Food and Public Health 2019, 9(5): 139-147
DOI: 10.5923/j.fph.20190905.01

# Degumming Alternatives for Edible Oils and Biodiesel Production

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**Abstract** Vegetable oils are predominantly composed of triacylglycerols (>95%), besides that, a wide variety of minor components including free fatty acids, phystosterols, tocopherols, colour pigments, metals and phospholipids are present in oils and fats. The presence of phospholipids in vegetable oils can cause oil darkening during the deodorization step and the inactivation of the lipases during the enzymatic transesterification process. The first step of the refining process is the degumming, which is designed to remove phospholipids. Traditional degumming processes such as water degumming and acid degumming cannot guarantee the low phosphorus content required for physical refining. Enzymatic degumming is a new process that uses enzymes known as phospholipases, which hydrolyze the phospholipids releasing fatty acids or diacylglycerols, thus increasing the oil yield. Moreover, due to the reduced reaction time and/or to the increased productivity, the ultrasonic technique has also been recently employed in association with degumming processes. Therefore, the purpose of this review is to present relevant studies on enzymatic degumming for edible oils and biodiesel production, from enzymatic catalysis, considering the most recent alternatives for product quality improvement and process costs reduction, with a focus on the simultaneous use of enzymatic degumming and ultrasonic technique.

**Keywords** Enzymatic degumming, Phospholipases, Ultrasound, Biodiesel

## 1. Introduction

Vegetable oils are mainly composed of triacylglycerols (TAGs), which are formed by a molecule of glycerol with three esterified fatty acids. In addition, crude vegetable oils contain up to 5% of non-glyceridic materials, formed by different amounts of free fatty acids (FFA), phospholipids and other compounds. To obtain edible oils from crude oils, a series of refining operations, including degumming, neutralization, bleaching and deodorization are needed [1-3].

Degumming is the first stage of the refining process and involves the removal of phospholipids, proteins, and colloidal substances. The oils used for the synthesis of biodiesel as well as the edible oils must be degummed for the removal of phospholipids and reduction of the final content of phosphorus to the specified limits (<10 mg/kg) [4]. The presence of phospholipids can cause oil darkening during the deodorization step and lipase inactivation during the transesterification process.

The degumming process can be classified as aqueous, acid, or enzymatic degumming. The aqueous or water degumming process is effective when applied to the hydratable phospholipids, since in the presence of water they become insoluble in oil, and can be easily separated by centrifugation. The non-hydratable phospholipids, which are phospholipids combined with calcium, magnesium or iron cations, can be removed by the addition of acids, such as phosphoric or citric [5-6]. The acid and aqueous processes, considered traditional techniques, have as disadvantages the excessive consumption of chemicals and a high generation of effluents, respectively [7].

According to Cesarini et al [8], the traditional degumming processes result in a loss of 2.5% of the total oil amount, for crude soybean oil containing 900 mg/kg of phosphorus, considering the current market price of US$ 1,100 per ton, it corresponds to a loss of US$ 27.5 per ton of oil. The oil loss is associated with the drag of neutral oil along with the phospholipids, as well as the removal of intact phospholipids.

Enzymatic degumming is a process for removing phospholipids from crude oil in which phospholipases are used. The enzymes hydrolyze the ester bonds present on the phospholipid molecules, resulting in diacylglycerols (DAGs)

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Published online at http://journal.sapub.org/fph

or FFA release, which will contribute to the oil yield [9]. A part from that, it has the advantage of lower generation of effluents, and reduction of operational costs [10].

Currently, the combination of techniques to increase the efficiency and productivity of the degumming process and biodiesel production has been studied in the literature. The combination of lipases and phospholipases for the production of biodiesel allows the use of crude oils, in which phospholipases have the role of degumming and lipases the function of transesterification, all taking place in a single step, thus reducing the production costs [8;11]. Another alternative to accelerate enzymatic processes is the use of ultrasound. The ultrasonic cavitation technique is used to intensify mass transfer rates and, in consequence, to increase the rates of biochemical reactions [7]. The association of enzymatic degumming with ultrasound would reduce the content of phospholipids, improve productivity, and reduce refining time [12].

Therefore, the goal of this review is to evaluate the degumming of vegetable oils for edible oils and biodiesel production focusing on the use of enzymatic processes and ultrasound technique, to increase productivity, improve product quality and reduce reaction time.

## 2. Vegetable Oils Production

The growing world population has resulted in a sharp increase in the demand of oils and fats. To meet this demand, the oil production has increased from 40.8 million tons in 1980 to more 200 million tons in 2019 [13-14]. As shown in Figure 1, the projection for the global vegetable oil consumption and production increases until 2020.

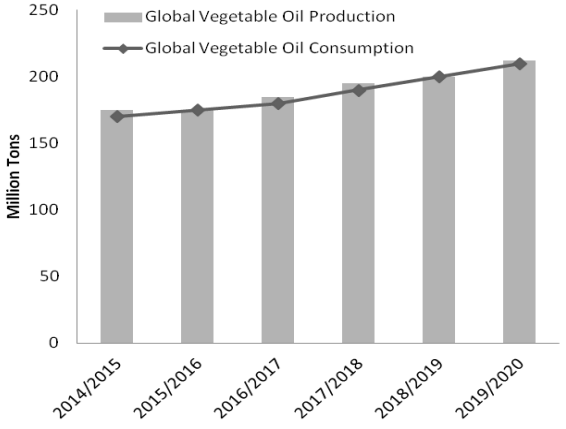


**Figure 1.** Global Vegetable Oil Consumption and Production [14]

The choice of the feedstocks to be used for biodiesel or edible oil production is based on the availability of the feedstock in a given region.

Brazil and USA are global soybean suppliers and represent 85% of global trade. In 2019/2020 is expected that Brazil lead soybean exporter in answer to a larger harvest and ascent access to China. Corn production together with soybeans account for about 80% of Brazil's grain production, although soybeans are more liquid on the international market. Nevertheless, corn can be a viable feedstock for biodiesel production given its importance in the Brazilian market [14].

Rapeseed oil production is expected to increase by 2.0 million tons in 2019/2010, with its largest production in Canada, the European Union and China. Sunflower oil has the most prominent production in Ukraine, Russia and the European Union. Sunflower oil imports are forecast at 8.8 million tons, the second highest ever recorded [14].

Kenaf oils (India and Africa) and Camellia oils (Korea and China) are most commonly used for the production of cosmetics and edible oils as they have properties beneficial to human health [15;16].

Palm oil major producers and exporters are Malaysia and Indonesia. The consumption of palm oil in food is expected to grow by 34%, while using it for non-edible purposes such as biodiesel production will increase production by 44%. Jatropha oil is an non-edible oil and used as an alternative for biodiesel production [14;17].

Crude vegetable oil needs to undergo a refining process before being consumed, so that many minor components, such as fatty acids, phospholipids and tocopherols are removed during this process. The first stage of refining is degumming, applied for removing phospholipids. Soybean lecithin is a phospholipid widely used in the food and pharmaceutical industries, because of its surfactant functionality. After acid degumming the oil is neutralized with a 5% NaOH solution, resulting in the production of soaps that are removed though centrifugation. The next step is bleaching, used to remove the soluble compounds that affect the oil color [18]. The last step is the deodorization, being removed in this step aldehydes, ketones, remaining FFA, among other compounds that cause an unpleasant odor to the final product.

## 3. Degumming

Crude oils usually have impurities (phospholipids, FFAs, metals and etc) which during the refining process may cause damage to the oil's stability, color and flavor, so that their removal is necessary [19].

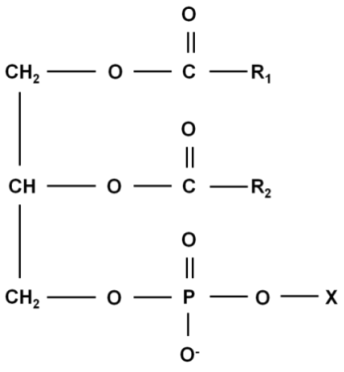


**Figure 2.** Phospholipids structure (*X=Head group)

Degumming is the first stage of refining, in which phospholipids are removed. Phospholipids (PL) are derived from phosphatidic acid, a compound obtained by the condensation of glycerol with phosphoric acid and two fatty acids, as shown in Figure 2. The phospholipids, also known as gums, have a hydrophilic part and a hydrophobic region and are therefore compatible with organic (apolar) and aqueous (polar) environments, being widely used as emulsifiers in the food industry [20].

Different types of crude vegetable oil have specific phosphorus content, as shown in Table 1.

**Table 1.** Content of phosphorus in different oils

| Type of oil | Phosphorus in oil, mg/kg | Reference |
|---|---|---|
| Soybean | 640 – 1140 | [3] |
| Rapeseed | 252 – 1155 | [3;18] |
| Sunflower | 170 – 544 | [3;19] |
| Corn | 360 – 951 | [2;3] |
| Camellia | 57 – 670 | [10;16] |
| Kenaf | 48 – 63 | [15;23] |
| Jatropha | 43 – 133 | [24;25] |
| Palm | 6 – 19 | [26;27] |

The presence of phospholipids in the subsequent stages of refining may damage the oil color and flavor. In fact, their presence at the high temperatures occurring during the deodorization step may cause the oil darkening [28].

The degumming process is considered the best option for the removal of phospholipids and for the production of gums from crude oils in an industrial scale. The degumming process consists of the following steps [19]:

- Formation of phospholipid micelles with polar solvents;
- Fast hydration of phospholipids at high temperatures;
- Conversion of non-hydratable phospholipids by acidification followed by neutralization.

The traditionally used techniques for the removal of phospholipids are water degumming and acid degumming. However, a new technology based on the enzymatic degumming, is emerging as an alternative process because increases the refined oil yield.

### 3.1. Water And Acid Degumming

Water degumming is effective when applied to the hydratable phospholipids, because when the water is added to the crude oil, the phospholipids are hydrated and can be separated by centrifugation [6]. This type of degumming still leaves about 80 to 200 mg/kg of phosphorus in the oil, depending on the quality and presence of non-hydratable phospholipids, which remain in the oil [19]. Sampaio et al. [2] performed the aqueous degumming of crude corn oil, which initially had a phosphorus content of 951.0 mg/kg, obtaining a final content of 67 mg/kg. Ye et al. [29] performed the aqueous degumming in crude rapeseed oil and obtained a decrease in phosphorus content from 690 mg/kg to 61 mg/kg. Both obtained a reduction of about 90% of the phosphorus content. However, the final phosphorus content is still higher than the level required for the physical refining.

In the acid degumming, the non-hydratable phospholipids, namely those phospholipids combined with calcium, magnesium, or iron cations, are removed. For this procedure, it is necessary to add acids, such as phosphoric or citric [5-6]. Citric acid is used not only to decompose the metal salts, but also as a chelating agent to keep the metals soluble in the aqueous phase [30]. Mei et al. [31] performed the acid degumming of *Silybum marianum* seed oil using different types of acids, such as citric, phosphoric, oxalic, and tartaric acids. The initial phospholipids content in the oil was 273 mg/kg. The degumming using citric, phosphoric, oxalic and tartaric acids obtained the following results for the phospholipids content, respectively: 113.87 mg/kg, 197.83 mg/kg, 185.49 mg/kg, and 125.1 mg/kg. It was observed that the citric acid allowed the best result regarding the decrease of phospholipids content. Acid degumming requires the use of large amounts of acid solutions, high temperatures and also generates a lot of wastewater [10;24].

Szydłowska-Czerniak; Łaszewska [33] and Jiang et al. [10], when evaluating the acid degumming applied to crude rapeseed and soybean oils, obtained a reduction of phospholipids content from 5655 mg/kg to 268 mg/kg and from 752 mg/kg to 32 mg/kg, respectively. Moreover, the final phosphorus content was still over 10 mg/kg, the maximal level usually considered appropriate for physical refining.

In view of this, conventional degumming should be replaced by other processes that have operates at mild temperature, lower energy consumption, reduction effluent generation and increased oil yield. Taking into account these demands, enzymatic degumming has emerged as a key technology.

### 3.2. Enzymatic Degumming

Enzymatic degumming is the process of removing phospholipids from crude oil using enzymes known as phospholipases. The main phospholipases types are $A_1$, $A_2$, and C, with their target sites varying according to their specificity (Figure 3).

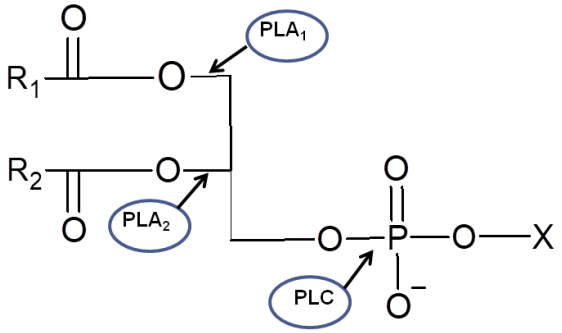


**Figure 3.** Phospholipases action sites in the phospholipid molecule (*X=Head group)

Currently, there are several types of enzymes for the treatment of plant oils, such as phospholipase $A_1$ ($PLA_1$) and phospholipase $A_2$ ($PLA_2$), which remove the fatty acid from positions 1 and 2, respectively, relative to glycerol [18;26]. Phospholipase C (PLC) has been recently introduced to the industry, and it hydrolyzes the bond between acylglycerol and the phosphate group, generating a molecule of diacylglycerol (DAG) [10].

Table 2 shows the most used commercial phospholipases types, their commercial names, manufacturers, and optimum processing conditions.

**Table 2.** Phospholipases characteristics

| Phospholipases Type | Commercial names | Manufacturer | Effective on phospholipids | End Products | Optimum pH | Optimum temperature | Reference |
|---|---|---|---|---|---|---|---|
| $PLA_1$ | Lecitase Ultra<br>Lecitase NOVO A-PLA | Novozymes | All | FFA and Lyso-phospholipids | 5.5<br>4.1 | 55 ℃<br>75℃ | [35] |
| $PLA_2$ | Rohalase PL-Xtra | AB enzymes | All | FFA and Lyso-phospholipids | 4.0 | 55 ℃ | [35] |
| | LysoMax | Danisco | All | FFA and Lyso-phospholipids | 5.0 | 50 ℃ | [36] |
| | $PLA_2$ | German AB | All | FFA and Lyso-phospholipids | 4.0 | 50 ℃ | [34] |
| PLC | Purifine PLC (1G) | DSM | PC and PE | DAG and Phosphate esters | 5.7 | 60 ℃ | [2] |
| | Purifine PLC (3G) | DSM | All | DAG and FFA | 5.5 | 60 ℃ | [35] |

Enzymatic degumming may reduce the phosphorus content more than 99%, independent of the initial phosphorus content in the crude vegetable oil. Lecitase Ultra ($PLA_1$) is the most used phospholipase in the available literature. Sampaio et al. [21] performed the enzymatic degumming in crude soybean and rapeseed oils using Lecitase Ultra ($PLA_1$), and the phosphorus content of the enzymatically degummed oils were reduced to less than 7.0 mg/kg and 3.9 mg/kg, respectively.

Lamas, Constenla e Raab [22] performed enzymatic degumming on crude sunflower oil, using $PLA_1$ (Lecitase Ultra) and $PLA_2$ (MAXAPAL $A_2$) and, after 180 min, obtained a final phosphorus content of 3.02 mg/kg and 5.81 mg/kg, respectively.

Recently, the use of PLC (Purifine PLC) has been tested in different vegetable oils. Ye et al. [29] used rapeseed oil for enzymatic degumming using phospholipase PLC, the initial phosphorus content was 690 mg/kg and after degumming they obtained a phosphorus content of 7.34 mg/kg. Sampaio et al. [2] also used Purifine C for the enzymatic degumming of crude corn oil and obtained a phosphorus reduction from 957 mg/kg to 27 mg/kg.

Jiang et al. [10] studied the association of phospholipases with citric acid in the degumming process of crude soybean oil. The association of citric acid (CA) with PLC + $PLA_1$ and CA with $PLA_1$ resulted in phosphorus levels in the degummed oils of 1.8 and 4.7 mg/kg, respectively. Experiments carried out without the addition of citric acid resulted in a higher final phosphorus content, suggesting that citric acid does not only help maintain the optimum pH of the enzyme, but it also opens micelles and facilitates the action of phospholipases.

### 3.3. Ultrasound Improved Degumming

Recently, the optimization of enzymatic degumming process has been studied combining the enzymatic process with other techniques, including ultrasound, which is a kind of cavitation technique.

Cavitation is the formation, growth, and collapse of microbubbles that occur in a short time, releasing energy and generating high temperatures (in the range of 1000 - 15000 K) and pressures (in the range of 500-5000 bar ) within a very restricted space region[29;30].One of the main effects of cavitation is the release of a significant energy magnitude and the generation of local turbulence, which can increase the mass transfer efficiency and play an important role in enzymatic reactions. The use of ultrasound can intensify the degumming process, hence reducing the reaction time, amount of chemicals, energy consumption, and increasing the productivity.

Jiang et al. [39] performed the enzymatic degumming of rapeseed oil in association with the ultrasound technique and obtained a reduction of the phosphorus content from 252 mg/kg to 6.5 mg/kg, reduction higher than 97%. Similar results were also found by More & Gogate [12] when evaluating the enzymatic degumming of soybean oil. Therefore, the use of ultrasound appears to be a good alternative for the optimization of enzymatic degumming.

In Table 3, the phosphorus content and different types of catalysts used for each type of degumming are shown.

**Table 3.** Content of phosphorus and types of catalyst for different degumming types

| Degumming Type | Type of Oil | Catalyst | Content of Phosphorus (mg/kg) | | Reference |
|---|---|---|---|---|---|
| | | | Crude Oil | After degumming | |
| Water | Crude *Crambe abyssinica* | water | 86.30 ± 15.45 | 39.4 ± 0.02 | [40] |
| | Crude rapeseed | | 690.03 ± 0.38 | 61.54 ± 1.57 | [29] |
| | Rapeseed | | 1176 | 204 | [41] |
| | Soybean | | 558 | 75 | [41] |
| | Crude sunflower | | 544.51 ± 19.83 | 63.21±6.99 | [22] |
| | Crude soybean | | 752.8 ± 10.2 | 80.5 ± 5.6 | [10] |
| | Crude corn | | 951.0 ± 8.53 | 67 | [2] |
| Acid | Crude Crambe abyssinica seed | 0.05 wt% of phosphoric acid | 86.30 ± 15.45 | 61.10 ± 2.69 | [40] |
| | Crude *Crambe abyssinica* seed | 25 wt% phosphoric acid solution | 86.30 ± 15.45 | 35.56 ± 11.8 | [40] |
| | Crude Soybean | Citric acid | 752.8 ± 10.2 | 32.5 ± 1.6 | [10] |
| Enzymatic | Crude rapeseed | PLC | 690.03 ± 0.38 | 7.34 ± 0.39 | [29] |
| | Crude sunflower | Lecitase Ultra ($PLA_1$) | 544.51 ± 19.83 | 3.02±0.20 | [22] |
| | Crude sunflower | MAXAPAL $A_2$ ($PLA_2$) | 544.51 ± 19.83 | 5.81±0.40 | [22] |
| | Crude soybean | Lecitase Ultra ($PLA_1$) | 875±8 | 0.7±0.0 | [21] |
| | Crude rice rran | Lecitase Ultra ($PLA_1$) | 390 | 10,1 | [42] |
| | Crude soybean | Lecitase Ultra ($PLA_1$) | 752.8 ± 10.2 | 46.4 ± 3.1 | [10] |
| | Crude soybean | PLC modified Bacillus cereus | 752.8 ± 10.2 | 68.2 ± 4.7 | [10] |
| | Crude soybean | $PLA_1$+PLC | 752.8 ± 10.2 | 39.6 ± 2.7 | [10] |
| | Crude corn | Purifine (PLC) | 951.0 ± 8.53 | 27 | [2] |
| Acid + Enzymatic | Crude soybean | Citric Acid (CA)+PLC+$PLA_1$ | 752.8 ± 10.2 | 1.8 ± 0.8 | [10] |
| | Crude soybean | CA+PLC | 752.8 ± 10.2 | 24.6 ± 0.8 | [10] |
| | Crude soybean | CA+$PLA_1$ | 752.8 ± 10.2 | 4.7 ± 0.4 | [10] |
| Enzymatic + Ultrasound | Rapeseed | Lecitase Ultra ($PLA_1$) / Ultrasonic = 0.07 W/$cm^3$ | 252.05 ± 0.91 | 6.49 ± 0.4 | [39] |

# 4. Biodiesel Production

Brazil is self-sufficient in the bioethanol business, with the production and distribution infrastructure as well as the domain of technologies involved in the sugarcane and ethanol production chain associated with the automotive sector. In addition, ethanol is a fuel that has low toxicity, is fully biodegradable and considered environmentally friendly. Bioethanol is obtained from renewable sources, thus being favorable for reducing the emission of greenhouse gases [43].

In 2008 the Brazilian government decided on an incentive program to produce biodiesel and established adding 2% of this biofuel to diesel, a percentage that has been increasing over the years. In May 2019, the addition of 11% of biodiesel to diesel was fixed, with an increase until 15% up to the 2023 year [44]. Thus, with the government incentive and the use of techniques that could reduce the production costs, should result in the production of cheaper and more competitive biodiesel in the biofuels business.

The production of biodiesel usually occurs through alkaline catalysis, with the use of refined vegetable oil as a source of triacylglycerols. However, the oil refining process generates high costs in biodiesel production. Therefore, the use of alternative processes that employs crude vegetable oils for biodiesel production is emerging as a feasible innovation, for example, through the use of enzymatic degumming for removal of phospholipids.

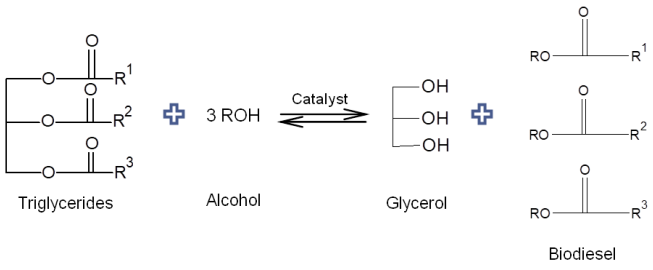


**Figure 4.** Transesterification reaction

Biodiesel is chemically defined as the monoalkyl esters of

long chain fatty acids, obtained by the transesterification reaction (Figure 4) (alcoholysis) of vegetable oils or animal fats (triacylglycerols - TAG), or by the esterification of free fatty acids (FFA) with an (methyl or ethyl) alcohol, by the use of acidic, basic or enzymatic catalysts, which can be of the homogeneous or heterogeneous kind [45].

The acid catalysts used are sulfuric and sulphonic acids, with sulfuric acid being more often used due to its low cost. This route has the disadvantage of a process sensitive to the presence of water, the need for a high molar ratio alcohol: oil and long reaction time. In addition, the use of acids can cause corrosion to the equipment [38;39].

Alkaline transesterification proceeds faster than the acid-catalyzed reaction, and is the most used transesterification route worldwide. The catalysts are mainly represented by sodium hydroxide (NaOH) and potassium hydroxide (KOH). However, when the alkaline catalyst reacts with the free fatty acids there is the formation of soap and water. The soap generated may inhibit the phase separation between glycerol and esters of fatty acids and the catalyst may accelerate the saponification reaction, thus decreasing biodiesel yield [40;41].

The enzymatic transesterification uses lipases as catalysts. They can esterify both fatty acids and triglycerides and there is no saponification process. In this way, no further washing or purification steps are required, thus reducing energy consumption and water and effluent treatment costs. In addition, the glycerol formed is of better quality than that generated in the acidic and alkaline processes [50].

Enzymatic catalysts may be used in immobilized or liquid form (free enzyme). The immobilized form of lipases increases its stability, simplifies the further processing of biodiesel and facilitates recovery and reuse of the catalyst. The liquid formulation is, in general, cheaper than the immobilized one, because the immobilization process increases the cost of producing enzymes. In addition, a liquid formulation prevents mass transfer through multiple phases which decreases the mass transfer influence, providing a faster reaction [51].

The best performing lipases achieve a conversion of above 90% in the transesterification process for temperatures ranging from 30 to 50°C. Some parameters, such as lipase origin, enzymatic activity, reaction temperature, form of presentation (liquid or immobilized) and molar ratio between alcohol:oil, influence the maximum yield of biodiesel production [50].

The most used enzymes for the transesterification processes are the immobilized lipases. Noureddini, Gao and Philkana [48] used the *Pseudomonas cepacia* lipase (immobilized) for the transesterification of soybean oil, were used 10 g of soybean oil, temperature of 35°C and 475 mg of lipase. As short chain alcohols methanol and ethanol were used in the molar ratios of 7.5:1 and 15.2:1 (alcohol:oil), respectively. The formulation containing methanol and ethanol gave a yield of 67% and 65% in 1h, respectively. Hernández-Martín & Otero [49] used lipase Lipozyme TL IM (immobilized) for the transesterification process and ethanol as the short chain alcohol. Were used 2g of oil (borage, soybean, olive or sunflower), 10% (w/w) of lipase, in different temperatures ranging for 25 to 60°C and for 24h. The molar ratio was 1:0.33 (alcohol:oil) and gave an ethyl ester yield of 84% in only 5-7h.

Other authors, such as Souza et al. [52], studied the transesterification of soybean oil, using as catalyst the Novozyme 435 (immobilized) lipase with a molar ratio of 1:1 (ethanol:oil), and obtained a yield of 83.5% in 90 min using 3 wt.% of enzyme and ethanol at 50°C. In contrast, Sangaletti et al. [53] studied the transesterification of soybean oil with Novozyme 435 (immobilized) lipase as the catalyst and a molar ratio of 4.5:1 (ethanol:oil) and obtained a yield of 85.4% of fatty acid ethyl esters. It is important to emphasize, that the mentioned studies obtained about 85% yield of fatty acid ethyl esters, a percentage still outside the ideal conversion, which would be above 96%, according to ANP (the Brazilian National Agency of Petroleum, Natural Gas and Biofuels).

The enzymatic transesterification processes using liquid lipases have been explored by other researchers with lipases from different sources. For instance, Kaieda et al. [54] used *Pseudomonas cepacia*, *Pseudomonas fluoresces* and *Candida rugosa* lipases in their free (liquid) form as the catalysts for the transesterification reaction of soybean oil. For the experiments were used 9.65g of soybean oil, 0.35g methanol, 0.5g enzyme and was incubated at 35°C, and obtained a yield of 80, 90 and 90%, respectively enzyme. Cesarini, Diaz, and Nielsen [55] studied the performance of *Callera Trans L*. lipase, in free (liquid) form, for the production of methyl esters of fatty acids from crude soybean oil in temperature at 35°C, 1% lipase, 3 to 15 wt.%, 16% (w/w of oil) of methanol, during 24h and obtained a yield of 96%. The conversion rates to biodiesel presented by the liquid enzymes were higher than the immobilized ones, besides showing a conversion near or equal to 96%, the value required by the ANP. Therefore, liquid enzymes can be a viable alternative for the production of biodiesel.

The use of enzymes for the production of biodiesel has been a path traced in recent times. The combination of the best parameters for the process generates a decrease in production costs, including the costs for treatment of chemical effluents. Thus, the use of the enzymes in their free form and vegetable oils in their raw state seems to be a suitable alternative of reduction costs for the production of biodiesel, with the preservation of the environment. In this case, the phospholipases will have the role of degumming and the lipases the function of transesterification, allowing the process to occur in a single step. Vegetable oils used to produce biodiesel are also submitted to the degumming step, since the presence of phosphorus in the oil can trigger the inhibition of the catalyst action during the reactive steps. Therefore, the presence of phosphorus inhibits the separation of glycerin and water, resulting in an emulsifying effect [6;48]. The ANP allows a maximum amount of 10 mg/kg of phosphorus present in the final biodiesel.

## 5. Association of Enzymatic Degumming with Enzymatic Transesterification

Recently, some researchers associated phospholipases with lipases, to perform the biodiesel production processing in a single step. According to the literature, in spite of being an enzymatic process, this combination can reduce costs compared to the traditional processes of biodiesel production [57].

Jang et al. [58] used the association of $PLA_2$ with the lipase C. rugosa and R. Oryzae (liquid form) in a proportion of 1:1, for the production of biodiesel in two steps from crude canola oil (10g), temperatura at 37℃, 10% (w/w of oil) of free lipase solution, 4.5 mL of methanol, during 60h. The results revealed a conversion to fatty acids methyl esters greater than 84.25% in 60 hours.

Cesarini et al. [8] used the lipase *Callera Trans L* in liquid form along with phospholipases $PLA_1$, PLC and LLPL-2 for the production of biodiesel from crude soybean oil and canola oil, were used temperature at 35℃, 1% of lipase, 2 to 3.5wt.%, 1.5eqs of methanol and reaction time was 24h. The authors obtained a content of fatty acid methyl esters higher than 95% and phosphorus content lower than 5 mg/kg in 10 hours. Li, Du & Liu [11] used the free lipase (NS81006) and *Aspergillus niger* modified $PLA_1$ for the production of biodiesel from the crude soybean oil and obtained a fatty acid methyl esters content of 94.9%. From the results, it is possible to verify that the use of phospholipase $A_1$ resulted in higher biodiesel yields. Thus, with the combination of phospholipases and lipases, in their optimum conditions, good results, in both conversion (>95%) and phosphorus content (<10 mg/kg), are obtained at the end of biodiesel production, as we can observe in the previous studies.

## 6. Conclusions

The use of enzymatic degumming as a step to remove phospholipids, which is a common goal of degumming, increasing the oil yield has attracted attention of the vegetable oil industry. The association of enzymatic degumming with the enzymatic transesterification can provide numerous and sustainable benefits, which includes the use of non-refined and cheaper vegetable oils, less chemicals and mild processes. Besides, the combination of both processes involves a great efficiency increasing in the new era of vegetable oil and biodiesel production at industrial scale.

The combination of the enzymatic degumming or the enzymatic transesterification with the ultrasound technique would potentiate the removal of phospholipids, reduce reaction time, energy consumption and increase productivity. Therefore, the mentioned processes and techniques are of great importance for the future studies.

## ACKNOWLEDGEMENTS

The authors thank the funding agencies: CNPq (429873/2018-2; 305870/2014-9; 308924/2017-7; 406963/2016-9; 132428/2018-0), FAPESP (2014/21252-0; 2016/08566-1). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Supeior – Brasil (CAPES) – Finance Code 001.

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