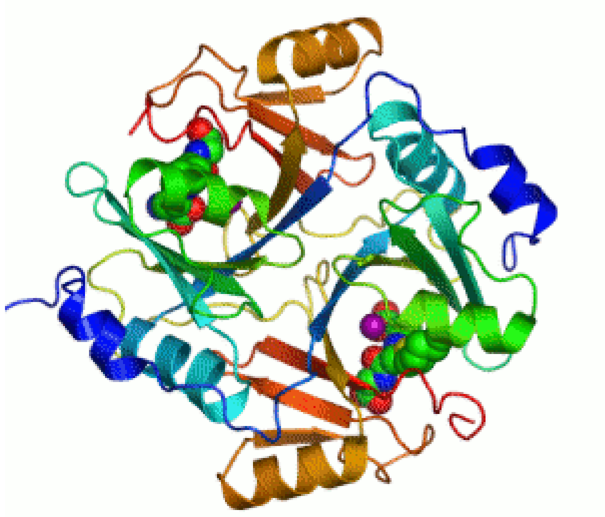
Enzyme Applications in Oil Refining



FE 461

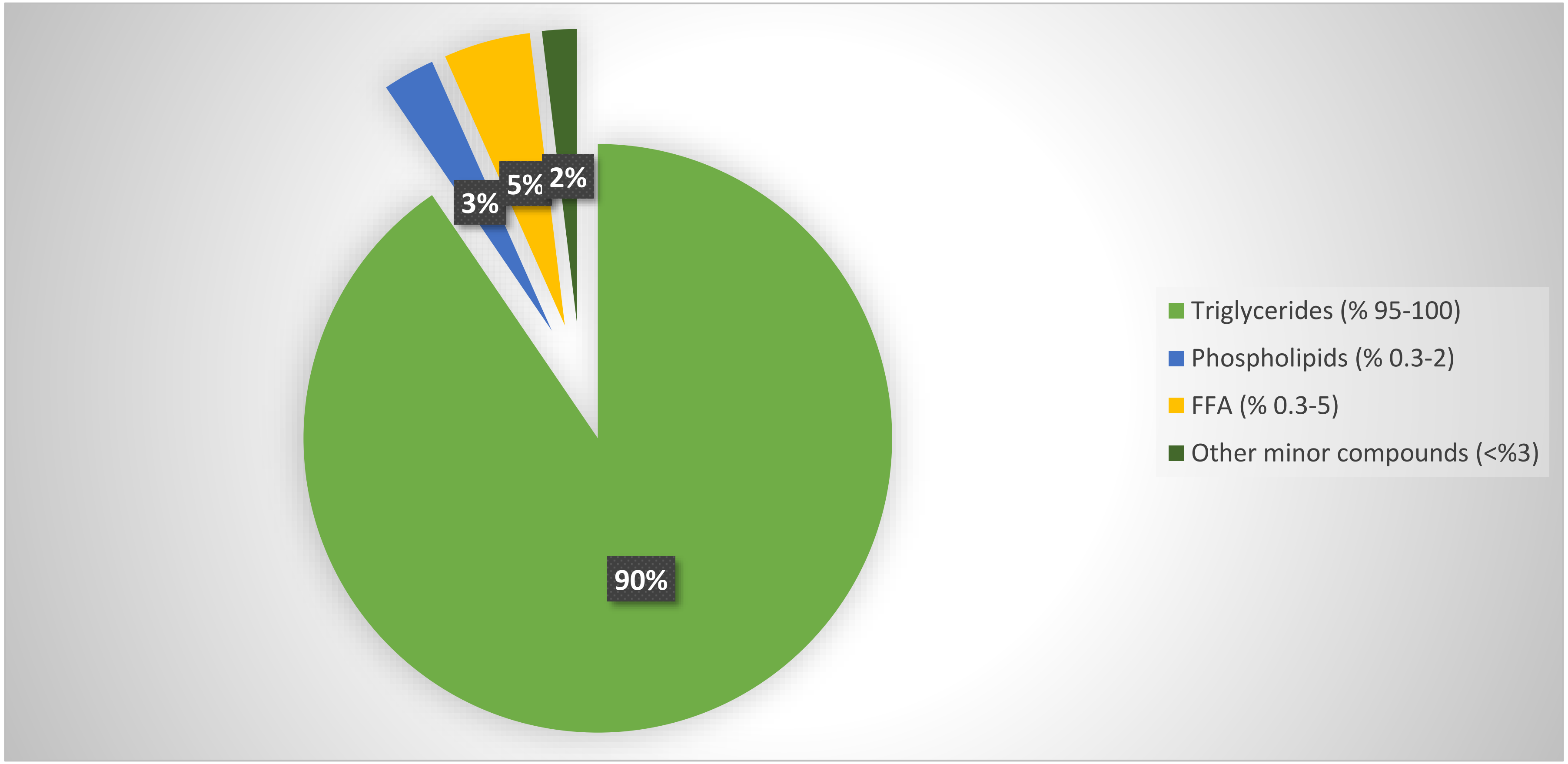
Enzymes in Fats and Oil Industry

Dr. Hasene KESKİN ÇAVDAR

Enzymes in Oil- and Lipid Based Industries

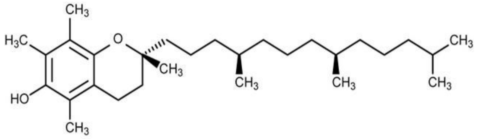
- Enzyme Application in Oil Extraction
- Enzyme Application in **Oil Refining**
- Enzyme Application in Lipid Modification

Fats and Oils

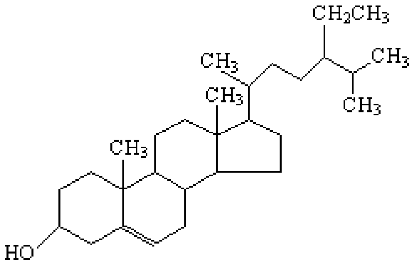


Fats and Oils (Continued)

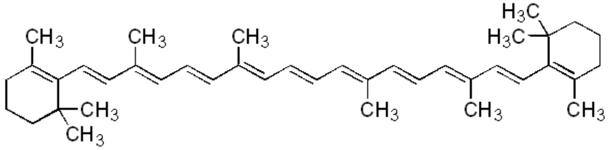
Minor Components in Vegetable Oils



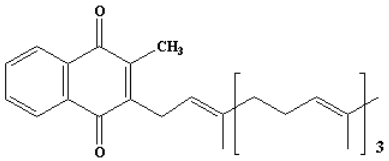
Tocopherols



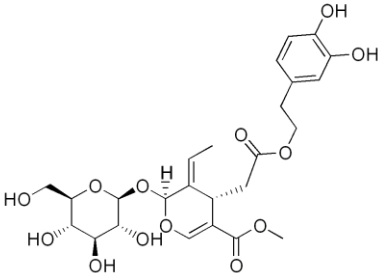
Sterols



Beta-carotene



Vitamins

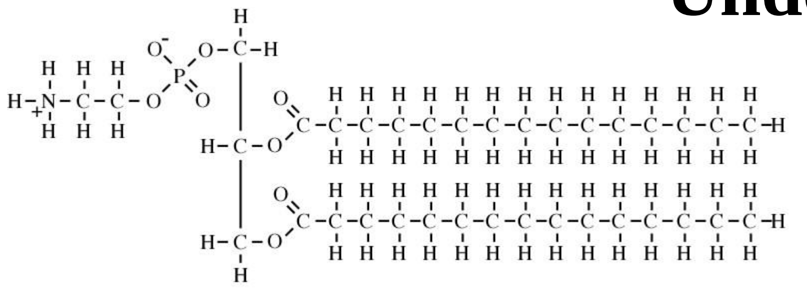


Phenolic compounds

- High functional values for human health
- Desirable in refined oil
- Try to be preserved during oil refining

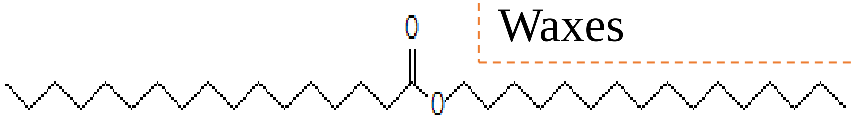
Fats and Oils (Continued)

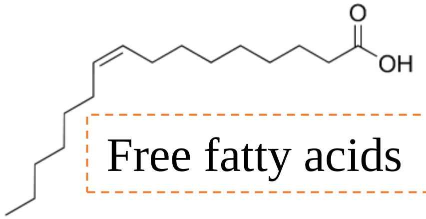
Undesirable Components in Vegetable Oils



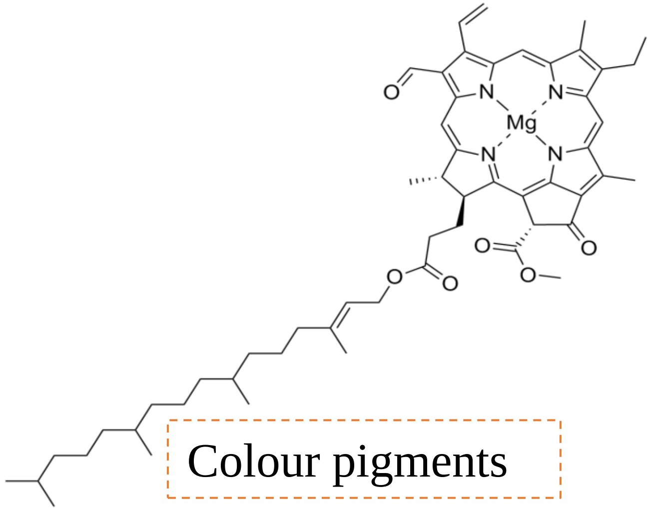
Phospholipids

Waxes

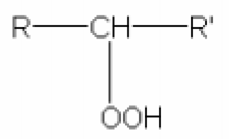


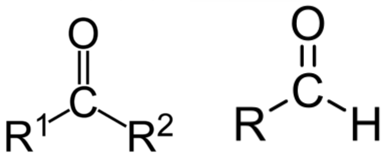


Free fatty acids



Colour pigments





Off-flavors

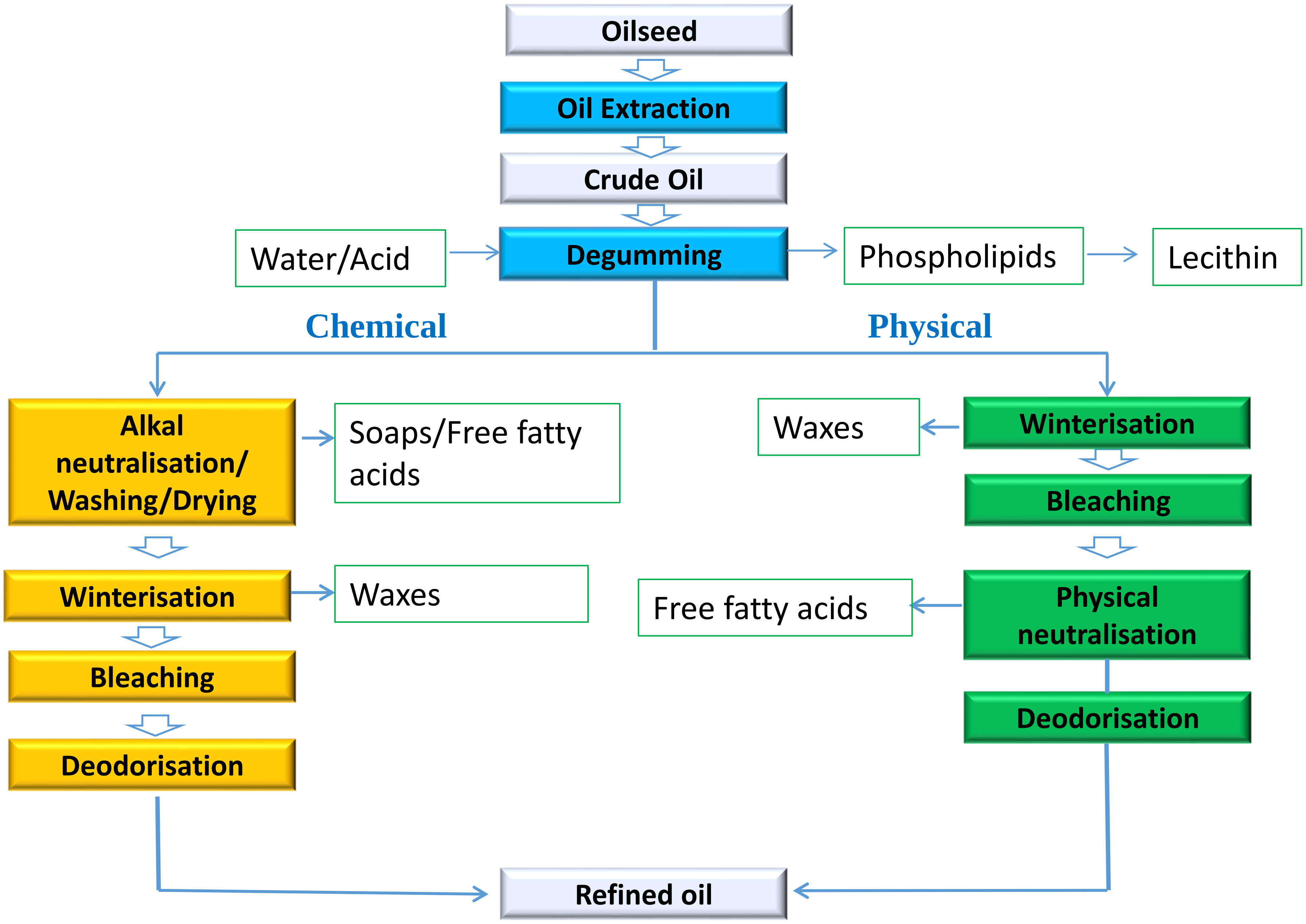
- Undesirable components in refined oil
- Decreases oil quality
- Try to be removed during oil refining

Fats and Oils (Continued)

Oil without refining is termed “crude” oil because it contains a number of impurities including phospholipids, FFAs, pigments, sterols, carbohydrates, proteins, and their degradation products.

Vegetable oils need to be refined, and this is partly to eliminate the phospholipids.

Refining: Chemical and Physical



Degumming

Degumming process is necessary for vegetable oil production. Phospholipids must be removed. Because, phospholipids that generally exist in crude oil :

- form insoluble and gummy precipitate.
- can cause oil darkening (or browning) during subsequent bleaching, hydrogenating, and deodorizing steps of refining.
- serve as a precursor of off-flavors.
- have a negative effect on the taste and shelf life of the oil.
- reduce oxidative stability of the oils

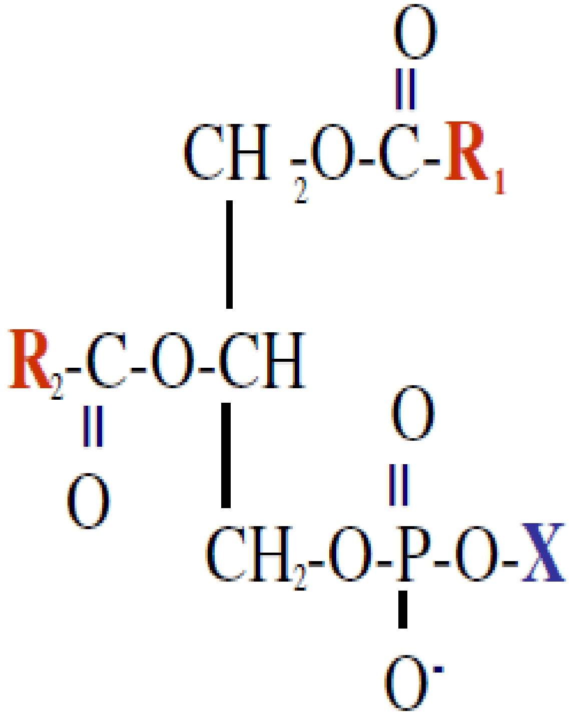
Gum Content of Various Oils

Oil Type	Phosphatides (%)	Phosphorus (ppm)
Soya	1-3	400-1200
Corn	0.7-2.0	250-800
Cottonseed	1.0-2.5	400-1000
Sunflower	0.5-1.3	200-500
Palm	0.03-0.1	15-30

What is a Phospholipid?

- Phospholipids are a kind of lipid containing triglyceride with two fatty acid radicals and one side chain formed by a phosphate ester.
- There are two main types of phospholipids:
  - hydratable phospholipids: easy to remove
  - nonhydratable phospholipids (NHP): hard to remove oil
- Some NHP removed with hydratables in water degumming requires the use of a acid to convert to hydratable for complete removal

What is a Phospholipid? (Continued)



R_1, R_2 : Fatty acids

X : H (**PA**), choline (**PC**), ethanolamine (**PE**), serine (**PS**), inositol (**PI**)

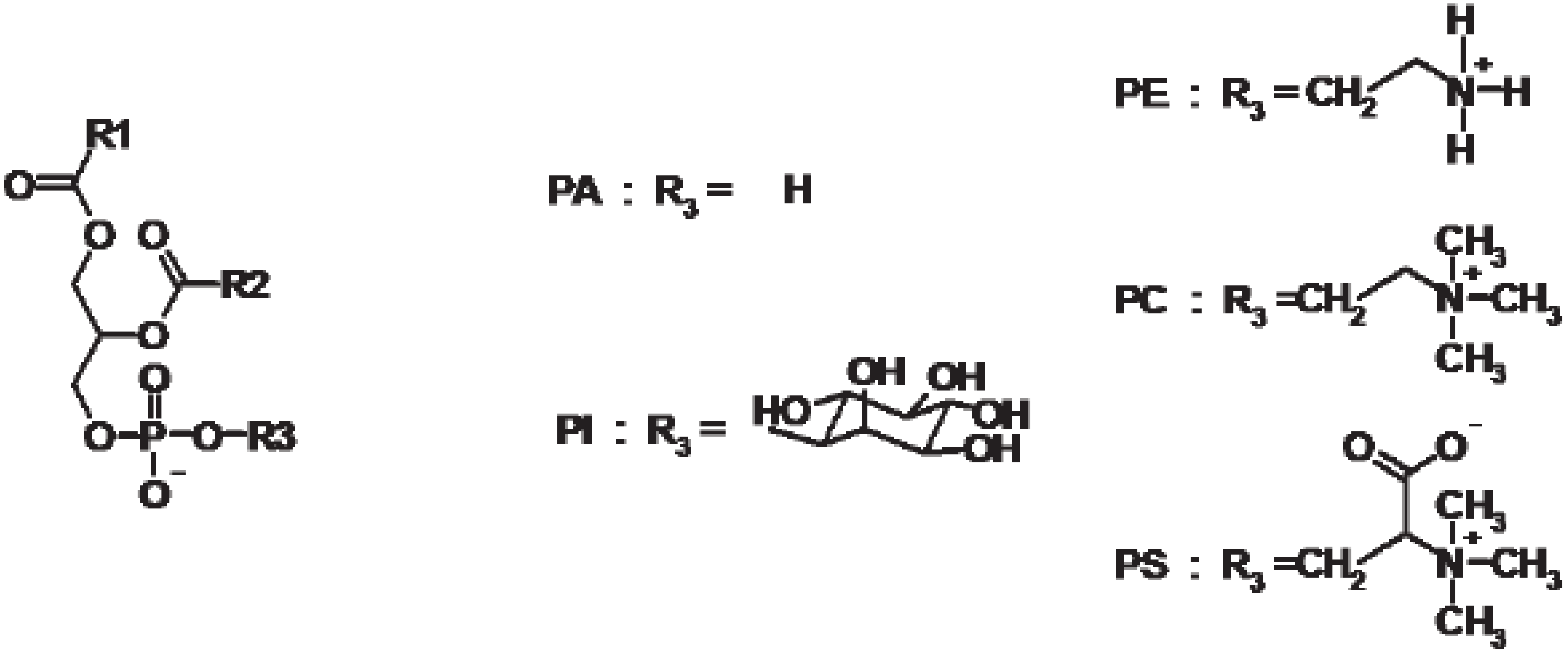
What is a Phospholipid? (Continued)

- Phospholipids are part of plant-cell walls including those containing the lipids.
- Phospholipid quantities and type are determined by oilseed growth, storage and handling conditions.
- The amount of phospholipids that are extracted together with the oil depends on the extraction temperature. Both the oil-extraction rate and the content of phospholipids in the oil are increased by increasing the extraction temperature.
- Thus, processing systems which maximize oil yield also increase the phospholipid level in the resulting oil.

Phospholipids in Vegetable Oil

- Several different types of phospholipids are in vegetable oils
- Phospholipids are molecules with an amphiphilic character.
- They have a high preference for distribution at the oil–water interface, and act as emulsifiers.
- An absence of water renders them able to dissolve in the oil. However, the presence of water makes them insoluble in oil.

Phospholipids in Vegetable Oils (Continued)



Structure of the most common phospholipids: R_1, R_2: Fatty acid residues,
PA = phosphatidic acid, PI = phosphatidylinositol, PE = phosphatidylethanolamine, PC = phosphatidylcholine, and PS = phosphatidylserine.

Removal of Phospholipids

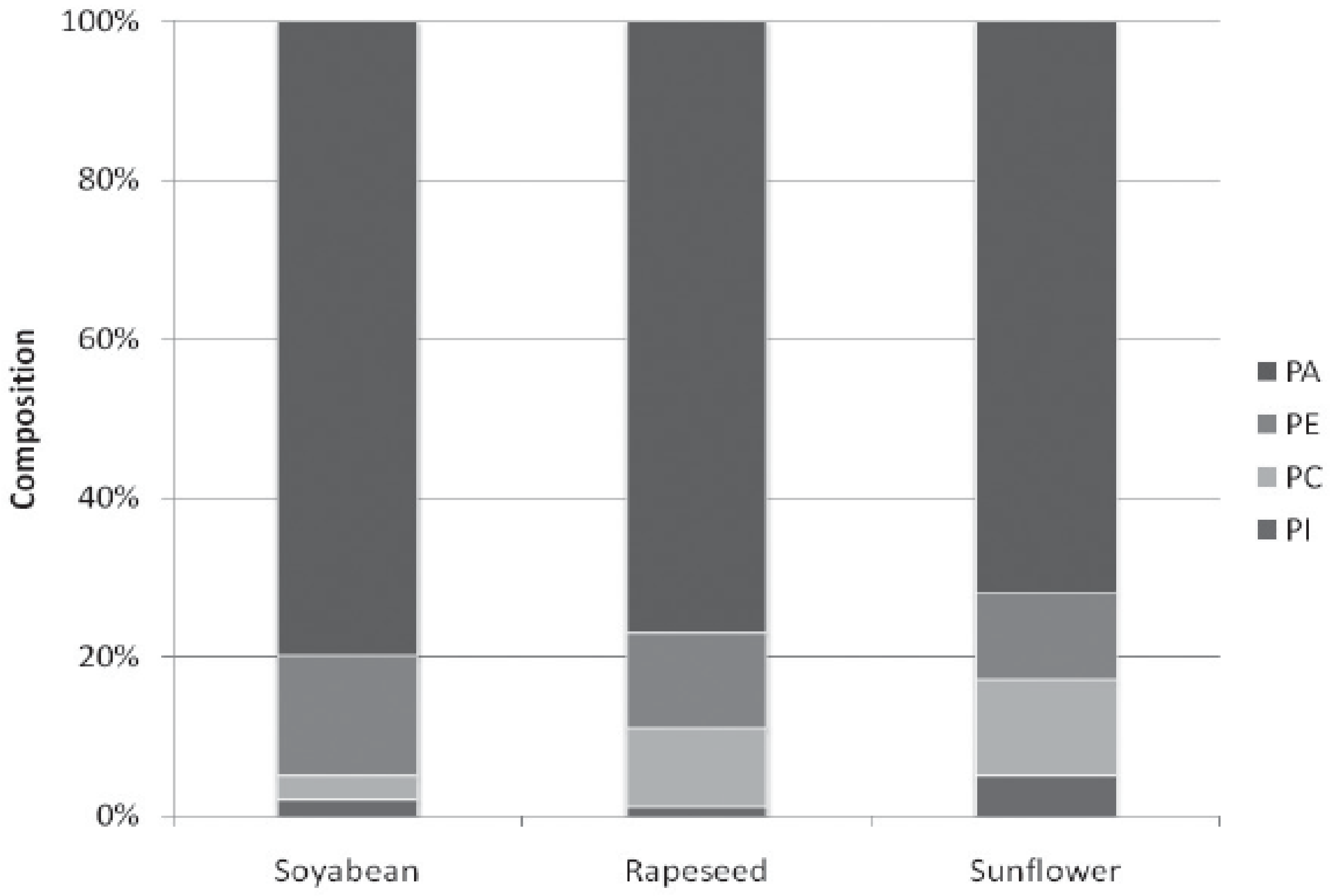
The hydratable phospholipids are effectively removed by water degumming. But nonhydratable phospholipids are hard to remove without special treatments.

Therefore, the major purposes of degumming are removing the nonhydratable phospholipids or transforming them into hydratable phospholipids and then eliminating them with water.

Relative Rates of Phospholipid Hydration

Phospholipid	**Relative Rate of Hydration**
Phosphatidylcholine	100
Phosphatidylinositol	44
Phosphatidylinositol (calcium salt)	24
Phosphatidylethanolamine	16
Phosphatidic acid	8.5
Phosphatidylethanolamine (calcium salt)	0.9
Phosphatidic acid (calcium salt)	0.6

http://lipidlibrary.aocs.org/processing/degum-enz/index.htm.

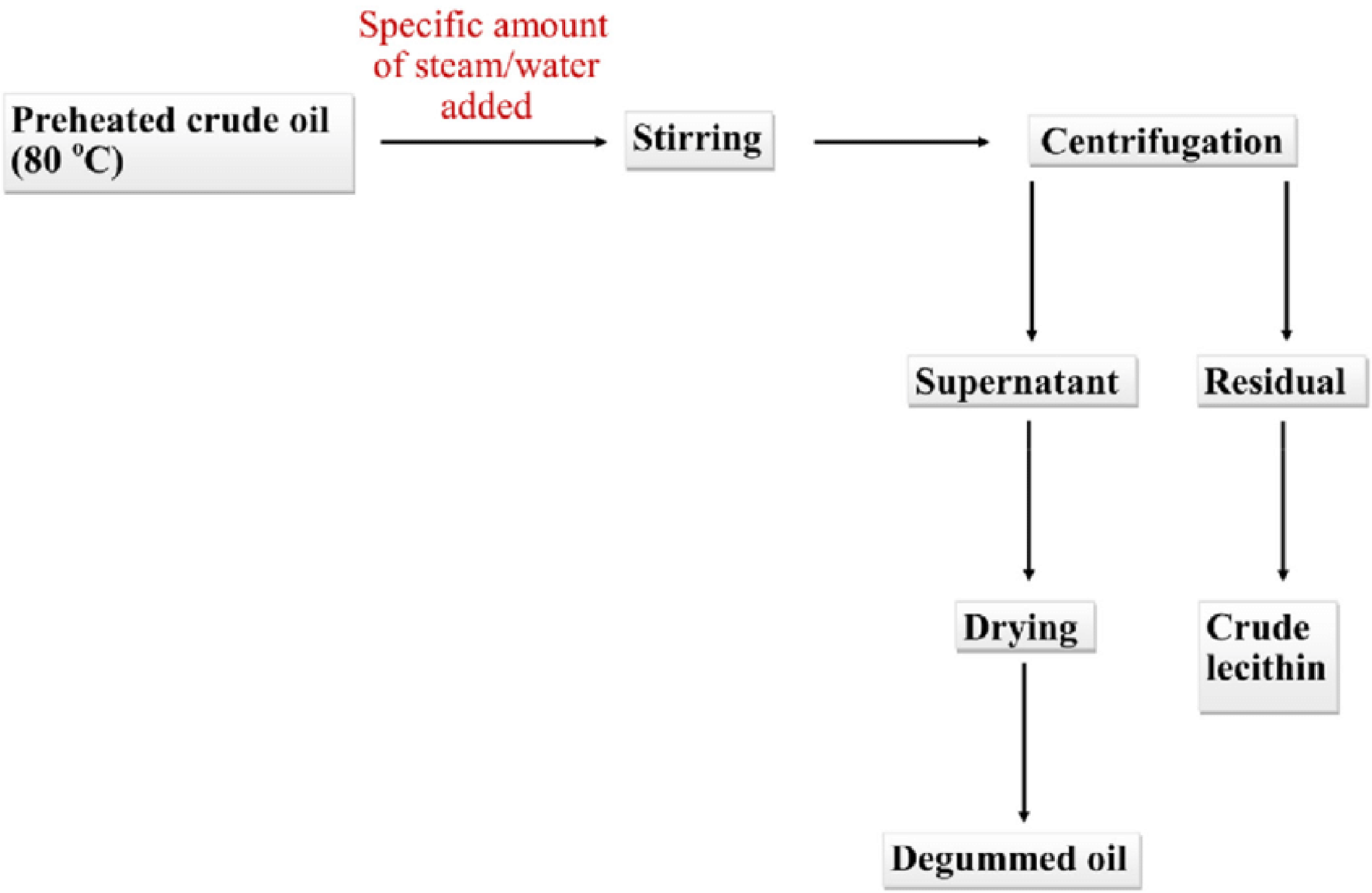


Composition of nonhydratable phospholipids (NHP) from different oils.
PI = phosphatidylinositol, PC = phosphatidylcholine, PE = phosphatidyl-ethanolamine, and PA = phosphatidic acid.

Types of Degumming

- Numerous different refining/degumming processes were developed, and are classified as physical or chemical processes.
    - Water degumming
    - Acid degumming
    - Membrane degumming
    - Enzymatic degumming
- One of the most recent methods is enzymatic degumming.
- All methods aim to reduce the phospholipid content of the oil, measured as phosphorus, to below 10 ppm.

Removal of Hydratable Phospholipids

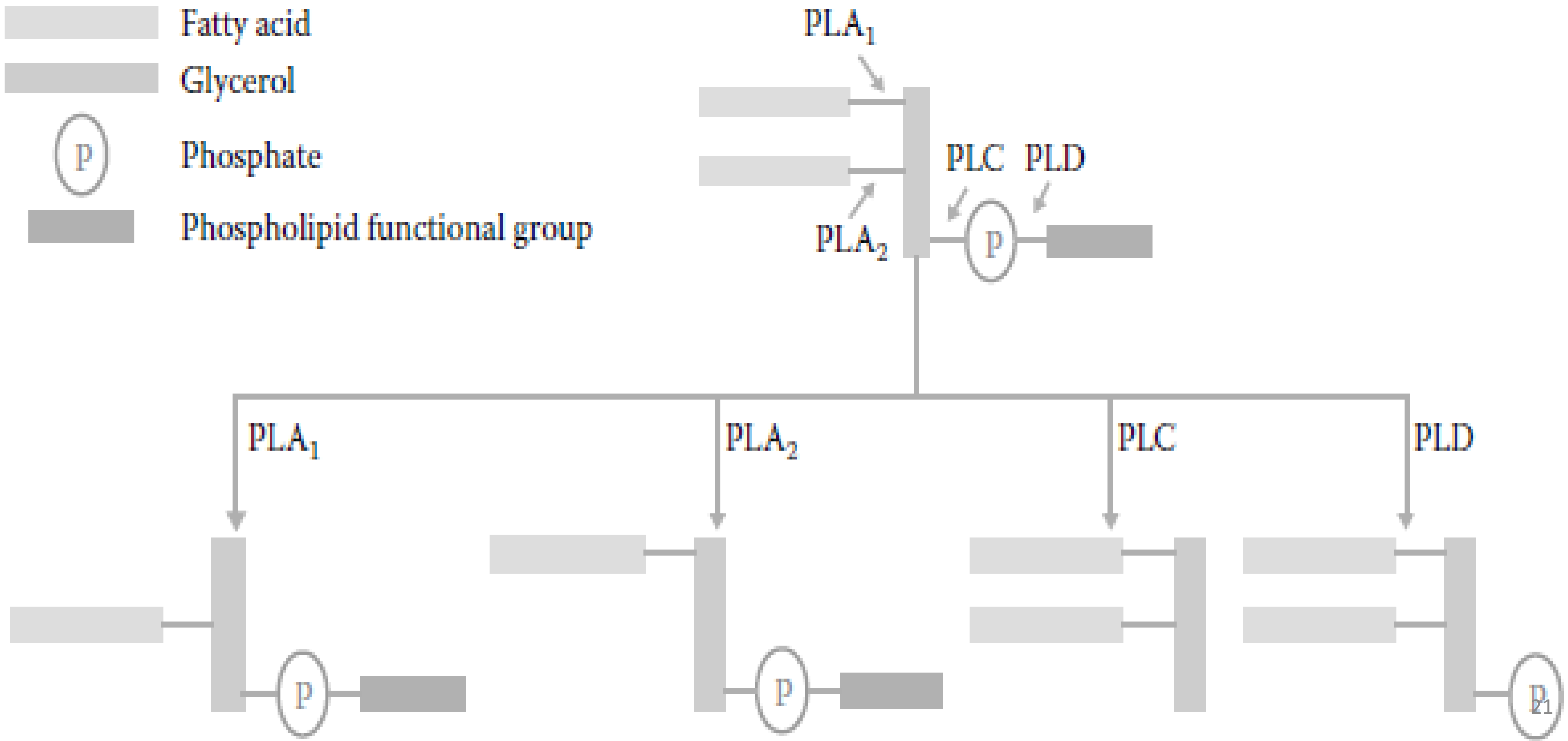


Enzymes Used in Removal of Phosholipids

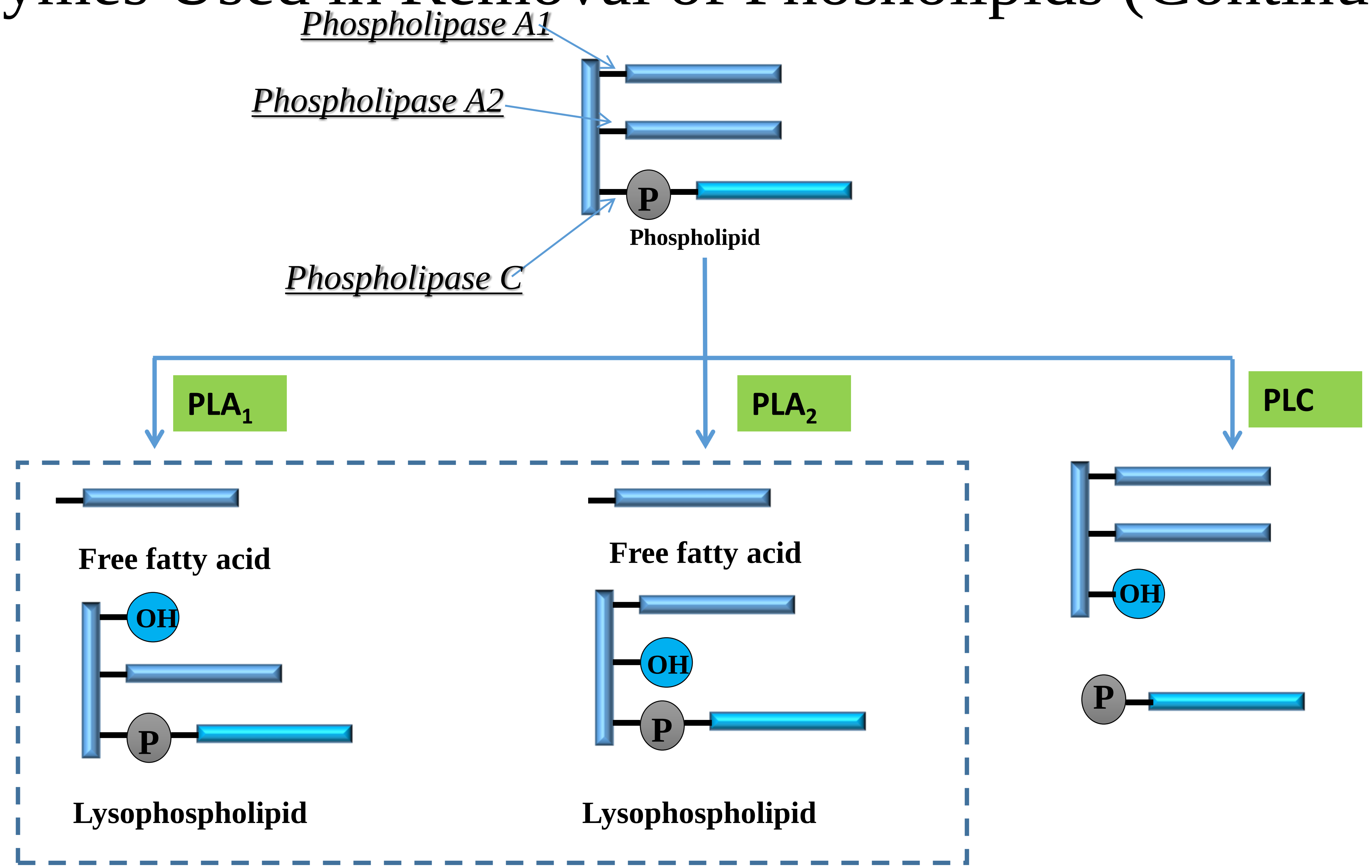
- Phospholipases are used to increase the hydratability of the phospholipids in the oil.
- Phospholipases break phospholipids into water-soluble and oil-soluble fragments, thereby reducing their ability to form an emulsion.
- Less emulsion formation means less yield loss due to entrained oil, and lower gum content enables cleaner separation of oil and heavy phases, with further reduction in yield loss.
- From a biochemical perspective, there are four main types of phospholipase.

Enzymes Used in Removal of Phosholipids (Continued)

Structure of phospholipids and reactions catalyzed by phospholipase enzymes



Enzymes Used in Removal of Phosholipids (Continued)



Hidratlanamayan fosfolipitler hidratlanabilir duruma getirilirler.

Enzymes Used in Removal of Phosholipids (Continued)

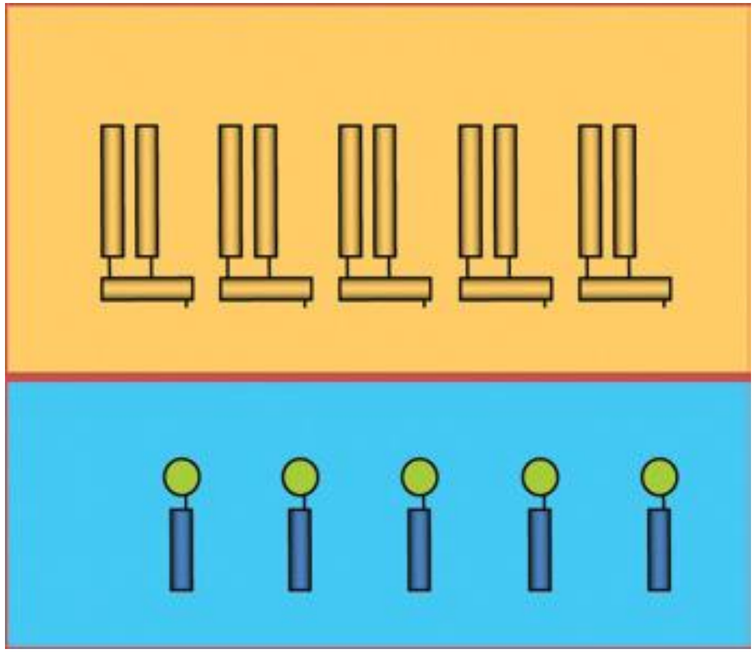
- Phospholipases (A1 and A2) that remove one of the fatty acids from the glycerol backbone to produce a lysophospholipid are the most commonly used in edible oil refining. Thus, nonhydratable phospholipids are converted into a more hydratable form, making it easy to remove by water as part of the physical degumming process.
- Degumming with PLA enzymes results in a significant reduction in yield loss due to the reduction in entrained oil. However, the fatty acids produced remain in the degummed oil and, for both edible oil and biodiesel production, have to be removed in later refining steps (usually) by conversion to soapstock, resulting in formation of an emulsion and loss of oil yield.

Enzymes Used in Removal of Phosholipids (Continued)

- Phospholipase D removes the phospholipid head group to produce a glycerophosphate, and it was found to play an important role in the formation of nonhydratable PA even at a temperature of 65°C in the presence of water-saturated hexane. However, in aqueous media, this enzyme is readily deactivated by heat.

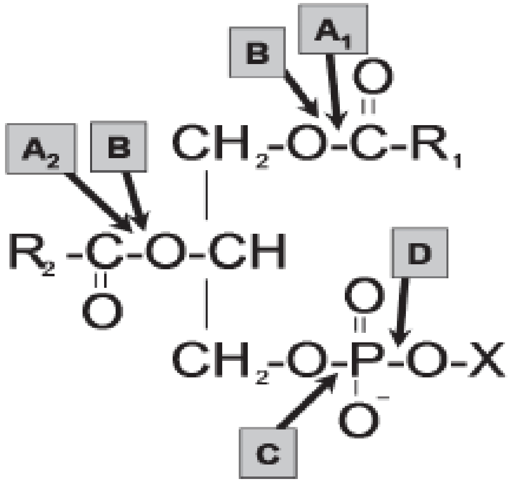
Enzymes Used in Removal of Phosholipids (Continued)

- Recently, phospholipase C has been introduced to oil degumming. The hydrolysis of the bond between the glycerol backbone and the phosphate group results in the formation of a diglyceride and a water-soluble portion containing the phosphorus and head group portions of the phospholipid.



Enzymes Used in Removal of Phosholipids (Continued)

- Different types of phospholipases are classified according to where the catalyzed hydrolysis reaction takes place on the phospholipid molecule.



Types of phospholipases (A_1, A_2, B, C, and D) distinguished according to which bond they are hydrolyzing. R_1 and R_2 represent fatty acids, and X is hydrogen, choline, ethanolamine, serine, or inositol.

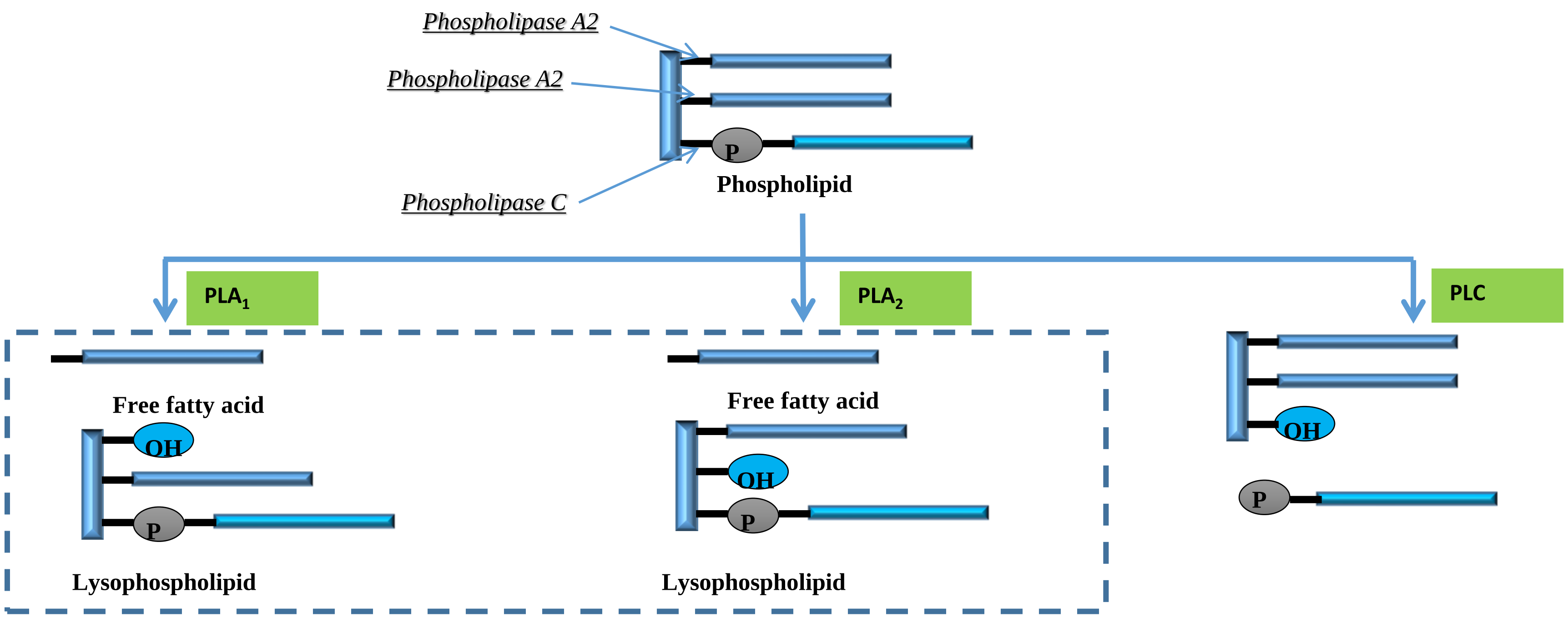
Enzymatic Degumming Process

The process equipment for enzymatic degumming must provide a system where the enzyme is able to work at optimal conditions. Enzymatic degumming is seen as a three-step operation in which it is necessary to:

1. Create an emulsion with the phospholipids distributed at the water–oil interface,
2. Provide the conditions for the enzyme to work efficiently, and
3. Separate the phospholipids/gums from the oil.

Enzymatic Degumming Process (Continued)

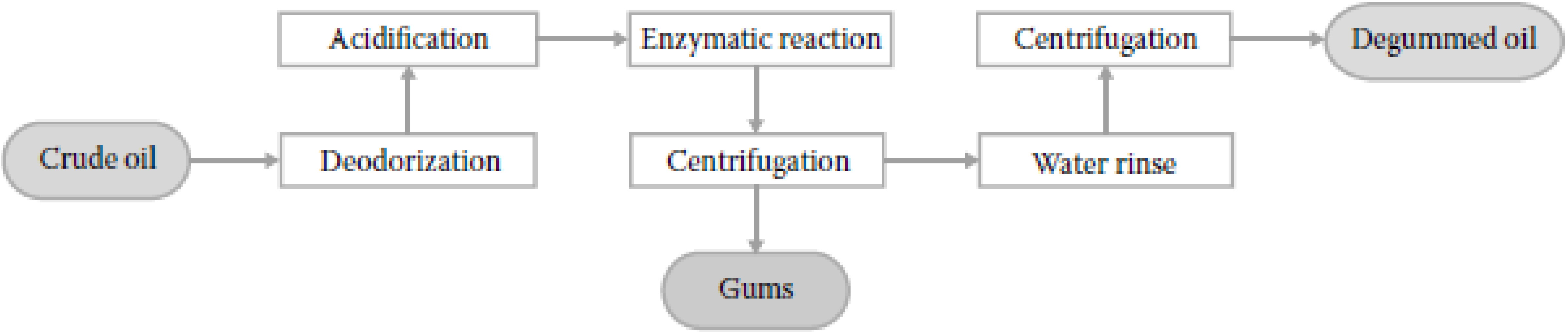
The enzymatic method is based on the conversion of nonhydratable phospholipids to hydratable lyso-phospholipids, which are easy to eliminate with the water phase by centrifugation. The method is applied to a wide range of oils, and the number has grown since the method was first introduced.



Conversion of nonhydratable phospholipids to hydratable lyso-phospholipids

Enzymatic Degumming Process (Continued)

Typical flow chart of an enzymatic degumming process.



Enzymatic Degumming Process (Continued)

Acid conditioning/pH adjustment of the crude or water degummed oil is to make the nonhydratable phospholipids more accessible for enzyme degradation at the oil–water interface and to bring the pH closer to the optimal pH of the enzyme.

↓

Enzyme is added. High shear mixing is applied to ensure optimal distribution in the oil. Enzyme dosing depends on the type of enzyme and on the phospholipid content of the oil, which usually varies between 50-200 ppm.

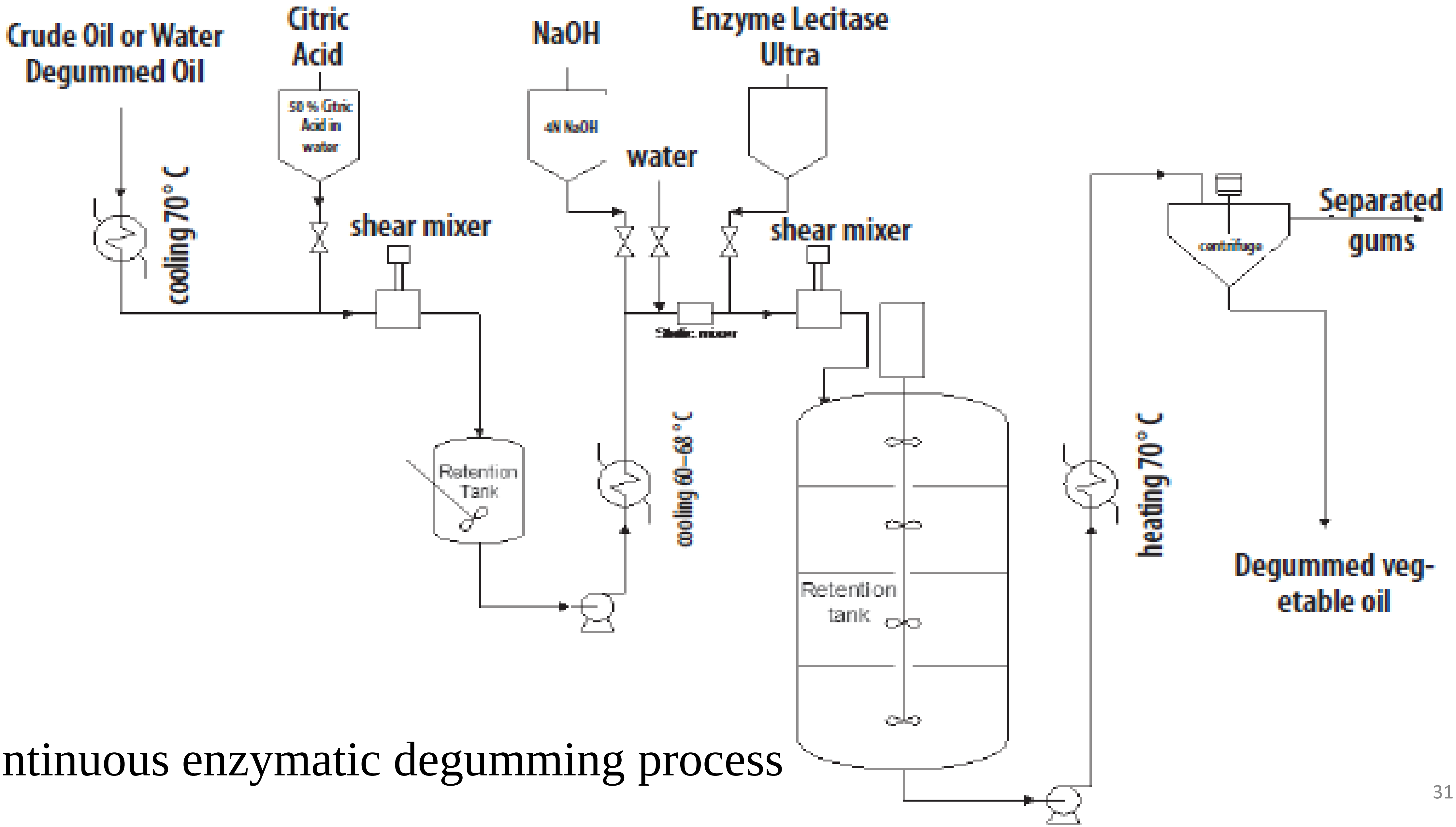
↓

The optimal reaction temperature is 50°C–60°C, and the required reaction time mainly depends on the enzyme dosage.

↓

The heavy phase consisting of water and gums or phosphate esters is separated by centrifugation from the degummed oil.

Enzymatic Degumming Process (Continued)



Continuous enzymatic degumming process

Enzymatic Degumming Process (Continued)

- The first part of the process is the citric-acid step, in which a small amount of citric acid (0.04–0.1%) is added to the oil as a concentrated solution. The citric acid is distributed with a high–shear mixer, and allowed to react in a holding tank with a retention time of 10–30 min at 70°C. The citric acid helps to chelate the metals in the oil, and opens the phospholipid micelles to allow enzyme action upon the phospholipids. After the citric-acid treatment, the oil is cooled to 50–55°C for the optimal level for the enzyme reaction.
- Sodium hydroxide (NaOH) is added to adjust the pH of the water phase. Water is added together with the enzyme, and includes the water added as part of the citric acid, NaOH, and enzyme solutions. A second high-shear mixer is employed to assure a complete distribution of the ingredients and the creation of a water-in-oil emulsion with the phospholipids distributed at the water–oil interphase.
- The use of a high-speed shear mixer to ensure that the enzyme is evenly distributed in very small water droplets throughout the oil is a key element in achieving good degumming. The conversion of the phospholipid to lyso-phospholipid takes place at the oil–water interface, and clearly, the more finely the droplets are divided, the greater the interface surface area will be, and the more efficient the degumming process.

Enzymatic Degumming Process (Continued)

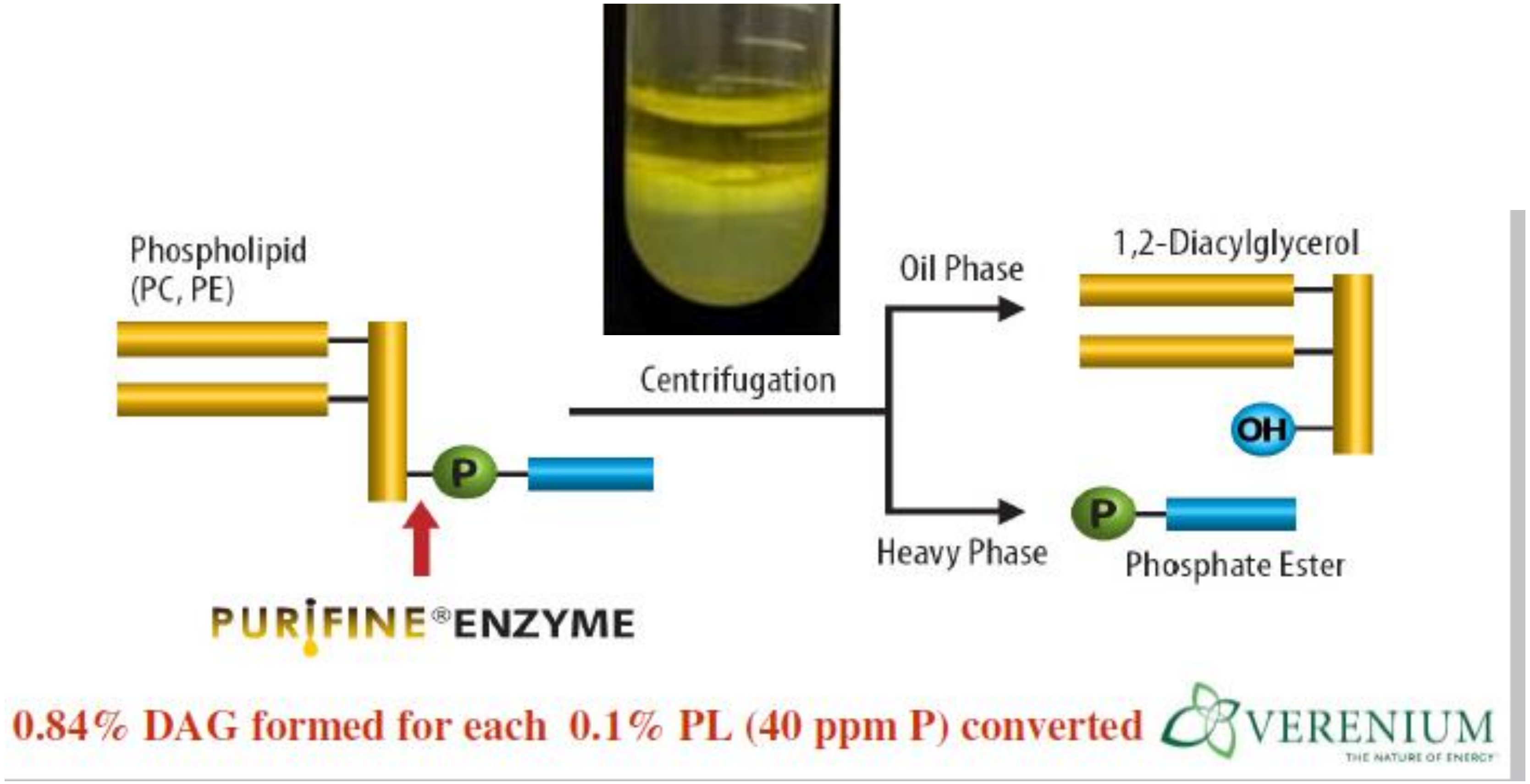
- The second phase of the process is to allow enough time (4-6 h) for the enzyme to react in a continuous stirred-tank system (CSTR).
- When the enzyme reaction is completed, the oil is heated to 70–80°C to inactivate the enzyme and to facilitate the aggregation of the gums and their removal by the centrifuge in the next step of the process.
- After the reaction, the phospholipids/gums are separated from the oil. As all the gums are now hydratable, they are eliminated with the water phase by the centrifugation.
- Following the centrifugal separation, the oil passes to bleaching and deodorization, where both silica and bleaching earth are used to remove any remaining phospholipid and adjust the color to the desired level.

Enzymatic Degumming Process (Continued)

Ingredients for Enzymatic Degumming of 10,000 kg of Oil per Hour

	Dosage	Kg per hour
Oil		10,000
Citric acid	0.065%	7.1 kg of citric acid monohydrate as a 45% solution
Sodium hydroxide (NaOH)	1.5 molar equivalents	2.03 kg of NaOH as a 4N solution
Water	2.5% of total water	226 kg beside what comes in with the other ingredients
Enzyme (Lecitase Ultra)	30 ppm	0.3 kg

Oil Recovery from Gums



Oil Recovery from Gums (Continued)

- In the conventional degumming and chemical refining process, gums work as emulsifiers and are responsible for the major part of the oil losses.

- In enzymatic degumming, the enzym*e* action eliminates the emulsification properties of the gums. Thus, oil saving increases.

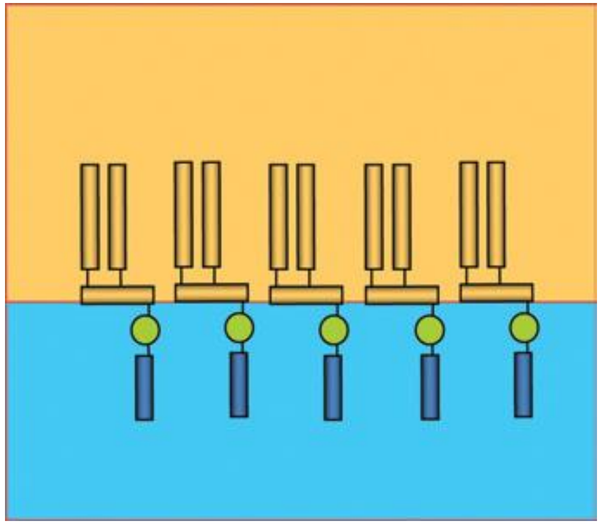
Comparison of Enzymatic and Other Degumming Methods

- The main difference between the chemical and physical refining is the way the free fatty acids (FFAs) are eliminated. In chemical refining, the FFAs are converted to soapstock by the addition of sodium hydroxide. At the same time, all the phospholipids are hydrated and are removed with the soapstock. The elimination of the soapstock requires several washing/centrifugation steps; this causes a significant oil loss.
- The key advantage for enzymatic degumming is that the process converts all the gums to a hydratable form, and therefore virtually eliminates oil losses. No oil is entrapped in the gums; therefore, the physical yield of oil can be 1.0–1.5% higher, depending on the process and the oil to be degummed. This makes enzymatic degumming the process with the lowest yield loss of all the different degumming methods.
- All of the different types of degumming have to reach a low content of phosphorus, but only enzymatic degumming is able to do this, while at the same time reducing oil losses to a minimum. Normally, physical degumming, while being preferred because of the use of less aggressive chemicals, is not seen as being robust enough to tackle as wide a range of conditions as chemical refining. Enzymatic degumming combines this with the ability to cope with a very wide range of oils and oil qualities

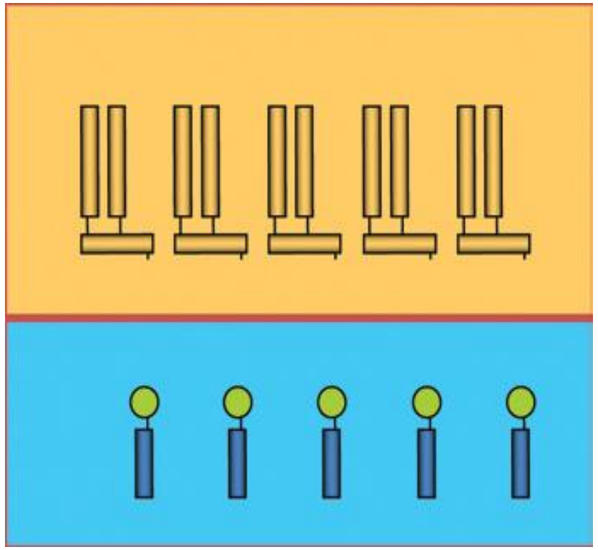
Comparison of Enzymatic and Other Degumming Methods (Continued)

- Compared with traditional degumming methods, enzymatic degumming could happen at mild reaction temperatures (40°C–50°C), much lower than that of traditional methods (85°C–90°C), thus helping to benefit oil quality

Comparison of heavy phases from water-degumming and enzymatic degumming and monitoring the reaction.



(A) Phospholipids are emulsifiers and in the resence of water will sit at the oil water interface, trapping oil in the process (left panel). The gum of water, phospholipid, and oil is removed in a centrifuge and is viscous and difficult to handle as it exits the separator (right panel).



(B) The action of a degumming enzyme like Purifine® PLC on phospholipids breaks the emulsion (left panel), reducing the amount of entrained oil; the resultant heavy phase is free-flowing as it exits the centrifuge (right panel). The change in gum viscosity with addition of enzyme to a water-degumming process allows easy visualization of a successful enzyme reaction by plant operators

Advantages of Enzymatic Degumming

- Increases oil yield
- No requirement for washing step
- Performed at lower temperatures
- Decreases process time
- Decreases use of chemicals

Overall Oil Output (Deodorized Oil) Obtained via Different Degumming Methods

Degumming method	Kg of oil obtained	Oil loss %
Water degumming + chemical refining	942.7	5.73
Water degumming + semi physical refining	944.3	5.57
Water degumming + acid degumming	945.9	5.41
Water degumming + enzyme degumming	948.2	5.18
Full enzyme degumming	952.1	4.79

Degumming with Sodium Hydroxide and Phospholipases

	Caustic refining	PLA Enzymatic refining	PLA/PLC enzymatic refining
Starting Phos level in crude oil	500 ppm	500 ppm	500 ppm
Phos level after centrifuge	2 ppm	2 ppm	2 ppm
Centrifuge discharge (dry%)	3.19	1.13	0.62
Yield of oil (%)	96.5	97.4	98.3

References

- Hitchman, T. (2009). Purifine® PLC: Industrial application in oil degumming and refining. Oil Mill Gazetteer. 115, 1-4.
- Zhang, W., Li, P. and Yang, R. (2016). Enzymes in Oil- and Lipid-Based Industries. In: Enzymes in Food and Beverage Processing (Chandrasekaran, M., Ed.) CRC Press, Boca Raton, Florida, USA, pp. 227-254
- Cowan, D. and Nielsen, P. M. Enzymatic degumming of edible oils and fats. In: Bleaching and Purifying Fats and Oils (List, G.R., Ed.) AOCS Press, Urbana, Illinois, USA, pp. 221-240.