Soybean Oil Degumming by Immobilized Phospholipase A_1

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ABSTRACT: *In the present study, we investigated the ability of an immobilized phospholipase A_1 (PLA_1) in degumming of soybean oil. The enzyme was immobilized by simple adsorption on bentonite without any further modification. The free and immobilized PLA_1 were characterized by Fourier Transform InfraRed (FT-IR) spectroscopy and X-Ray Diffraction (XRD). The immobilization of PLA_1 lowered the Energy of Activation (EA) from 155.64 to 27.13 kJ/mol, resulting in higher catalytic efficiency of PLA_1. Thermal stability of the immobilized enzyme was found to be higher compared to free PLA_1. Moreover, under the condition of pH 5.5 and T = 50 °C, the phosphorus content was reduced to less than 10 ppm after 4-5 h for free PLA_1 and after 7 h for immobilized PLA_1.*

KEYWORDS: *Enzymatic degumming; Immobilization; Bentonite; Phospholipase; Soybean oil.*

INTRODUCTION

During dietary crude oil refining, the degumming process is a preliminary important step in the treatment of crude vegetable oil [1]. This process removes phosphatides by converting them to hydrated gums, which are then easily separated from the oil by centrifugation [1]. Enzymatic degumming using phospholipases A1 has emerged, when the traditional processes are not optimally suited for oil with a high content of Non-Hydratable Phosphatides (NHP) [1,2]. It can reduce the phosphorous content to less than 10 ppm, heavy metals, improve the oil yield and obtain less oil in the gums [3,4]. Moreover, enzymatic degumming is considered as an environmental-friendly process, because of the mild condition in which it is carried out and the amounts of acid and base used, wastewater discharged and the cost in the process of enzymatic degumming were reduced during refining oil [5].

Although, the use of enzymes in an immobilized state offers many advantages, like improvement of stability and activity of the enzyme, until today, many factories employ the phospholipase A_1 in a soluble form and use it only once [1,6]. Its non-reusability causes high operating costs and rending its application in the industry no profitable. Thus, to overcome this inconvenience, it would be advantageous to recycle the enzyme by immobilizing it on a solid support and try to reuse it several times [7,8]. A recent report including the use of PLA_1 in industrial processes [6,9], and its immobilization [8-10] was recently published. These findings corroborate the vast potential of the phospholipase in terms of their application in the field of foodstuffs, and also indicate the importance of the immobilization in the reduction of its economical cost and the profit that can be generated. Generally, lipases are immobilized by entrapment, covalent binding, adsorption, or membrane confinement [11,12]. The adsorption method is easy, rapid, economic, cheap and also gentle toward the enzyme [11]. Moreover,

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the choice of a support is also a key parameter to take in the account.

The literature points out that from among supports, the bentonite has a high potential to be used as adsorbents, due to its low cost and a high surface area [12,14]. Also, such supports present many advantages: low toxicity, good chemical reactivity, a bacterial resistance, available locally (Algerian bentonite) and important thermal stability. On the other hand, some works presented in the literature have shown that immobilization of enzymes using activated bentonite as a support, has been successfully achieved [14-16]. Indeed, its absorption capacity has been exploited to remove excess proteins from wine [13]. Similarly, this attractive interaction between bentonite and proteins has been exploited in the immobilization of enzymes, such as lipase [15-17], α-amylase [18] and *Catalase* [19]. However, there is no reported study focusing on the immobilization of PLA1 by adsorption on the bentonite.

The objective of this study was to immobilize the phospholipase A1 (PLA_1) onto activated bentonite by using adsorption method. The mineral support is quite cheap and biocompatible. The immobilized enzyme is found to be thermally more stable by some authors [10,15-18]. The free and immobilized PLA_1 characteristics were studied, this was done through a comparison of the pH and temperature optimum of the free and immobilized enzyme. Furthermore, the thermal stability of the immobilized enzyme was also investigated. Our study was completed by an application of the free and immobilized phospholipase PLA_1, on the degumming of soybean oil.

EXPERIMENTAL SECTION

Materials

Liquid phospholipase A_1, noted PLA_1 used in this study was provided from Novozymes A/S, (Bagsvaerd, Denmark). The PLA_1 was claimed to have an enzyme activity of 10,000 $U.mL^{-1}$. The support of immobilization is bentonite called Activated Bleaching Earth (ABE), obtained from Taiko Clay Group, Malaysia. The ABE is principally composed of montmorillonite and its BET surface area is 167 $m^2.g^{-1}$. The main constituents of this clay, expressed as percentage of oxides by weight, are as follows: SiO_2 (76.2 %), Al_2O_3 (11.2 %), Fe_2O_3 (2.7 %), MgO (0.8 %), CaO (2.3 %), Na_2O (0.6 %), K_2O (0.6 %). Soybean oil with a phosphorus content of 200 ppm, soybean lecithin was used as substrate. All of the cited products were kindly donated by Cevital factory (Bejaia, Algeria). Other chemicals were of analytical grade and all aqueous solutions were prepared with distilled and bi-distilled water.

Methods

Characterization of materials

The X-Ray Diffraction (XRD) spectra of the bent and PLA_1 immobilized on bent have been obtained with monochromatic CuKa radiation using an X'Pert³ Powder Pro Panalytical's Diffractometer (PANalytical. the Netherlands). The FT-IR spectra were carried out with FT-IR 8400 spectrophotometer (Shimadzu, Japan), in the range 4000–400 cm^{-1}.

Immobilization of the PLA_1 onto bentonite

The immobilization procedure for the enzyme was as follow: 0.5 g of the bentonite was dispersed in 5 mL of 0.05 M citric-acid buffer (pH 4), and then 1mL of enzyme solution was added to the suspension. The mixture was stirred at 250 rpm at room temperature for 3 h. At the end of this time, the suspension was filtered through Whatman filter paper (Grade 42, diam. 240 mm, pore size 2.5µm, and supplied by Sigma-Aldrich), to separate the solid phase (Bent-PLA_1) from the liquid phase (filtrate); then washed two times with distilled water to remove impurities. Until use, the immobilized PLA_1 thus obtained was dried at room temperature and stored in a refrigerator (4 °C).

Protein determination

The amount of protein was determined by Bradford method at a wavelength of 595 nm using Bovine Serum Albumin (BSA) from Sigma-Aldrich, as standard [19]. The Protein concentrations were calculated using standard curves of BSA.

Phospholipase activity of the free and immobilized PLA_1

The methods described by literature [20,21] with some modifications were used to measure the phospholipase activity of the free and immobilized PLA_1 onto bent. Phospholipase activity was measured using soybean lecithin as a substrate under the following condition. A soybean lecithin emulsion was prepared by mixing a 10 % of

phospholipids and 4 % polyvinyl alcohol solution at a volume ratio of 1:4 at 15 000 rpm for 20 min. The reaction mixture, consisting of 5 mL of lecithin emulsion, 5 mL of 0.05 M citric acid buffer and 1 mL of enzyme solution (0.5 g of immobilized PLA_1), was incubated for 15 min (30 min for immobilized PLA_1) at 40 °C under mechanical stirring (250 rpm). The reaction was ended by adding 15 mL of 95 % ethanol. The fatty acids released by free and immobilized PLA_1 were titrated with 0.05 M NaOH. Blanks were measured with a heat-inactivated enzyme solution which was kept at 100 °C for 15 min. One unit of phospholipase activity (U/mL) was defined as the amount of enzyme that liberated 1 µmol of fatty acid under the assay conditions. All activity assays were repeated three times.

The phospholipase activity and amount of protein per mL of Lecitase Ultra were respectively 1450 U/mL and 14.3 mg/mL. All the enzyme activities were reported as a relative activity defined as fellow:

$$\text{Relative Activity (\%)} = \frac{\text{Enzymed activity}}{\text{Maximum enzyme activity}} \times 100$$

Where; "maximum enzyme activity" was the highest enzyme activity in each set of experiments.

Effect of pH and temperature on free and immobilized PLA_1 activities

The optimum pH for free and immobilized PLA_1 were determined by measuring the activity with varying pH values of the reaction buffer (3.0, 4.0, 5.0, 6.0, 7.0 and 8.0) using the 0.05 M citric acid buffer. The optimum temperature for the free and immobilized PLA_1 was investigated within the temperature range 30 - 95 °C.

Enzymatic degumming process of soybean oil

Batch degumming of soybean oil experiments were performed with free and immobilized PLA_1, according to the published procedure in previous studies with some modifications [20-22]. These essays were performed in 500 mL Erlenmeyer flask fitted with a laboratory model stirrer, containing 200 g of soybean oil with 200 mg/kg as the initial content of phosphorus. The flask was placed in a water bath at 80 °C, and then 0.2 mL of citric acid solution (45%) was added. The mixture was mixed at high speed for 2 min and then incubated at 80 °C for 20 min. For the free form of PLA_1, at the end of this period, the oil was cooled to 40 °C and then adjust to pH 5.5 and 7 with 4% NaOH. Afterward, deionized water (2.5 % of oil mass) and 40 µl of PLA_1 were added. The mixtures were then mixed at approximately 3000 rpm for 1 min and incubated at 40 and 55 °C under mechanical stirring at approximately 250 rpm for a time t (h). Every 1h, 2 g of oil samples are drawn for phosphorus analysis. For the immobilized form of PLA_1, the essays were performed similar to the procedures of free enzyme, except that 1 g of immobilized PLA_1 (187 U/g) was added to start the reaction. the residual phosphorus content in the oil was determined by applying both methods reported by *Yang et al.* [20] and AOCS Official Method Ca 12-55 [1, 23] with some modifications.

RESULTS AND DISCUSSION

Analysis of bent and Bent-PLA_1 by XRD

The X-Ray Diffraction (XRD) method provide a powerful tool to understand the changes in the interior of the clay microenvironment and thus to evaluate the different types of nanocomposites formed. Fig. 1, shows the XRD patterns of the (a) (bent) and (b) immobilized PLA_1 onto bentonite (bent-PLA_1).

Comparing the X-ray diffractograms of the bent and bent-PLA_1, they show the difference of the form of the spectra in the area 2θ [2, 10°] by the reduction of the intensity of the reflection, thus, the parent bent (Fig 1a) was structurally different from the bent-PLA_1 (Fig 1b). Moreover, these results show a shift of the d-value. Indeed, the positions of the peaks displace to the right, the first peak (d_{001}) related to the basal spacing, is equal to 20.72 Å and 14.95 Å , but when the enzyme was immobilized, the basal spacing increases to 28.4 Å and 16.16 Å respectively. This is probably caused by the immobilization of the enzyme, even at low enzyme loading. This means that the immobilization of the enzyme leads to partial exfoliation (a beginning of layer destruction).

Similar results were observed among many authors. Gopinath and Sugunan, [12], report an increase of the basal spacing from 10 Å for montmorillonite to 19.8 Å for invertase immobilized by adsorption and suggest that the side chains of the enzymes penetrate into the inter-layer space of the clay mineral particles, and not the whole of the enzyme. Moreover, *Tzialla et al.* [24], reported that enzyme immobilization leads to effective destruction of the ordered structure of the layered mineral to total exfoliation of the clay even at low enzyme loadings.

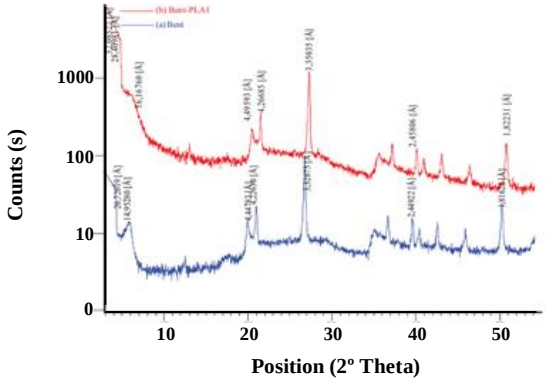

Fig. 1: XRD of (a) bentonite, and (b) PLA_1 immobilized onto bentonite.

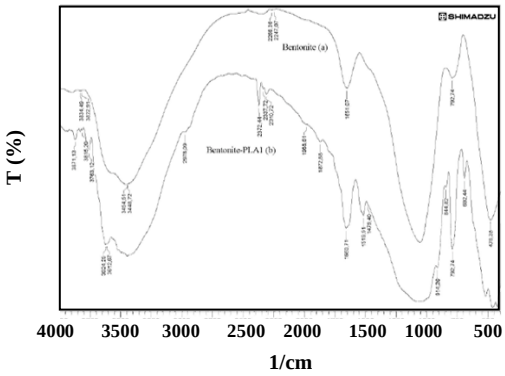

Fig. 2: The FTIR spectra of (a) bentonite and (b) immobilized PLA_1 (bentonite-PLA_1).

FT-IR spectra of bent and bent-PLA_1

The FT-IR spectra of bentonite and bentonite-PLA_1 are presented in Fig.2a and Fig.2b respectively.

It is well known that FT-IR spectroscopy is regarded as a powerful technique for the determination of the secondary structure of the protein [16]. The analysis of the Amide I band in the range of 1600-1700 cm^{-1} originate mainly from C=O stretching vibration makes it possible to obtain information on the effect of the immobilization on the secondary structure of the protein, and amide II at 1510-1580 cm^{-1} with weaker intensity is largely due to the N-H bending with a contribution of the C-N stretching vibration, and this band is associated with the side chain vibrations and the changes of hydrogen bonds in the protein [16, 24].

The bands at 1650-1660 cm^{-1} were assigned to α-helix. The component due to α-helix structures is a good indicator of the folding of the enzyme [24]. As shown in Fig 2b the presence of amide I peak (1650 cm^{-1}) and amide II peak (1519 cm^{-1}) of immobilized enzyme was observed, that confirms us the intercalation of the PLA_1 enzyme into the interlayer space of bentonite support. Moreover, the intensity of the band at 1650 cm^{-1} (amide I peak of the immobilized PLA_1) was increased. It was probably disturbed by the peak of bending vibrations of hydroxyl of bentonite [16], or it may be due to an increase in the α-helix content and leads to a properly-folded functional enzyme fraction enhancing thus the biocatalytic activity.

Adsorption of the PLA_1 on the bent and yield of immobilization

Table 1 presents the results in terms of adsorbed PLA_1 and yield of immobilization, using optimum pH, time and agitation rate for immobilization.

From these results, the amount of PLA_1 adsorbed on the bent was 13.22 mg/g of the support. These results testify that bent has excellent adsorption properties due to its large specific surface area (167 m^2/g). It may be related to the higher chemical affinities of the PLA_1 by the bent, which allowed the adsorption of a high amount of enzyme [16]. The high immobilization yield (about 92%), confirms the strong affinity between the support and the enzyme. Furthermore, the obtained results (Table 1) showed, the maximum loading of phospholipase on bent was obtained at pH 4.0, indicating that the electrostatic attraction originated from the opposite charged to support and PLA_1 was the main force to adsorb the phospholipase [16].

Phospholipase activity of the PLA_1

Effect of pH

Fig. 3 shows the effect of the pH on the relative activity of the PLA_1 on its immobilized and free forms. As can be seen, by the curves, the optimal pH for maximum activity of Bent-PLA_1 was estimated to be 7.0, Interestingly this value is similar to the one obtained for the free PLA_1. It can be seen also that the phospholipase activity of the PLA_1 was strongly influenced by the pH, as showed by the decline in the activity beyond the optimum. The activity of immobilized PLA_1 was suppressed at acidic pH (4.0–5.0) but increases significantly at pH 6.0 while the activity of free PLA_1 was suppressed only at pH 4.0 and expressed activity of 300 units.mL^{-1}, at pH 5.0. After pH 7, the relative activity was decreased for both forms of the biocatalyst.

The same value of the optimum pH (7.0) was founded for the free and immobilized Lecitase® ultra onto a Polystyrene DA-201 Resin by *Ning Liu et al.* [25].

Table 1: Amount of the PLA_1 adsorbed on the bent and yield of immobilization.

Amount of protein loaded (mg/g bentonite)	Immobilization yield (%)	Optimum pH for immobilization	Optimum time for immobilization (h)	Optimum of agitation rate for immobilization (rpm)
13.22	92	4.0	3	250

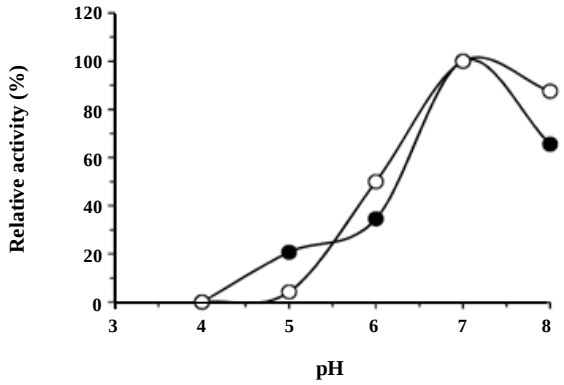

Fig. 3: Effect of pH on phospholipase activities of (filled circles) free PLA_1 (100% = 1150 units/mL) and (empty circles) immobilized PLA_1 (100% = 600 units/mL). Phospholipase activities measured in 0.05 M citric-acid buffer (pH 4.0 – 8.0) at 40 °C.

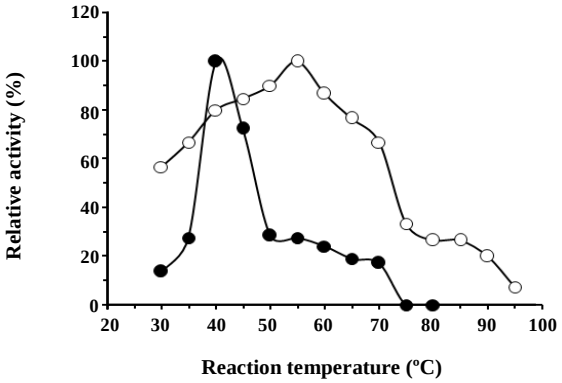

Fig. 4: Effect of temperature on phospholipase activities of (filled circles) free PLA_1 (100% = 1200 units/mL) and (empty circles) immobilized PLA_1 (100% = 750 units/mL) Phospholipase activities were measured in 0.05 M citric-acid buffer (pH 7.0).

Therefore, the effect of pH on the immobilized enzyme activity was caused by altering the enzyme conformation or by promoting enzyme precipitation [25]. In a similar work, evaluating the effect of the pH on the activity of Lecitase Ultra, *Jianfeng Zhan et al.* [26] had founded an optimum value of activity at pH 7.5. *Sheelu et al.* [10], showed a broad pH activity profile between 6.5 and 8.5 for the free enzyme while the immobilized PLA_1 has an optimum pH at 7.5. For *Dianyu Yu et al.* [8, 18], the optimum pH of the free and immobilized enzyme were 5 and 6.0 respectively. At pH 5.5, the activity of the free enzyme was 85.2%. The activity of the free PLA_1 dramatically decreased at pH values above pH 5.0. In the same work, the immobilized enzyme maintained more than 90% activities at pH of 4.5 - 6.5. The authors suggest that the free PLA_1 was more sensitive to environmental pH compared to the immobilized enzyme.

To conclude; the equilibrium point between enzyme activity and pH stability could be found at the pH 7.0, at which the effect of the enzyme activity is the best.

Effect of temperature

Since the stability of the enzyme is of prime importance in industry, the effect of temperature on the enzyme activity in an aqueous assay solution was studied. The enzyme activity was dependent on temperature, as illustrated in Fig. 4. The results show that the temperature optima for the free PLA_1 and the immobilized PLA_1 were 40 and 55 °C, respectively. The free PLA_1 retained more than 70 % of its initial activity over a range of temperature from 40-50 °C. Moreover, an increase in temperature above its optimum point (40 °C) resulted in lower activities due to thermal denaturation. This is may be attributed to multiple attachment points for enzyme guaranteed by the supports which limit their conformational alterations under higher temperature. *Kharrat et al.* [27] have noted that silica aerogels used as support for lipase, immobilization may be protecting the enzyme against thermal denaturation.

A similar result was reported in the previous study. *Sheelu et al.* [10] were used gelatin hydrogel, as support for PLA_1 immobilization, and were found that Beyond 40 °C, the relative activity of the free PLA_1 declined rapidly.

The immobilized PLA_1 was retained more than 60 % of its initial activity over a range of temperatures from 55 °C to 70 °C. After its optimum point (55 °C), the increase of the temperature until 70 °C, the phospholipase activity decreases gradually, because of immobilization process

could increase stability and form the enzyme substrate complex which can hinder the access of substrates to the active site [26]. After immobilization, structure stabilization of the PLA_1 may be occurred and thus improve its temperature resistance.

The immobilization of PLA_1 could protect the active sites of PLA_1, and also inhibit a part of the activity of he enzyme at high temperatures, compared to the free enzyme [10, 11, 18, 28].

Finally, at pH 7.0, the equilibrium point between enzyme activity and the optimum of temperature could be found at 40 °C for free PLA_1 and 55 °C under its immobilized form. At these values of temperature, the effect of the enzyme activity is the best.

The apparent activation energy (Ea) values were calculated by using the regression equations for Arrhenius plots of immobilized PLA_1 [y=-1.419 x+6.436 (R2 = 0.998)] and free PLA_1 [y= -8.143x +27.97 (R2 = 0.967)]. The Ea values were estimated to be 27.13 kj/mol[1] and 155.64 kj/mol, respectively.

The immobilization of PLA_1 lowered the Ea from 155.64 to 27.13 kj/mol, resulting in higher catalytic efficiency of PLA_1. It is quite obvious that the value of the Ea was significantly lowered with immobilization. The lower value of activation energy obtained for the immobilized PLA_1 indicating that the immobilized form of the enzyme was less sensible to high temperature and become hard to deactivate. Our results were in accordance with those obtained previously by *Dong et al.* [16], where the activation energy of immobilized PLA_1 was lower than the free form. This decline of Ea was attributed to the promotion of mass transfer of the substrate from solution to the biocatalyst surface [16].

Thermal stability

The thermal stability of free and immobilized PLA_1 is shown in Fig. 5. The results in Fig. 5 show that immobilized PLA_1 retains 72 % and 60 % of its catalytic activity at 65 °C and 70 ° C respectively, after 1 h of incubation, while the free PLA_1 retains only 46.81% and 30 % of its original activity at 65 °C and 70 ° C respectively.

These results suggest that the immobilization improves significantly, the stability of enzymes against heat denaturation. Similar results, for the PLA_1 immobilized, were reported by many authors [9, 10, 27, 28] and it is often found that immobilized enzyme has higher thermal stability than free enzyme due to the restriction of its conformational flexibility attributed to its multiple attachment points of the enzyme on the support which limits the conformational alterations and movements under various temperatures [27]. In addition, they announced that an increase in thermal stability implies a reduction in the denaturation reaction [27, 28]. Indeed, the immobilization of enzyme in a carrier often limits its freedom to undergo drastic conformational changes, thus resulting in increased thermal stability [18, 29].

Batch degumming process

Our choice on conditions degumming (pH and Temperature °C) is as follow: the optimum values obtained previously (pH 7.0, T= 40 °C for free PLA_1 and pH 7.0, T= 55 °C for immobilized PLA_1) were used for soybean oil degumming experiments. For the better investigation, pH and temperature applied in the Cevital refinery were also used; (pH=5.5, T=50 °C).

According to the previous studies [3, 18, 22, 30], the effectiveness of the enzymatic degumming process was evaluated by determining the phosphorus content in the treated oil. The monitoring of this parameter of the process was conducted in order to meet the quality requirements of the refined oil. The standards require phosphorus levels below 10 mg/kg [1-5].

The initial phosphorus content in the soybean oil samples was 212 ppm. When citric acid was added to the oil, the phosphorus content decreased to 128.04 ± 2.6. The decrease in phosphorus content observed after addition of citric acid was attributed to the coagulation and precipitation of part of phosphatides [21,30]. From the results obtained in Fig. 7, the following notices were observed:

- Free PLA_1 exhibited a pronounced decrease in phosphorus content rather than the immobilized PLA_1.
- For the free form of the enzyme, when the optimal pH and temperature used for the degumming essays was 5.5 and 50 °C; 12.5 ppm of phosphorus content was obtained after 4 h, and 8.2 ppm after 5 h, while 28.00 ppm after 4 h was obtained when using optimum conditions (pH 7.0 and T= 40 °C) of activity of PLA_1. In addition, the optimum conditions (pH 7.0, T= 40 °C and 55 °C) in which, the enzyme (free and immobilized PLA_1) exhibits a maximum activity doesn't automatically cause the maximum removal of phosphorus levels.

To conclude about these points, the better the conditions

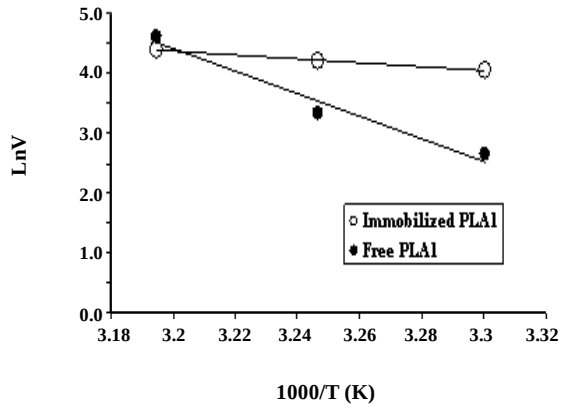

Fig. 5: Arrhenius representation of the apparent activation energy.

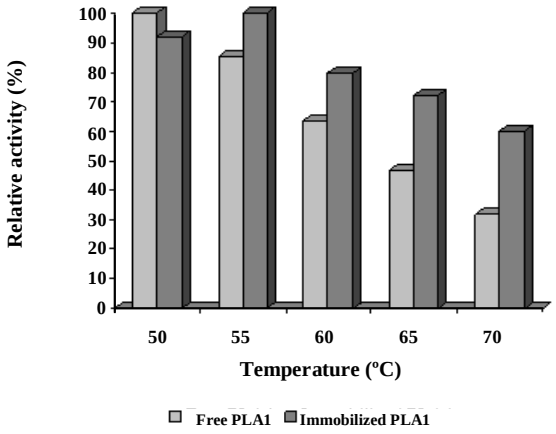

Fig. 6: Thermal stability of the free and immobilized PLA_1.

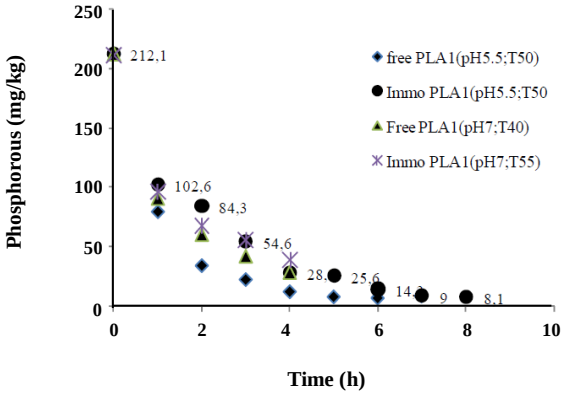

Fig. 7: Phosphorus content as a function of time in soybean oil samples degummed by free and immobilized PLA_1.

to reduce phosphorus content by free PLA_1 were pH 5.5 , temperature 50 °C and time 4-5 h. Similar results have been reported by *Yang et al.* [20]. For the immobilized PLA_1, it appeared that when using the selected optimal conditions for the degumming essays, the difference in the phosphorous content has not been significant after 4 h of the treatment. Indeed, the phosphorus was reduced from 212 ppm to 38.6 ppm at pH 7.0, T= 55 °C.

However, the phosphorus reduction becomes significant (9 ppm) after 7 h of incubation at pH 5.5 and a temperature of 50 °C. Similar times have been obtained by *Dianyu Yu et al.* [9, 18]. Immobilization improved thermal stability and resulted in the formation of the enzyme substrate complex which hindered the access of substrates to the active site [9, 18]. The immobilized PLA_1 process at 50 °C has the advantages of better operational stability.

Xiaofei Jiang et al. [22] has used PLA_1 for soybean oil degumming, the residual phosphorus content was reduced to 8.2 mg/kg at 50 °C and a pH of 5.0 within 4 h. Also, the contents of Fe, Ca and Mg were significantly decreased after the degumming treatments. *Dianyu Yu et al.* [18], using free and magnetic immobilized phospholipase A_1, the phosphorus content was reduced from 138 ppm to 8.7 mg/kg with free PLA_1 (pH 5.0, T= 50 °C) and to 7 ppm after 7 h with immobilized PLA_1 (pH 6.0, T= 55 °C). In the other study [8], the authors were found that the phosphorus content was reduced from 142.2 mg/kg to 10.4 ppm and 10.1 ppm after 3 h and 7 h with free PLA_1 and immobilized on calcium alginate respectively. The temperature and pH values were maintained at 50–58 °C and pH 5.5–6.0.

A number of other studies have used free phospholipase A_1 and immobilized PLA_1 to reduce the phospholipid content of vegetable oil to a final phosphorus level less than 10 mg/kg [5, 21-22,26,29] .

CONCLUSIONS

The objective of this study was to develop a new material for immobilization of phospholipase A_1 onto bentonite, by physical adsorption and in order to apply it for the degumming of soybean oil. Optimal enzyme activity was obtained when the immobilization of PLA_1 onto the bentonite had been performed under the following conditions: pH 4.0, agitation rate 250 ppm and time 2 h. The amount of protein loaded was 13.22 mg/g, at the optimized immobilization conditions. From

the FT-IR analysis of peak position and their intensities, it appeared that after immobilization, the conformation of PLA_1 is retained and the aggregation between the immobilized PLA_1 molecules are maximally avoided and importantly, the bentonite provided protection for the immobilized PLA_1 which exhibits better thermal stability than the free form. XRD analysis confirmed the grafting of PLA_1 onto the bentonite. In the batch oil degumming (pH 5.5, T 50 °C) the phosphorus content was reduced to less than 10 ppm with free PLA_1 after 4-5 h while the immobilized PLA_1 was only able to reduce it to 9 ppm after 7 h. Although immobilized PLA_1 was less efficient at hydrolyzing phospholipids compared to free PLA_1, it has the advantage of reusable enzymes and can be considered as a prospective biocatalyst for the industrial applications.

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