





Isolation of Lipase from Soyabean Seeds and Its Immobilization in Calcium Alginate Beads

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Abstract	Article History
<p>In general, lipase enzymes are used in the form of free lipase enzymes which can only be used once and are less effective, so that their use is costly. Therefore, to maximize the use of the lipase enzyme, immobilization of the enzyme that allows multiple use is a choice. The purpose of this study was to determine the activity of the soyabean (<i>Glycine max</i>) lipase in its free and immobilized form. The enzyme was partially purified by ammonium sulphate precipitation and DEAE ion exchange chromatography. The partially purified lipase from soyabean seed was immobilized by entrapment method using Calcium alginate beads. The immobilized lipase enzyme was characterized to determine the protein content, enzyme activity, effect of temperature, effect of pH, the effect of substrate concentration and kinetic parameters (Km and Vmax). The investigation showed that the crude lipase extract from soyabean seed sprouts had an activity of 10.71 U/mL and a protein concentration of 0.27 mg/mL. The purification profile revealed that the 40-80% ammonium sulphate precipitation and DEAE ion exchange had the highest protein content of 1.98 mg/mL and 2.04 mg/mL respectively with enzyme activity of 5.19 U/mL and 3.44 U/mL at 1.32 and 0.83 purification folds, respectively. The SDS PAGE profile of the soyabeans extracted lipase revealed a molecular weight above 100 kDa and less than 150 kDa. The protein concentration of the Ca-alginate immobilized lipase was estimated to be 0.1 mg/mL with an enzyme activity of 3.37 U/mL. The optimum temperature for the immobilized lipase enzyme was 60 °C and the optimum pH was 8.0. These results indicate that the immobilized lipase enzyme from seed sprouts of soyabean (<i>Glycine max</i>) can be used with little or no purifications and immobilization by entrapment method in calcium alginate bead can greatly improve its activity.</p> <p>Keywords: Lipase, <i>Glycine max</i>, activity, Immobilization, Calcium alginate</p>	<p>Received: 29 Dec 2025 Accepted: 18 Jan 2026 Published: 24 Jan 2026</p> <p>Scan QR code to view*</p>  <p>License: CC BY 4.0*</p>  <p>Open Access article.</p>

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Introduction

Lipases (triacylglycerol ester hydrolase, EC3.1.1.3) are enzymes that catalyze the total or partial hydrolysis of fats and oils, releasing free fatty acids, diacylglycerol, monoglycerol and glycerol (Al-Haidari *et al.*, 2020). Lipases are the industrially important biocatalysts, which are envisioned to have tremendous applications in the manufacture of a wide range of products. Their unique properties such as better stability, selectivity and substrate specificity position them as the most expensively used industrial enzymes (Sarmah *et al.*, 2017). The purified enzyme is used in a wide range of food industries, detergent industries, personal care products and cosmetics industries (Al-Haidari *et al.*, 2020). Also, it is used in biodiesel production (Gashaw *et al.*, 2015), pharmaceutical industry (Cancino *et al.*, 2008), polyesters industry, and fatty acids production (Al-Haidari *et al.*, 2020). Lipases can be extracted from microorganisms including bacteria (Yang, *et al.*, 2016) and fungi (Ayinla *et al.*, 2017) and from animals

(Zhou *et al.*, 2013) and plants (Mazou *et al.*, 2017). Plant lipases are extracted from different parts of the plant, such as fruits, latex and seeds. However, seeds, especially oil-seeds, are the main parts which contain higher concentrations of lipases. The lipases in seeds play an important role in the growth of the embryo, providing it with energy for growth and development. Therefore, lipases are present in higher levels during the period of seeds germination (Al-Haidari *et al.*, 2020). Recently, seed lipases have been the focus of much attention as biocatalysts. In some cases, these enzymes present advantages over animal and microbial lipases due to some quite interesting features such as specificity, low cost, availability and ease of purification, representing a great alternative for potential commercial exploitation as industrial enzymes (Barros *et al.*, 2010). Therefore, exploration of lipases from seeds is growing, for example indigenous lipases of walnut seeds (Djarkasi *et al.*, 2017) avocado seed sprouts (Sya'bani *et al.*, 2017) durian seed sprouts (Dzulkarnain, 2018)

and cocoa beans (Permana *et al.*, 2013). Enzyme production requires a fairly high cost and is generally used only once (Uba, 2019). Therefore, to carry out further reactions, a new lipase enzyme is needed so that the resulting product is more optimal. The use of lipase enzymes that can only be used once is less effective and costs a lot (Mulko *et al.*, 2019). Therefore, to maximize the use of the lipase enzyme, enzyme immobilization can be carried out. Enzyme immobilization is a method of binding enzymes to a solid material or matrix that is not soluble in water. It aims to separate the enzymes at the end of the reaction, without contaminating the reaction products so that they can be used sustainably.

Enzyme immobilization can be done by several methods, one of which is the adsorption method because it is easy to operate (Enemchukwu *et al.*, 2026). However, this method produces immobilized enzymes with weak immobilization bonds, immobilized enzymes are very sensitive to changes in temperature and pH, and the amount of immobilized enzymes is very small (Zhao *et al.*, 2015). Therefore, the method used in this research is the trapping method. When compared with the adsorption method, this method is relatively stable. Besides being easy to do, this method is inexpensive, produces stable immobilized enzymes and is able to trap enzymes in large quantities and with high enzyme activity (Bilal *et al.*, 2019). The support matrix commonly used in this method is calcium alginate beads. In the present study, lipase was isolated from germinating seeds of *Glycine max*, partially purified and immobilized by entrapment in calcium alginate beads.

Materials and Methods

2.1 Seed germination

Seeds of soya bean seeds were soaked in water for 6 hr and were allowed to germinate for 24 hr at room temperature (28 °C) on moist filter paper placed in a Petri dish for germination studies (Gadge *et al.*, 2011; Anukam *et al.*, 2020a; 2020b; Egurefa *et al.*, 2020; Uba *et al.*, 2020a; 2020b; 2020c; 2020d; Uba *et al.*, 2021; Chude *et al.*, 2021; Ubani *et al.*, 2024; 2025; Ekwenze *et al.*, 2025; Mere *et al.*, 2025).

2.2 Extraction and isolation of crude extract of lipase from germinating soya bean seeds

After 48 hr of seed germination, the seed coat was removed manually and 20 g seed cotyledon were homogenized in chilled acetone at 4 °C. The suspension was centrifuged at 4000 rpm and residue obtained was dissolved in 100 mL distilled water followed by centrifugation at 4000 rpm. Supernatants (crude extracts) were stored in aliquots for protein concentration determination, lipase activity, purification, immobilization and characterisation (Dokubo and Uba, 2023; Gadge *et al.*, 2011; Okafor *et al.*, 2023; Uba *et al.*, 2024; Uba and Anidu, Uba and Obiefuna, 2023; Uba and Okonkwo, 2025).

2.3 Determination of protein concentration

Protein concentration was determined by Biuret method as described by Nasry *et al.* (2022) and Ubajekwe *et al.* (2025). Bovine serum albumin (BSA) was prepared at 150 g/L stock protein standard and after which different concentrations range 0-150 g/L were made. To all the tubes, 4 mL of 3 % NaOH solution were added to make it up to 5 mL solution. Also 1 mL

of Biuret solution were added, shaken, and incubated at room temperature for 15 mins. Similar procedure was repeated for all samples. The absorbances were measured spectrophotometrically at 540 nm wavelength using water blank. The amount of protein was determined from the BSA standard curve.

2.4 Determination of lipase activity

In this study, lipase activity was measured using Jaganmai *et al.* (2023), Ubajekwe *et al.* (2025) and Okolo *et al.* (2025) methods where 4- para nitro phenol acetate (pNPA) solution was prepared freshly by mixing 10 mL of 0.03 % pNPP in isopropanol, and 90 mL of 0.1 % gum arabic solution in 50 mM Tris-HCl buffer (pH 8.0) containing 0.4 mL of olive oil under constant stirring conditions until the whole solution gets suspended. To 0.9 mL of pNPA solution (freshly prepared), 0.1 mL of the lipase enzyme (Crude/Purified) was added and incubated at 37 °C for 30 min. The colour formation was measured at 410 nm (Uba *et al.*, 2018).

Activity (U/hr/mg of protein) = (Absorbance at 410nm/18.3) X Dilution factor

2.5 Purification protocol

2.5.1 Ammonium sulphate precipitation

The crude lipase was partially purified using ammonium sulphate within 0 – 100 % saturation (Popoola and Olateru, 2021). The filtrates were treated with 24.3, 28.5 and 15.7 g of ammonium sulphate to 0-40, 40 - 80 and 80 – 100 % saturation respectively. The mixture for each batch of percentage saturation was stirred continuously for 20 min until the ammonium sulphate dissolved. The mixture was kept at 4°C overnight in a refrigerator after which it was centrifuged at 4,000 rpm for 1 hr. The sediments were used to determine the total protein content and lipase activity and the concentration range with the highest specific activity was then made available for the next application, ion exchange chromatographic technique.

2.5.2 Ion exchange chromatography

The pooled and concentrated enzyme fraction of the dialysate were run on a preactivated Sepharose FF equilibrated with 50 mM pH 8.0 Tris-HCl buffer, containing 0.5 M (NH₄)₂SO₄. Before loading onto the column, the (NH₄)₂SO₄ concentration of the solution was adjusted to 0.5 M and unbound proteins were removed from the column at a flow rate of 60 ml/h with the same buffer. Then the column will be eluted with a reverse gradient from 0.5 M to 0.05 M (NH₄)₂SO₄ in 50 mM pH 8.0 Tris-HCl buffer. The eluents from the chromatographic column fractions were also analysed for lipase activity. The fractions that showed the highest lipase activity were pooled and assayed for protein content. The specific activity of the purified enzyme was compared with that of the crude enzyme and the purification fold will be calculated. The active fractions were stored at 4 °C until used for polyacrylamide gel electrophoresis and further enzyme characterization (Bakir and Metin, 2016).

2.6 Determination of native molecular weight using Polyacrylamide Gel Electrophoresis

The subunit molecular weight of purified lipase extract was analyzed with sodium dodecyl sulphate polyacrylamide gel electrophoreses (SDS-PAGE) according to the method of Laemmli (1970) in 12 % slab gel. About 0.1 mL of purified protein was loaded onto the gel. The gel was stained with 0.1 % Coomassie brilliant blue R-250. The molecular weights of the purified lipase was determined in comparison with standard molecular weight markers (molecular mass range: 14.4 – 116.3 kDa)(Okoye *et al.*, 2020).

2.7 Preparation of lipase immobilized in calcium alginate gel microspheres

The preparation of lipase immobilized in calcium alginate gel microspheres was carried out according to the method of Garba *et al.* (2018) with slight modifications. Exactly 5 g of sodium alginate was dissolved in 30 mL distilled water and autoclaved at 121 °C for 15 mins. After cooling 20 mL of sodium alginate was mixed with 5 mL of purified lipase and then allowed to stand for 10 mins. The enzyme/alginate mixture was pumped drop wise in 0.4 M CaCl₂ and kept for 1 hr to ensure its complete hardening. The beads were washed with distilled water. The lipase / calcium alginate beads were kept in 0.05 M tris-HCl buffer of pH 8.0 at 4 °C until used. The method of Yucel *et al.* (2011) was used to calculate the protein content and lipase activity of the immobilized lipase.

2.8 Effect of temperature and pH on lipase activity

For the determination of optimum temperature for lipolytic relative activity, lipase was assayed at temperatures ranging between 30 - 80 °C in a temperature-controlled water bath using the Eze and Ezema (2012) standard method of assay. Enzyme solutions were incubated at 30 - 80 °C for 30 min and then cooled at 4 °C for another 30 min. The lipase relative activity was determined as earlier described as described by Eze and Chilaka, 2010; Uba *et al.* (2019) and Dibua *et al.* (2025). Lipase activity was assayed for in the pH range of 3.0 - 9.0 using the following buffer system: sodium acetate (pH 3.0 - 4.5); sodium phosphate (pH 5.0 - 6.5), and Tris-HCl (7.0 - 9.0) for 1 hr at optimum temperature to determine the optimum pH.

2.9 Determination of enzyme kinetics (V_{max} and K_m)

The Tripathi *et al.* (2014) method of measuring enzyme kinetics was adopted in this study. Lipase activity at olive oil substrate concentrations (10 - 140 mg/mL) was determined at the optimum temperature by measuring absorbance at 410 nm. The specific activity of lipase was tested with increasing concentrations of substrate. The kinetic parameters, K_m and V_{max} were estimated from Michaelis-Menten equation. The data was fitted in curve of the single rectangular hyperbola (equation $y = \frac{ax}{b + x}$). From this, V_{max} and K_m values were derived. The rate of reaction (V_{max}) was expressed as the number of micromole of substrate changed /hr/mg of protein in the sample. One Unit specific activity was defined as one micromole substrate conversion per mg per hr.

Results and Discussion

The catalytic versatility of lipases underscores the potential for discovering new lipases with specific selectivity and substrate tolerance. This suggests a continual exploration for lipases that

meet specific industrial requirements and environmental safety considerations (Golani, 2024).

The results of the protein contents and enzyme activities of the crude lipase from soya bean seeds is shown in Figure 1. The result indicates that the crude lipase from soya bean seeds have a protein content of 0.27 mg/mL and a lipase activity of 10.71 U. The result of the purification profile of the extracted lipases is shown in Table 1. The result indicated that the ammonium sulphate 0 – 40 % fraction had the best specific activity of 3.39 U/mg and a purification fold of 2.40 and yield of 43.70 % than 40 – 80 % and 80 - 100 % fractions, respectively. The molecular weight of the purified lipase enzymes against the standard protein marker was found to be above 100 and less 120 KDa using SDS – PAGE assay (Figure 2). Comparatively, Figure 3 revealed that calcium alginate bead immobilized lipase has the higher enzyme activity of 13.37 U/mL than the free/test lipase with 10.71 U/mL. Also, the protein content here showed clearly that the calcium alginate immobilized lipase had the least protein concentration of 0.22 mg/mL. The results obtained from the study of effects of temperature shown in Figure 4 indicated that the two forms of lipases under studied (calcium alginate bead immobilized lipase and the free lipase) have an optimum temperature between 50 and 60 °C. Okunwaye *et al.* (2015) reported that the optimum temperature of lipase from *Raphia mesocarp* was 40°C. Activity was rapidly lost at temperatures greater than 50°C. From Figure 5, it can be seen that Calcium alginate bead immobilized and free lipases showed relatively high enzyme activity at both acidic and alkaline pH but optimum pH at 8.0 which contradicted the findings of Okunwaye *et al.* (2015) who reported that the maximum activity of lipase from *Raphia mesocarp* was observed to be at pH 7 which dropped onwards. In Figure 6, it can be seen that at all substrate concentrations under studied, calcium alginate bead immobilized lipase and the free lipase showed progressive increase in enzyme activity with calcium alginate bead lipase having a higher enzyme activity at any point of the substrate concentration. From Figure 7, it can be seen that Calcium-alginate bead immobilized lipase had the lesser K_m values (higher affinity) over the free/test lipase, confirming an improved catalytic efficiency.

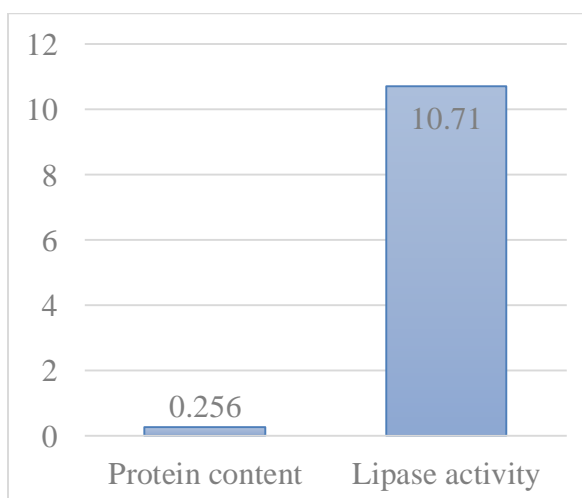
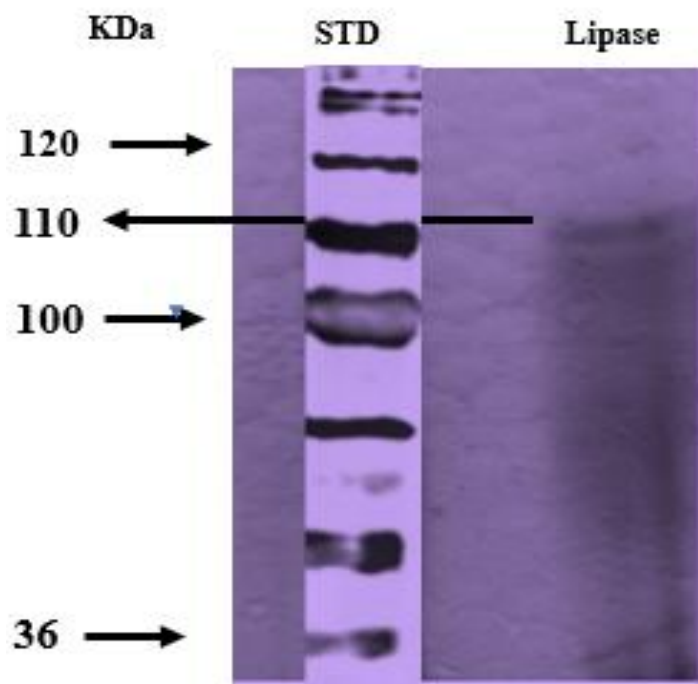
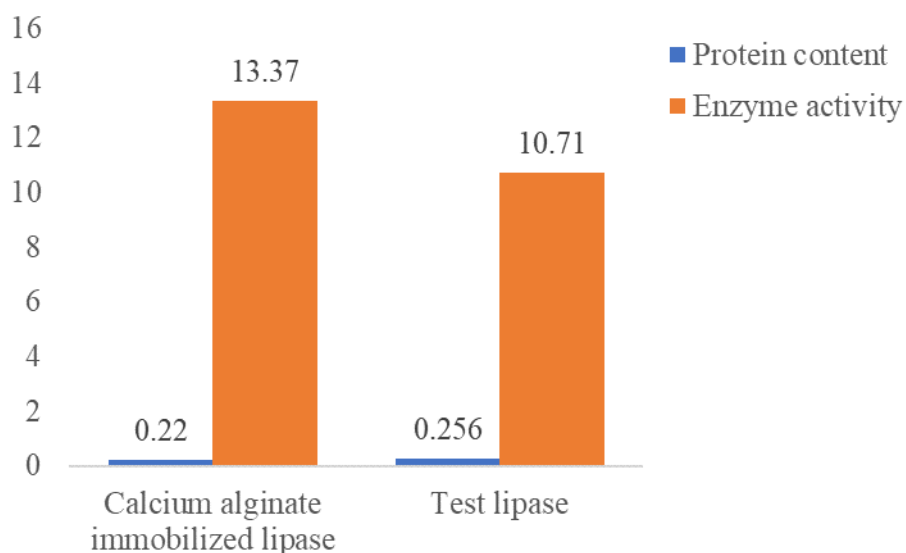


Figure 1: Protein content and enzyme activities of the lipase produced by soya beans

Table 1: Purification profile of extracted lipase from Soya beans

Purification step	Total protein (mg/mL)	Total activity (U)	Specific activity (U/mg)	Fold purification	Yield (%)
Crude	0.27	10.71	39.67	1.00	100
Ammonium sulphate precipitation (0 – 40 %)	1.38	4.68	3.39	2.40	43.70
Ammonium sulphate precipitation (40 – 80 %)	1.98	5.19	2.62	1.32	48.46
Ammonium sulphate precipitation (80 – 100 %)	1.37	3.95	2.88	2.10	36.88
DEAE ion exchange chromatography	2.04	3.44	1.69	0.83	32.12

**Figure 2:** SDS PAGE profile of the extracted lipase produced by soya beans against standard protein marker**Figure 3:** Protein content and enzyme activities of the test and immobilized lipase produced by soya beans

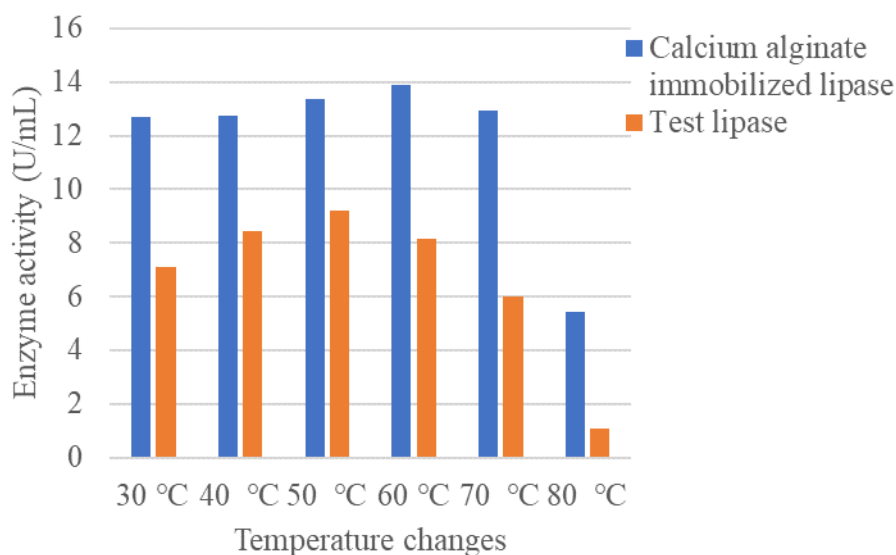


Figure 4: Temperature effect on the enzyme activities of the test and immobilized lipases produced by Soya Beans

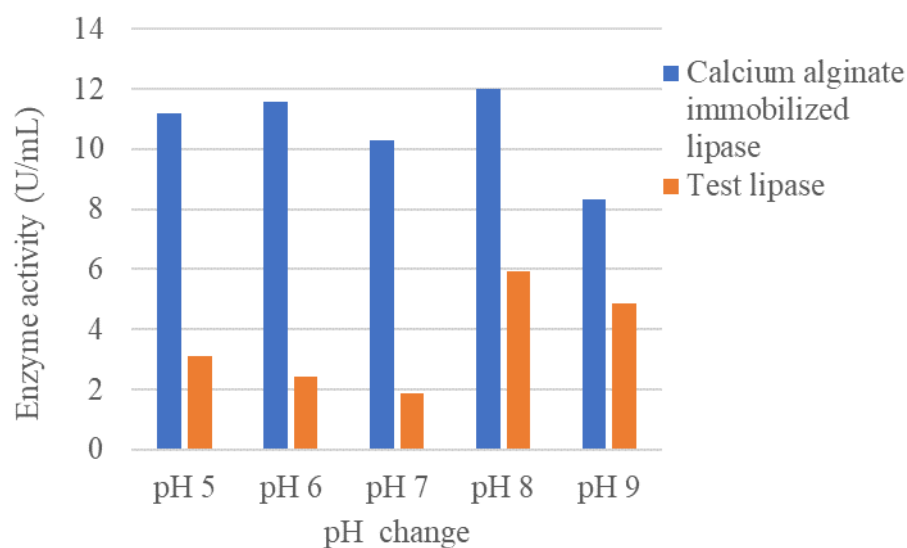


Figure 5: Effect of pH on the enzyme activities of the test and immobilized lipases produced by Soya Beans

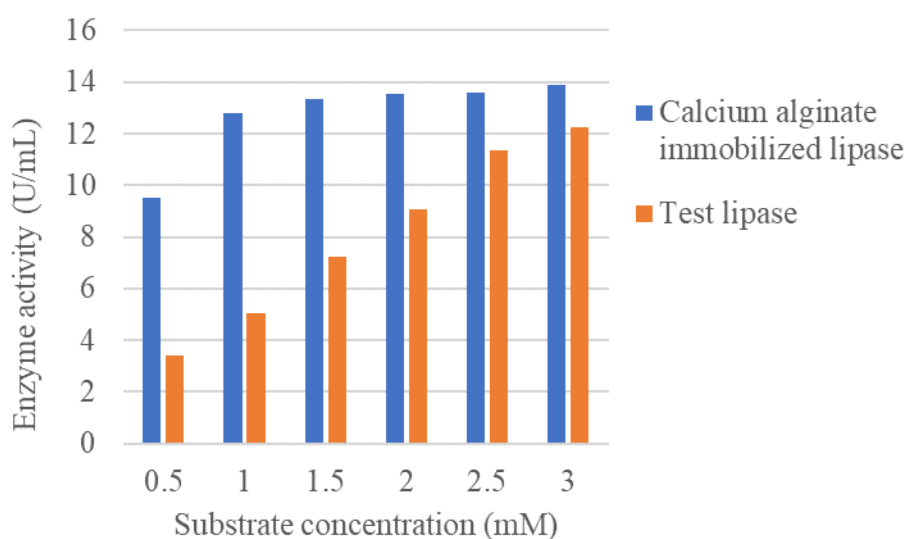


Figure 6: Substrate effect on the enzyme activities of the test and immobilized lipases produced by Soya beans

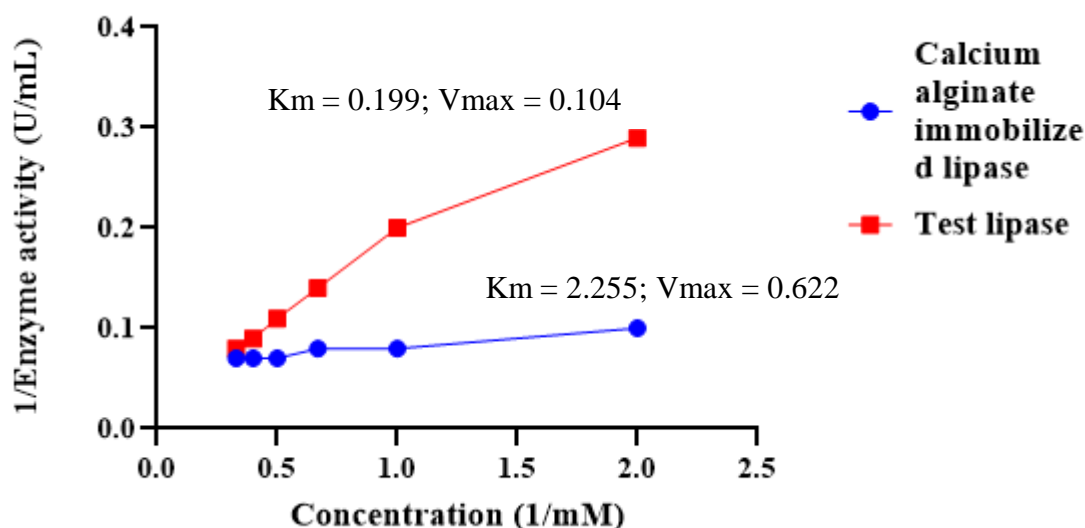


Figure 7: Km and Vmax of the test and immobilized lipases produced by Soya beans

Conclusion

These results indicated that the immobilized lipase enzyme from seed sprouts of soyabeans (*Glycine max*) can be used with little or no purifications and immobilization by entrapment method in calcium alginate bead can greatly improve its activity and catalytic efficiencies.

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