Optimization and comparison of water degumming and phospholipase C degumming for rapeseed oil

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ABSTRACT
In the present study, the phospholipase C (PLC) degumming and water degumming process were studied, respectively, and each was optimized by orthogonal array experimental design; the minimum phosphorus content of PLC degumming was 7.34 ± 0.39 mg/kg, while that of water degumming was 61.54 ± 1.57 mg/kg. Oxidation stability of different oils were analyzed by testing the induction time, and the induction time of degummed oils were a little shorter than crude oil due to the removal of natural phospholipids which acted as antioxidants. Diacylglycerol and triglyceride analysis results showed that PLC degumming was superior to water degumming in increasing the oil yield and decreasing the neutral oil loss during the degumming process. According to the phospholipids composition analysis of degummed oil gums, it could be concluded that PLC was effective to remove the phosphatidylcholine and phosphatidylethanolamine, while having no activity on phosphatidylinositol or phosphatidic acid.

Introduction
Rapeseed (i.e. Canola) is one of the most widely used oil-bearing seed crop in the world. The double-low rapeseed oil (a kind of rapeseed with low content of erucic acid and sulfur glucoside) contains 51–70% oleic acids, 15–30% linoleic acids and 5–14% linolenic acids according to GB/T 1536-2004. The content of unsaturated fatty acid is over 80%, and the balanced fatty acid composition, stability during cooking and low price make it a valuable part of the food chain and the human diet (List, 2015), it is a kind of popular oil, especially in China.

To get edible oil from most oil-bearing seeds, for example soybean, rapeseed, it is necessary to conduct a series of refining operations, such as degumming, neutralization, decoloration, deodorization etc. (Jiang, Chang, Wang, Jin, & Wang, 2014). The purpose of these refining operations is to remove undesirable impurities that affect the quality (taste, smell and appearance) and shelf life of the edible oils. Various refining methods have been developed, which can be divided into physical refining and chemical refining, the former involves degumming, bleaching and steam distillation, while the latter consists of degumming, neutralization, bleaching and deodorization (Čmolík & Pokorný, 2000; Yu et al., 2013).

Chemical refining is still the most important and generally practiced method in the edible oil refining industries, although it causes a large quantity of industrial waste-water and high neutral oil losses. As we know, physical refining offers many advantages such as improving oil yields, reducing chemicals and water use over chemical refining. Instead of using acid or water in oil degumming process, physical refining can use enzymatic method, which makes use of phospholipase (Clausen, 2001; Jahani, Alizadeh, Pirozifard, & Qudsevali, 2008) and it is more environmentally friendly.

Oil degumming is the initial step in the refining process of crude vegetable oils, and most phospholipids, protein and mucilaginous gums can be removed during this process.
These impurities need to be separated from crude oils because they usually serve as precursors of off-flavors and bring problems to the latter processing procedures of the oil (Jiang, Chang, Jin, & Wang, 2014), therefore, nearly complete removal of the phospholipids is essential for the production of high-quality oil. Phospholipids in oils are usually present in hydratable and nonhydratable forms, by adjusting the pH of reaction system, the nonhydratable phosphatides, including phosphatidic acid and calcium or magnesium salts of lyso phosphatide, can be disintegrated and then removed by centrifugation during degumming process. So far, many traditional degumming processes have been designed to remove these phospholipids and mucilaginous gums (Yang, Zhou, Yang, Wang, & Wang, 2008) which include acid degumming (Pan et al., 2001), water degumming (Indira, Hemavathy, Khatoon, Krishna, & Bhattacharya, 2000), super degumming, total degumming, ultrafiltration degumming (Manjula & Subramanian, 2006; Sengar, Kaushal, Sharma, & Kaur, 2014) etc.

Enzymatic oil degumming is a suitable process for oil physical refining. It was first developed in the 1990s in initial industrial plant trials by the German Lurgi Company, as the 'EnzyMax process' (Yang, Wang, & Yang, 2006), in which a phospholipase was used to convert nonhydratable phospholipids into their hydratable forms, which were then removed by centrifugation. The several necessary steps for this degumming process were as follows: adjusting the pH of the oil with buffer, adding the enzyme solution and carrying out the enzyme reaction and separating the gum/sludge from the oil (Jahani et al., 2008). In comparison with traditional degumming processes, enzymatic degumming can not only achieve the very low phosphatide content required for physical refining, that is, a phosphatide content of less than 15 mg kg\(^{-1}\), but also has advantages of reduced acid use, wastewater generation and operating cost, as well as improved product yield. Thus, enzymatic degumming offers a safe biological route and eco-friendly solution for the industrial process (Jiang et al., 2011).

The phospholipases can be classified into phospholipase \(A_1\) (PLA\(_1\)), phospholipase \(A_2\) (PLA\(_2\)), phospholipase C (PLC) and phospholipase D (PLD) according to their action sites toward phospholipid molecule; the action sites of the different phospholipases were shown in Figure 1. In recent years, researchers have studied enzymatic degumming with different phospholipases; in the case of Lecitase Ultra, the phosphorus contents of rice bran and soybean oils were enzymatically degummed to 6.86 and 4.1 mg/kg after 4.07 and 4 h, respectively (Jahani et al., 2008; Jiang et al., 2014). In the case of Lecitase 10 L, the phosphorus contents of soybean oil was enzymatically degummed to 4.9 mg/kg after 5 h (Jiang et al., 2011). However, from Figure 1, it should be noted that PLA\(_1\) and PLA\(_2\) both catalyze the production of free fatty acids (FFA), and this may affect the quality of degummed oil and the latter oil refining process. PLC does not cause the formation of FFA because it hydrolyzes the bond between the acylglycerol and the phosphate group. Accordingly, it liberates diacylglycerol (DAG), which was recognized as a part of oil and will not be removed and, therefore, will contribute to the refined oil yield (Hitchman, 2009). Whereas, PLC cannot achieve a complete phosphorus removal of vegetable oils as it only catalyzes the splitting off of the phosphate group from phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) (and from phosphatidyl serine, if present) and thus converts these phosphatides into DAG and phosphate esters of choline and ethanolamine, respectively. It has no activity with respect to phosphatidyl inositol (PI) or phosphatidic acid (PA) (Dijkstra, 2010).

In present study, the primary objective was to optimize the PLC and water degumming process by orthogonal array experimental design to find the optimal operating conditions that minimize the residual phosphorus content, respectively. Then the different indexes related to oil process and oil quality were analyzed by testing oil oxidation stability, oil glyceride content and phospholipids composition of two degummed oil gums with the purpose of making a comparison of water and PLC degumming process. All the present work was to help to provide some theoretical foundations for the utilization of PLC in oil degumming and making the oil suitable for physical refining (residual phosphorus < 10 mg/kg), while maintaining the maximal oil yield and not significantly decrease the oil quality.

Materials and methods

Materials

The experimental oils used in present study were kindly supplied by Hubei OKing Star Grain Industry Co., Ltd. (Xiangyang, China); the original phosphorus content of crude rapeseed oil was measured as 690.03 ± 0.38 mg/kg oil. PLC was provided by College of Life Sciences & Technology, Huazhong Agricultural University, and the activity was determined to be 9000 U/g by College of Life Sciences & Technology, Huazhong Agricultural University, and the activity was determined to be 9000 U/g by p-nitrophenylphosphorylcholine method according to Jiang, Chang, Wang et al. (2014) and Yang, Wang, Yang, Mainda and Guo (2006). The PLC was prepared by the laboratory using submerged fermentation of a kind of genetically modified bacillus, which cultivated in the State key laboratory of agricultural microbiology. The reference materials used in TLC-FID (thin layer chromatography/flame ionization detector) analysis were all chromatographically pure (>99%) which purchased in Sigma Chemical Ltd. (St. Louis, MO, USA) and Anpel Laboratory Technologies (Shanghai) Inc.; n-hexane, anhydrous ether and glacial acetic acid were chromatographic grade bought in Honeywell (China) co., LTD (Beijing, China). All

Figure 1. Structure of phospholipids and the various sites of attack of the various phospholipase, where X = H, choline, ethanolamine, inositol etc. The various sites of attack of PLA\(_1\), PLA\(_2\), PLC and PLD are shown with arrows.

Figura 1. Estructura de fosfolipidos y varios lugares de ataque de varias fosfolipasas, donde X = H, colina, etanolamina, inositol, etc. Los varios lugares de ataque de PLA\(_1\), PLA\(_2\), PLC y PLD se muestran con flechas.
other reagents and chemicals were all of analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China), and the water used in present study was prepared by ultrapure water generator (Milli-Q Integral, USA).

**PLC degumming process**

The PLC degumming process was modified according to Jiang, Chang, Jin and Wang (2015): Crude rapeseed oil (100 g) was heated to 45°C in a water bath, as the temperature was maintained at 45°C, 2.4 mL/kg of citric acid solution (500 g/L) was added to achieve a suitable pH (about 5.0); after being homogenized (10,000 rpm) for 1 min, the oil and water mixture was incubated for 20 min at 45°C and 200 rpm in the water-bathing constant temperature vibrator. Whereafter, about 12 μL/kg dilute PLC liquid (the original PLC was diluted to 1000 times for use) was added in, and the mixture was subjected to a high shear rate (15,000 rpm) for 2 min, a suitable amount of water (around 30 mL/kg) was added to the mixture before the enzyme degumming reaction under the conditions that the degumming time was 90 min, the water-bath temperature was 45°C and the oscillation rate was 200 rpm, and then, heated to 95°C for 10 min to inactivate the enzyme. After the reaction, the oil mixture was quickly centrifuged at 10,000 rpm for 10 min (HITACHI, model CR21G). The supernatant was collected and dried by the rotary evaporator at 0.09 MPa, 80°C for oil yield, residual phosphorus and other quality analysis.

**Water degumming process**

The water degumming process of crude oils was modified based on the method of Zhan et al. (2015): 100 g oil sample were stirred (250 rpm) and heated to 70°C, and 3% of hot distilled water was added dropwise. The mixture was then stirred at 70°C for 30 min. The hydrated gum from the hot oil was separated by centrifugation (10,000 rpm, 10 min). Afterward, the top layer of oil was collected and dried by rotary evaporation at 0.09 MPa, 80°C for oil yield, residual phosphorus and other quality analysis.

**Optimization of the PLC degumming conditions**

Since various factors potentially affect the PLC degumming efficiency (the phospholipid removal efficiency is represented as the residual phospholipid content in rapeseed oil), it is necessary to optimize the degumming process conditions to develop a feasible degumming method. In the PLC degumming process, several operating parameters, including citric acid dosage, degumming temperature, amount of water addition and degumming time, were considered to have large impact on PLC degumming efficiency according to the single factor experiments investigated by Zhan et al. (2015); in their study, all operating parameters were tested over a wider range on the basis of single-factor experiments so as to help to narrow down the ranges of the tested parameters. The orthogonal array experimental design L₉ (³) was performed to optimize the four parameters to obtain the minimum phospholipid content of the oil. Factors and levels for the orthogonal test are shown in Table 1, and the orthogonal array design and results for the four variables are presented in Table 2.

**Range analysis of PLC degumming and water degumming process**

$K_i$ and $R_j$ are the two key parameters in range analysis, $K_i$ is defined as the sum of the evaluation indices of all levels $i (i = 1, 2, 3)$ of each factor $j (j = A, B, C, D$ in PLC degumming range analysis, $j = E, F, G, H$ in water degumming range analysis), and $k_i$ (mean value of $K_i$) is used to determine the optimal level and the optimal combination of factors. The lower residual phosphorus content means higher phospholipid removal efficiency, so the optimal level for each factor will get when $k_i$ is smallest. $R_i$, which is defined as the range between the maximum and minimum values of $k_i$, used to evaluate the importance of each factor, that
The significance of each factor was evaluated by calculating the F value. Differences in degumming efficiency were considered significant at $P \leq 0.05$.

**Acid value, peroxide value and oil color determination**

Acid value (AV) of the oil samples was determined in accordance with GB/T 5530-2005. Peroxide value (PV) of the oil samples was determined in accordance with GB/T 5538-2005. The oil color was analyzed in accordance with GB/T 22460-2008, which uses Lovibond method.

**Analysis of phosphorus content**

Phosphorus content analysis was carried out by heating 0.5 g of zinc oxide with 3 g of oil in a porcelain crucible on a gas burner until it becomes a black without smoking, which was then heated at 550–600°C in ceramic fiber muffle furnace (Model TM0910, Beijing Michem Instrumentation Co., Ltd) for 2 h until it turned into off-white ash. The phosphorus content of the ash was determined by the molybdenum blue spectrophotometer method according to GB/T 5537-2008. All experiments were carried out in triplicate for the calculation of the mean value.

**Oxidation stability analysis**

Oxidative stability of the rapeseed oils was analyzed by the Rancimat method using a Metrohm 892 Rancimat (Herisau, Switzerland) instrument, the analysis method was in accordance with GB/T 21121-2007. Samples of 3.5 g were analyzed under a heating block of 120°C at a constant air flow of 20 L/h, and the conductivity range was 0–500 µS/cm.

**Glyceride content assay**

The TLC-FID (Shimadzu, Japan) was used for the quantitative determination of glyceride content of the crude oil and the two degummed oil, respectively. A volume of 0.02 mL oil sample was dissolved in 1 mL of n-hexane in a glass test tube, 1 µL of the diluted sample was pointed on the TLC rod and was spread in the n-hexane/anhydrous ether/glacial acetic acid (55:15:1, v/v/v) solvent system, the sample then was analyzed in the FID apparatus, the analysis conditions were as follows: the hydrogen flow rate (90 mL/min); the air flow rate (2.0 L/min); the scanning speed (30 s/rod).

**Analysis of phospholipids composition**

The phospholipid was first purified by acetone, then TLC-FID method was adopted for the quantitative determination of lyophospholipids. A quantity of 0.30 g purified phospholipid sample was dissolved in 1 mL n-hexane in a glass test tube, 1 µL of the diluted sample was pointed on the TLC rod before it was spread in the chloroform/methyl alcohol/water (65:35:5, v/v/v) solvent system. Afterward, the sample was analyzed in the FID apparatus, the analysis conditions of the TLC-FID were the same as the analysis of glyceride content.

**Experimental design and statistical analysis**

All experiments were performed in triplicate with all data expressed as mean values ± standard deviations of...
independent triplicate experiments. Statistical analysis was performed with SPSS 16.0 software (IBM SPSS software, USA), and all the figures were drawn by using Origin 8.5 software (Origin Lab Ltd., USA). One-way ANOVA was carried out and Tukey adjustment was used to determine the significant difference between treatments. Significant differences were declared at \( P \leq 0.05 \).

Results and discussion
Optimization of PLC degumming and water degumming process

All representative combinations are distributed uniformly in the research area and can greatly reflect the situation of the whole area selected for examination, and orthogonal array experimental design has proven to be a cost-effective optimization strategy that can obtain the optimal conditions of each parameter in a limited number of experimental trials (Mei et al., 2013; Wu et al., 2012).

Optimization of PLC degumming process conditions

In orthogonal array, experimental design of PLC degumming process, experiments with four factors and three levels were employed to assign the considered factors and levels as shown in Table 1. Nine experiments were carried out according to the orthogonal array (Table 2) to complete the optimization process. The data (expressed as mean values \pm standard deviations) in Table 2 were taken as the original data and used in range analysis. The mean values of \( k_{ji} \) (expressed as \( K_\text{ji} \)) for different factors at different levels in the range analysis are shown in Table 5. As is mentioned that the lower mean value (\( k_{ji} \)) indicates the larger effect on phospholipid removal efficiency, therefore, the best level for each factor can be determined according to the highest mean value (\( k_{ji} \)) of the experimental condition. The optimal combination of the PLC degumming operation parameters was clearly distinguished according to Table 5, that is, a citric acid dosage of 6.0 mL/kg, PLC dosage of 11.00 μL/kg, a degumming temperature of 45°C and a degumming time of 120 min, since \( k_{ji} \) was highest with this combination (A,B,C,D). The verification test was conducted under the optimal conditions, and the residual phosphorus content was 7.34 ± 0.39 mg/kg of independent triplicate experiments.

As mentioned before, the range value (\( R \)) indicates the significance of a factor’s effect, the larger \( R \) means the bigger impact of the factor on the phospholipid removal efficiency. Thus, as seen from Table 5, according to the \( R \) values, the influence of different factors on the phospholipid removal efficiency decreases in the order A (citric acid dosage) > B (PLC dosage) > C (degumming temperature) > D (degumming time). The largest range value, \( R_\text{A} \), indicates that a small change in citric acid dosage can produce a significant change in phospholipid removal efficiency of the PLC degumming process. The smallest range value \( R_\text{D} \) indicates that the phospholipid content changes only slightly with a change in degumming time.

ANOVA analysis of PLC degumming process conditions

The significance of each factor was evaluated by calculating the \( F \) value and the results are summarized in Table 7. The factor effect of degumming time is very small and is regarded as the experimental error. As seen from Table 7, under the inspection level (\( \alpha = 0.05 \)), the critical value can be found in the distribution table of \( F \) values: \( F_{0.05}(2, 2) = 19.00 \). It is clear to conclude that \( F_A > F_B > F_C > F_D \), which indicated that factor A (citric acid dosage), factor B (PLC dosage) and factor C (degumming temperature) are the prominent factors affecting the phospholipid content of during the PLC degumming process (at 95% confidence level); however, factor D (degumming time) is not a significant factor affecting the phospholipid removal efficiency (at the range of 60–120 min, at 95% confidence). The value of \( F \) ratio reflects the impact of the factor on the degumming efficiency, so the results are consistent with the range analysis above.

Optimization of water degumming process conditions

Range analysis data of orthogonal experimental results of water degumming was shown in Table 6, from the results, it was clear to get the optimal combination of the water degumming operation parameters, which was a ratio of water to phospholipid to be 3.25, a degumming temperature to be 75°C, and a degumming time to be 4.5 h, since \( k_p \) was highest with this combination (\( E, F, G \)). In the verification test, three independent experiments were conducted under the optimal conditions, and the residual phosphorus content was 61.54 ± 1.57 mg/kg.

As seen from Table 6, from the \( R \) values, it can be concluded that the influence of different factors on the phospholipid removal efficiency in water degumming process decreases in the order \( E \) (ratio of water to phospholipid) > \( F \) (degumming temperature) > \( G \) (degumming time), and the largest range value was \( R_E \), however, the range value of the vacant column was the smallest, which was to say that range analysis results of the three factors were persuasive.

ANOVA analysis of water degumming process conditions

ANOVA of the three factors of water degumming process were shown in Table 8. As seen from Table 8, under the inspection level (\( \alpha = 0.05 \)), the critical value of \( F_{0.05}(2, 2) \) was 19.00, it can be concluded that \( F_B > F_A > F_C \), which indicated factor \( E \), is the key factor affecting the phospholipid content of during the water degumming process (at 95% confidence level); however, factor \( F \) and factor \( G \) are not significant factors affecting the phospholipid removal efficiency (at the range of 75–95°C and 3–6 h respectively, at 95% confidence).

Table 7. ANOVA of four factors of PLC degumming process.

| Factor | Sum of squares | d | F ratio | \( F \) critic value | Significance level |
|--------|----------------|---|--------|----------------------|-------------------|
| A      | 6.40           | 2 | 106.03 | 19.00                | Significant (\( P < 0.05 \)) |
| B      | 3.79           | 2 | 62.81  | 19.00                | Significant (\( P < 0.05 \)) |
| C      | 1.38           | 2 | 22.84  | 19.00                | Significant (\( P < 0.05 \)) |
| D      | 0.06           | 2 | 1.00   | 19.00                | Not significant    |
| Error  | 0.06           | 2 |        |                      |                   |

\( ^a \) A: Citric acid dosage; \( ^b \) B: PLC dosage; \( ^c \) C: degumming temperature; \( ^d \) D: degumming time.

\( ^a \) The \( d_f \) of each factor was defined as: \( d_f = n - 1 \), \( n \) represented the levels of each factor (\( n = 3 \) in present design).

\( ^a \) Dosis de un ácido cítrico; \( ^b \) dosis de PLC; \( ^c \) temperatura de desgomado; \( ^d \) tiempo de desgomado.
Table 8. ANOVA of three factors of the degumming process with water.

| Factor | Sum of squares | df | F ratio | F critic value | Significance level |
|--------|----------------|----|---------|----------------|--------------------|
| E      | 2221.50        | 2  | 25.60   | 19.00          | Significant (P < 0.05) |
| F      | 1635.06        | 2  | 18.84   | 19.00          | Not significant     |
| G      | 614.79         | 2  | 7.08    | 19.00          | Not significant     |
| Error  | 86.77          | 2  |         |                |                    |

**Note:** Different letters in the same row indicate significant differences (P ≤ 0.05).

**Table 9.** Overview of the phosphorus content, FFA, PV and oil color of different rapeseed oils.

| Samples | Phosphorus content (mg/kg) | AV (mg KOH/g) | PV (mmol/kg) | Oil color |
|---------|----------------------------|---------------|--------------|-----------|
| R1      | 690.44 ± 4.12e             | 3.56 ± 0.23b  | 5.80 ± 0.04c | 27.8 ± 0.8d |
| R2      | 0.43 ± 0.07a               | 0.02 ± 0.00a  | 0.83 ± 0.20a | 1.8 ± 0.1a  |
| R3      | 22.65 ± 0.93c              | 0.17 ± 0.03a  | 3.66 ± 0.1b  | 4.7 ± 0.2c  |
| R4      | 61.54 ± 1.57d              | 0.18 ± 0.03a  | 3.47 ± 0.15b | 4.3 ± 0.2c  |
| R5      | 7.34 ± 0.39b               | 0.19 ± 0.04a  | 3.49 ± 0.07b | 3.8 ± 0.1b  |

**Note:** Different letters in the same row indicate significant differences (P ≤ 0.05). (a’ means the lowest value, ‘e’ means the highest value).

**Overview of the phosphorus content, FFA, PV and oil color of different rapeseed oils.**

The phosphorus content, AV, peroxide value and color of different rapeseed oils were shown in Table 9, from the table, it was clear that the phosphorus content of PLC degummed rapeseed oil has a significant difference (P ≤ 0.05) compared with crude rapeseed oil and water degummed oil. It was also easy to know that PLC degumming was more effective means to remove phospholipid, and it could produce degummed oil whose phosphorus content was below 10 mg/kg. Studies have shown that for a successful physical refining, an efficient removal of the phospholipids during the degumming process (phosphorus content < 10 mg/kg) is crucial (Sampaio et al., 2015). There was no significant difference about the AV and peroxide value between the two degummed oils and the national standard fourth grade rapeseed oil. This result was consistent with the fact that PLC degumming would not generate additional FFA during the degumming process, and this is because that PLC only hydrolyzes the bond between the acylglycerol and the phosphate group (Dayton et al., 2015). While the color of PLC degummed oil was better than water degummed oil and fourth grade rapeseed oil. During the formation of phospholipid micelle, the coloring matters could be adsorbed by the phospholipids colloidal particles and then be removed by centrifuge along with phospholipids. For the PLC degumming was more inclined to convert nonhydratable phospholipids into hydratable form, it was more efficient to remove phospholipids than water or acid degumming, and more coloring matters also could be removed, in contrast.

**Oxidation stability analysis**

The oxidation stability analysis of PLC degummed oil, water degummed oil, crude oil and refined oil were conducted by Rancimat, the test results were shown in Figure 2.

From Figure 2, it can be known that the induction time of refined oil, PLC degummed oil, water degummed oil and crude oil were 3.68 ± 0.01 h, 3.84 ± 0.01 h, 4.48 ± 0.10 h and 4.99 ± 0.02 h, respectively. It was clear that the induction time of crude rapeseed oil was the longest among the four oils, while induction time of refined rapeseed oil was the shortest. From the one-way ANOVA analysis results, significant difference about induction time can be observed among the four oils (P ≤ 0.05). The research of Jiang, Chang, Wang et al. (2014) showed that PLC degumming could remove of metal ions in crude oil; it is well known that the removal of metal ions could retard the automatic oxidation of oil and improve its oxidative stability. However, in present study, the oxidative stability of degummed oils showed a slight decrease compared with crude oil, the main reason might be explained by phospholipids acting as natural antioxidants that postpone the rate of oil oxidation (Kashima, Cha, Isoda, Hirano, & Miyazawa, 1991; Martin et al., 2014). And the entrainment loss of some original antioxidants in crude oil during the formation of phospholipid micelle also reduced the oil stability at some extent.

**Diacylglycerol and triglyceride analysis in three oils**

Diacylglycerol, triglyceride and FFA in PLC degummed rape- seed oil, water degummed rapeseed oil and crude rapeseed oil were determined by TLC-FID methods, respectively, the results are shown in Figure 3.

As seen in Figure 3, in present study, diacylglycerol of crude oil, water degummed oil and PLC degummed oil were 3.96% ± 1.63%, 4.02% ± 1.26% and 7.03% ± 1.31%, respectively. The letters above the 3-bar charts of diacylglycerol showed that it had significant difference between PLC
degummed oil and the other two oils \((P \leq 0.05)\), this was due to the fact that PLC hydrolyzed the glycerophosphate key of Sn-3 point in phospholipid molecule (Figure 1) and produce additional diacylglycerol and phosphatidic acid. The phosphatidic acid then was separated by water washing, while the diacylglycerol was recognized as a part of oil and would not be removed, which would increase the yield of refined oil; moreover, the ingestion of diacylglycerol has also been found to have many nutritional and medical functions in human body according to some related studies (Tada et al., 2005).

Figure 2. The induction time of the four kinds of rapeseed oil.

**Figure 2.** El tiempo de inducción de cuatro tipos de aceite de colza.

Note: The letter (a, b, c and d) behind standard derivations of the experimental data above the bars represents the differences among different induction time of the four rapeseed oils; different letters indicate significant differences \((P \leq 0.05)\).  
Nota: La letra (a, b, c y d) detrás de las derivaciones estándar de los datos experimentales sobre las barras representan las diferencias entre los distintos tiempos de inducción de los cuatro aceites de colza; las distintas letras indican diferencias significativas \((P \leq 0.05)\).

Figure 3. Percentage distribution of diacylglycerol and triglyceride of PLC and water degummed rapeseed oil and crude rapeseed oil.

**Figure 3.** La distribución de los porcentajes de diacilgliceroles y triglicéridos del aceite de colza y el aceite crudo de colza desgomados con PLC y con agua.

Note: The letter (a, b, c and d) behind standard derivations of the experimental data above the bars represents the differences among different induction time of the four rapeseed oils; different letters indicate significant differences \((P \leq 0.05)\).  
Nota: La letra (a, b, c y d) detrás de las derivaciones estándar de los datos experimentales sobre las barras representan las diferencias entre los distintos tiempos de inducción de los cuatro aceites de colza; las distintas letras indican diferencias significativas \((P \leq 0.05)\).
The triacylglycerol in PLC degummed oil was the highest among the three oils, this would be explained by the fact that PLC degumming could lead to an overall reduction in mass of gums and recovery of most of this yield loss, while water degumming would result in neutral oil losses (Hitchman, 2009). This also indicated that PLC degumming was superior to water degumming in increasing phospholipid removal efficiency and decreasing the neutral oil losses.

**Analysis of phospholipids composition**

Composition of phospholipids of two different degummed oil gums were analyzed by TLC-FID method, the results were shown in Figure 4.

As seen in Figure 4, in the water degummed oil gum, the content of PC was the highest (33.05%), followed by PI and PE (24.64% and 10.50%, respectively) while the content of PA was lowest (1.90%) due to its nonhydratable property, it could be concluded that the hydrophilic nature of the four phospholipid were PC, PI, PE and PA in decreasing order, these results were consistent with many previous studies (Galhardo & Dayton, 2012). In the PLC degummed oil gum, the content of PC and PE were 41.53% and 36.22%, respectively. However, the content of PI and PA were 5.31% and 4.28%, respectively, according to the double sample variance average inspection results, it showed that there were significant difference about the four phospholipids between the water degummed oil gum and PLC degummed oil gum (P ≤ 0.05). That the high content of PC and PE, while low content of PI and PA could be explained by the specificity of PLC (Galhardo & Dayton, 2012; Mueller, Pascal, Olempska-Beer, Leblanc, & Meyland, 2009), as the content of PC and PE increased in PLC degummed oil gum, the relative content of PA and PI decreased. These results showed that the PLC used in this study could only catalyze the phosphate group from PC and PE while have no activity on PI or PA, and the research of Dijkstra (2010) also supported this result.

**Conclusion**

A relatively completely study of water degumming and PLC degumming was showed in the present work. The degumming efficiency, the composition of degummed gels, the oxidation stability of different oils and the hydrophilic nature of the four phospholipids were all showed and analyzed in present study. One of the most important conclusions showed that PLC employed for rapeseed oil degumming was more effective than water degumming and could reduce the phosphorus content to less than 10 mg/kg that suitable for oil physical refining. Meanwhile, the PLC degumming process was found to be superior to water degumming for that it not only could be more effective to remove phospholipids, but also could decrease the neutral oil losses, which had been be proven by previous studies (Barton, 2008; Jiang, Chang, Wang et al., 2014). However, the removal of natural phospholipids in crude rapeseed oil might lead to the decrease of oil oxidative stability, and this result could be helpful for the storage of degummed oil in industrial scale and remind the producers to conduct the subsequent process of deacidification, decoloration and deodorization in a reasonable time. The hydrophilic nature of PC, PI, PE and PA were confirmed according to the phospholipids composition analysis after degumming process, the hydrophilic characters of different phospholipids could also provide a basis for the separation and utilization of phospholipids.

![Figure 4. Percentage distribution of phospholipids gums content.](image-url)

**Figure 4.** Percentage distribution of phospholipids gums content.

**Figura 4.** Distribución de los porcentajes de contenido de gomas de fosfolípidos.

Note: The "*" behind standard derivations of the experimental data above the bars represents there were significant differences between the two groups ("*" , "**" and "***" represent the significance under P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001, respectively).

Nota: La "*" detrás de las derivaciones estándar de los datos experimentales sobre las barras representan diferencias significativas entre los dos grupos ("*" , "**" y "***" representan la significancia con P ≤ 0.05, P ≤ 0.01 y P ≤ 0.001, respectivamente).
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Disclosure statement
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Compliance with ethics requirements
This article does not contain any studies with human or animal subjects.

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