Optimization of Enzymatic Degumming Process for Sunflower Oil using Response Surface Methodology: Efficiency and Oil Yield

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Abstract

Response surface methodology was used to study the optimum processing conditions for enzymatic degumming of sunflower oil. Degumming assays were performed using a phospholipase A1 (Lecitase® Ultra) and an acyltransferase (LysoMax® Oil). A 2^{k-1} fractional factorial design was carried out to simultaneously study the effects of pH, temperature, enzyme concentration, buffer/substrate ratio and time on the phosphorus content. For the response obtained, a polynomial model was developed through multiple linear regression analysis. Lecitase® Ultra was affected by all studied factors while the buffer/substrate ratio did not significantly influence the performance of LysoMax® Oil.

The optimum conditions to achieve the lowest phosphorus content were reached at 50 °C, pH 5 and an enzyme dosage of 200 U/kg of oil during 90 minutes using Lecitase® Ultra and 59 °C, pH 5.9 and an enzyme dosage of 197 U/kg of oil during 89 minutes using LysoMax® Oil. Under optimal conditions, the phosphorus content decreased to less than 3 mg/kg with both enzymes. The oil yield was 96.80 g/100g and 96.85 g/100g with the treatment using phospholipase A1 and acyltransferase respectively.

Keywords: Enzymatic Degumming, Experimental Design, Response Surface Methodology, Oil Yield, Optimization.

Introduction

One area in which the development of biotechnology has had a major impact is the application of enzymatic technology to industrial processes as an alternative based on efficient and secure methodologies with minimum environmental damage.

Degumming is the first step in the refining process of vegetables oils and it removes phospholipids and mucilaginous gums that affect quality and storability. Among the types of degumming processes, enzymatic method is probably the best process available for reducing the phosphorus content of vegetable oils below 10 mg/kg.[5]

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Some enzymatic degumming processes have been suggested on laboratory and pilot plant scales.[6,7,26,27]

The different enzymes that are commercially available for processing vegetable oils vary in the way they act on phospholipids.[11] The phospholipase A1 (PLA1) and phospholipase A2 (PLA2) remove the fatty acid from positions 1 and 2 with respect to glycerol, the phospholipase C (PLC) catalyzes the hydrolysis of phosphate-glycerol bond in phosphatidylcholine and phosphatidylethanolamine and the lipid acyltransferase (LAT) and transfers a fatty acid to a sterol present in the oil in order to convert it into a sterol ester. All enzymes lead to lower oil retention by the gums, which contributes to an improved oil yield.[8,10,11]

Oil degumming standard laboratory test comprises treating with a buffer solution to achieve the desired pH, regulates the reaction temperature and additional dosage of the suitable enzyme.[6] Therefore, enzymatic degumming process parameters need to be optimized for each oil and enzyme type to achieve low phosphorus content and minimal oil loss. To optimize processes, it is necessary to identify the variables that significantly influence the system under study and how they affect. One of the popular used methods is the one-factor at a time. However, this method requires many experiments and it is difficult to consider interactions between variables. Regarding to this, the design of experiments allows simultaneously studying the effects of all factors of interest in a given process and the possible interaction between them.[21] Moreover, response surface methodology has shown to be an interesting tool for the optimization of degumming process.[1,13,14,20,26]

Initially research works on enzymatic degumming focused on rapeseed, rice bran and soybean oils.[6,23,26-28] On the other hand, the effect of enzymatic degumming on the physicochemical characteristics of sunflower oil has previously been reported.[17,18] However, the optimization of the necessary parameters to degum using sunflower oil as raw material has not been reported.

The objective of this work has been to study the effect of pH, temperature, enzyme dosage, time and buffer/substrate ratio of enzymatic degumming of sunflower oil using phospholipase A1 and acyltransferase. A statistical experimental design and response surface methodology analysis was employed to find the optimum operating

conditions that minimize the residual phosphorus content. The oil yield of the degummed oil samples was evaluated in order to assess its efficiency and chemical properties and compare it with water degumming.

Material and Methods

Materials: All reagents used were of analytical reagent grade. Citrate buffer (pH 5.0 and pH 6.0) was prepared by mixing citric acid solution (0.1 M) and sodium hydroxide solution (0.1 M), both made with twice-distilled water stock solutions achieve to yield a desired pH value in each case.

The crude sunflower oil extracted by hexane was provided by OMSHA, Argentina. The oil was stored in amber vessel protected from oxygen at 5°C until used. Lecitase® Ultra an acidic phospholipase A1, (EC 3.1.1.32) from *Thermomyces lanuginosus* expressed in *Aspergillus oryzae* was acquired from Novozymes (Bagsvaerd, Denmark) and LysoMax® Oil, a microbial lipid acyltransferase (EC 2.3.1.43) was provided by Danisco and Genencor (Arroyito, Córdoba, Argentina).

Oil degumming assay system: The assay system consisted of a jacketed reactor fitted with lid, a propeller stirrer and a thermometer. The reactor was connected to a water bath with water pump and a flexible tube. Sunflower oil (about 100 g) was loaded in the reactor which was kept at about the temperature needed for the specific experiment.

The buffer and enzyme solutions were added, the mixture was stirred with mechanical mixer to provide a safe large surface area through emulsification. The stop reaction was carried out during 30 min at 100 ºC. To recover oil and water phases, a centrifuged step was applied (10 min at 2400 *x g*).

For water degumming process, 100 g of sunflower oil was placed into the same reactor. The temperature was heated at 65° C and 3mL/100g of distilled water was added. The mixture was kept under stirring during the process. Then it was centrifuged (10 min at 2400 *x g*) to separate the gums to yield water degummed oil.

Experimental design- Optimized and validated procedures: Response surface methodology was performed to optimize the process of enzymatic oil degumming. A 2^{k-1} fractional factorial design k=5 with two replicates using Design Expert 7.0 software (Stat-Ease, Inc.) was used to screen significant factors with respect to their effects on the operating condition of enzymatic degumming process. The five selected factors were pH (A), temperature (B), enzyme dosage (C), time (D) and buffer/substrate ratio (E). The coded and uncoded (actual) levels of the independent variables are given in table 1.

The input variables lower and upper limits were specified together with phosphorus content as the response variable to generate the experimental matrix. Sixteen treatments were established using a computer simulation and they were carried out under homogenous conditions while the experimental sequence was randomizing in order to minimize the effects of uncontrollable factors.

An Analysis of Variance (ANOVA) was conducted to determine the significant effects of process variables on the response. By applying multiple regression analysis to the experimental data, a polynomial equation was generated representing the phosphorus content in the oil as a function of variables.

In the graphical optimization, the response models were visualized as three dimension surface plots to better understand the relation and interaction effects of independent variables. In the numerical optimization, the exact optimum level of independent variables was achieved by applying response optimizer. Desirability functions were used to the fitting model optimization by minimizing the phosphorus contents.

The adequacy and efficiency of predicted response surface models were verified by comparing experimental data and predicted ones. In order to check the validity of the models obtained, additional experiments were examined under optimal conditions. These enzymatic processes were compared with the water degumming process.

Table 1
Selected factors and levels for the experimental design applied to the enzymatic degumming of sunflower oil.

FACTORS	LEVELS	
A. pH	5 (-1)	6 (+1)
B. Temperature (°C)	50 (-1)	60 (+1)
C. Enzyme concentration (U/kg of oil)	100 (-1)	200 (+1)
D. Time (min)	30 (-1)	90 (+1)
E. Ratio Buffer/Substrate (mL/100g v/m)	1 (-1)	3 (+1)
Response: Phosphorus content (mg/kg)		

Numbers in parentheses are coded factors

Analytical Methodology: To characterize the crude and degummed oil samples, phosphorus content was measured by Standard Official Method Ca 12-55.[2] The sample is calcined in the presence of zinc oxide. Moisture and volatile matter were determined using Ca 2d-25 method[2], the content of insoluble impurities was evaluated by Ca 3a-46 method using hexane as solvent[2] and the acid value was measured by Ca 5a-40 method.[2]

To evaluate the efficiency of enzymatic degumming processes, the residual phosphorus content in crude and degummed oil optimized samples was used. The efficiency of each degumming process was estimated based on its ability to reduce the phosphorus content using the following equation:

$$\text{Efficiency} = (\text{Pi-Pr}) / \text{Pi} \quad (1)$$

where Pi = initial phosphorus content of the crude oil (mg / kg) and Pr = actual content of phosphorus in degummed oils (mg / kg).

In order to calculate the yield of each degumming process, the percentage phosphorus content reduction was calculated using the following equation:

$$\text{Yield process (g/100g)} = \text{Efficiency} \times 100 \quad (2)$$

The oil yield of each degumming processes was experimentally obtained by measuring the recovery of degummed oil. The crude oil was weighed before the treatment into a container. After the degumming and centrifugation processes, the upper oil phase was weighed. The degummed oil recovery was calculated using the following equation:

$$\text{Oil yield (\%)} = (\text{m2} / \text{m1}) \times 100 \quad (3)$$

where m1 = mass of crude oil sample and m2 = mass of degummed oil.

All experiments and measures were performed in duplicate. Duplicate determinations were per independent replicate of degumming treatments. The results were expressed as mean value ± standard deviation. The differences in mean values between samples were assessed with Student's t-test, being statistically different at significance level of 5 %.

Results and Discussion

Fitting model for Lecitase® Ultra degumming assays*:* For trials performed with Lecitase® Ultra, the phosphorus content ranged between 2.86 ± 0.32 and 19.27 ± 0.54 mg/kg (Table 2). Even though the elemental phosphorus content does not specify the quantities of each of the different phospholipids present in the vegetable oil, it is known historically as a good indicator of the presence of phospholipids and its contents allow to the estimation of the total phospholipid concentration.

The ANOVA and the regression coefficients are shown in table 3 for phosphorus content as response function. Obtained data did not require transformation for statistical analysis. ANOVA results indicate that the model was significant ($p < 0.0001$) and phosphorus content was significantly influenced by pH (A), temperature (B), enzyme concentration (C), time (D), buffer/substrate relation (E) and interactions between two factors: AB, AC, AD, AE, BC, CD and DE, being the p value < 0.0001 in all cases. BE, BD and CE interactions were not significant.

By applying multiple regression analysis to the experimental data, the following equation model was generated. It represents the phosphorus content in the oil as a function of significant variables in terms of actual factors:

$$Phosphorus\ \left(\frac{mg}{kg}\right) = -110 + 37.0 \times A + 2.78 \times B - 3.22 \times 10^{-1} \times C - 1.93 \times 10^{-1} \times D - 25.0 \times E - 7.65 \times 10^{-1} \times A \times B - 4.02 \times 10^{-2} \times A \times C + 4.87 \times 10^{-2} \times A \times D + 3.45 \times A \times E + 1.07 \times 10^{-2} \times B \times C\ 1.07 \times 10^{-2} \times B \times D + 3.71 \times 10^{-2} \times B \times E - 1.02 \times 10^{-2} \times C \times D + 3.86 \times 10^{-2} \times C \times E + 4.29 \times 10^{-2} \times D \times E$$

As observed in table 3, predicted R^2 comparable to adjusted R^2, low PRESS and adequacy precision higher than 4, show that the model fitted is adequate to predict the phosphorus content in degummed oils by Lecitase® Ultra.

Fitting model for LysoMax® Oil degumming assays: Table 2 shows the levels for each factor and the result of the response study for degummed oils by LysoMax® Oil. The matrix test is generated by Design Expert and the response is the phosphorus content obtained experimentally. The phosphorus content ranged from 2.61 ± 0.04 and 17.90 ± 0.08 mg/kg.

The coefficients of the predictive model obtained to study of enzymatic degumming reaction with acyltransferase enzyme were analyzed by ANOVA (Table 5). This predictive model is the result of analysis of the significant effects of each factor and the interactions between them. Results of ANOVA indicate that the model was significant ($p < 0.0001$) and phosphorus content was significantly influenced by the pH, temperature, enzyme concentration, time and by interactions between two factors: AB, AC, AD, AE, BC, BD, BE, CD, CE and DE being p value < 0.0001 in all cases.

The buffer/substrate ratio (factor E) expressed in mL/100g v/m was the only factor that resulted insignificant. For this factor, the p-value was 0.1520 (Table 3). The buffer provides the aqueous phase for these enzymatic reactions. The non-significant influence of the buffer/substrate ratio for the reaction catalyzed by LysoMax® Oil may be due to the acyltransferase primary activity of this preparation. Lecitase® Ultra has principally hydrolase activity and requires water as substrate for its catalytic activity being the reaction significantly influenced by the buffer/substrate ratio.

Table 2
Phosphorus contents for the different combinations of experimental conditions in the sunflower oil degummed

FACTORS					Phosphorus*(mg/kg)	
A	**B**	**C**	**D**	**E**	**Lecitase® Ultra**	**LysoMax® Oil**
6	50	200	90	1	3.98 ± 0.03	2.72 ± 0.04
6	60	100	30	3	8.20 ± 0.11	8.14 ± 0.08
6	60	100	90	1	7.48 ± 0.08	3.80 ± 0.17
5	50	100	90	1	13.12 ± 0.05	7.77 ± 0.15
5	50	200	30	1	15.55 ± 0.12	8.70 ± 0.06
5	60	100	30	3	17.01 ± 0.24	17.07 ± 0.21
6	50	200	30	3	9.99 ± 0.31	11.90 ± 0.11
5	50	100	30	3	8.14 ± 0.50	11.40 ± 0.17
5	60	200	30	1	19.27 ± 0.54	13.10 ± 0.12
5	50	200	90	1	2.86 ± 0.32	17.90 ± 0.08
6	50	100	30	3	16.30 ± 0.49	3.51 ± 0.10
6	60	200	90	3	11.81 ± 0.33	8.31 ± 0.04
5	60	100	90	3	10.14 ± 0.43	7.49 ± 0.03
5	60	200	90	1	15.91 ± 0.12	2.61± 0.04
6	50	100	90	3	19.11 ± 0.58	13.1 ± 0.10
6	60	200	30	1	14.24 ± 0.41	6.13 ± 0.06

* Mean values ± standard deviation (n = 2)

Table 3
p-Values (ANOVA) and regression coefficients estimated by the factorial model

Source	Lecitase® Ultra*		LysoMax® Oil**	
	p-value	**CE ± CI**	**p-value**	**CE ± CI**
Model	< 0.0001		< 0.0001	
Intercept		12.6 ± 0.27		8.98 ± 0.08
Principal Factors				
A. pH	< 0.0001	-0.65± 0.27	< 0.0001	-0.58 ± 0.08
B. Temperature	< 0.0001	0.97± 0.27	< 0.0001	-0.54 ± 0.08
C. Enzyme dosage	< 0.0001	-0.39 ± 0.27	< 0.0001	-1.97 ± 0.08
D. Time	< 0.0001	-1.48 ± 0.27	< 0.0001	-2.81 ± 0.08
E. Buffer/substrate ratio	< 0.0001	-0.88 ± 0.27	0.1520	0.028 ± 0.08
Interactions				
AB	< 0.0001	-1.91 ± 0.27	< 0.0001	-2.47 ± 0.08
AC	< 0.0001	-1.00 ± 0.27	< 0.0001	-0.38 ± 0.08
AD	< 0.0001	0.73 ± 0.27	< 0.0001	0.20 ± 0.08
AE	< 0.0001	1.72 ± 0.27	< 0.0001	0.73 ± 0.08
BC	< 0.0001	2.68 ± 0.27	< 0.0001	1.08 ± 0.08
BD	0.0328	-0.15 ± 0.27	< 0.0001	0.15 ± 0.08
BE	0.0117	0.19 ± 0.27	< 0.0001	-0.21 ± 0.08
CD	< 0.0001	-1.54 ± 0.27	< 0.0001	-0.12 ± 0.08
CE	0.0092	0.19 ± 0.27	< 0.0001	0.72 ± 0.08
DE	< 0.0001	1.29 ± 0.27	< 0.0001	0.69 ± 0.08

CE= Coefficient Estimate in terms of coded factors, CI= Confidence Interval (95%)
*R2=0.9972, Adjusted R2=0.9945, Predicted R2=0.9886, Adequacy Precision=62.869, PRESS = 8.72
**R2=0.9997, Adjusted R2=0.9995, Predicted R2=0.9990, Adequacy Precision= 204.502, PRESS = 0.72

Table 4
Characterization of sunflower oils, crude and degummed by enzymes under optimal conditions to minimize the phosphorus contents according to experimental design

Sample	Moisture and volatile matter (g/100 g)	Acid value (mg KOH/g)	Hexane insoluble (g/100 g)	Phosphorus (mg/kg)
Crude oil	0.0810 ±0.007[a]	2.20 ± 0.030[b]	0.087 ± 0.002[a]	487 ± 0.003[c]
Degummed oil by Lecitase® Ultra	0.1975 ± 0.005[c]	4.97 ± 0.025[c]	0.046 ± 0.001	2.42 ± 0.002[b]
Degummed oil by LysoMax® Oil	0.1605 ± 0.006[b]	1.65 ± 0.020[a]	0.047 ± 0.001	2.30 ± 0.004[a]

Results are mean values ± standard deviation. (n = 2).
The means within a column followed by the same letter are not significantly different ($\alpha = 0.05$)

Applying multiple regression analysis to the experimental data, an equation model that represents the phosphorus content in the oil as a function of significant variables was generated. The fitted model, expressed in coded significant variables, is represented by:

$$Phosphorus\ \left(\frac{mg}{kg}\right) = -217 + 51.7 \times A + 4.70 \times B - 2.17 \times 10^{-1} \times C - 2.55 \times 10^{-1} \times D - 9.26 \times E - 9.87 \times 10^{-1} \times A \times B - 1.52 \times 10^{-2} \times A \times C + 1.34 \times 10^{-2} \times A \times D + 1.46 \times A \times E + 4.31 \times 10^{-3} \times B \times C + 9.71 \times 10^{-4} \times B \times D - 4.20 \times 10^{-2} \times B \times E - 7.96 \times 10^{-5} \times C \times D + 1.45 \times 10^{-2} \times C \times E + 2.31 \times 10^{-2} \times D \times E$$

The high fitting goodness of the proposed model for the enzymatic degumming assay of sunflower oil using LysoMax® Oil is fundamentally based on predicted R^2 - adjusted $R^2 < 0.2$ (absolute value) in a reasonable agreement and adequacy precision > 4.

Optimization for Lecitase® Ultra degumming assays: The numerical optimization indicates the minimum phosphorus content predicted by the model (2.86 mg/kg) obtained at 50 °C, pH 5 during 90 minutes using a 200 U/kg enzyme dosage, 3mL/100g buffer/substrate ratio for degumming oil by Lecitase® Ultra. These results indicate the high efficiency of the enzyme to achieve low phosphorus contents.

Figure 1 shows the negative effect of increase temperature and pH on residual phosphorus content. Higher values of pH and temperature reduce the removal of phosphorus from oil. Increasing of phosphorus at temperatures over 50°C can be attributed to partial denaturation of the enzyme and loss of its hydrolytic activity.[13]

Thus, the increase in pH values above the optimal value of the enzyme (5) results in higher phosphorus content. Lecitase® Ultra is an acidic lipase that exhibits maximal activity at pH 5.0. Its phospholipase activity predominates when the temperature is over 40 °C and its maximum is exhibited at 50 °C.[26]

Among the individual variables, reaction time has the maximum effect on phosphorus content. Marrakchi et al[20] using a 2^{7-4} fractional experimental design in acid degumming of olive oil found that time was an influence main factor. This parameter usually depends on oil type. Jiang et al[15] reported that using this enzyme needs 3 h to reduce the phosphorus content below 10 mg/kg in rice bran oil, but in soybean and camellia oil, these conditions were not enough. During enzymatic treatments, the time interacts with enzyme dosage. As shown in figure 2, the phosphorus content decreased by increasing reaction time and enzyme dosage.

Manjula et al[19] reported that with an enzyme dosage of 2520 U/kg oil, the rice brain oil reduces its phosphorus content from 390 to 10 mg/kg. In addition, the authors concluded that increase in enzyme dosage beyond 2520 U/kg did not improve the degumming efficiency. Jahani et al[13] optimized enzyme dosage of Lecitase® Ultra as 480 U/kg oil for enzymatic degumming of rice bran oil and reported a reduction in phosphorus content from 197 to 10 mg/kg. The phosphorus content of the crude soybean oil was reduced to less than 10 mg/kg after 120 min time using 30 mg/kg of enzyme dosage.[25]

Jahani et al[13] reported that in the case of rice bran oil, applying desirability function method, the optimal operating conditions were reaction time of 4.07 h, enzyme dosage of 50 mg/kg and added water of 1.5 mL/100 g. At this optimal point, phosphorus content of degummed oil was 8.86 mg/kg. Therefore, time and the enzyme dosage may vary depending on the initial phosphorus content and type of oil besides the type of phospholipase and the enzyme assay conditions.

Optimization for LysoMax® Oil degumming assays: There are several numerical solutions for the optimization of the minimum required phosphorus, all of them are at maximum concentration of enzyme and higher reaction time. The desirability function for all solutions was 1.

The minimum phosphorus content predicted by the model (about 2.61 mg/kg) for LysoMax® Oil degummed oil was determined as 90 minutes for reaction time, 190 mg/kg for enzyme dosage, pH 5.2, 54 °C of temperature and 1.5 mL/100g buffer/substrate ratio. Other optimal solution was 90 minutes for reaction time, 197 U/kg for enzyme dosage, pH 5.9, 59 °C of temperature and 1.5 ml/100g buffer/substrate ratio according to numerical parameters.

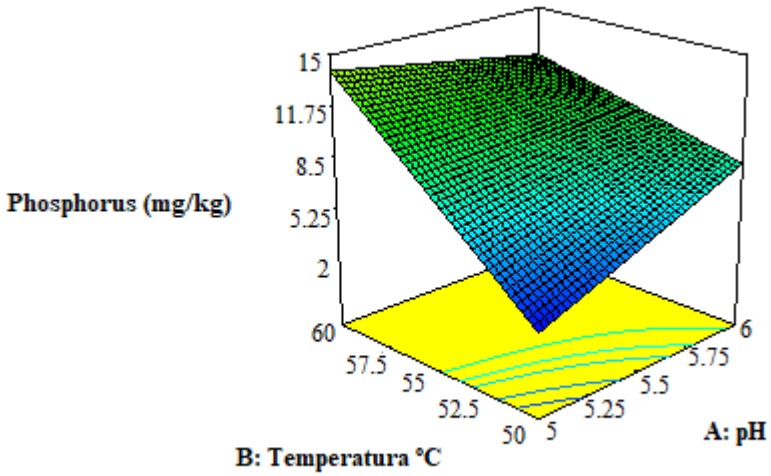

Figure 1: Measures of the change in phosphorus content and its regressed surface as a function of Temperature (°C) and pH for degumming oil by Lecitase® Ultra. Enzyme dosage = 200 U/kg of oil, time = 90 min, buffer/substrate ratio (mL/100g v/m) = 3

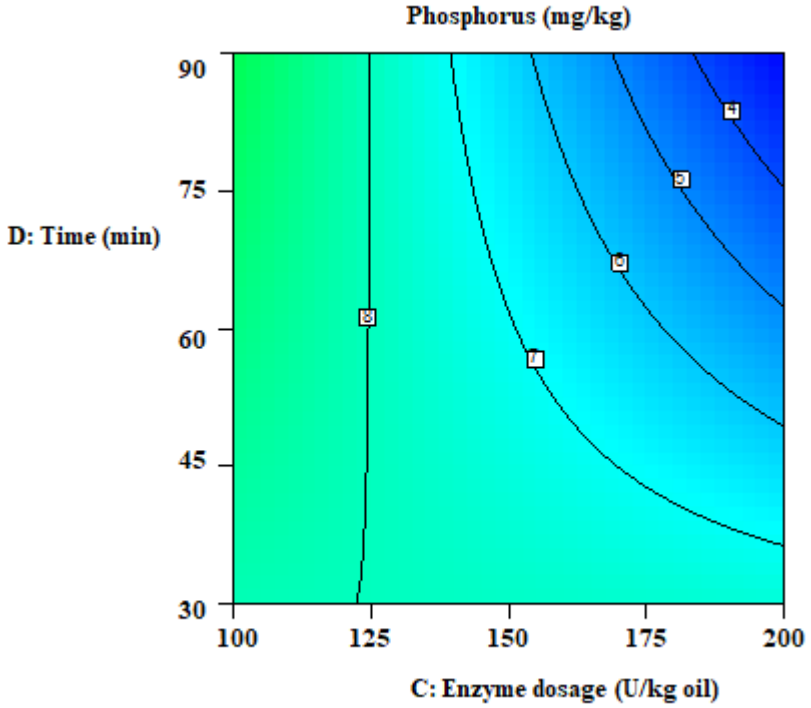

Figure 2: Contour diagram for degumming oil by Lecitase® Ultra as a function of Time (min) and Enzyme dosage (U/kg of oil). pH 5, Temperature = 50 °C, buffer/substrate ratio (mL/100g v/m) = 3

A further advantage of lipid-acyltransferase enzymatic process is that pH does not need to be adjusted. In the water degumming process, it can work in the range of pH from 5.0-5.5 to about 6.5-7.0. This pH results in a high reactivity of the lipid acyltransferase.[24] Figure 3 shows the effect of temperature and pH on the residual phosphorus content. When both factors are at higher or lower end coded value, the phosphorus content achieves the lowest level. However, acyltransferase works fine in all the range of pH and temperature selected in this work.

In the same way as for Lecitase® Ultra, among the individual variables, reaction time has the maximum effect on phosphorus content and it decreases by increasing reaction time. As shown in figure 4, the phosphorus content decreased by increasing reaction time and enzyme dosage.

Compared with others works, time and the enzyme dosage may vary depending on the initial phosphorus content and type of oil besides the type of enzyme.[13,15,19] All the main effects were negative contributions within the model. When

one of these main factors increases in the range of the study, the phosphorus content of the degummed oils decreased.

Crude and degumming oil samples: Table 4 summarizes characterization results of crude, water degummed and enzymatic degummed oil samples under optimal conditions. The moisture content of the enzyme and water degummed oil samples is higher than that of crude oil. There are no significant differences between the moisture content of water and phospholipase A1 degummed oil samples. Both treatments used 3mL/100g aqueous solution/substrate ratio. Therefore, the source of this variability could be due to the addition of the aqueous solution amount.

The acidity of the crude oil is consistent with the values detected in a previous study for this type of oils[3]. The increase in acid value from 1.09 to 2.00 g of oleic acid/100 g in degummed oil by Lecitase® Ultra was due to the fatty acids released during enzymatic hydrolysis of the phospholipids. However, obtained free acidity does not represent drawback for its reduction during the next steps of the refining process. Similarly, Yang et al[26] reported that enzymatic degumming process using phospholipase A1 in rapeseed and soybean oils caused an increase (about 0.15 g/100g) of free acidity in the degummed oils.

For enzymatic degumming testing using LysoMax® Oil, it was observed a slight decrease of acid value compared to crude oil. This enzyme can extract a fatty acid fraction of a phosphatide and transfer it to a present sterol in the oil to become a sterol ester.[9] Subsidiary transacylase activities involving the transfer of a free fatty acid to a lyso-phospholipid in order to form a new phospholipid have been suggested for this enzyme.[12,16]

These activities could be responsible for the decrease in acidity content observed. Water degummed revealed a slight decrease of acid value compared with crude oil. This is in accordance with previous work in sunflower oil extracted with hexane that informed a reduced from 1.60 to 1.35 g of oleic acid/100 g after water degumming[3].

The content of hexane insoluble impurities was significantly reduced in all processes. These impurities are non-lipid nature substances that could be entrained more efficiently in the gum phase and removed from oil phase during the centrifugation step.

The average phosphorus content value of crude sunflower oil (487.1 ± 9.8 mg/kg) was in accordance with the range from 342 to 657 mg/kg measured in crude oils extracted by hexane produced in the same processing plant.[4] The experimental phosphorus content of the degummed oil obtained using Lecitase® Ultra and LysoMax® Oil, was less than predicted by the model. Under these conditions, it was possible to achieve phosphorus contents less than 3 mg/kg of oil.

These results are consistent with the values detected in previous of sunflower oil using the same enzymes under similar process conditions.[17] These findings were also informed for other authors who worked with both enzymes using other type of oil.[9,11,24,26] Values of 3 mg/kg of residual phosphorus are generally low enough for the physical refining process.[26]

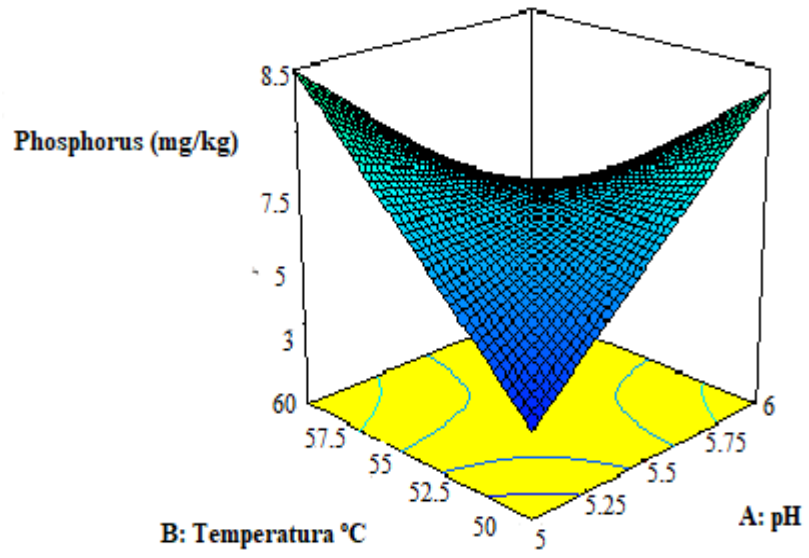

Figure 3: Measures of the change in phosphorus content and its regressed surface as a function of Temperature (°C) and pH for degumming oil by LysoMax® Oil. Enzyme dosage = 197 U/kg of oil, time = 89 min, buffer/substrate ratio (mL/100g v/m) = 1.5

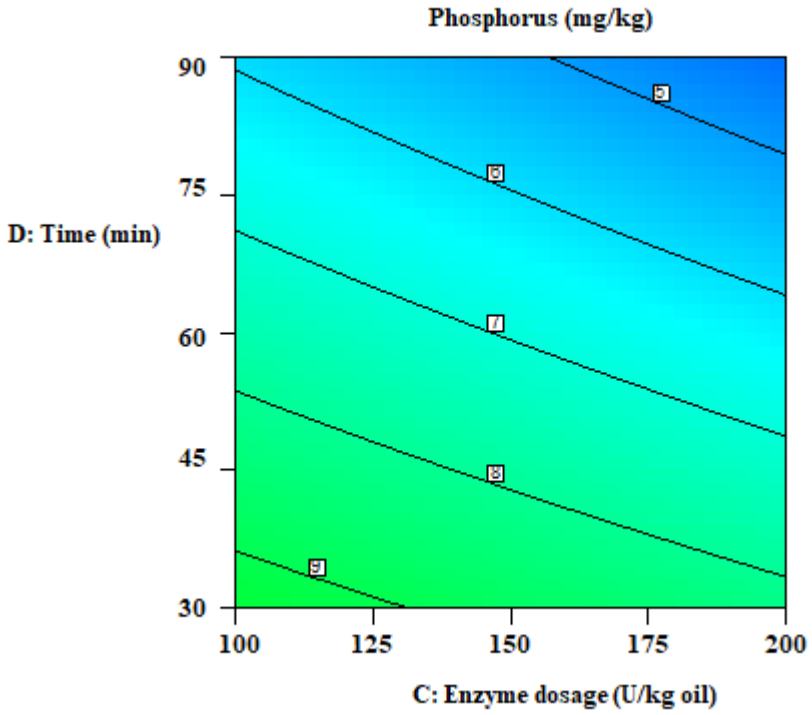

Figure 4: Contour diagram for degumming oil by LysoMax® Oil as a function of Time (min) and Enzyme dosage (U/kg of oil). pH 5.9, Temperature = 59 °C, buffer/substrate ratio (mL/100g v/m) = 1.5

Table 5
Yield reaction and oil yield of water and enzymatic degummed samples under optimal conditions

Sample	Yield reaction (g/100g)	Oil yield(g/100g)
Degummed oil by Lecitase® Ultra[1]	99.50 ± 0.02[b]	96.80 ± 0.16[b]
Degummed oil by LysoMax® Oil[2]	99.54 ± 0.01[b]	96.85 ± 0.25[b]
Water degummed oil[3]	81.23 ± 1.44[a]	93.75 ± 0.35[a]

Results are mean values ± standard deviation of 2 replicates analyzed by duplicate
The means within a column followed by the same letter are not significantly different (test t, $\alpha = 0.05$)
("a" means the lowest value, "b" means the highest value)
[1] pH = 5, temperature = 50 °C, enzyme dosage = 200 U/kg, time = 90 min, buffer/substrate ratio = 3mL/100g v/m
[2] pH = 5.9, temperature = 59 °C, enzyme dosage = 197 U/kg, time = 89 min, buffer/substrate ratio = 1.5mL/100g v/m
[3] temperature = 60 °C, time = 90 min, water/substrate ratio = 3mL/100g v/m

The residual phosphorus content after 90 min of water degumming was 91.38 mg/kg. During the water process, the hydratable phospholipids are removed and the phospholipids remained after treatment can be considered as non-hydratable.[29] The phosphorus content of crude rice bran oil was reduced from 390 mg/kg to 128 mg/kg with 5h of water degumming treatment.[19] Jiang et al[15] found that after water degumming, the phosphorus of soybean, rapeseed oil and camellia oil samples was above 10 mg/kg. These results are in accordance with the present work and suggested that water degumming is not effectively in order to degum sunflower oil for physical refining.

Efficiency of reaction and oil yield: The efficiency of reaction and oil yield of degumming processes are listed in table 5. Compared to the water degumming process, higher efficiency was observed by using enzymes treatments. The reaction yield was higher than 99 g/100g using both enzymes. The results of oil yield showed that there would be approximately 6.75g/100g of oil loss during the water degumming process and less oil loss was found in enzymatic degumming samples. The oil loss in water process can be attributed to the non-hydratable phospholipids, which would be retained and separated with the gums phase. Several authors reported high yield for enzymatic oil degumming.

Jiang et al[15] reported a high oil yield in soybean, camellia and rapeseed oil being 97.14g/100g, 96.80g/100g and 96.42 g/100g respectively using phospholipase A1. Münch[22] using the same enzyme for soybean, sunflower and rapeseed referred a high oil yield without quantifying it. Soe and Brown[24] revealed that the yield of soybean oil can be increased 2 g/100g, using the acyltransferase compared with

the same oil treated by water process. These results are consistent with the values obtained in this work.

Conclusion

The optimal conditions to achieve the minimum residuals phosphorus content in the oil were determined. Time was the most significant variable on enzymatic degumming process for both enzymes. Lecitase® Ultra is affected by all factors studied. LysoMax® Oil is not significantly affected by the buffer/substrate ratio and requires less water in the reaction medium to have high reactivity. The reaction with acyltransferase showed minimal phosphorus contents practically in all evaluated ranges of pH and temperature indicating that these factors need not be controlled.

Considering only the main factors, the phospholipase A1 enzyme requires lower temperatures while the acyltransferase enzyme works better at higher temperatures. Under optimal conditions, degumming process with both enzymes efficiently decreases the phosphorus content in crude sunflower oil below 3 mg/kg, which is comparable with predicted response value by the proposed models. This low phosphorus content guarantees good quality oil suitable for physical refining. The results suggest that design expert combined with the response surface modeling has been shown to be effective in determining the optimum conditions for enzymatic degumming of sunflower oil. Compared to the water degumming process, higher efficiency and oil yield were observed by using enzymes. The results revealed that both enzymes are a good prospect for degumming sunflower oil. It is feasible to standardize reaction conditions using any of the two enzymes in the same oil refining plant.

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