Investigating Lipase Activity in Ungerminated *Colocynthis citrullus lanatus* (Egusi Melon) Seeds

Jonathan Bege
Department of Biochemistry
Kaduna State University
Kaduna, Nigeria
begejonathan@gmail.com

Markus Victor
Department of Biochemistry
Kaduna State University
Kaduna, Nigeria
markusvictor8@gmail.com

Gaiya Daniel
Department Biochemistry
Ahmadu Bello University
Zaria, Nigeria
gaiyadaniel@gmail.com

***Abstract*- Activity of lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) was investigated in ungerminated *Colocynthis citrullus lanatus* (Egusi Melon) seeds. Titrimetric analytical method with 0.01 M NaOH using phenolphthalein as indicator was the method employed. The lipase activity was expressed as the percent free fatty acids (FFAs) liberated. The results obtained showed that ungerminated seeds of Egusi melon (*Colocynthis citrullus lanatus*) have lipase activity. It was observed that 1g of the enzyme preparation was enough to optimally act on 5g oil in 2.5ml hexane. The optimum conditions for lipolysis were found to be 30^{o}C and 60 min period of incubation. This shows that *Colocynthis citrullus lanatus* seeds will deteriorate with storage. The egusi melon seed lipase could be exploited commercially for industrial applications.**

***Index Terms*— *Colocynthis citrullus lanatus*, ungerminated seed, lipase activity.**

I. Introduction

Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are carboxylesterases that catalyze the hydrolysis, esterification and transesterification of acylglycerides with acyl chains with more than ten carbon atoms at an oil-water interface [22]. Lipase activity has been generally found to be prominent in germinating seeds [15], although with few exceptions in ungerminated seeds of castor bean [28], *Jatropha curcas* [2] and African oil bean seed [11] where it has shown activity.

Oilseed lipases have immense potential for commercial application as industrial enzymes, especially in those oilseeds that are considered under-utilized [11]. Egusi melon is one of the members of the group of crops considered to have been neglected, more than other members of its group that most botanical description of the crop are made with reference to other closely related members of the family such as the water melon (*C. lanatus*) which has received more attention of researchers [25][26]. *Colocynthis citrullus lanatus* (Egusi Melon), a member of the family Cucurbitaceae [5], originated from Africa but introduced to many parts of the world like Europe and Asia during the last 2000 years [31]. It is a very important crop in Nigeria and many parts of Africa. Nigeria is the largest producer of the crop [4][33]. The seed of egusi melon contains approximate 53 % oil and 28 % protein [10], vitamins (A, B1, B2 and C) and minerals such as S, K, P, Ca, Mg, Fe and Zn [24]. The crop is cultivated for its seeds, in which oil is extracted and used for cooking and other industrial purposes, while the residue is used for soup [29][18][25]. The seeds have also been discovered to be useful in the production of biodiesel [12].

Oilseeds are a major source of protein and oil for food and feedstuff, constituting an indispensible part of our industrial raw materials [21][32]. But, a number of these seeds deteriorate before they could find application due to many of factors, among which are fungal infections, causing damages raging from reduction in seed germination, increase in the peroxide value, decrease in nutritive value, change in colour and mycotoxin production [3][6][7][8][9]. Another deteriorating factor aside fungal infection is the moisture content [10]. It was observed that, a 1% decrease in moisture increases the life of seeds twice [13]. Reports had also shown that improper storage and post-harvest handling in ungerminated (dormant) African oil bean seed initiate lypolytic activity, causing deterioration and depreciation in the commercial values of the seed oil [11]. This study therefore examines the activity and some properties of lipase in ungerminated egusi melon seed.

II. MATERIALS AND METHODS

A. Materials

Dry seeds of Egusi melon were purchased from the Central Market in Kaduna City. The seeds were carefully dehiscence and kept at 4^{o}C prior to analysis to avoid early lipolysis. Shea

butter oil was obtained from Alpha Chemicom Limited, Yoruba road, Oriapkater, Kaduna Nigeria. All the chemicals and reagents used were of analytical grade from E. Merck AG, Darmstadt, Germany.

B. Preparation of enzyme

The preparation of the enzyme was carried out according to the modified method of Hassanien and Mukherjee (1986) [14]. The shells of the seeds were carefully removed to avoid bruising the endosperm. Twenty five grams (25 g) of the seed cotyledons were ground with 30 ml of cold acetone using an electrical Blender. The acetone extract was filtered through a cheese cloth and washed four times, with 20 ml each time, of cold acetone (4^{o}C). The residue was then air dried at room temperature (26±1^{o}C) to yield the acetone powder, which was kept at 4^{o}C until ready for assay.

C. Assay of enzyme activity

The lipase activity was assayed using a modified titrimetric method of Khor *et al.*, (1986) [19]. The assay mixture, containing 5 g of substrate, 2.5 ml of hexane to solubilize the oil, and 1 g of the crude enzyme, was incubated at 30^{o}C for a period of 1 h with continuous stirring using a magnetic stirrer. At the end of the incubation, 25 ml of acetone–ethanol (1:1 v/v) were added to stop the reaction and to extract the free fatty acids (FFAs) liberated. The FFAs in the mixture were then estimated by direct titration with 0.01 M NaOH using phenolphthalein as indicator. Lipase activity was expressed as the percent FFAs liberated after 1 h incubation. Corrections were made for endogenous fatty acid production (assay mixture without substrate) and nonenzymatic fatty acid production (assay mixture without enzyme preparation).

D. Effects of temperature and time

A thermostat water bath was used for determination of the of lipase activity at various temperature. The assay mixtures, containing 5 g of substrate, 1 g of enzyme preparation and 2.5 ml of hexane, were incubated at different temperatures (30–45^{o}C) for 1 h and the activity measured. The effect of time on lipase activity was determined at the same temperature (30^{o}C) by varying the time of incubation (15, 30, 45, 60 min) of the assay mixture.

E. *Effect of concentration of substrate on lipase activity*

Different concentrations (2, 3, 4, 5 g) of the substrate (Shea butter oil) were used in the assay mixture with in 2.5 ml hexane and 1g of enzyme preparation. The rate of lipolysis on the substrate concentration at 30^{o}C for 1h was determined by assaying lipase activity as described above.

F. Effect of enzyme concentration on lipolysis

Different concentrations of the enzyme preparation (0.25, 0.50, 0.75, 1.00, 1.50, 2.00 g) were used in assay mixture with 5 g of substrate in 2.5 ml of hexane. Enzyme assay was carried out at 30^{o}C for 1h as described for lipase activity above. The control assay mixture was incubated without added enzyme preparation.

III. RESULTS AND DISCUSSION

Studies of lipase activity in ungerminated seeds of Egusi melon (*Colocynthis citrullus lanatus*) showed that the seeds have lipase activity. This finding comes in agreement with some past studies done on ungerminated seeds. Lipase activity has been generally found to be noticeable in germinating seeds [15]. Studies carried out on oilseeds revealed that ungerminated seeds may have little or no lipase activity [16] [34]. However, reports have shown that ungerminated seeds of castor bean [28], *Jatropha curcas* [2] and the African oil bean [11] have shown lipase activity.

Figure 1 shows the effect of different enzyme concentration on the activity of the ungerminated egusi melon seed lipase incubated at 30^{o}C for 1 h. The relationship between varying enzyme concentrations with constant substrate concentration (5g) was not linear. However, there was steady but slow lipolysis at enzyme concentration 0.25g. Between enzyme concentrations 0.25 and 0.5g, and 0.75 and 1g, there were high rate of lipolysis. This is comparable to other researchers' finding where the relationship is also not linear for African oil bean lipase preparation [11]. This study showed that the highest rate of lipolysis was noticed at enzyme concentration 1g. Beyond this, rate of lipolysis decreased. The result showed that 1g of enzyme is enough to optimally act on 5g oil.

Figure 2 shows the effect of different substrate concentration on the activity of the ungerminated egusi melon seed lipase incubated at 30^{o}C for 1 h. The logarithmic relationship between substrate concentration and rate of lipolysis was observed to be inversely proportional. This is in contrast to the finding where the relationship is linear for African oil bean [11]. In this study, Shea butter oil was the substrate used. It has been found to contain predominantly Oleic acid (55.54-57.63%) [27]. In the finding with African oil bean lipase, lipolysis was more pronounced in palm kanel and coconut oils with short-chain fatty acids than with palm oil and Raphia oil with long-chain fatty acids [11]. Other studies have also shown that lipases are more active on triacylglycerol containing short-chain fatty acids [17] [23]. The idea is that short-chain fatty acids have higher solubility than long chain fatty acids, thus have smaller inhibitory effect in the lipid phase of the triglyceride emulsion [11][23]. This perhaps explains the inversely proportional relationship observed between substrate concentration and rate of lipolysis in this study as the increasing concentration of the Shea butter oil (substrate) containing long-chain fatty acids may have caused more inhibition in the lipid phase.

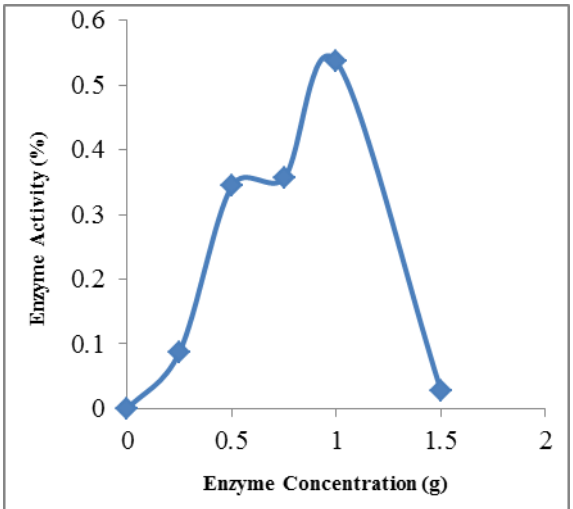

Fig.1. Effect of enzyme concentration on the activity of the ungerminated Egusi melon seed lipase incubated at 30°C for 1 h

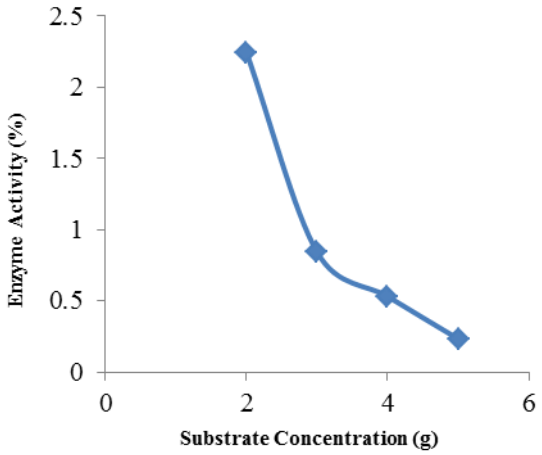

Fig.2. Effect of enzyme concentration on the activity of the Lipase from ungerminated Egusi melon seed incubated at 30°C for 1 h

Figure 3 shows the effect of period of incubation on the activity of lipase from *Colocynthis citrullus lanatus* seed. There was a linear logarithmic relationship between the time of incubation and rate of lipolysis. This finding is in agreement with other reports of researchers using coconut, pea nut, oil palm and other sources that the rate of lipolysis increased with period of incubation in a linear logarithmic relationship [1][11][19][20][30]. In this study, no apparent lipase activity was observed at 15 min. This finding came in contrast to the work conducted on coconut oil with short-chain fatty acids (lauric oils) [11]. This contrast could be because, the predominantly long chain fatty acids present in Shea butter oil, having a comparatively lower water solubility, may have been inhibitory in the lipid phase of the triglyceride emulsion at that time of incubation. Thus, more time was needed to overcome it as show in Fig. 3. In this study, the optimum incubation period was 60 min.

The effect of temperature on the activity of lipase from *Colocynthis citrullus lanatus* seed is shown on Fig. 4. The optimum temperature for the lipase activity was found to be 30°C. Beyond this, a steady decline was observed. In the work with palm oil and African oil bean, it was also discovered that the optimum lipase activity was at 30°C [1][11].

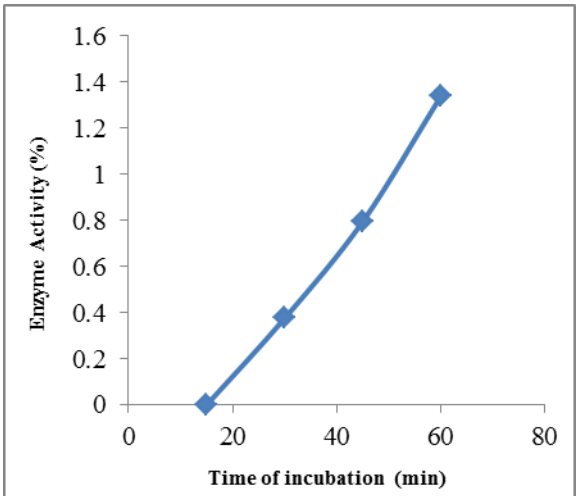

Fig.3. Effect of incubation time on the activity of the Lipase from ungerminated Egusi melon seed incubated at 30°C

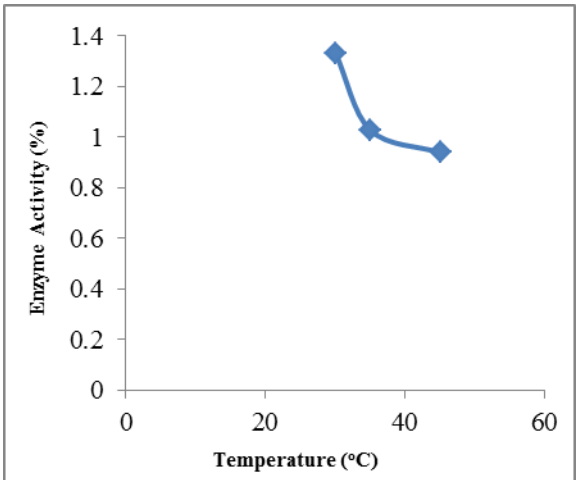

Fig. 4. Effect of Temperature on the activity of the Lipase from ungerminated Egusi melon seed incubated for 1 h

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