegetal oils, it is necessary to remove impurities that affect the yield and moreover the taste, smell, visual appearance, and storage stability of the oil. The most important class of impurities are phospholipids, often referred as “gums”. For decades, the industry has been using thermochemical processes to remove those phospholipids (PL) from crude oil, and more recently different enzymes solutions (based on phospholipases) have been proposed by the enzyme companies. The hydrolysis of the PL by phospholipases at the water-oil interface will inhibit the emulsion and release the oil trapped in the gum phase. Flowcharts of non-enzyme assisted processes vs. enzyme assisted process are presented in Figure 5. Enzymatic degumming can be applied to oils from rapeseed/canola, soyabean, ricebran, corn, sunflower seeds, and palm with the following benefits:

- higher oil yields (up to 2% increase);
- energy savings (operates at 50–60 °C vs. 85 °C for conventional process);
- very low oil losses;
- easier pumping and separation operations;
- limited formation of soaps;
- lower water consumption.

Three main phospholipases (PL) used by the industry are categorized according to their fatty acid-glycerol cleavage pattern and are referred as PLA1, PLA2 and PLC (Fig. 6). For optimum performance, enzymatic oil degumming can also involve the use of PL combinations, either in blends or in sequential additions.

It is important to note that besides their different cleavage patterns, each PL developed by the enzyme industry has unique properties in terms of pH and temperature profiles according to their origin and mode of production.

PL used in the oil industry are from microbial origin (*Trichoderma*, *Aspergillus*, *Pichia*...) or still from porcine pancreas. Leading suppliers for the PL range of enzymes are Novozymes, DSM, Dupont, AB Enzymes (Tab. 3).

As the oil industry is implementing the enzyme assisted degumming, PL product ranges from the leading suppliers are in constant evolution. Product innovation is driven by the need for enzymes with improved performance, higher temperature resistance and ability to operate at lower pH.

For instance, Novozymes introduced in 2000 the Lecitase® Ultra 10L (pancreatic PLA2) and the Lecitase® (microbial PLA1), followed in 2003 by Lecitase® Ultra (thermostable microbial PLA1) and recently announced the launch of a new acid tolerant and thermostable PLA1 from *Talaromyces leycettanus* (Longin, 2016).

AB Enzymes also recently introduced an acid tolerant PLA2 named Rohalase® PL-Xtra (Schroegel-Truxius, 2016) while DSM is now proposing Purifine® 3G, a combination of distinct PL (Muntendam, 2016).

## 4 Conclusion

This review showed the potential of lipases as biocatalysts for a broad range of industries with a specific focus on food

**Table 3.** Overview of different PL from the leading enzyme suppliers (adapted from Galhardo, 2012).

Commercially available phospholipases for enzyme degumming			
Supplier	Brand	Activity	Organism of origin
AB enzymes	Rohalase <sup>®</sup> MPL Rohalase <sup>®</sup> PL Extra	PLA2	<i>Trichoderma reesei</i>
Danisco	Lysomax <sup>®</sup>	PLA2	<i>Streptomyces violaceoruber</i>
DSM	Gumzyme <sup>®</sup>	PLA2	<i>Aspergillus niger</i>
Novozymes	Lecitase <sup>®</sup> 10L	PLA2	Porcine pancreas
Novozymes	Lecitase <sup>®</sup> Novo	PLA1	<i>Fusarium oxysporum</i>
Novozymes	Lecitase <sup>®</sup> Ultra	PLA1	<i>Thermomyces lanuginosa/Fusarium oxysporum</i>
DSM (ex. VERENIUM range)	Purifine <sup>™</sup> PLC Purifin <sup>®</sup> 3G	PLC nc	<i>Pichia pastoris</i> nc

applications. While lipases market is still a small portion of the global food enzymes market, it is expected to grow with new products and application developments. The example of enzymatic oil degumming shows that almost 20 years after the technology has been developed, new enzymes are still being developed for better performance and benefits for the end user. The development of high value products such as human milk fat substitutes as well as other functional lipids in the food and nutraceutical segments will also drive the lipases market in the coming years.

However, the speed and importance of industrial implementation of enzymatic processes are still limited by three main factors:

- time and cost to develop new enzymes;
- new technology acceptance (in other words, time to move from a current technology to the enzyme assisted technology);
- acceptance of enzymes produced by the mean of genetically modified micro-organisms.

Recent progresses in new enzymes development and enzymes optimisation (strain engineering, protein engineering...) will most likely fasten and lower the cost of new enzymes development. Moreover, enzyme application in the food sector is usually seen as a sustainable and safe technology.

For these reasons, industrial enzymes in general and lipases in particular will be continuously implemented in a broad range of applications as a an alternative to chemical based processes,

delivering superior performance and yield, improved product functionalities with a lower impact on energy resources.

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