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An enhanced fat extraction from *Pycnanthus angolensis* (African nutmeg) seeds using cellulase from *Aspergillus niger* strain BC23

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Abstract

A promising extraction method for obtaining fat from *Pycnanthus angolensis* seeds through *Aspergillus niger* strain BC23 enzyme pretreatment and particle size reduction is presented in this work. The employed *A. niger* enzyme was produced from corn bagasse waste material, which served as the only source of carbon. *P. angolensis* seeds were dried, pulverized and subsequently sieved into two different particle sizes of 1.0 mm and 1.4 mm. This was followed by enzyme pretreatment prior to fat extraction. Results demonstrated that while the peak enzyme activity (121.036 U/mL) occurred on day 4 of fermentation, the maximum cellulase activity could be achieved at 60% saturation of ammonium sulfate purification. Compared with the results of water-treated and untreated seed samples, the fat yield, free fatty acid and iodine values of the enzyme-treated seed samples were considerably higher ($P < 0.05$). As peroxide value was less in the enzyme-assisted extracted fat, which pointed to a good-quality oil, the decreased refractive index suggested peak unsaturation. Despite considerable differences ($P < 0.05$) in refractive indices, fat's specific gravity matched ($P > 0.05$) when enzyme-treated, water-treated and untreated samples were compared. On the whole, size reduction of *P. angolensis* seeds pretreated with *A. niger* cellulase enzyme to improve both fat quality and yield implied it as a useful inventory for confectionary as well as cosmetic industries.

Keywords: acid value; *Aspergillus niger*; cellulase; peroxide value; *Pycnanthus angolensis*; saponification

Introduction

Pycnanthus angolensis (Welw) Warb. (family Myristicaceae) is a widely distributed species of tree in Central and West Africa, with its different parts traditionally serving in management and treatment of wide range of ailments such as mouth ulcer, anemia, malaria, schistosomiasis and fungal infections (Gustafson *et al.*, 2013; Mapongmetsem, 2007; Sofidiya and Awolesi, 2015). The seeds of the plant resemble those of true nutmeg (*Myristica fragrans*), although the latter belongs to a different genus; hence *P. angolensis* is literally known as African nutmeg. Its bitter-tasting inedible seeds serve as spice ingredient in Central Africa (Mapongmetsem, 2007). The seeds have an ample fat content of roughly

56–61.6%, with myristic (58.1–64.4%) and myristoleic (19.4–26.3%) acids as their main fatty acid components (Gustafson *et al.*, 2013). The fat, also called kombo butter, is a useful component of cosmetic/soap production, as it is a potential source of cetylmyristoleate (Gustafson *et al.*, 2013) and some terpenoid quinonic acids (Mapongmetsem, 2007), with antioxidant/anti-inflammatory properties. That is why, kombo butter is used extensively in cosmetic/pharmaceutical industries to make ointments used to relieve pain of body joints and muscles. Nonetheless, as common to most plant seeds, fats from *P. angolensis* seeds is extracted through mechanical and solvent (aqueous, organic) methods (Gustafson *et al.*, 2013; Lourith *et al.*, 2016; Mu *et al.*, 2021; Sirisompong *et al.*, 2011).

However, owing to ever-increasing demand of fats from vegetable sources (Borawski *et al.*, 2016), and compounding urbanization, civilization, and industrialization, there is a requirement to maximize fat yield, especially from oil seeds. A potential strategy is the use of enzymatic pretreatment of seeds and feedstocks for improved yields of fat and oil (Díaz-Suárez *et al.*, 2021; He *et al.*, 2020; Li *et al.*, 2012). Enzymatic breakdown of structural polysaccharides covering cells of fat-bearing seeds would enhance the release of fats/oil stored in the cells, and that also with less energy (Nhi and Tuan, 2016). Hence, this would enhance the penetration of extracting solvent and eventual release of fats into the medium.

As an environment-friendly approach, the enzyme-assisted extraction process would produce fat that would possess high oxidative stability and low phospholipid concentrations. This approach/strategy would minimize product loss during downstream processing (Kalia *et al.*, 2001; Zhang *et al.*, 2011). Despite having advantages, the application of enzyme-assisted fat extraction in relevant industries, especially in developing countries such as Nigeria, has been hampered by long processing time and high cost of enzyme purification. Hence, there is a requirement to explore the use of crude or partially purified enzymes for extraction of fat from seeds (Liu *et al.*, 2016). Studies utilizing enzyme pretreatment for extracting lipids from various plant materials have shown enhancement in lipid recovery. Enzymes such as cellulases, hemicellulases and pectinases, individually or in combination, have been employed in the pretreatment of plant materials for the hydrolysis of cell wall components, thereby increasing their accessibility to extracting solvents and improving the yield of fats/oil (Didia *et al.*, 2018; Nhi and Tuan, 2016; Qiu *et al.*, 2019; Tano-Deborah and Ohta, 1994; Zhang *et al.*, 2018).

Globally, agro-waste remains among the major solid wastes, which include corn cob, corn bagasse, sugarcane bagasse, grape vines, etc., and their use as an alternative source has become the focus of interest for many researchers (Ashori *et al.*, 2014; Atoyebi *et al.*, 2019). Production of amylase from *Aspergillus niger* has been previously reported through the fermentation of biomass bagasse waste substrates (Aliyah *et al.*, 2017). To the best of our knowledge, no study has investigated how cellulase produced from the fungus *A. niger* grown from waste material like corn baggase can help to enhance extraction of fat specifically from *P. angolensis* seeds through the context of particle size of seeds. To supplement the existing information, therefore, the present work investigated the use of partially purified cellulase from *A. niger* for enzymatic pretreatment of *P. angolensis* seeds prior to fat extraction, specifically aimed to improve (fat) quality and yield. The hypothesis is that fat yield can be improved based on the use of different particle size seeds. More so and as a cocktail of enzymes, the use of near-crude cellulase extract would play an important role, particularly to avoid the loss of (some) enzyme components that can happen during the extensive purification processes.

Materials and method

Schematic overview of the experimental program

Figure 1 shows the schematic overview of the experimental program, from the microbial isolation and culturing,

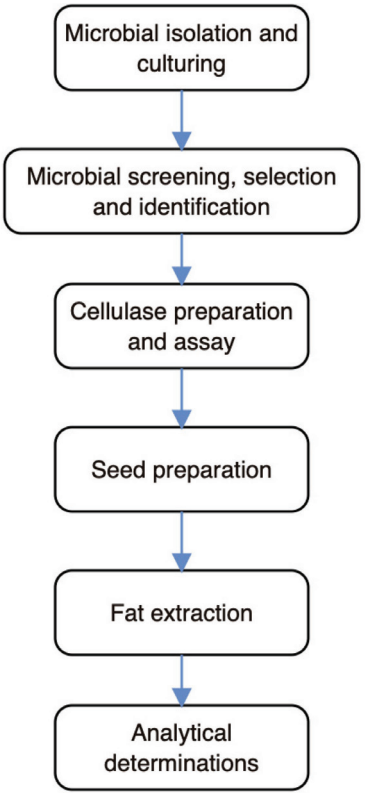

Figure 1. The schematic overview of the experimental program from the microbial isolation and culturing, microbial screening, selection and identification, cellulase preparation and assay to analytical determination.

microbial screening, selection and identification, cellulase preparation and assay to analytical determinations. For emphasis, the present work was purposed to investigate the use of partially purified cellulase from *A. niger* for the pretreatment of *P. angolensis* seeds prior to fat extraction for improving yield and quality of fat. Besides, cellulase was to be produced from *A. niger* using the corn bagasse—a domestic waste which is to serve as the only carbon source. Additionally, the temperature and pH optima for enzyme activity were also ascertained. In all cases, the measurements were performed thrice using different samples. In addition, all chemicals and reagents used were of analytical grade, and were procured from certified retailers.

Isolation and culturing of microorganism

Soil samples were collected from dumpsites in Nsukka area of Enugu State. The soil was serially diluted and used to inoculate sterilized agar plates using spread plate method. About 0.1 mL of each sample was aseptically transferred to Sabouraud dextrose agar (SDA) plates using a micropipette. Samples were evenly distributed around the plate using a sterilized spreader, and incubated at 30°C for 4 days for fungi growth. Distinct colonies were sub-cultured repeatedly on SDA to get pure fungi isolates.

Screening, selection and identification of cellulase producing fungi

Basal salt medium was prepared according to the method adopted by Pointing (1999). The media were sterilized in an autoclave at 121°C for 15 min, transferred aseptically to sterilized petri dishes and allowed to solidify. The test organisms were inoculated into the media and incubated at 30°C in darkness for 5 days. In order to identify the cellulase-producing fungi, the plates were covered with 2% (w/v) aqueous Congo red stain and left for 20 min. The stain was poured off, and the agar surface was washed with distilled water (Bradner *et al.*, 1999). Thereafter, the plates were inundated with 1-M NaCl for 20 min to remove the stain. The positive result of colonies of cellulase-producing fungi was identified as a yellow-opaque region with red background. Isolated fungi (*A. niger*) were identified based on cultural and morphological characteristics using lactophenol cotton blue staining method and strain BC23 (species *C. elegans*) in accordance with Pitt and Hocking's (2009) taxonomic descriptions.

Culture medium

Culture medium was prepared using dried and ground corn bagasse as the sole source of carbon to produce cellulase from *A. niger*. Basal salt media prepared according to the Pointing (1999) method were put into separate conical flasks (250-mL capacity), each containing basal medium (100 mL). This was autoclaved at 121°C for 15 min. After cooling, *A. niger* was introduced. Replicate cultures were left incubated at 30°C for several days. Crude enzyme was harvested from flasks every day, and assays of cellulase activity were conducted for determining the day of maximum enzyme production. In addition, cultures of different concentrations of corn bagasse were incubated to obtain the optimum concentration of substrate for cellulase production.

Cellulase preparation and activity assay

Solid-state fermentation

Cellulase was produced by solid-state fermentation. In all, 25 g of corn bagasse was placed in different flasks. The Pointing (1999) medium was added to each flask and autoclaved. After cooling, the flasks were inoculated and incubated at 30°C for 4 days before extraction of crude enzyme, which was done by adding 0.1-M sodium phosphate buffer (50 mL, pH 6.0) into each flask. This was followed by agitation at 150 rpm and 30°C for 30 min. Slightly wet cheese cloth was used for extract filtration, and the filtrate was centrifuged at 6,000 rpm for 15 min to get clear supernatant (crude enzyme), which was kept at 4°C prior to use.

Cellulase activity assay

The partially purified cellulase was mixed with substrate, 1% carboxymethylcellulose (CMC) in phosphate buffer (pH 6.5) at 50°C for 15 min. At 540 nm, dinitrosalicylic acid (DNS) assay was applied to determine enzyme activity by quantifying the level of reducing sugar produced per milliliter. Similarly, protein content was determined by the Lowry method (Lowry *et al.*, 1951) using BSA as protein standard.

Ammonium sulfate precipitation

Ammonium sulfate precipitation was performed to ascertain the percentage of salt required to precipitate maximum protein from the stock (crude) of enzyme solution. Various concentrations of salt (20% w/v to 100% w/v) were added to crude enzymes (10 mL each) in nine test tubes. After thorough mixing of the content in each tube, they were kept aside at 4°C for 30 h. Thereafter, they were centrifuged at 4,000 rpm for 30 min, followed by decanting of supernatants and redissolution of pellets in equal volume of 0.1-M phosphate buffer (pH 6.0). Estimation of cellulase activity was done in each tube. The percentage of salt for which the activity of cellulase was highest was noted, and was scaled up to 1 L for mass precipitation of the enzyme for dialysis.

Temperature and pH optima of cellulase activity

The activity of cellulase from *A. niger* was determined through incubation of the enzyme with CMC solution (1% w/v) through 30–90°C range, at an interval of 10°C for 15 min and at pH 6.0 (0.1-M sodium phosphate buffer). The optimum temperature was obtained from a plot of activity of cellulase against temperature. In addition, the effect of pH on cellulase activity was estimated using 0.1-M sodium acetate buffer, pH 3.5–5.5, phosphate buffer, pH 6.0–7.5, and Tris-HCl buffer, pH 8.0–10.0 at intervals of 0.5. The optimum pH was estimated from a plot of activity of cellulase against pH.

Collection and preparation of the seeds

The *P. angolensis* seeds used in this study were collected from Umana Ndiuno in Ezeagu Local Government Area of Enugu State, Nigeria. The seeds were screened to remove the damaged ones; thereafter their shells were carefully removed to obtain kernels. The kernels were dried to a constant weight in an oven at 60°C for 96 h, ground using mechanical grinder and sieved into two particle sizes of 0.1 mm and 1.4 mm. Each of the two particle sizes of *P. angolensis* seeds was divided into three portions of 100 g each. The first portion was treated with 50-mL buffer and 50-mL partially purified enzyme (cellulase). The contents were thoroughly mixed for 1 h before incubation. The second portion was treated with 50-mL buffer and 50-mL distilled water to serve as control and thoroughly mixed for 1 h before incubation. The third portion was left without any treatment. The treated portions were incubated at 50°C for 24 h while the pH values of the treated samples were adjusted to 6.5 using sodium phosphate buffer. At the end of incubation, the treated samples were dried to constant weights in an oven and stored in a desiccator.

Extraction of fat from *P. angolensis* seeds

Fat extraction from *P. angolensis* seeds was carried out by the Soxhlet extraction technique using n-hexane as solvent. The percentage fat yield of *P. angolensis* seeds was determined after triplicate run (each at 65°C for 6 h) and the mean value was calculated. The percentage fat yield was calculated using the following equation below:

$$\%\text{Fat yield} = \frac{\text{Weight of extracted oil (g)}}{\text{Weight of dried sample (g)}} \times 100$$

Physicochemical properties of fat

Physiochemical properties of fat were determined using the method mentioned by Association of Official Analytical Chemists (AOAC, 2005), which included the following: acid value, free fatty acids, saponification value, iodine value, peroxide value, refractive index and viscosity.

Acid value and determination of free fatty acids

For determining acid value (AV), 5.0 g of fat was taken in a conical flask containing 25-mL absolute ethanol; to this was added 2 drops of phenolphthalein indicator. The mixture was heated with continuous shaking in a water bath at 65°C for 10 min. The resulting solution was cooled and titrated against 0.1-N KOH until a pink color appeared. The AV was estimated following equation below:

$$\text{AV} = \frac{\text{Vol. of KOH} \times \text{Normality of KOH}}{\text{Weight of sample (g)}} \times 56.1$$

The amount of free fatty acids (FFA) was calculated by the following equation below:

$$\text{FFA} = \text{Acid value} \times 0.503.$$

Determination of saponification value

In order to determine the saponification value (SV) of fat, 25 mL of 0.5-N alcoholic potassium hydroxide was first added to 2-g fat in a 250-mL capacity conical flask. Thereafter, a reflux condenser was attached and the mixture was heated for 1 h in a boiling water bath, with intermittent shaking. Following the addition 3 drops of phenolphthalein indicator to the warm solution, titration was carried out against 0.1-N HCl. In addition, titration was done against HCl using the blank sample (25-mL alcoholic KOH). Saponification value was estimated using the following equation below:

$$\text{Saponification value (SV)} = \frac{56.1(\text{B–S}) \times \text{Normality or HCl}}{\text{Weight of sample (g)}}$$

where B = amount of HCl in milliliter required for the blank sample, and S = amount of HCl in milliliter required for sample.

Determination of iodine value

Determination of iodine value (IV) was done by adding 20-mL carbon tetrachloride and 25-mL Wijs reagent to 0.25-g fat in a 250-mL capacity Erlenmeyer flask. The flask was stoppered, swirled to mix the contents, and then stored in dark for 30 min. Thereafter, 10-mL 30% potassium iodide solution and 100-mL distilled water were added and the mixture was titrated against sodium thiosulfate solution. Following the disappearance of yellow color, 1-mL starch indicator was added and the solution (blue in color) was titrated until the endpoint (solution becomes colorless). Similarly, the procedure was repeated for blank solution (without fat) and the iodine value was calculated following the equation below:

$$\text{IV} = \frac{(\text{B–S}) \times \text{Normality of } Na_2S_2O_3 \times 0.127}{\text{Weight of sample (g)}} \times 100$$

where B = amount of sodium thiosulfate solution in milliliter required for blank sample, and S = amount of sodium thiosulfate solution in milliliter required for sample.

Determination of peroxide value

For determining peroxide value (PV), 30-mL acetic acid–chloroform solution was first added to 5-g fat in a 250-mL capacity stoppered Erlenmeyer flask. The mixture was swirled until the complete dissolution of fat. Thereafter, 0.5-mL solution of saturated potassium iodide was added, and the flask was stoppered and contents swirled for 1 min. Followed with immediate addition of 30-mL distilled water, the flask was restoppered and vigorously shaken to release iodine from the chloroform layer. A deep red-orange colored mixture was then titrated against 0.1-N sodium thiosulfate solution until the disappearance of color. This was followed by the addition of 1-mL starch solution, and the mixture was further titrated with more of 0.1-N sodium thiosulfate solution until the disappearance of blue-gray color in the upper layer. The procedure was repeated for blank as well as PV (expressed in milli-equivalent of active oxygen per kg of sample) calculated using the following equation below:

$$\text{PV} = \frac{(\text{S}-\text{B}) \times \text{Normality of } \text{Na}_2\text{S}_2\text{O}_3 \times 1{,}000}{\text{Weight of sample (g)}}$$

where S = amount of sodium thiosulfate solution in milliliter required for sample, and B = amount of sodium thiosulfate solution in milliliter required for blank sample.

Determination of refractive index and specific gravity

Refractive index was determined by using Abbe refractometer. In addition, specific gravity (SG) of fat was determined by using a 25-mL capacity density bottle at 50°C. It was calculated as the ratio of the weight of fat to that of equal volume of water at a specific temperature, depicted in the equation below:

$$\text{SG} = \frac{\text{Density of fat (Kg/m}^3\text{)}}{\text{Density of water (Kg/m}^3\text{)}}$$

Determination of viscosity

Viscosity (η_1) was determined using Oswald viscometer. The stated viscometer has two marks, one at top of the bulb and another at the bottom. The sample was drawn from one arm to fill the bulb, after being poured from the opposite arm. On getting to the top mark, a stopwatch was set and the time the sample took to flow down to the bottom mark from the top mark was recorded as t_1. This was used to calculate the viscosity using the following the equation below:

$$\eta_1 = \frac{\eta_2 \times \rho_1 \times t_1}{\rho_2 \times t_2}$$

where η_1 = viscosity of sample, η_2 = viscosity of water = 1.005, ρ_1 = density of sample and ρ_2 = density of water.

Statistical analysis

SPSS version 22 for Windows was used to run the collected data. One way analysis of variance (ANOVA) was applied to the emergent data derived from three measurements taken from different samples. The results were presented in terms of mean ± standard deviation (SD) where appropriate, and the level of statistical significance was set at $P < 0.05$.

Results and discussion

Enzyme production, ammonium sulfate precipitation profile, and cellulase activities

Incubation time and substrate concentration were the critical factors considered for the efficiency of enzyme production, which Gregg and Saddler (1996) considered while reviewing the factors affecting cellulose hydrolysis and potential of enzyme recycle to enhance efficiency of integrated wood-to-ethanol process. The initial aspect of this study, which was performed to determine the day of peak enzyme production, could be considered to be a pilot feature. Figure 2 shows the effects of (a) incubation period and (b) substrate concentration on enzyme production. The peak enzyme activity (121.036 U/mL) was recorded (Figure 2A) specifically on day 4 of fermentation. This situation suggested a feasible harvest (enzyme) at about 96 h (on day 4) of fermentation, similar to the findings published elsewhere (Abubakar and Oloyede, 2013; Costa *et al.*, 2021; Maftukhah and Abdullah, 2018; Okoye *et al.*, 2013). The incubation period, however, would depend on a number of factors, such as the availability/level of nutrients, fermentation environment, nature of fermenting organisms and the physiological conditions influencing the (fermentation) process (Patil and Dayanand, 2006; Shivasharanappa *et al.*, 2014). With the concentration of substrate (corn bagasse) being optimized, the cellulase activity peaked (121.331 U/mL) when the concentration of the corn bagasse reached 25% w/v (Figure 2B). It is important to observe that the crude cellulase production was conducted by growing the fungi for 4 days in a mineral medium that contained 25% w/v corn bagasse, the latter being the sole carbon source. Afterwards, the medium containing crude cellulase was harvested and purified partially, subsequently.

The ammonium sulfate precipitation profile is shown in Figure 3. Given the ammonium sulfate purification at 60% saturation, a maximum cellulase activity seemed apparent, which enabled its use for subsequent stages. After precipitation, the enzyme was dialyzed to remove excess salt. Based on the highest activity because of changes in temperature and pH, the dialyzed enzyme was interpreted. The effects of (a) temperature and (b) pH

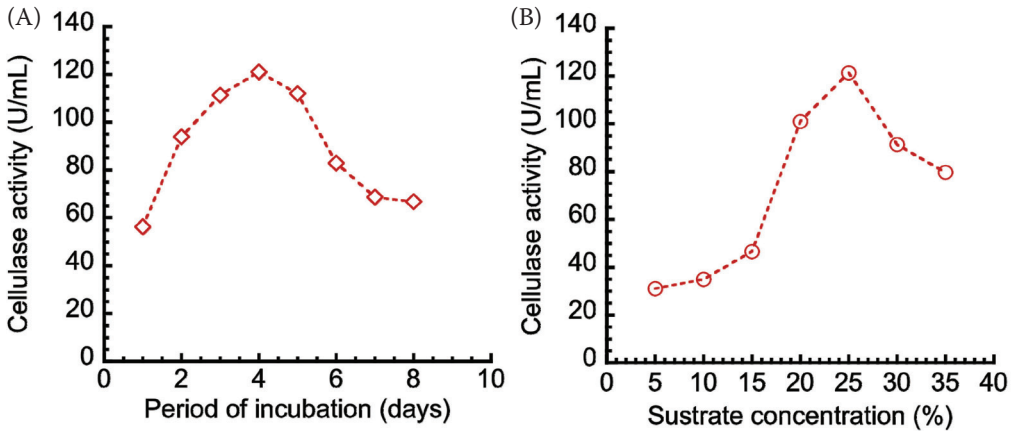

Figure 2. Effects of (A) incubation period, and (B) substrate concentration on enzyme production.

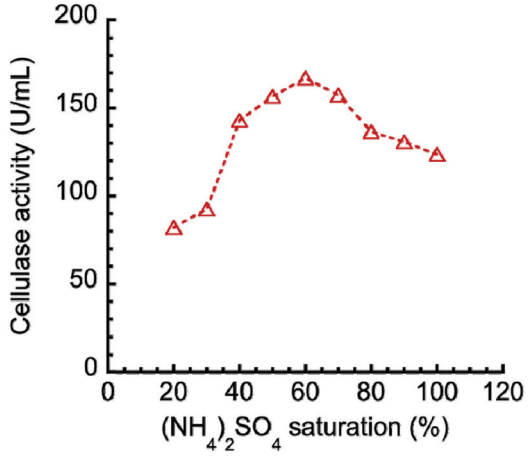

Figure 3. Ammonium sulfate precipitation profile.

on cellulase activities are shown in Figure 4. The temperature increased with enzyme activity to attain the peak of 50°C (Figure 4A), which declined subsequently. Temperature increased with average kinetic energy and reaction rate of enzyme molecules. The enzyme activity was inclined to drop gradually after reaching an optimum temperature, suggesting change in enzyme conformation. Possibly, the disulfide bond interactions between hydrophobic domains might be disrupted, which might be followed by denatured/inactivated enzyme. Equally, the optimum cellulase activity (264.73 U/mL) was observed at pH 6.5 (Figure 4B), which suggested enzyme functioning in an acidic environment. Previous studies (Okoye *et al.*, 2013; Srivastava *et al.*, 2017) obtained optimum temperature of 50°C for other cellulase produced from *Emericella variecolor* and *A. niger*. Gilna and Khaleel (2011) and Srivastava *et al.* (2022) also obtained pH 6.5 as the optimum value for other cellulase produced from *Cladosporium cladosporioides* and *Rhizopus oryzae* coculture and *A. fumigatus*. In the present work, crude fractions, and precipitated and dialyzed enzymes were assayed for cellulase activities and protein contents. Comparisons of protein concentrations and activities of crude fractions, and precipitated and dialyzed cellulases are shown in Figure 5. Protein concentration decreased from 33.65 to 10.20 mg/mL after precipitation of ammonium sulfate, and further decreased from 10.20 to 9.84 mg/mL after dialysis, the latter being attributed to the removal of proteins of lower molecular weight. In addition, decrease in cellulase activity from 394.18 to 159.22 U/mL after precipitation but subsequent increase from 159.22 to 333.98 U/mL after dialysis could be due to the removal of impurities to alter enzyme activity.

Purification profile of cellulases from *A. niger*

The purification profile of cellulases from *A. niger* is shown in Table 1. These were the result of protein and enzyme assays of crude as well as precipitated and dialysed enzymes used to determine their respective activities and purification fold. Effect of particle sizes and enzyme treatment on fat yield is shown in Table 2. The percentage fat yield of *P. angolensis* seeds of particle size 1.0 mm demonstrated the peak of enzyme-treated sample (88.15 ± 0.82%), followed by water-treated sample (76.75 ± 0.42%) and untreated sample, which produced the least yield (72.08 ± 0.95%). The percentage fat yield of enzyme-treated sample appear considerably higher ($P < 0.05$) compared to water-treated and untreated samples. The same pattern seemed apparent when the particle size of *P. angolensis* seeds increased to 1.4 mm. The enzyme-treated sample obtained a fat yield of 83.91 ± 0.33%, significantly higher ($P < 0.05$) than the water-treated sample (74.87 ± 0.55%) and the untreated sample (68.34 ± 2.36%). Fat extracted from the enzyme-treated sample was also higher than the fat yield of 74.13% earlier obtained by Nagre *et al.* (2011), which asserted enzyme pretreatment with capacity to provide enhanced fat extraction. Similarly, pretreatment of biomass with

Table 1. Purification profile of cellulase from *A niger*.

Purification steps	Vol (mL)	Protein concentration (mg/mL)	Activity (U/mL)	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification fold
Crude enzyme	1,000	33.65	394.18	33,645	394,176	11.72	1
60% $(NH_4)_2SO_4$	82	10.20	159.22	835.99	13,056	15.62	1.33
Dialysed enzyme	101	9.84	333.98	993.84	33,732	33.94	2.89

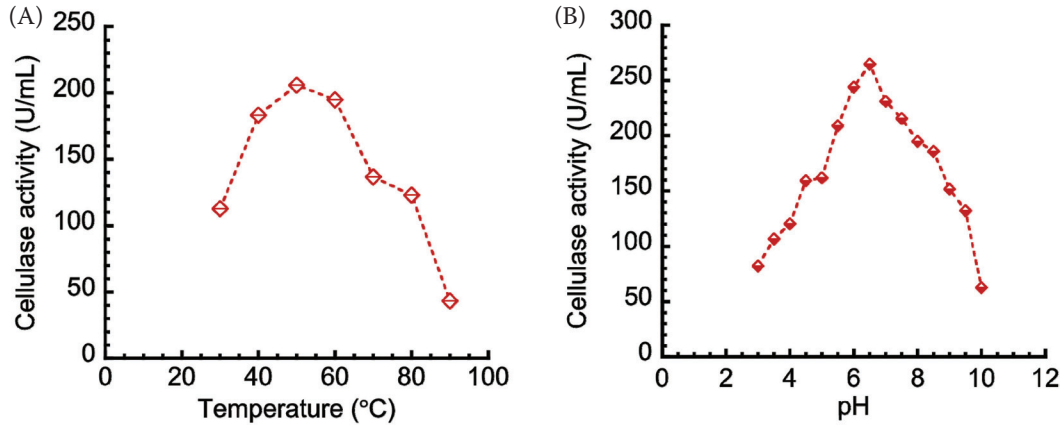

Figure 4. Effect of (A) temperature, and (B) pH on cellulase activities.

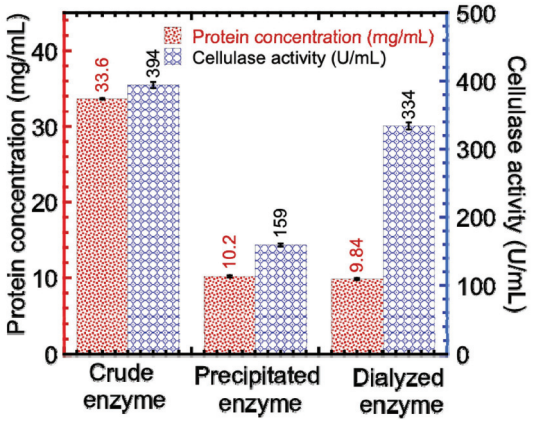

Figure 5. Comparisons of protein concentrations and activities of crude, precipitated, and dialyzed cellulase.

enzymes prior to lipid extraction increased lipid yield relative to non-enzymatic pretreated samples (Díaz-Suárez *et al.*, 2021; He *et al.*, 2020; Liu *et al.*, 2019). In another study, Otu *et al.* (2015) obtained a 4% increase in fat yield when the shea tree seeds were treated with crude enzyme. In the present study, the enzyme-treated samples depicted enhanced fat yield in the investigated *P. angolensis* seeds of two particle sizes. Probably, this could be due to the presence of enzymatic hydrolysis that digested complex cell walls of oil-bearing seeds, which by enhancing the permeability of solvent (n-hexane), favored the oil extraction. Furthermore, the smaller particle size of 1.0 mm, as shown in Table 2, produced higher fat yield in each group of enzyme-treated, water-treated

Table 2. Effect of particle size and enzyme treatment on fat yield.

Particle size	Untreated sample	Fat yield (%) Water-treated sample	Enzyme-treated sample
1.0 mm	72.08 ± 1.65^a	76.75 ± 0.72^b	88.15 ± 1.42^c
1.4 mm	68.34 ± 4.09^a	74.87 ± 0.96^b	83.91 ± 0.57^c

Values were mean ± standard deviation of triplicate run. Different superscripts alphabets ($^{a, b \text{ and } c}$) across the rows indicate significant difference between mean values at $P < 0.05$.

and untreated samples. Reduction in particle size of seeds to have increased fat yield could be anticipated, given the enhanced surface area of smaller particle size (Dash *et al.*, 2021; Ishak *et al.*, 2021).

Physicochemical properties of extracted fat from *P. angolensis* seeds

The physicochemical properties of extracted fat from *P. angolensis* seeds are shown in Table 3. For emphasis, the physicochemical properties involved measurement of acid value, free fatty acid, specific gravity, viscosity, refractive index as well as iodine, saponification and peroxide values. The acid value of fat of the enzyme-treated sample (7.33 ± 0.06 mg KOH/g) was considerably ($P < 0.05$) higher than that of the water-treated sample (5.95 ± 1.22 mg KOH/g) and the untreated sample (5.72 ± 0.11 mg KOH/g). A considerable increase ($P < 0.05$) in free fatty acid of the enzyme-treated sample (3.69 ± 0.03%) occurred when

compared with the water-treated (2.99 ± 0.11%) and untreated samples (2.88 ± 0.06%). Probably, the cellulase's hydrolytic action on the cell walls of oilseeds exposed the lipid bilayer of cell membranes, which might have enhanced the secretion of native seed enzyme lipase. Prolonged activities of lipase to degrade lipid bilayer would likely increase the release of free fatty acids (Kumar *et al.*, 2021).

Saponification value revealed the average molecular weight of triacylglycerol components of fat. Higher saponification values indicated shorter chain fatty acids in ester bonds with glycerol backbone. Despite that no noticeable difference ($P > 0.05$) occurred between the saponification values of fat of the enzyme-treated (236.46 ± 2.81 mg KOH/g) and water-treated (227.11 ± 7.06 mg KOH/g) samples, that of untreated (223.37 ± 3.24 mg KOH/g) sample appeared considerably ($P < 0.05$) lower (Table 3). Besides that Nagre *et al.* (2011) reported a saponification value of 238.0 mg KOH/g fat in *P. angolensis* seeds, other workers showed virgin coconut oil extracted using different processes would produce higher saponification values (254.10–264.04 mg KOH/g) highest at the enzyme-assisted method produced the highest values (Mohammed *et al.*, 2021). Since the saponification values in this study were lower than those reported for virgin coconut oil, those from *P. angolensis* fat would accumulate in lower proportions of low molecular weight fatty acids; this suggests a smaller acidity content, and hence better feedstock for soap and confectionary applications.

Table 3 also reveals that, despite resemblances ($P > 0.05$), the iodine value of fat of the enzyme-treated samples was slightly higher (65.36 ± 2.21 g I_2/g) than the water-treated (64.18 ± 1.06 g I_2/g), followed thereafter by untreated (62.99 ± 0.78 g I_2/g) sample. Further, these iodine values compared well with the data (64.9 g I_2/g) of *P. angolensis* seeds reported by Nagre *et al.* (2011). Similarly, a higher iodine value was indicated for enzyme-assisted oil extracted from *S. mukorossi* seed kernels (113.15 ± 3.01 g I_2/100 g) compared with the iodine value of solvent-extracted oil (107.39 ± 2.64 g I_2/100 g) (Liu *et al.*, 2019). Owing to low iodine values observed in the present study, the fat of *P. angolensis* seeds could be considered as nondrying lipid with promising storability. Relatively low degree of unsaturation of fat would depict an improved thermo-oxidative stability, which can potentially serve as biolubricants and bioplasticizers (Cecilia *et al.*, 2020; Ikhuoria and Maliki, 2007).

The peroxide values of fat of enzyme-treated (14.63 ± 1.53 meq/kg), water-treated (27.97 ± 2.08 meq/kg) and untreated (32.63 ± 1.53 meq/kg) samples differed considerably ($P < 0.05$) (Refer to Table 3). The less peroxide value of the enzyme-assisted extracted fat suggested higher resistance to oxidative rancidity, and thus a good quality oil (Mohammed *et al.*, 2021; Nandi and Bhattacharyya, 2015). Resistance to oxidative deterioration of enzyme-treated sample would help to substantiate the oxidative stabilities (Elagbar *et al.*, 2016), given the presence of natural antioxidants. Moreover, the above situation is compared with onion oil extracted with cellulase with high phenolic content, which is an antioxidant, as reported by Salina *et al.* (2013). Thus, the enzyme used to enhance fat extraction could help liberate antioxidants (Nhi and Tuan, 2016). Besides the high antioxidant content of *P. angolensis* fat (Nagre *et al.*, 2011), natural antioxidants could help to reduce the peroxide value of oil/fat (Ullah *et al.*, 2003; Zhang *et al.*, 2021).

Additionally, the specific gravity of fat resembled ($P > 0.05$) across enzyme-treated (0.856 ± 0.10), water-treated (0.864 ± 0.10) and untreated (0.893 ± 0.09) samples (Table 3). Specific gravity values of fat observed in the present work were less than the data reported by Nagre *et al.* (2011) for *P. angolensis* (0.922), which had been obtained via solvent extraction. Reduced specific gravity of fat of the enzyme-treated sample could be due to its lower molecular weight, considering its saponification values. At the temperature of 50°C, the viscosity of fat differed significantly ($P < 0.05$) between enzyme-treated (1.369 ± 0.02 cP), water-treated (1.520 ± 0.04 cP) and untreated (2.498 ± 0.04 cP) samples (Table 3). The least value of viscosity of fat of the enzyme-treated sample could be due to its slightly higher degree of unsaturation, reinforced by its iodine value. This phenomenon demonstrated that oil viscosity depended on the structure of molecules and increased with the saturation of fatty acid (Talkit *et al.*, 2012). Additionally, the refractive index of fat of enzyme-treated (1.481 ± 0.0019), water-treated (1.485 ± 0.0005)

Table 3. Physicochemical properties of the extracted fat of *P angolensis* seeds.

Parameters	Untreated sample	Water-treated sample	Enzyme-treated sample
Acid value (mg KOH/g)	5.72 ± 0.11^{a}	5.95 ± 1.22^{a}	7.33 ± 0.06^{b}
Free fatty acid (%)	2.88 ± 0.06^{a}	2.99 ± 0.11^{b}	3.69 ± 0.03^{c}
Saponification value (mg KOH/g)	223.37 ± 3.24^{a}	227.11 ± 7.07^{a,b}	236.46 ± 2.81^{b}
Iodine value (gI_2/g)	62.99 ± 0.78^{a}	64.18 ± 1.06^{a}	65.36 ± 2.21^{a}
Peroxide value (meq/kg)	32.63 ± 1.53^{a}	27.97 ± 2.08^{b}	14.63 ± 1.53^{c}
Specific gravity	0.893 ± 0.010^{a}	0.864 ± 0.010^{a}	0.856 ± 0.010^{b}
Viscosity (cP)	2.498 ± 0.000^{a}	1.520 ± 0.037^{b}	1.369 ± 0.002^{c}
Refractive index	1.4927 ± 0.04^{a}	1.4856 ± 0.04^{b}	1.4811 ± 0.02^{c}
Color	Reddish-brown	Reddish-brown	Reddish-brown

Values were mean ± standard deviation of triplicate run. Different superscripts alphabets ($^{a, b \text{ and } c}$) across the rows indicate significant difference between mean values at $P < 0.05$.

and untreated (1.493 ± 0.0003) samples also differed considerably ($P < 0.05$). Decrease in the refractive index of enzyme-treated sample was probably at the highest level of unsaturation, not only as reflected by the iodine value but also by shortened fatty-acyl chain components as well as higher saponification number (Bayisa and Bullo, 2021). By increased refractive index of enzyme-treated samples, the fat hydrolysis of native enzyme lipase indicated a higher free fatty acid. Compared to their constituent free acids, however, the triacylglycerols possessed higher refractive indices (Ibeto *et al.*, 2012).

Conclusions

The use of partially purified cellulase from *A. niger* for the enzymatic pretreatment of *P. angolensis* seeds prior to fat extraction, targeting improvement of (fat) yield and quality, was investigated. For emphasis, the fat yield was aimed to be enhanced based on the use of seeds of different particle size. The study demonstrated that *P. angolensis* seeds were high fat-yielding seeds, which could serve as a commercially rich source of fat. Pretreatment of *P. angolensis* seeds with cellulase and reduction in their particle size were found to improve fat yield. The physicochemical properties of enzyme-treated, water-treated and untreated seed samples differed significantly, indicating a promising impact of processing methods on quality of extracted fat. The fat obtained from enzyme-treated seeds was high, with appreciable saponification value and high oxidative stability, and thus could find applications in confectionery, soap and cosmetic industries.

We believe that this was the first study to specifically employ partially purified cellulase from *A. niger* for enzymatic pretreatment of *P. angolensis* seeds prior to fat extraction, thus targeting improvement in fat yield and quality. The work also established that corn bagasse, a common domestic waste, could be used as a sole carbon source of cellulase production from *A. niger*. The decreased refractive index of enzyme-treated samples would suggest unsaturation at its peak, supported by their saponification and iodine values, and shorter chain fatty acid components. Size reduction and treatment of *P. angolensis* seeds with *A. niger* cellulase improved both fat quality (improved saponification number, iodine value and refractive index) and yield, which suggested their potential application in cosmetic and confectionary processes. Considering the high levels of acidity, the extracted fat is not suitable for direct human consumption, and the future studies must target to ease this challenge.

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Conflict of interest

The authors declared that they had no competing interest to disclose.

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