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ISOLATION AND CHARACTERIZATION OF CRUDE LIPASE FROM SPROUTED *HIBISCUS SABDARIFFA* (SORREL) SEEDS

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Abstract: Lipases (triacylglycerols acylhydrolase, EC. 3.1.1.3) are part of the family of hydrolases that act on carboxylic ester bonds. Lipase enzyme was isolated from sorrel (Hibiscus sabdariffa) seeds and characterized. The seeds were sprouted, dried at room temperature, ground to powder and homogenized in phosphate buffer to extract the crude enzyme. Lipase activity was determined using egg yolk as substrate. The activity of the enzyme was found to value with optimum pH 6.0 and temperature, 37°C, while the activation energy was 10.2 kJ/mol. The K_M and Vmax were 1.70 mg/ml and 3.92 µmol/ml respectively. The enzyme was inhibited by mercury in form of $HgCl_2$. Preliminary kinetic data for the lipase have been obtained and it could be useful in assessing its overall suitability in industrial and domestic uses.

Keywords: *Hibiscus Sabdariffa*, Seed Lipases, Sprouting, Germination

INTRODUCTION

Sorrel (*Hibiscus sabdariffa*) is a vegetable that belongs to the family malvaceae and is cultivated for its leaves and young shoots which are eaten either raw or as cooked vegetable (Mukhtari, 2008). Sorrel is an annual or perennial herb or woody based sub shrub, growing to 2-2.5 m tall. The leaves are three to four lobes, 8-15 cm long, arranged alternatively on the stems (Burton-Freeman and Britt, 2010). The flowers are 8-10 cm in diameter, white to pale yellow with a dark red spot at the base of each petal, and have a stout fleshy calyx at the base, 1-2 cm wide enlarging to 3-3.5 cm fleshy and bright red as the fruit matures (Barbara, 1993). *Hibiscus sabdariffa* is easy to grow in most well drained soils but can tolerate poor soil as well (Rose and Pike, 2006). Sorrel is an economically important plant particularly in the sahel zone of West Africa. The leaves, seeds and the calyces are valued for their nutritional and medicinal uses. The most exploited part of sorrel plant is the calyces, which may be green, red and dark red (Elibo, 2000). The calyces are used as a refreshing drink or beverage and in the preparation of preservative and jellies (Tindal, 1983). The sorrel seeds are valuable food resource on account of its protein, calory and fat, and also substantial amount of fibre and other valuable nutrients. The seeds are subjected to a meat substituted condiment (Mohammed et al, 2007). In the southern parts

of Borno state (Biu), the seeds are fermented, ground and used as soup ingredient. Lipases (triacylglycerols acylhydrolases with EC.3.1.1.3) are enzymes belonging to the group of serine hydrolases which hydrolyse esters of long chain aliphatic fatty acid from their glycerol backbone only; and differ from esterases by their ability to hydrolyse insoluble fatty acyl esters (Macrae and Hammond, 1985). Lipases are widespread in nature and have been found in animals, higher plants and microorganisms. In plants, lipase activity has been identified in various tissues but relatively high concentration is found in seeds (Gadge et al, 2011). Recently, seed lipases have been the focus of much attention as biocatalyst. In some cases, these enzymes present advantages over animal and microbial lipases. Therefore, purification of the enzyme represents a great alternative for potential commercial exploitation as industrial enzymes (Njidda, 2011). In the present study we report the isolation and partial characterization of crude lipase from sprouted sorrel seeds.

METHODS

Sorrel Seeds and Sprouting

Sorrel (*H. subdarifa*) seeds were purchased from a local market in Maiduguri (*Monday* Market) and authenticated by a plant taxonomist from the department of Biological Sciences, University of Maiduguri. Exactly 25 g of the seeds was washed 4-5 times with distilled water, and then steeped (in distilled water) for 24 h and covered with jute sack and allowed to germinate for 3 days. The sprouted seeds were allowed to dry at room temperature within 5 days. The dried seeds were ground into powder using pestle and mortar and was stored in clean container and kept in refrigerator until required.

Crude Enzyme Extraction

The crude enzyme was extracted according to Uvere and Orji (2002). Exactly, 1.0g of the ground sprouted seeds was homogenized in 4 ml 0.05 M cold phosphate buffer pH 6.0. The homogenate was then filtered with cheese cloth and centrifuged at 5000 xg. The supernatant was collected and kept on ice while the pellet or debris was resuspended in 5 ml of the extraction buffer. This homogenate was similarly treated and supernatant collected. The combined supernatants were used for the lipase activity assay.

Enzyme Activity Assay of Lipase

The lipase activity from the supernatant was assayed by modification of the egg yolk coagulation method described by Habermann and Newmann (1954). The reaction mixture contains 1ml substrate (egg yolk 100 mg/ml) mixed with 1 ml of buffer and 100 µl of crude enzyme. The mixture was incubated at 37°C for 10 min and the reaction was stopped by immersing the test tubes in boiling water for 2 min. The fatty acids released by the enzyme were titrated against 20 mM Na0H using

phenolphthalein as indicator. Lipase activity was derived by calculation based on the 1:1 molar relationship between a fatty acid and NaOH during a neutralization reaction and expressed as μmol of fatty acid released per minute.

Determination of Optimum pH

The optimum pH was determined by preparing the enzyme solution at various pH conditions ranging from 4 to 8.

Determination of Optimum Temperature

The effect of temperature on crude lipase activity was determined by assaying the enzyme activity at varying temperature, from 20°C to 60°C.

Determination of K_M and Vmax

The kinetic parameters, K_M and Vmax, of crude lipase were determined by assaying for lipase activity using increasing substrate concentration from 10 mg/ml to 100 mg/ml with a fixed enzyme concentration to get the corresponding V_0. The double reciprocal plot was plotted from which the K_M and Vmax were deduced.

Determination of Activation Energy

The activation energy of the crude lipase was determined from Arrhenius plot of Log V_o against reciprocal of temperature (1/T) in Kelvin.

Determination of the Effect of Inhibitor

Solutions of 10, 20 and 50 μl of 20 mM $HgCl_2$ were used to determine the effect of mercury on the enzyme activity. The effect of Hg^{2+} was determined by assaying the enzyme activity in the absence and presence of the inhibitor.

RESULTS AND DISCUSSION

In the present study, we have isolated and characterized lipase from sorrel (*Hibiscus sabdariffa*) seeds. The activity (Vmax) and activation energy of the enzyme were 3.92 μmol/ml and 10.2 kJ/mol, suggesting that the lipase activity was strong after sprouting the seed. Lipase activity has been identified in the various tissues of plants but relatively high concentration is found in seeds (Vajanti and Mumtaz, 2002.In germinated oilseeds, mobilization of the stored fatty acid is essential to supply energy and carbon for embryonic growth (Barros et al, 2010). These metabolic events are under control of lipolytic enzymes like lipases to catalyze lipid mobilization during and after germination period (Quettier et al, 2009).In most cases, lipolytic activity is not observed in the non-germinated seeds, but only in post germinated seeds (Hassanien and Mukherjee, 1986; Villeneuve, 2003).

Figure 1 shows the effect of pH on the activity of the enzyme. The assay was carried out using the pH range pH 4–8 with constant substrate concentration and at temperature 37°C. The enzyme also produced an optimum pH of 6.0 using egg yolk as the substrate. The optimum pH of most lipases studied showed that it is either in alkaline pH region or in the acidic pH region, suggesting two types of lipases exist Ejedegba et al, 2007). The optimum pH 6.0 of the lipase shown in the present research, suggested a preference for acidic environments. Indeed the acidic microenvironment is an indication that this environment will contribute to the enhancement of the enzymatic activity such as in the sprouting period.

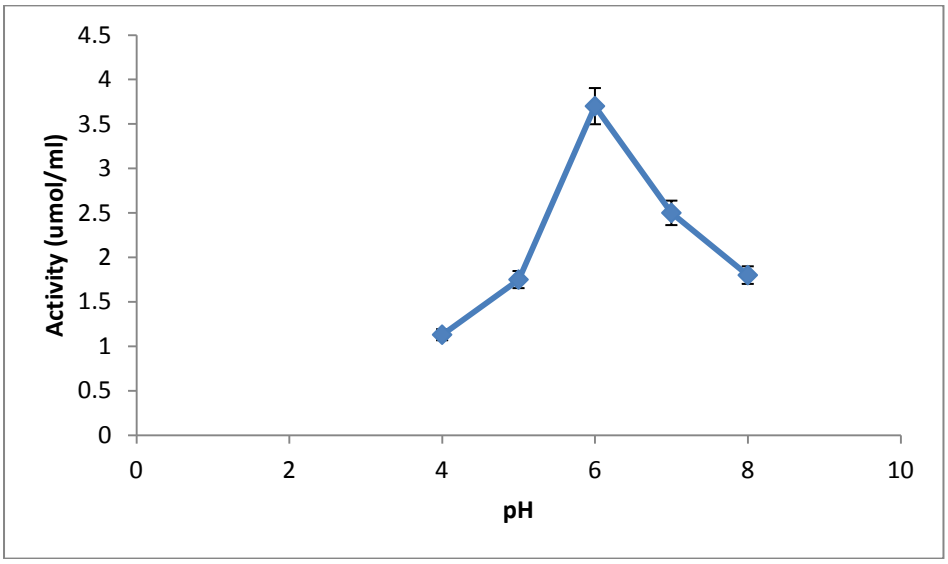

Figure 1. Effect of pH on activity of crude lipase.

The enzyme was optimally active at 37°C (Figure 2) with Ea of 10.2KJ/mol (Figure 3). Lipases have been found to be active over a wide range of temperature. Abigor et al, (2002) reported a temperature of 37°C seed lipases from *Jatropha curcas* L, purified wheat seed lipase had optimum temperature of 37°C as reported by Kapranchikov et al (2004). Higher temperatures up to 80°C optimum have been reported for rice seed using triolein as substrate Bhardwaj et al, (2001). Ea of 10.2 Kj/mol is considered low and thermodynamically favourable, implying less frequency of collision required to surmount the activated complex and from the product Wurochekke et al, (2008).

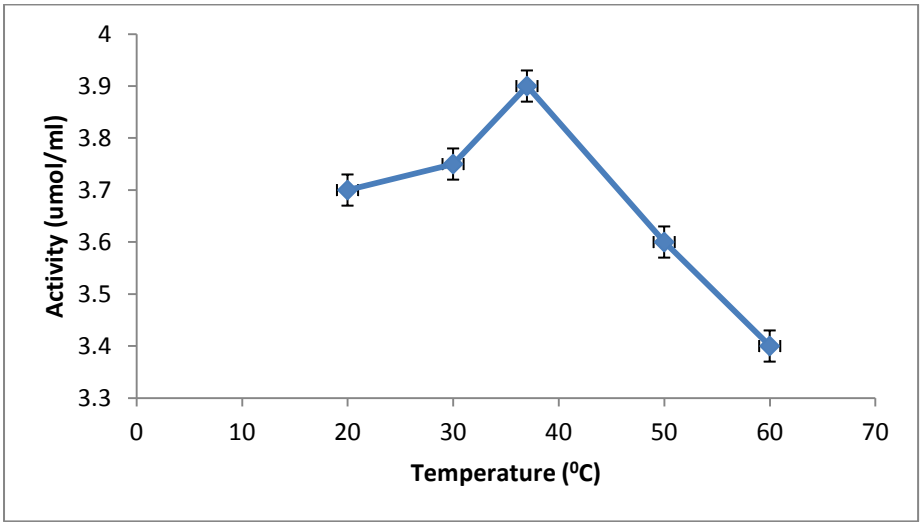

Figure 2. Effect of temperature on activity of crude lipase.

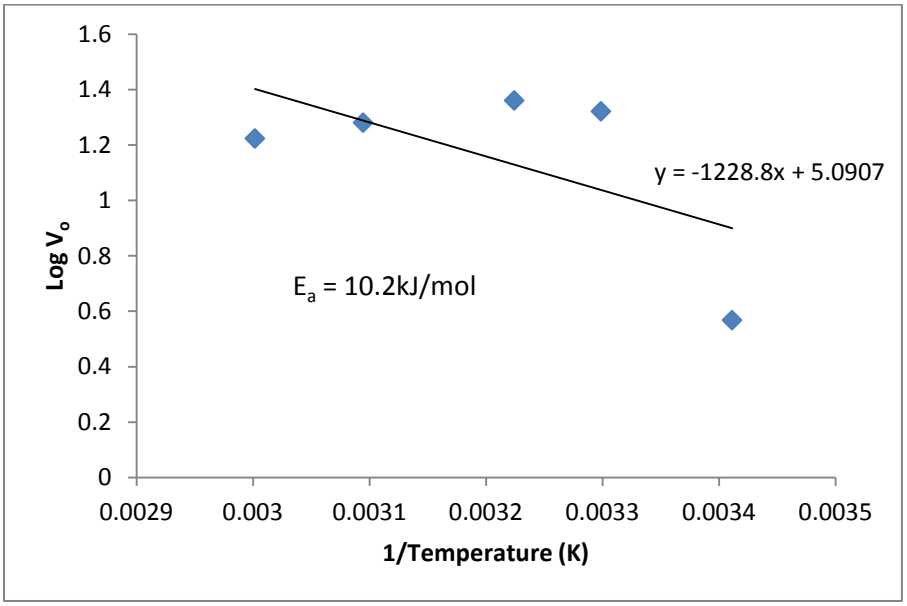

Figure 3. Arrhenius plot for crude lipase.

The K_M and Vmax values indicate the physiological efficiency of the enzyme because a Vmax of 3.92µmol/ml (Figure 4) presupposes that at least 235.2 mmol free fatty acid (FFA) as product will be released within an hour. This amount of product can serve as a compact source of energy for the sorrel seeds to sprout or for embryonic growth (Barros et al, 2010).

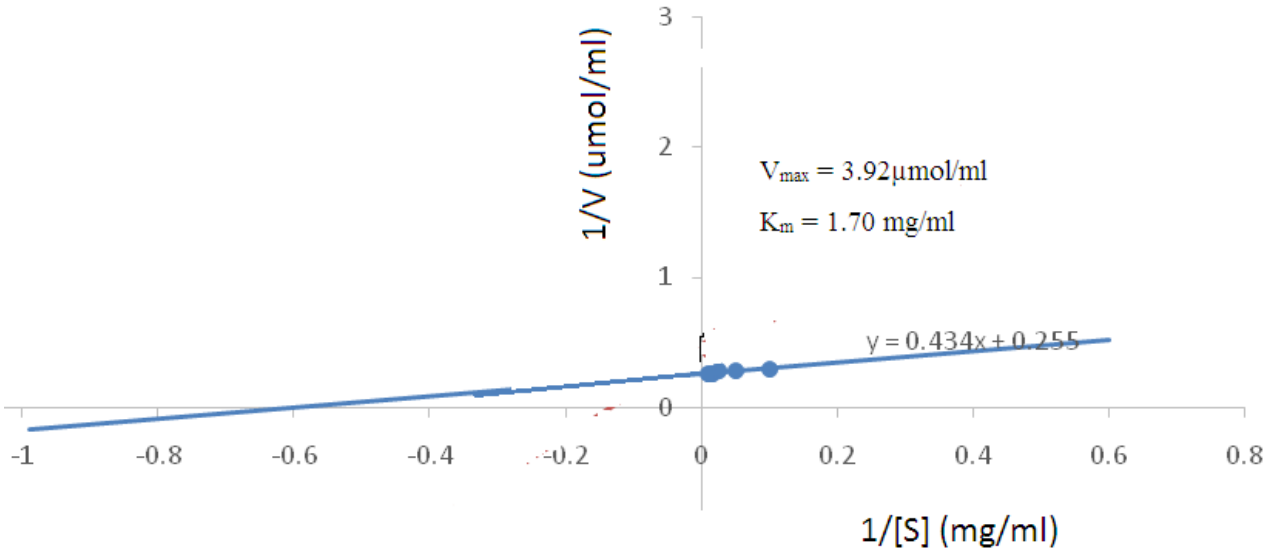

Figure 4. Lineweaver-Burk plot for crude lipase.

Figure 5-7 present the result for the effect of Hg^{2+} on the crude lipase activity. The pattern of inhibition shows a mixed type of inhibition. Similarly, effects of metal ions and organic inhibitors (Mercuric ion and p-chloromercuricbenzoic acids) on castor bean lipase activity has shown that only sulfhydryl group reactants inhibited the acid lipase at very low concentrations (Allen and Robert, 1983). EDTA and Hg^{+2} were also reported to inhibit the lipase activity (Gadge et al, 2011). Although secondary plot was not carried out in the present study, it is conceivable that the lipase extracted might therefore be a sulfhydryl sensitive enzyme with cysteine in its active site. Mercury has a strong affinity for sulfhydryl groups of proteins, including enzymes. Because of the abundance of sulfhydryl groups in the active sites of many enzymes, it is difficult to establish exactly which enzymes are affected by Hg^{2+} in biological systems. Inhibition of enzymes is of considerable importance in the food processing industries. The investigation of enzyme inhibition by chemicals (inhibitors) is of importance for the elucidation of the structure of enzymes, or at least the structure of the enzyme. But in food applications, it is rarely impossible to inhibit enzymes in this manner because of the toxic nature of this inhibitor.

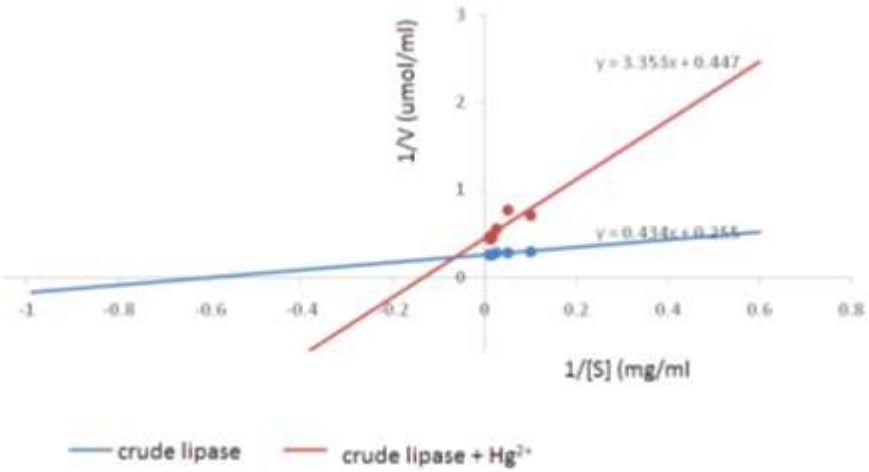

Figure 5. Lineweaver-Burk plot for inhibition of crude lipase by Hg at 10ul.

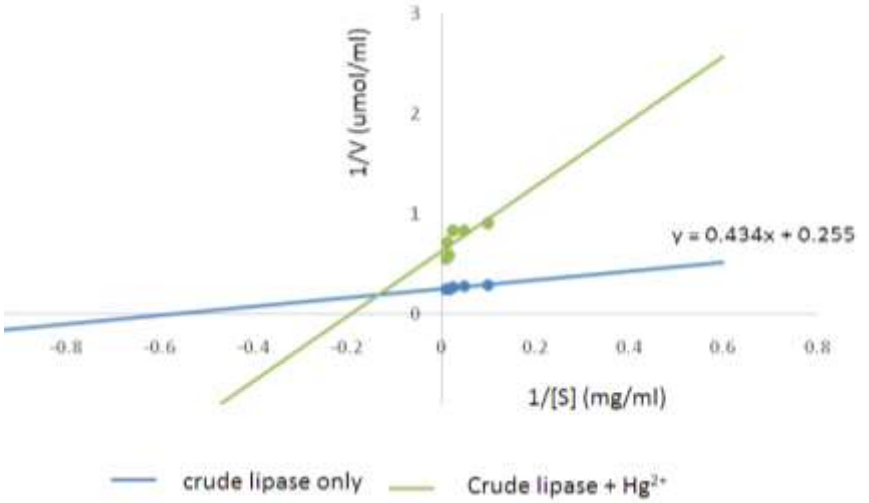

Figure 6. Lineweaver-Burk plot for inhibition of crude lipase by Hg at 20ul.

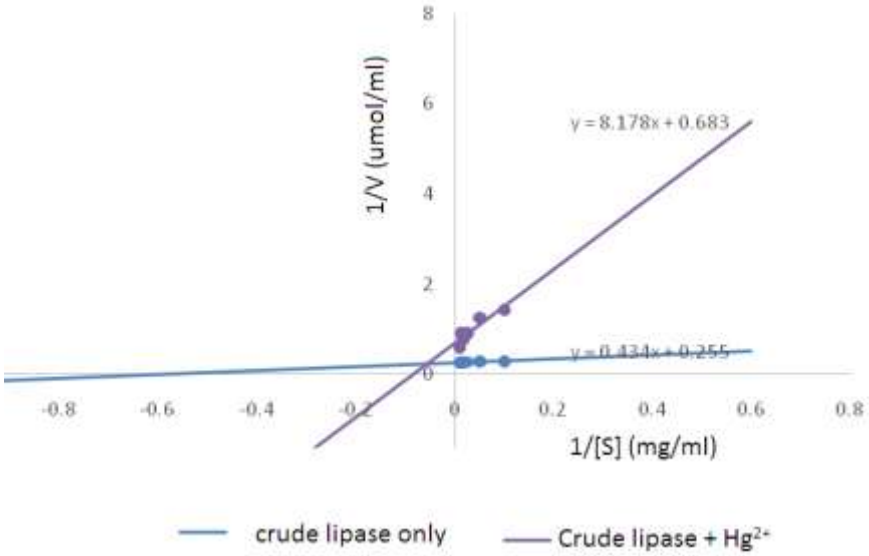

Figure 7. Lineweaver-Burk plot for inhibition of crude lipase by Hg at 50ul.

CONCLUSION

Preliminary kinetic data for the lipase have been obtained and it could be useful in assessing its overall suitability in industrial and domestic uses.

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