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Partial Purification of Indigenous Lipase from Germinated Bambara Groundnut *(VoandzeiaSubterrenea)*

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Abstract: *Lipase is an enzyme that catalyzes the hydrolysis of ester bonds in substrates such as phospholipids, triglycerides and cholesteryl esters. Lipase (E.C. 3.1.1.3) was extracted from the 5th day of germination of bambara groundnut (Voandzeia subterrenea) using 0.1M phosphate buffer pH 7.8. The enzyme was purified using 60% ammonium sulphate saturation via precipitation followed by dialysis and column chromatography using carboxymethyl cellulose. Lipase from germinated voandzeia subterrenea has various industrial applications hence alternative source of the enzyme is crucial especially from plant.*

Keywords: *Germination, plant source, lipase, and purification.*

1. INTRODUCTION

Lipase (Triacylglycerol acylhydrolase) (E.C. 3.1.1.3) is an enzyme which catalyzes the hydrolysis of ester carboxyl bonds in acylglycerol to release free fatty acids and glycerol. It is capable of catalyzing esterification reaction at water restricted environment. Generally, lipase has a catalytic triad which consists of amino acid: serine, histidine and aspartate or glutamate residues. Lipases from microbial source can have their character influenced by its medium where it grows, while lipases from plant and animal sources are not influenced by their environment. Lipases from plant sources have specific properties, such as high affinity with triacylglycerol of the plant content. This property is not found in lipases from microbial sources (Huang *et al.,* 2008). Lipases have been isolated from many species of plants, animals, bacteria, fungi, and yeast. It have been widely used in the food and other industrial applications and thus there is an increasing demand in discovering new lipase sources. Lipases are one of the important groups of biocatalysts used in biotechnological applications (Benjamin and Pandey, 2008). Permana*et al.,* (2012) found that lipase from germinated seeds; have higher activity, the highest activity being dependent on length of germination. Lipases have been found to be present in seeds rich in oils (Beisson*et al.,* 2000). Although there have been studies on lipase from germinated seeds especially groundnut seeds. However, studies on lipase from germinated bambara groundnut are still limited and unexploited. Hence, this study is aimed at purifying the indigenous lipase of germinated bambara groundnut using olive oil as substrate.

2. MATERIALS AND METHODS

2.1. Materials

Chloroforms used was a product of May and Baker Ltd, olive oil-Arista chemicals, triethanolamine hydrochloride crystal – M & M laboratory chemicals Ltd, Bovine Serum Albumin (BSA) - BDA England, Folin – ciocalteu-BDH England and Carboxymethyl cellulose –Sigma chemical company. Other chemicals used were of analytical grade.

2.1.1. Collection of Plant Materials

Bambara groundnut seeds (*voandzeiasubterrenea*) were bought locally from Ogige Main Market Nsukka, Enugu State, Nigeria.

2.2. Methods

2.2.1. Germination of Bambara Seeds

Bambara groundnut seeds were immersed in water and viable seeds settled at the bottom. The viable seeds were imbibed in water for 10hrs and aerated for 2hrs followed by another 10hrs imbibitions. At

the end of the imbibitions, the seeds were germinated on already prepared germination beds for 7 days. The beds were watered every two days (Onyekwelu and Olawole, 2007).

2.2.2. Extraction of Lipase

2, 3, 4, 5, 6 and 7 days germinated seeds as well as un-imbibed seeds (day zero) were decoated and the roots removed. The seeds (51.5g each) were homogenized for 15minutes with cold 0.1M phosphate buffer (pH 7.8) using Philips blending machine (R2000) as described by Afiukwa*et al.*, (2009) after washing them severally with distilled water. The homogenate was filtered using cheese cloth and the filtrate centrifuged for 10mins at 5000g. The supernatant containing the crude enzyme was collected and used as the crude extract while the pellets were discarded. Lipase activity was determined for each of the extracts as in the assay section below.

2.2.3. Purification of Lipase

The crude lipase (100mls) was precipitated with ammonium sulphate by the method of Doonan, (1996) at various ammonium suphate saturation (10, 20, 30 – 100%). After addition of ammonium sulphate, crude lipase (100ml) was precipitated for 12hrs and the resulting precipitate was collected by centrifugation at 5000g for 30mins at 4^0C. The precipitate was dissolved in 20mls of 0.1M phosphate buffer (pH 7.8). The lipase activity and protein content of this solution were then determined.

Crude lipase (300mls) was then initially brought to 20% ammonium sulphate $(NH_4)_2SO_4$) saturation and was allowed to stand for 12hrs at 4^0C. This was centrifuged at 5000g for 30mins. The supernatant was made up to 60% ammonium sulphate saturation and allowed to stand for 12hrs at 4^0C. The precipitate was recovered by centrifugation at 5000g for 30mins. This was dissolved in 0.1M phosphate buffer (pH7.8) to a final volume of 50ml and dialyzed for 12hrs against the same buffer.

The dialysate was applied into a gel filtration column ($2.5\times62cm^3$) with carboxymethylcellulose and pre-equilibrated with 0.1M phosphate buffer (pH 7.8) and the eluting buffer of 5ml/min was collected. The protein content (λ = 250nm) and lipase activity (440nm) were monitored for every fraction (3ml). The fractions which had the highest lipase activity were pooled and used as partially purified enzyme.

2.2.4. Assay of Lipase Activity

Hydrolytic activities of lipase were detected by the method of Marseno *et al.*, (1998). Olive oil 50% in isooctane (5ml) was mixed with 250µl lipase. It was then incubated in a shaking water bath (100 strokes/min) at 35^0C for 60mins. The resulting oil layer was taken (3ml) and added with 0.6ml of pyridine Cu-acetate (pH 6.0). After thoroughly mixing, the mixture was centrifuged at 750g for 5min and then read the absorbance of the oil layer at λ = 715nm. Oleic acid was used as a standard at concentration of 0, 2, 4, 6, 8 and 10mM. One unit of lipase activity was described as an amount of the enzyme which produced 1µmol fatty acid per min under standard conditions.

2.2.5. Protein Concentration

Protein concentration in all enzyme extracts was determined as described by Lowry *et al.*, (1951) with bovine serum albumin as a standard protein.

3. RESULTS AND DISCUSSION

3.1. Variation of Protein and Lipase Activity with Days of Germination

The result of this study shows that germination started 2 days after imbibitions and was completed in 7 days. The result showed a steady increase in lipase activity from day zero through day 2 to the 5^{th}day of germination. Maximum lipase activity was detected at the 5th day and at pH 7.8 (table 1.0). The lipase activity started decreasing after day 5 through the 7^{th} day after imbibitions. This is possible because it has been found that lipase from germinated seeds has higher activity being dependent on the length of germination (Permana*et al.*, 2012). In a similar study it was found that germination of cocoa bean had highest lipase activity at day 3 after imbibitions (Permana, 2011). During germination lipase activity increases in the endosperm to hydrolyse the lipids present in endosperm to release free fatty acid as the germination progresses.

3.2. Purification of Lipase from Germinated Bambara Groundnut

Solid ammonium sulphate was used to precipitate the crude lipase extract in various concentrations (10% - 100%). The higher the concentration of ammonium sulphate, the more protein precipitated. The lipase activity likewise increased as the ammonium sulphate concentration increases and became relatively constant from 60% concentration. Consequently, two steps precipitation (20 – 40%, meaning discarding precipitate at 20% saturation and continuing precipitation with 40% saturation) were selected for the study. The 60% ammonium sulphate saturation and protein precipitated when dialyzed removed impurities from the sample raising the specific activity to 0.21U/mgwith 4.38 purification fold.

The purification fold of the lipase increased to 115.76after applying carboxylmethyl cellulose G-200 column chromatographic technique. The overall purification scheme is summarized in table 2.0. This result agrees with that of Deepak, 2012 who reported that 60% saturation was proved to be effective for maximum specific activity of lipase from *Bacillus PumilusRK 31;* and that of Permana, (2011) who also reported that lipase activity increased during solid ammonium sulphate precipitation with increasing ammonium sulphateconcentration and became constant at the concentration of 60%.

4. CONCLUSION

Crude lipase was extracted from germinated Bambara groundnut (*Voandzeia subterrenea)* and purified partially using 60% ammonium sulphate saturation, raising the enzyme specific activity to 0.21U/L with 4.38 purification fold. The results of this research show that lipase enzyme could be sourced alternatively especially from Bambara groundnut.

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Table1. *Variation of protein and lipase activity with days of germination*

Days	Lipase activity
1	0.0123
2	0.134
3	0.197
4	0.256
5	0.442
6	0.315
7	0.201

Table2. *Purification Table*

Purification step	**Total volume (ml)**	**Total activity (u)**	**Total protein (mg)**	**Specific activity u/mg**	**Purification fold**
Crude lipase	300	19.14	393.76	0.05	1.00
Ammonium sulphate precipitation	300	7.25	34.11	0.21	4.38
Dialysis	50	3.94	14.43	0.46	28.41
Carboxymethyl cellulose G-200	25	1.86	0.70	5.59	115.75

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