

Green Synthesis of Eco-Friendly Potassium Nanoparticles Immobilized Lipase Enzyme and its Potentials in Biodiesel Production

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ABSTRACT

Potassium Chloride and *newbouldia laevis* leaves extract were used for the synthesis of green potassium Nanoparticles (K-NPs) which were used to immobilize lipase from the germinating seeds of soya beans (*Glycine max*). The synthesized K-NPs were characterised by UV-visible spectrophotometry, Fourier transform infrared (FTIR), Scanning electron microscopy (SEM) and Energy dispersive X-ray (EDX). The UV-VIS spectroscopy absorbency peaks at 350 nm wavelength and 0.0275 absorbance. The FTIR revealed nine distinctive absorption bands which peaked at 799 -1273cm⁻¹, 1273 -1407 cm⁻¹, 1610 -1847 cm⁻¹, 2029 -2212 cm⁻¹, 2454 -2539 cm⁻¹, 2710 -2828 cm⁻¹, 2997 -3063 cm⁻¹, 3208 -3347 cm⁻¹, and 799 -3818 cm⁻¹. The SEM revealed particles that are amorphous in shape with no cluster or colony formation. The EDX showed high concentrations of elements like Tin, Vanadium and Titanium, moderate concentrations of Calcium, Sodium, Magnesium and Potassium, and a very low concentrations of Iron, Aluminium, Phosphorus, Silicon and Sulphur. The synthesized K-NPs were used to immobilize the lipase obtained from germinating seeds of soya beans. The K-NPs immobilized lipase was used in biodiesel production. The quality of the biodiesel produced by the K-NPs immobilized lipase mediated transesterification were investigated using the refractive index (RI), specific gravity (SG), Boiling point (B.Pt), and acid value (AV). The investigation revealed 1.4, 0.9 g/mL, 300 °C, and 0.6% m/m for RI, SG, B.Pt, AV respectively. The quantity of the biodiesel produced was investigated using Gas chromatography flame ionization detection (GC/FID) and the result showed 68.5% content of the entire reaction mixture to be fatty acid methyl ester (FAME/Biodiesel). These results indicate that the biosynthesized K-NPs to be a complex molecule with high molecular mass; the quality and quantity of the biodiesel produced with K-NPs immobilized lipase competed with the world standard (ASTM 6751) for biodiesel.

Keywords: Biodiesel, Enzyme immobilization, *Glycine max*, *Newbouldia laevis*, Potassium nanoparticles (K-NPs), Transesterification.

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1. INTRODUCTION

Following the advancements in nanotechnology and its expansion to different sciences, including biology and biochemistry, extensive research with remarkable results has recently been conducted on stabilizing different enzymes on chemical nanoparticles to promote their quality and efficiency. Because of their short life, low efficiency, and instability of their structure, enzymes are stabilized on nanoparticles and used for industrial, medical, and pharmaceutical purposes (Darvishi *et al.*, 2024). In this study, an enzyme was immobilized on green nanoparticles and the biochemical parameters were examined. So far, enzyme immobilization has mainly been performed on chemical nanoparticles. The question was, “Can nanoparticles synthesized by using plants be a good substrate for the stabilization of enzymes?” Enzymes are protein-based catalysts that accelerate biological reactions by reducing the activation energy and remain unchanged after the reaction (Islam *et al.*, 2008). The enzyme used in this study was *Glycine max* lipase.

Lipases are multipurpose hydrolase enzymes of plant, animal, or microbial origin (Darvishi *et al.*, 2019). They have various applications, but their stability in different environments is very low. Also, they are expensive, and their use in free form is not cost-effective. Because their half-life is short and there are limitations to their repeated usage, the stabilized form of the enzyme is used (Darvishi *et al.*, 2024). Enzyme stabilization means stabilizing the enzyme on the surface. This is performed with various carriers and by using different methods. Usually, a method that incurs the most minor damage to the enzyme, is more accessible, safer, and less costly is preferred (Ranjbakhsh *et al.*, 2012). In this study, the enzyme was stabilized on a

nanoparticle using covalent bonds. The covalent bond of the enzyme to the carrier has the advantage of firmly fixing the enzyme, so that the possibility of purging in an aqueous environment is minimized, and there will be no protein contamination in the product. Nanoparticles are one of the most important substances for this purpose.

In addition, using green synthesized nanoparticles is very attractive and rather unknown (Zuridah *et al.*, 2011). Following chemical methods, some toxic reagents usually remain on the nanoparticles. This is why the use of plants as stable and accessible resources in preparing biocompatible nanoparticles has attracted the attention of many researchers in recent years. The merits of this method include its non-toxicity, biocompatibility, low cost, and high purity nanoparticle production (Larsen *et al.*, 2008; Uba and Okonkwo, 2025; Uba and Obiefuna, 2023; Ele *et al.*, 2024; Uba *et al.*, 2024). Compared with free enzymes, immobilized enzymes have recently found more applications due to their remarkable merits such as higher stability, continuous use, retrieval, lower reaction volume, reduced environmental contamination, and cost-effectiveness (Ding *et al.*, 2015; Enemchukwu *et al.*, 2026). This study aimed to immobilize *Glycine max* lipase on green synthesized K-NPs, determine the protein concentration and activity of the stabilized enzyme, and apply the immobilized lipase in transesterification for biodiesel production.

2. MATERIALS AND METHODS

2.1 Seed germination

Seeds of soyabean 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; 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 studies (Gadge *et al.*, 2011).

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 Synthesis of potassium nanoparticle (K-NPS)

2.5.1 Preparation of aqueous leaf extracts

The Fresh leaves of *Newbouldia laevis* were washed with de-ionized water to remove foreign materials, air-dried for 5 days, ground and were sieved to obtain a fine particle mesh size. Thereafter, 50 g of these fine particles were weighed and mixed with 250 mL of de-ionized water in a 500 mL beaker. It was then set to boil at 60 °C for 15 mins in a water bath. The extract was filtered using Whatman No. 1 filter paper. The filtered extract was stored in a refrigerator at 4 °C for further experiments (Shweta *et al.*, 2022).

2.5.2 Biosynthesis of potassium nanoparticles (K-NPs)

In this study, 14.5 g potassium chloride was transferred into a 100 mL volumetric flask and dissolved to the mark. Later, 50 mL of it was transferred into a beaker containing 50 mL of the prepared *Newbouldia laevis* leaves extract. The mixture was heated to 80 °C and stirred at 3000 rpm for 30 mins until the mixture become intense. Thereafter, it was placed on aluminium foil and dried in an oven at 90 °C for 18 hr, and later transferred into an amber bottle and refrigerator at 4 °C for further analysis (Ruiz Romero *et al.*, 2018).

2.6 Physical and chemical characterization of potassium nanoparticles (K-NPs)

2.6.1 UV-VIS spectrophotometric analysis

In order to measure the amount of discrete wavelength of UV or visible light that are absorbed by or transmitted through the K-NPs UV-visible spectrophotometric analysis was conducted using a UV-visible spectrophotometer (Apel 3000UV) (Uba and Obiefuna, 2023; Ele *et al.*, 2025).

2.6.2 Fourier transform- infra red (FTIR) spectroscopic analysis

In order to further confirm the functional group on the green synthesized potassium nanoparticle (KNPs) formation, Fourier transform infrared (FTIR) spectroscopic analysis was carried out. (Uba and Obiefuna, 2023; Okafor *et al.*, 2023; Dokubo and Uba, 2023).

2.6.3 EDX Analysis

EDX spectra was analyzed to identify the elements and estimate their concentrations, and elemental distribution on the nanoparticles' surface (Egerton, 2011; Uba *et al.* 2024).

2.6.4 SEM analysis

SEM images of the nanoparticles was captured to visualize their morphology and surface characteristics (Ubani *et al.* 2024)

2.7 Preparation of K-NPs Immobilized Lipase

In this study, 20 mL of 10% glutaraldehyde was added to the nano crystals of K-NPS under stirring at 25 °C for 2 hr. After 2 hr, the particles were washed thrice with Tris-HCl buffer. Thereafter, 10 mL of the purified enzyme solution was added and stirred under magnetic stirrer for 1 hr and later at 4°C for 12 hr. The precipitate was washed with Tris-HCl buffer thrice. The protein and enzyme activity before and after immobilization steps were measured (Malar *et al.*, 2019).

2.8 Characterization of the Immobilized Lipases

2.8.1 Temperature Profile of the Free and Immobilized Enzymes

To determine the effect of temperature on lipase activity, samples of free and immobilized enzymes were incubated in the vicinity of the pNPP substrate at 30, 40, 50, 60, 70, 80, and 90 °C in a water bath for 10 min. Then, the absorption of the samples was read at a wavelength of 410 nm in the spectrophotometer, and the lipase activity in these samples was determined as described by Uba *et al.* (2019).

2.8.2 Effect of pH on Free and Immobilized Enzymes

Changes in the activity of free and immobilized enzymes were examined at different pH levels, from 3 to 9 (3, 4, 5, 6, 7, 8, and 9). In this method, the substrate is prepared at different pH levels as described by Uba *et al.* (2019) and Dibua *et al.* (2025). The substrate is poured onto the enzyme and incubated for 10 min at optimal temperature. At the end of the reaction, their absorption is read using a spectrophotometer at 410 nm.

2.9 Production of Biodiesel

In this study, transesterification reaction was carried out in 250 mL conical flasks according to the methods of Tripathi *et al.*, (2014) and Garba *et al.*, (2018) as modified. The transesterification process was performed in a 100 mL reaction mixture containing waste vegetable oil: methanol in molar ratio (3:1), NaOH/KOH (1 %), 100 mg alginate immobilized lipases (four beads), and 1 mL of free enzymes catalyst K-NPs and alginate immobilized lipases was shaken for 3 hrs at 200 rpm and 45 °C. These treatment mixtures were then poured into different separating funnels and then allowed to stay overnight to ensure the completion of the reaction, and for the mixture to completely separate into two layers of biodiesel and the denser glycerol settling at the bottom. The denser glycerol was then drained off, while the biodiesel was washed with distilled water, with the possible impurities that can affect combustion and exhaust emission all removed (Canakci and Van Gerpen 1999). The biodiesel was then allowed to settle for 1 hour to separate into two layers of the pure biodiesel and hydrated methanol, and these were separated using separating funnel.

2.10 Physicochemical Characterization of Clarified Biodiesel

The physicochemical properties were determined using saponification value, iodine value, free fatty acid, peroxide value, specific gravity, smoke point, flash point, boiling point, acid value, refractive index and thiobarbituric acid contents, respectively as described by Onyegbula *et al.* (2020).

2.10.1 Gas chromatography flame ionization detection (GC-FID)

In this study, the organic compounds contained in the biodiesel are identified and quantified using GC-FID according to the methods of Uba and Anidu (2023).

2.10.2 Fourier transform-infra red (FTIR) technique

The FTIR analysis was performed on the biodiesel in order to confirm the internal molecular structures in diverse range of fields, (Uba and Anidu, 2023).

RESULTS

The results of the green synthesis and characterization of potassium nanoparticles are presented in Figures and Tables. Figure 1 shows a pronounced absorption maximum at 350 nm with an absorbance of 0.0275, corresponding to strong surface plasmon resonance of the synthesized nanoparticles.

From the Figure 2 result, it showed the spectral profile with about 10 (more than 5) absorption bands which is indicative of a complex molecule with high molecular mass.

Table 1 showed the elemental composition and their atomic concentration in the biosynthesized green potassium nanoparticles. The result highlights the level of possible contaminants like sulphur to be very small and insignificant, the moderate concentrations of lipase co-factors (calcium, sodium, magnesium, potassium) and complete absence of possible lipase inhibitors (mercury, zinc, lead, copper).

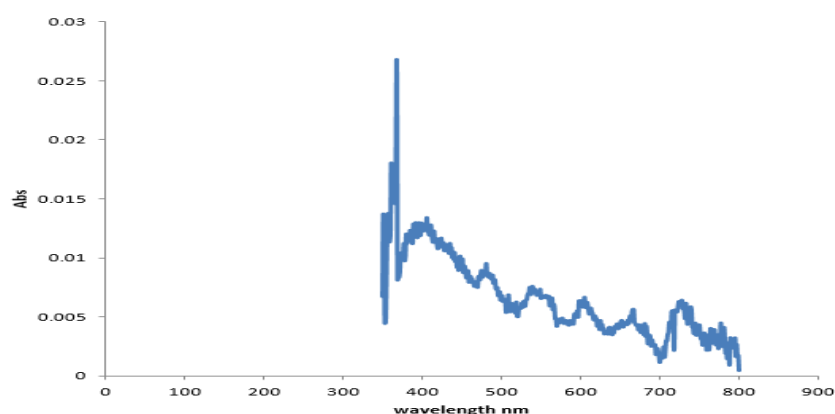


Figure 1: UV-VIS spectroscopy absorbency peaks of *Newbouldia laevis* synthesized potassium nanoparticles (KNPs).

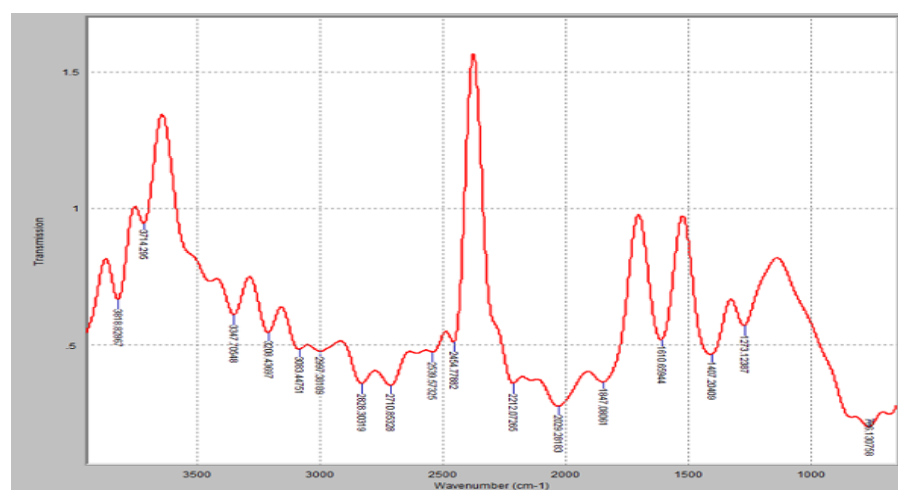


Figure 2: Fourier Transform Infra-red spectral profile of biosynthesized potassium nanoparticle

Table 1: Element composition of dispersive X – ray profile of biosynthesized potassium nanoparticle

Element number	Element symbol	Element name	Atomic concentration	Weight concentration
19	K	Potassium	52.43	55.82
50	Sn	Tin	3.37	8.76
11	Na	Sodium	5.14	2.59
22	Ti	Titanium	2.60	2.22
12	Mg	Magnesium	3.83	2.03
14	Si	Silicon	3.31	2.03
13	Al	Aluminum	2.84	1.67
15	P	Phosphorus	2.41	1.63
17	Cl	Chlorine	11.51	11.17
16	S	Sulphur	1.37	0.96
20	Ca	Calcium	5.90	5.79
23	V	Vanadium	0.28	0.32
26	Fe	Iron	5.00	5.00

From the Figure 4 result, the scanning electron micrograph revealed that the particles are amorphous in shape as there was clearly no cluster or colony formation of the biosynthesized green potassium nanoparticles.

From the Figure 3 result, showed very high peaks of potassium, tin, chlorine, calcium, and iron indicating crystalline structure while the others (of low peaks) indicated amorphous structural composition.

Comparatively, potassium nanoparticles immobilized lipase had a higher activity of 10.93 U/mL than the free/test lipase with 10.71 U/mL. Also, the protein content here showed close concentrations for the K-NPs immobilized lipase and the free/test lipase with 0.26 mg/mL for K-NPs and 0.256 mg/mL test lipase

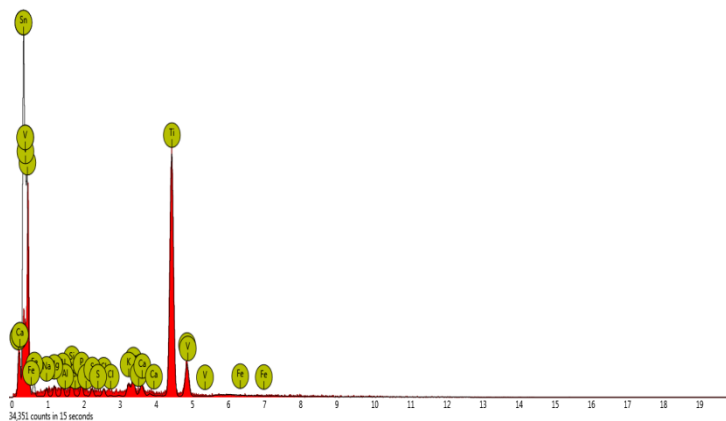


Figure 3: Energy dispersive X – ray profile of biosynthesized potassium nanoparticles

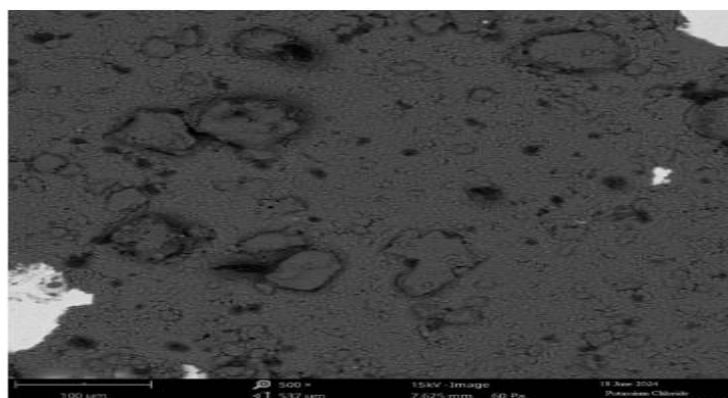


Figure 4: Scanning electron micrograph profile of biosynthesized potassium nanoparticles (X500 magnification).

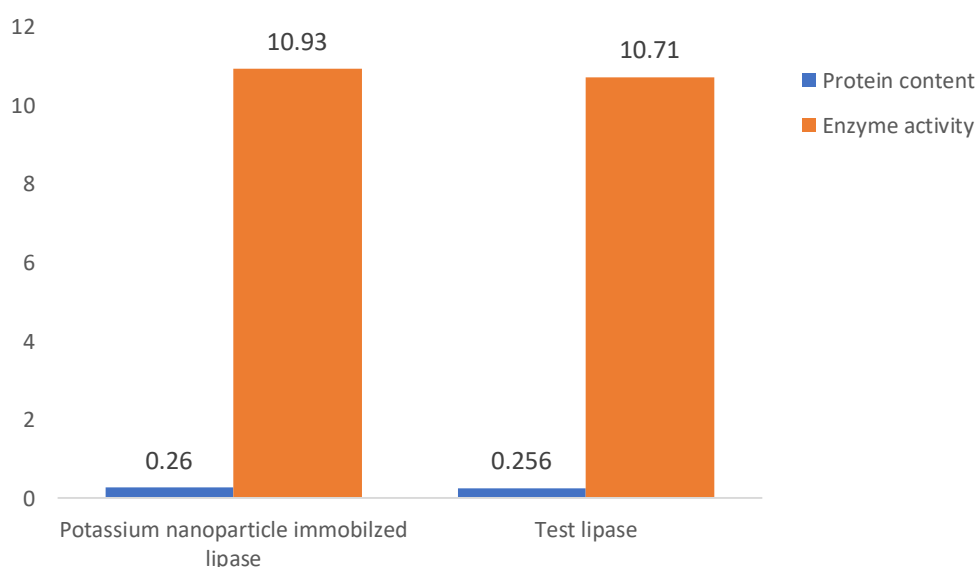


Figure 5: Protein content and enzyme activities of the test and potassium immobilized lipase produced by soyabeans.

The Figure 6 result indicated that the two forms of lipases under studied (potassium nanoparticle immobilized lipase and the free lipase) have an optimum temperature between 50 and 60 °C.

From the Figure 7 result, it showed relatively high enzyme activity at both acidic and alkaline pH while the two forms of enzyme under studied (K-NPS immobile lipase and free lipase) had optimum pH at 8.0.

From the Figure 8 result, it showed the FAME profile of the biodiesel under the same set up. From these, a similar trend occurred as found in the control/NaOH set up. The amount of the FAME in the transesterification mixture is 69 % (65.41707) of the total reaction mixture which is significantly higher than the amounts of the other by products.

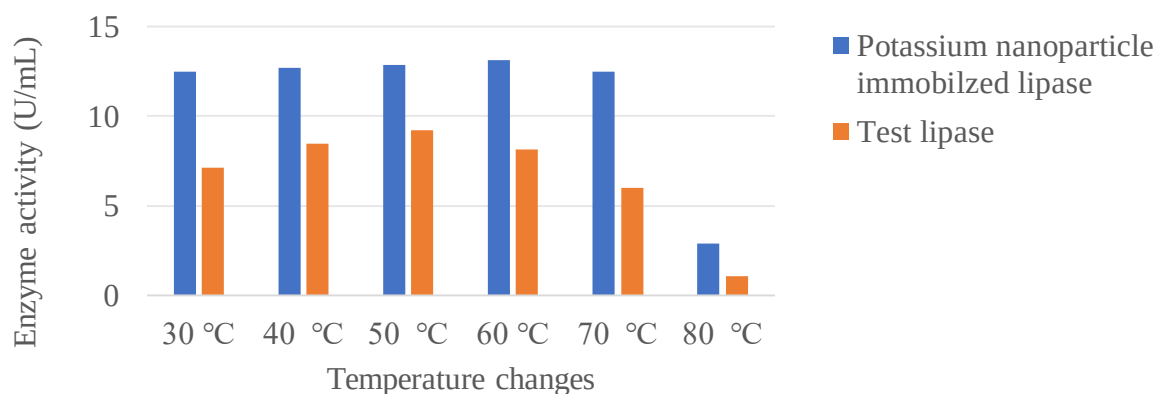


Figure 6: Temperature effect on the enzyme activities of the test and immobilized lipases produced by Soyabeans.

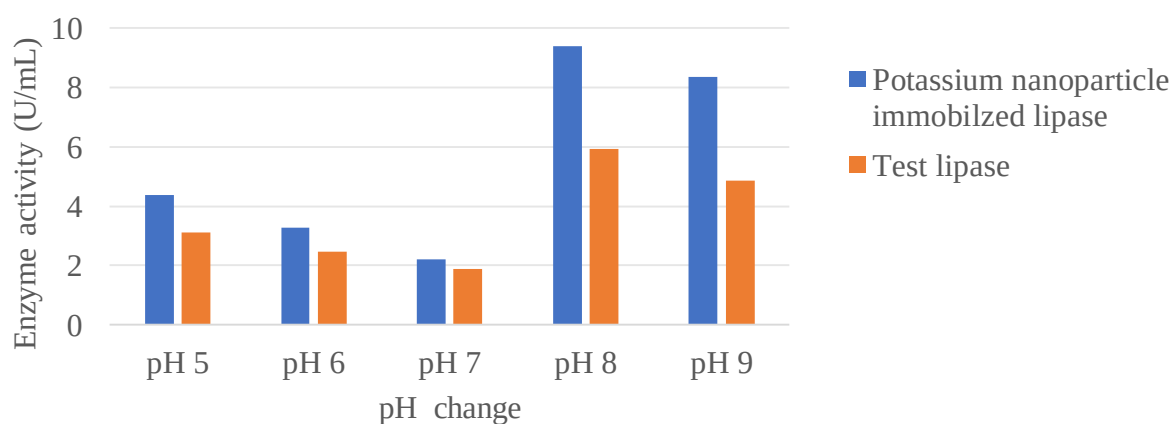


Figure 7: Effect of pH on the enzyme activities of the test and immobilized lipases produced by Soya beans.

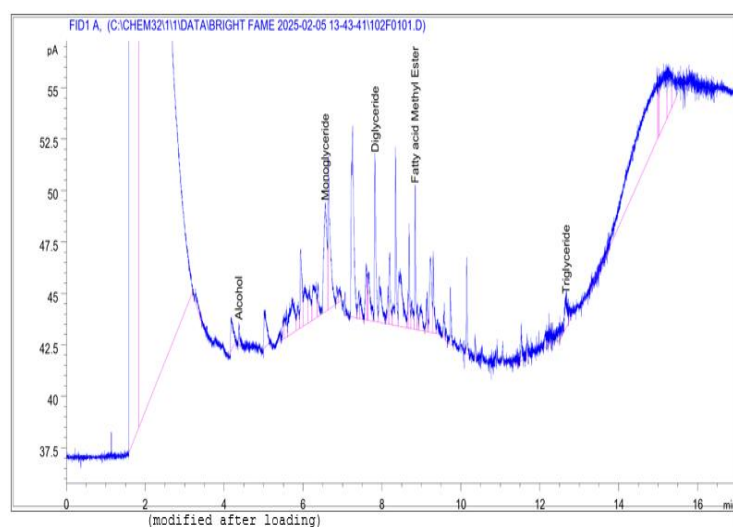


Figure 8: GCFID Chromatogram of the biodiesel produced under free lipase set up.

Table 2 presents the GC-derived fatty acid methyl ester (FAME) profile of the biodiesel produced under the free lipase setup, showing the distribution of reaction products and intermediates. The chromatogram confirms FAME as the dominant component, alongside residual glycerides and alcohol.

From the Figure 9 result, it showed the FAME profile of the biodiesel under the same set up. From these, a similar trend occurred as found in the control/NaOH set up. The amount of the FAME in the transesterification mixture is 68 % (75. 73765); this is comparatively higher than the amounts of the other by products in the mixture.

Table 2: Fatty acid methyl ester profile of the biodiesel produced under free lipase set up

Retention times (Min)	Area (pA*s)	Amount/ Area	Amount	Name
4.370	2.15940	1.26152	2.72414	Alcohol
6.566	27.40544	1.28513	5.21968	Monoglyceride
7.823	20.04156	1.28432	5.73982	Diglyceride
8.844	12.02299	1.28230	65.41707	Fatty acid Methyl Ester
12.674	12.13038	1.28268	15.55937	Triglyceride

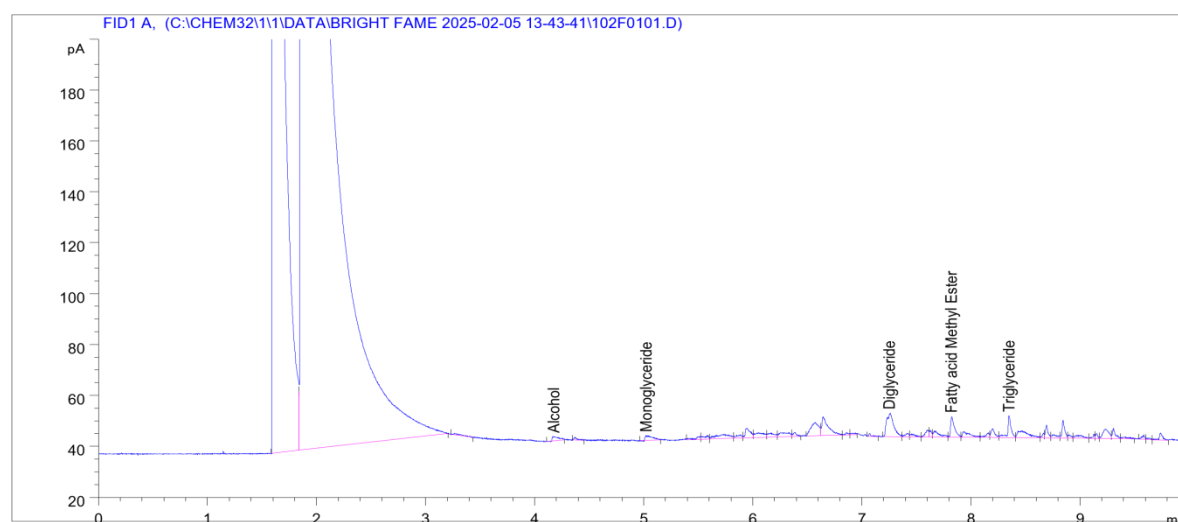


Figure 9: GCFID Chromatogram of the biodiesel produced under potassium nanoparticles immobilized lipase set up

Table 3: Fatty acid methyl ester profile of the biodiesel produced under potassium nanoparticle immobilized lipase set up

Retention times (Min)	Area (pA*s)	Amount/ Area	Amount	Name
4.177	5.97786	1.27826	7.64127	Alcohol
5.022	8.30491	1.28044	10.63392	Monoglyceride
7.258	40.41662	1.28580	1.96779	Diglyceride
7.823	20.04156	1.28421	75.73765	Fatty acid Methyl Ester
8.349	19.10220	1.28447	14.53617	Triglyceride

From the Figure 10 result, the wave number peaks recorded indicated presence of C-O stretch and primary or secondary alcohol in plane bend; carbonate ion, vinyl C-H in plane bend and organic nitrates; five-membered ring anhydride; thiols S-H stretch; methyl C-H asymmetric or symmetric stretch and vinyl C-H stretch; hydroxyl group, H-bonded, and normal 'polymeric' OH stretch.

From the Figure 11 result, it showed the spectral profile of the set up with K-NPs as catalyst. The wave number peaks recorded indicated the presence primary or secondary alcohol in plane bend and methyl C-H asymmetric or symmetric bend; C=C aromatic ring stretch and isothiocyanate; cyanide ion, thiocyanate ion and related ions; ammonium ion and methyl C-H asymmetric or symmetric stretch; aliphatic secondary amine N-H stretch and primary non-bonded hydroxyl OH stretch.

Table 4 summarizes the physicochemical properties of the biodiesel produced using free and K-NPs-immobilized lipase, compared with ASTM D6751 specifications, highlighting variations in fuel quality and compliance with standard limits.

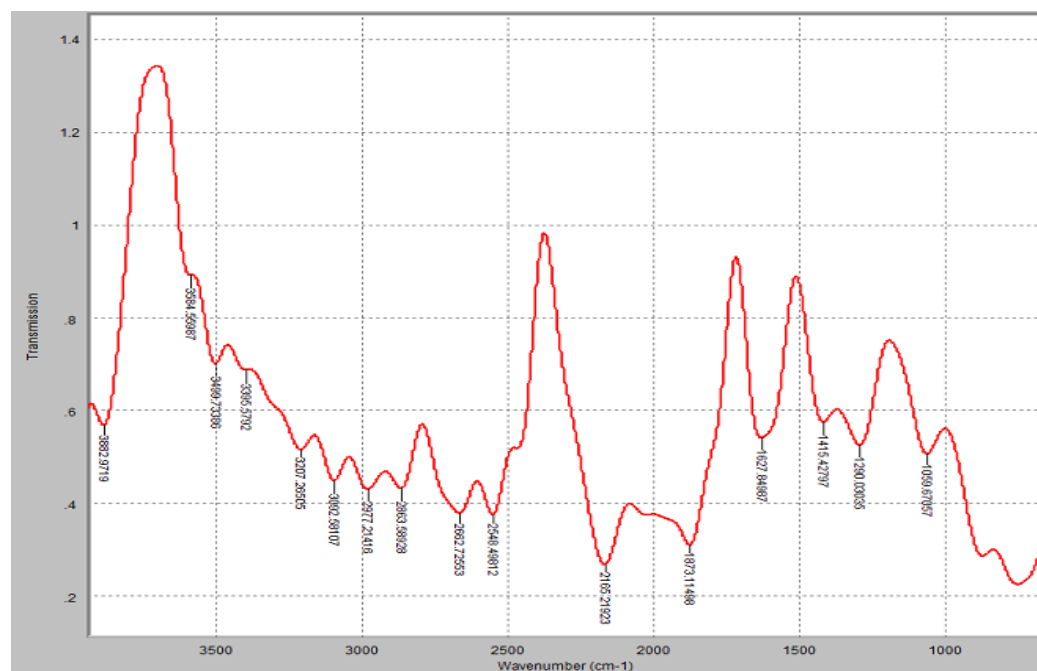


Figure 10: FTIR Analysis of the biodiesel produced with free lipase set up

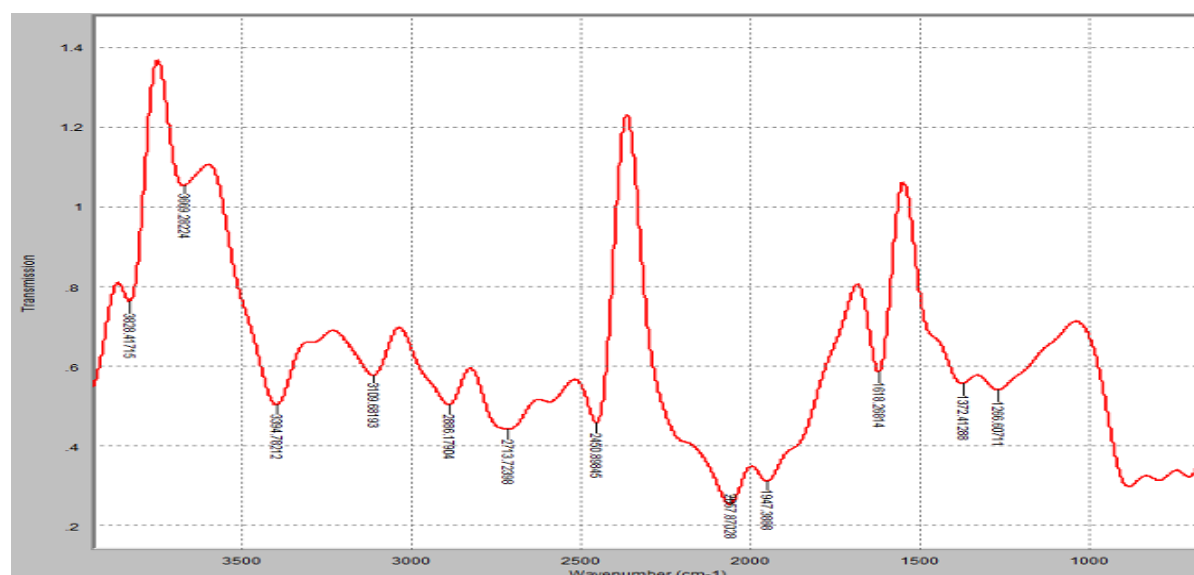


Figure 11: FTIR analysis of the biodiesel produced with Potassium nanoparticles immobilized lipase set up

Table 4: Physicochemical profile of the biodiesel produced

Parameters	Free Lipase	K-NPs Lipase	ASTM D6751 Test limit
Saponification value (mg/kg)	262.8298	287.3794	185 -196
Iodine value (mg I ₂ /100)	104.904	101.52	≤ 120
Free fatty acid (KOH mg/kg)	0.273	0.2785	0.50
Peroxide Value (mg/kg)	10.8	12.4	4.0 - 7.5
Specific gravity (g/mL)	0.926	0.936	0.85 – 0.90
Smoke point (° C)	195	232	NA
Flash point (° C)	128	140	120 – 140
Boiling point (° C)	325	300	330 – 375
Acid value (KOH mg/kg)	0.546	0.557	0.50
Refractive index @ 40 °C	1.415	1.422	1.2 - 1.8
Thiobarbituric acid test (mg MDA/kg)	5.289	4.920	0.80

Key: NA = Not available; MDA = Malondialdehyde; mg = Milligram; Kg = Kilogram; °C = Degree centigrade; KOH = Potassium hydroxide; g = Gram; mL = Millilitre; Mpas.Sec = Millipascal per second; I₂ = Iodine

DISCUSSION

Figure 1 showed the UV-VIS spectroscopy absorbency peaks of *Newbouldia laevis* synthesized potassium nanoparticles (KNPs). From the result, the maximum peak was at 350 nm wavelength and 0.0275 absorbance which is high surface plasmon resonance which was comparable with the UV value of 360 nm obtained in the published work of Ekwenze *et al.* (2025). Figure 2 showed the spectral profile of Fourier Transform Infra-red spectral profile of biosynthesized potassium nanoparticle with about 10 (more than 5) absorption bands which is indicative of a complex molecule with high molecular mass. The detection of the hydroxyl group in the FTIR spectral regions could be attributed to the presence of phenolics which have been reported for their reducing and stabilizing potentials on metal nanoparticles by some researchers (Okafor *et al.* 2023; Ubani *et al.* 2025; Ele *et al.*, 2025). Table 1 showed the elemental composition and their atomic concentration in the biosynthesized green potassium nanoparticles. The result highlighted the level of possible contaminants like sulphur to be very small and insignificant, the moderate concentrations of lipase co-factors (calcium, sodium, magnesium, potassium) and complete absence of possible lipase inhibitors (mercury, zinc, lead, copper). Figure 3 showed the energy dispersive X – ray profile of biosynthesized potassium nanoparticles with very high peaks of potassium, tin, chlorine, calcium, and iron indicating crystalline structure while the others (of low peaks) indicated amorphous structural composition. The detection of high peak of potassium element further confirm the successful synthesis of potassium nanoparticles. Figure 4 showed the scanning electron micrograph profile of biosynthesized potassium nanoparticles (X500 magnification). The scanning electron micrograph revealed that the particles are amorphous in shape as there was clearly no cluster or colony formation of the biosynthesized green potassium nanoparticles and corroborated with the published work of Constantino-Alcazar *et al.* (2021). Figure 5 showed the protein content and enzyme activities of the test free lipase enzyme produced by Soya beans and potassium nanoparticles immobilized lipase. Comparatively, potassium nanoparticles immobilized lipase had a higher activity of 10.93 U/mL than the free/test lipase with 10.71 U/mL. Similar observation was reported by Enemchukwu *et al.* (2026) who reported higher enzyme activity in immobilized than the free enzyme. Also, the protein content here showed close concentrations for the K-NPs immobilized lipase and the free/test lipase with 0.26 mg/mL for K-NPs and 0.256 mg/mL test lipase. Figure 6 showed the temperature effect on the enzyme activities of the test and immobilized lipases produced by Soya beans indicated that the two forms of lipases under studied (potassium nanoparticle immobilized lipase and the free lipase) have an optimum temperature between 50 and 60 °C. The result also revealed that at higher temperatures, free lipase is easily denatured, whereas immobilized lipase is protected from denaturation. Therefore, because of the stability of the enzyme structure, its catalytic activity is also maintained (Darvishi *et al.*, 2024). 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. The effect of pH on the enzyme activities of the test and immobilized lipases produced by Soya beans showed relatively high enzyme activity at both acidic and alkaline pH while the two forms of enzyme under studied (K-NPS immobile lipase and free lipase) had 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. The change in pH may be due to strong interactions between the lipase enzyme and the substrate surface and corroborated the published work of Darvishi *et al.* (2024). Figure 8 showed the GCFID chromatogram of the biodiesel produced under free lipase set up showed the FAME profile of the biodiesel under the same set up. From these, a similar trend occurred as found in the control/NaOH set up. The amount of the FAME in the transesterification mixture is 69 % (65.41707) of the total reaction mixture which is significantly higher than the amounts of the other by products. Figure 9 showed the GCFID chromatogram of the biodiesel produced under potassium nanoparticles immobilized lipase set up showed the FAME profile of the biodiesel under the same set up. From these, a similar trend occurred as found in the control/NaOH set up. The amount of the FAME in the transesterification mixture is 68 % (75.73765); this is comparatively higher than the amounts of the other by products in the mixture. Figure 10 showed the FTIR profile of the biodiesel produced with free lipase set up. The wave number peaks recorded indicated the presence of C-O stretch and primary or secondary alcohol in plane bend; carbonate ion, vinyl C-H in plane bend and organic nitrates; five-membered ring anhydride; thiols S-H stretch; methyl C-H asymmetric or symmetric stretch and vinyl C-H stretch; hydroxyl group, H-bonded, and normal 'polymeric' OH stretch. The FTIR profile of the biodiesel produced with Potassium nanoparticles immobilized lipase set up showed the spectral profile of the set up with K-NPs as catalyst. The wave number peaks recorded indicated the presence primary or secondary alcohol in plane bend and methyl C-H asymmetric or symmetric bend; C=C aromatic ring stretch and isothiocyanate; cyanide ion, thiocyanate ion and related ions; ammonium ion and methyl C-H asymmetric or symmetric stretch; aliphatic secondary amine N-H stretch and primary non-bonded hydroxyl OH stretch. The the quality and quantity of the biodiesel produced with K-NPs immobilized lipase competed with the world standard (ASTM 6751) for biodiesel and agreed with the published works of Garba *et al.* (2018) and Suresh *et al.* (2025) who reported that biodiesel produced under optimized reaction conditions met ASTM D6751 and EN 14214 standards.

CONCLUSION

In this work, green synthesis of potassium nanoparticles was carried out using *Newbouldia laevis* extract and lipase enzyme from *Glycine max* was immobilized covalently on the nanoparticles. The results of electron microscope imaging showed the successful immobilization of the enzyme. The comparison of free and immobilized enzyme activity showed an optimal temperature from 30 °C to 70 °C and also the optimal pH of the immobilized enzyme to both acidic and alkaline range can make it a suitable candidate for use in industries that require acidic conditions such as paper, leather making and food industry. From the quality of the biodiesel produced, it appears that the immobilized lipase has improved characteristics for application in different industries.

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