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Full Length Research Paper

Characteristics of lipase isolated from coconut (*Cocos nucifera* linn) seed under different nutrient treatments

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Lipase (triacyglycerol acyl hydrolase, E. C. 3.1.1.3) activity was demonstrated on acetone powder prepared from coconut seeds, under different nutrients condition. Conversion of the free fatty acid released to copper salt enabled the activity of the crude enzyme extract on coconut oil, triolein, tripalmitine and olive oil to be determined colorimetrically. Lipase from coconut plant grown under complete nutrient conditions showed high affinity for the native substrate coconut oil. The enzyme acted maximally at 35°C and had a broad optimum pH of 7.5 – 8.5.

Key words: Lipase activity, coconut oil triolein, tripalmitine.

INTRODUCTION

The coconut palm is a member of the Palmae family, order Palmales, subfamily Cocoidae which is characterised by a three celled ovary drupaceous fruit with a woody or stony endocarp provided with three germ pores, while the fruit is usually one seeded (Smit, 1970).

The enzyme lipase (Triacylglycerol acyl hydrolase, E.C.3.1.1.3) catalyses the hydrolysis of various forms of fatty acyl esters and in contrast to other esterases needs an oil-water interface for optimum activity. No bonds other than carboxyl ester bonds have been found to be hydrolysed by lipases and in this respect, lipases are considered as carboxyl esterase (Sonnet, 1988). Lipases are widespread in nature and have been found in animals, higher plants and microorganisms (Ohnis et al., 1994). Germinating seeds have been shown to have an exceptionally high lipolytic activity probably in order to meet the energy requirements.

However, the best characterised plant lipase, castor bean lipase is also present in dormant seeds (Ory, 1969). Lipase activity has been reported in several oil plant extracts which include castor bean, palm seeds, oleifera seed, sunflower seed, and peanuts, just to mention a few (Abigar et al., 1985; Haderson and Osborne, 1991; Kham et al., 1991; Afolabi et al., 1991; Teirssene et al., 1995; Hoppe and Theimer 1997). Lipase from various sources have been used in flavour promotion in processed cheese and in rapid maturing and introduction of flavour in chocolate crumb, in improvement of egg white whipping properties (Alan, 1975) as well as in the analysis of triacyl glycerols (Forgia et al., 1975).

In recent years the growing demand of lipolytic enzymes has been increased due to its potential use in the various manufacturing processes of industrial goods such as detergent industry, food industry and in pharmaceutical industry (Boland et al., 1991; Gandh, 1997; Savendsen, 2000). There are many plants seeds, especially of tropical origin, upon which no work have been reported. The aim of the present study, therefore, is to establish the lypolytic activity of coconut seed lipase under different nutrient treatments in order to evaluate the effects of macronutrients on enzyme induction, properties and catalysis.

MATERIALS AND METHODS

Sample preparation

The coconut seeds used in this study were obtained from the NIFOR substation Abak, Akwa Ibom State of Nigeria. The experiment was randomised complete block designed form with 10 replicates per treatment and each treatment comprises a subtractive series of the major elements nitrogen (N), potassium (K) and mag-

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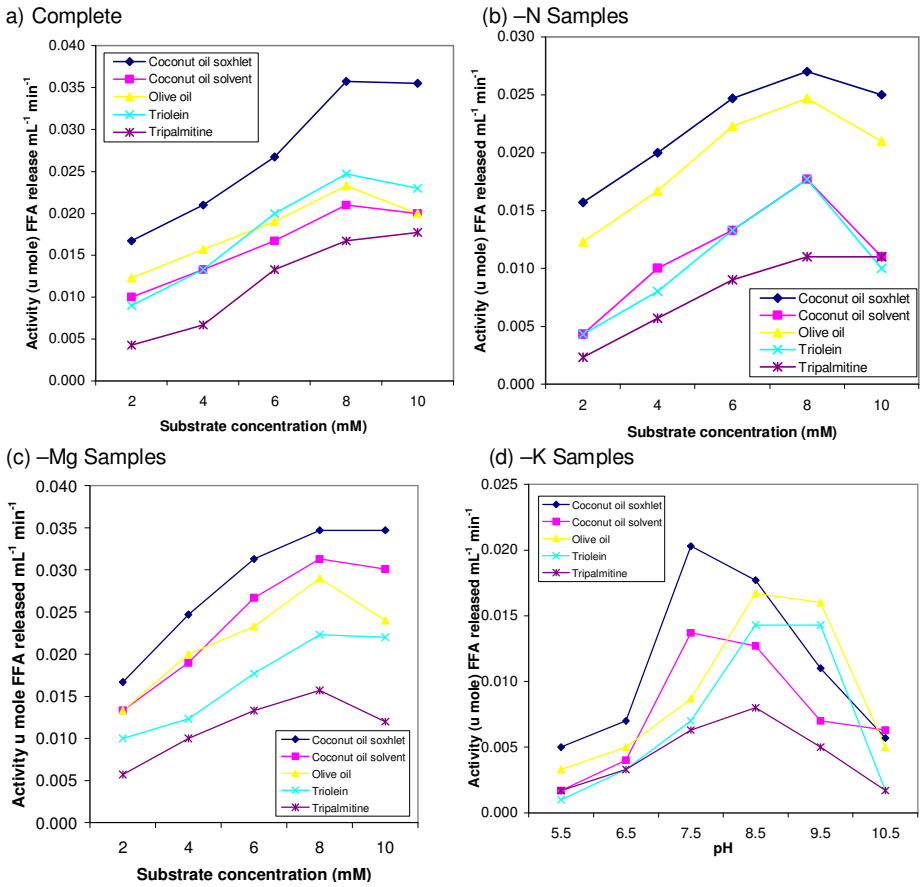

Figure 1. Effect of substrate concentration on activity of lipase isolated from coconut.

nesium (Mg). 2.5 kg of fertilizer was applied in two doses of 1.25 kg to each palm early in the raining season and towards the end of the raining season.

Lipid extraction

Lipids were extracted from the coconut seed endosperm by Folch et al. (1957) method and also by soxhlet method after drying to constant weight at 40°C. The extracted oil was stored at 40°C until required.

Preparation of fatty acid standard curve

This was done by the method as described by Schmidt et al. (1974) using stearic and oleic acids as standards. The colorimetric method is based on the conversion of free fatty acids into their copper salts and determination of the copper colorimetrically with diethyldithio carbamate at 440 nm.

Preparation for lipase acetone powder

The acetone powder was prepared as described by Wether (1957). This method involves the removal of the endosperm, and then washed with distilled water. The endosperm was then blended in cold acetone in a blender for 5 min. The suspension was kept at about 0°C and stirred for 5 min. This was then filtered and washed free of oil with cold acetone. The finely ground residue was dried and stored in the cold.

Preparation of enzyme solution

The enzyme solution was prepared by weighing out 2 g of the acetone powder and homogenised for 2 min with 20 ml cold 0.9% NaCl. The suspension was then centrifuged for 5 min at room temperature and the supernatant which contains the enzyme was stored at 4°C. The enzyme solution was prepared immediately before the assay.

Assay of enzyme activity

The method described by Schmitt et al. (1974) was used for the assay. This involves the separation of free fatty acids released in the form of their copper salts using chlorofarm extraction and which is subsequently determined colorimetrically at 440 nm.

RESULTS AND DISCUSSION

Figure 1 (a-d) shows the effect of substrate concentration on lipase enzyme activity. The enzyme activity assay

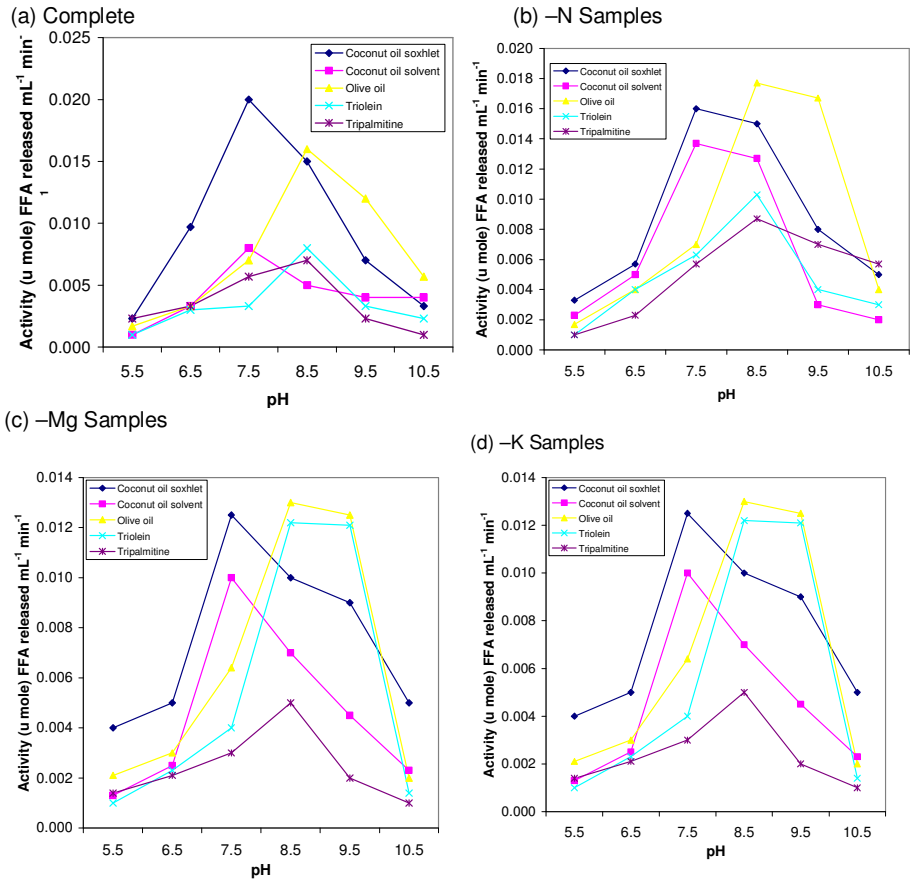

Figure 2. Effect of pH on activity of lipase isolated from coconut.

was carried out at pH 8.5 and room temperature with substrate emulsion at concentration 2–10 mM. The enzyme appears to become saturated at a substrate concentration of about 8 mM. Figure 2 (a-d) shows the effect of pH on the activity of the enzyme. The assay was carried out at pH 5.5–10.5 with constant substrate concentra-tion and at room temperature. pH for hydrolysis of the native substrate (i.e coconut oil irrespective of the method of extraction) was 7.5. The enzyme also exhibited the same optimum pH of 8.5 for other substrates. Figure 3(a-d) shows the effect of temperature on the activity of the enzyme. An optimum temperature of 35°C was displayed by all the treatments for the different substrates except the – N treatment which showed a broad optimum temperature of 35–40°C with triolein and the control showing an optimal temperature of 40°C with coconut oil obtained from solvent extraction.

Lipase from coconut seed hydrolysed different triacylglycerols emulsion at different rates. It was observed that the best substrate for the enzyme is its native substrate coconut oil. Olive oil also acted as a good substrate for the lipase in all the samples investigated. The enzyme has a lower affinity for triacylglycerol emulsion, triolein, than for the saturated acylglycerol tripalmitin. This might be due to the stearic hindrance as a result of double bond found in oleoyl chain triolein using natural and modified fat. Berner and Hammond (1970) found that the lipase from oat showed preference for splitting fatty acids in 1 and 3 positions of the triacylglyerol and the hydrolysis of natural fats was non specific but showed activity towards long chain fatty acid ester.

The effect of substrate concentration investigated also shows that there was an increase in enzyme activity until saturation point of about 8 mM. The decline after this concentration may be due to the effect of enzyme substrate concentration ratio or enzyme inhibited by the excess of substrate concentration or change of physiochemical characteristics (Khan et al., 1997). Similar result was also obtained for lipase from caesalpinia bonducella L. seeds where an optimum substrate concentration of 10% was obtained (Vajanti et al., 2001).

The coconut lipase acted maximally in mildly alkaline pH, a broad optimum pH of 7.5 with the native substrate to 8.5 with other acyl glycine emulsions. The effect of pH on the activity of lipase from many sources has been studied. The optimum pH of most lipases studied showed that it is either in alkaline pH region or in the acidic pH region, which shows that two types of lipases exist. Some of the acid lipase includes castor bean lipase at pH 4.0 – 4.2 (Ory et al., 1960) germinating seedling of *Cucumero-*

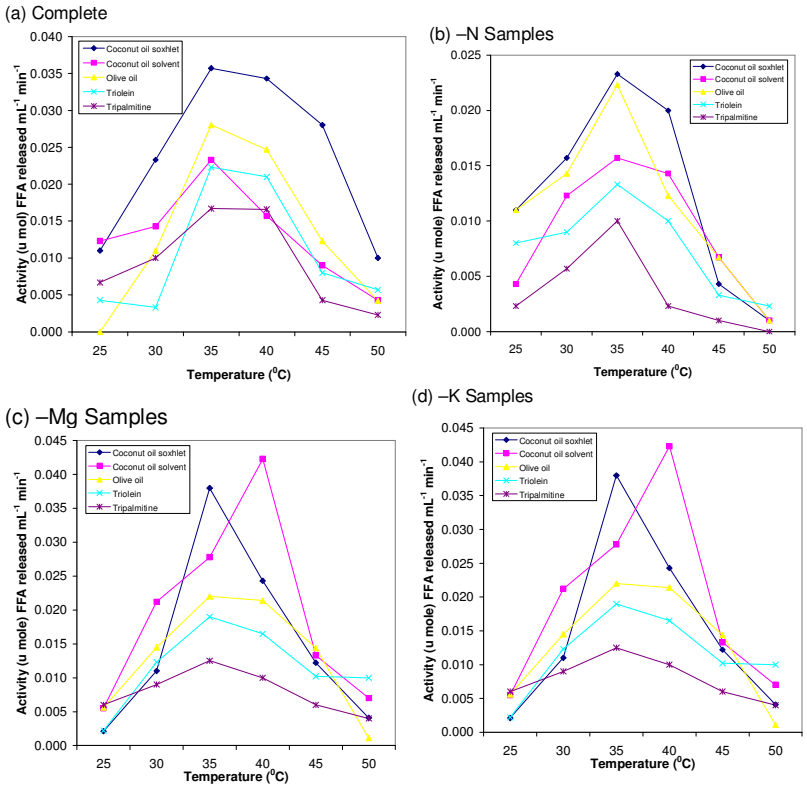

Figure 3. Effect of temperature on activity of lipase isolated from coconut.

ropsis edulis with pH 5.0 (Opute, 1975) and *Aspergilus* lipase with pH 6.5 (Fu et al., 1995). Alkaline lipases occurs in germinating rape seed at pH 8.5 (Wetter, 1957; Hoppe and Theirmer, 1997) rice bran pH 7.5 – 8.0 (Funatsu et al., 1971), F. Oxyspprium lipase at pH 8.6 and 7.0 depending on the substrate (Maria de Mascena et al., 1991), *C. bonducella* seeds at pH 7.0 (Vajanti et al., 2001), *Hibiscus canabinus* seeds pH 7.0 (Kausar and Akhtar, 1979), the coconut lipase studied belongs to this last class of lipase.

Lipases have been found to be active over a wide range of temperature. Some microbial lipases act at –20°C (Brokenheff and Jensen, 1974). *Sesanum indicum* lipase act at 37°C (Kumar and Murphy, 1966), melon lipase with optimum temperature of 37°C (Opute, 1975) and *Aspergillus* sp. lipase with optimum temperature of 30 – 40°C (Fu et al., 1995). Others include *Cajanus cajan* L. seed lipase at 30°C (Kham et al., 1991; Dahot et al., 1989), *Carissa carandas* fruit lipase at 30°C (Mala and Dahot, 1995), *C. bonducella* L. seed lipase at 30°C (Vajanti et al., 2001). In this study an optimum temperature range of 30– 40°C was observed.

Conclusion

From the study it was found that lipase grown under different nutrient conditions showed high affinity for native substrate coconut. The enzyme acted maximally at 35°C and had a broad optimum pH of 7.5 – 8.5. However further studies will be carried out on the effect of enzyme concentration and metallic ion on enzyme activity.

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