

Lipase Activity in Fermented Oil Seeds of Africa Locust Bean, (*Parkia Biglobosa*), Castor Seeds (*Ricinu Communis*) and African Oil Bean (*Pentaclethra Macrophylla*).

A.A. Liman*, P. Egwin, M.A. Vunchi and C. Ayansi

Department of Science Laboratory Technology, School of Applied Arts and Sciences. Federal Polytechnic, P.M.B. 55, Bida, Niger State
 [*Tel. 08036902291, E-mail: auduliman@yahoo.com]

ABSTRACT: The activity of lipase in fermented Africa locust bean, Castor seed, and Africa oil bean was determined. The peak lipase activity for fermented Africa locust bean, Castor seed, and African oil bean were $13.3 \times 10^{-3} \mu\text{mol}/\text{sec}$, $7.2 \times 10^{-3} \mu\text{mol}/\text{sec}$ and $10.6 \times 10^{-3} \mu\text{mol}/\text{sec}$ at day 5, 4 and 4 respectively. The optimum temperature and pH were 30°C and 7.0 respectively for all the fermented seeds above. Increasing NaCl concentration decreases the activity of Lipase indicating that NaCl is an inhibitor of lipase. The effect of substrate concentration on lipase activity in the fermenting oil seeds shows the normal Michaelis-Menten curve. The K_m for African locust bean is 0.065664mM, Castor seed is 0.067625mM, and African oil bean is 0.075848mM. While the V_{max} are: $15.229 \times 10^{-3} \mu\text{mol}/\text{sec}$, $14.787 \times 10^{-3} \mu\text{mol}/\text{sec}$ and $13.184 \times 10^{-3} \mu\text{mol}/\text{sec}$ respectively. The findings of this work indicates that fermented African locust bean, Castor seed and Africa oil bean could be a source of lipase for industrial application.

Key words: Fermentation, Lipase activity, *Pentaclethra macrophylla*, Daddawa

INTRODUCTION

The acceptability of a substance as food is based on various attributes which the substance must possess. Such substances must be attractive in appearance and taste, having good flavouring and colouring properties. Flavour also increases the nutritional quality of the food to which it is added (Adewakun, 1988).

Fermentation is one of the oldest methods of food processing and preservation known to man. In Africa, the art of fermentation is wide-spread including the processing of fruits and non alcoholic beverages (Adewusi *et al.*, 1992).

Food fermentation is basically aimed at producing important nutrients or eliminating anti-nutrient. This is necessary therefore to improve the prevailing cases of malnutrition in Nigeria (Steinkraus, 1995).

Significant contributions has been made in microbiology and biochemistry of fermentation of legumes and oil seeds leading to the production of fermented condiments such as “iru” from African locust bean, (Achinewhu and Barber, 1992); ogiri from melon seed (Odunfa, 1986), ‘Soumbala’ from African locust bean (Ouoba *et al.*, 2003), “daddawa” from soybean (Omafuvwe *et al.*,

2000), “afitin”, “iru” and “sonsu” from African locust bean seed (Azopkpota *et al.*, 2005), and ugba from African oil bean seed “Ogiri” from castor seed (Odunfa, 1985).

Lipases are glycerol ester hydrolases (E.C. 3.1.1.3), which hydrolyze ester linkages of glycerides at water-oil interface. It is well known that lipases are the most widely used enzymes in organic synthesis and more than 20% biotransformation are performed with lipases (Gitlesen *et al.*, 1997). In addition to their role in synthetic organic chemistry, these also find extensive applications in chemical, pharmaceutical, food and leather industries (Gulati *et al.*, 2005; Gunstone, 1999). Promising fields for the application of lipases also include the biodegradation of Plastics (Gombert *et al.*, 1999) and the resolution of racemic mixtures to produce optically active compounds (Muralidhar *et al.*, 2001).

In view of the variety in applications, there has been a renewed interest in the development of sources of lipases.

Parkia biglobosa (African locust bean) belong to the family of Leguminosea, which includes trees and shrubs that are rarely herbaceous. Other species such as those that yield timber, those cultivated for ornamental purposes and

those that yield gum are of economic importance (Dupriez, 1984).

Ricinus communis (Castor oil seed) is of the spurge family widely cultivated as an ornamental. Due to its ability to heal wounds and ailments, it is called Palm of Christ, or *Palma Christ*. Castor oil is also extracted from the seed (Roger and Gadfer, 1999).

Pentaclethra macrophylla (African oil bean) is an annual plant. It grows best under mild weather condition. The fruits are produced in the rainy season but get matured in the dry season. When grown on fertile ground with good rainfall, it grows to a height of 5-6 meter.

MATERIALS AND METHODS

Biological Materials: African locust bean (*Parkia biglobosa*), were bought from the Post office market in Bida, Niger State; the castor bean seeds were bought from Ogbete market in Enugu, Enugu State, while the African oil bean seeds were bought from Ekata market in Ibitolu local government area of Imo State.

Fermentation of Locust bean seeds, Castor seeds and African oil seeds: Three kilograms (3000g) of each raw seeds were cooked at boiling temperature, separately for 6 hours on a hot plate. Thereafter the seeds were soaked in the boiled water for another 6 hours after which the excess water was drained. The seeds were dehulled by rubbing between the palms after cooling and washed with water. The cotyledons were again cooked for another 30 minutes. The excess water was drained off and the seeds spread on stainless pot lined with sterilized banana leave and left to ferment at 35^oC for 6 days.

Sample Preparation for Crude Enzyme Extract: The fermented seeds were chopped into small pieces and then pulverized into fine powder in a blender. The materials were air dried at room temperature in fume hood, then stored at 20^oC until needed.

Lipase assay: Lipase activity was assayed titrimetrically on the basis of olive oil hydrolysis (Macedo *et al.*, 1997).

Calculation of Lipase activity: This was calculated by first determining the concentration of fatty acid used during the titration using the formula:

$$\frac{CaVa}{CbVb} = \frac{na}{nb}$$

Where

Ca = Concentration of acid used in mol/dm³,
Va = Volume of acid used,

Cb = Concentration of base used in mol/dm³,
Vb = Volume of base used,
na = number of mole of acid
nb = number of mole of base

$$Ca = \frac{CbVbna}{Vanb} \text{ (mol/dm}^3\text{)}$$

Then Ca obtained was then substituted in the following formula to calculate the enzyme activity.

$$\text{Enzyme activity} = \frac{\text{mole of fatty acid (mol/sec)}}{30 \times 60}$$

Determination of Lipase Activity with days:

The activity of lipase was determined using the method of Macedo (1995) described above, each day, as the oil seeds undergo fermentation for 6 days.

Effect of pH on the Activity and Stability of Lipase:

Phosphate buffer of pH 5.5 to 8.0 were prepared. Crude enzyme was extracted at the different pH and enzyme activity assayed using method of Macedo (1995), to obtain the optimum pH.

Effect of Temperature on the Activity and Stability of Lipase:

The activity of lipase was assayed at different temperatures ranging from 20^oC to 60^oC using method of Macedo (1995).

Effect of substrate concentration on Lipase Activity: Enzyme assay was repeated with varying substrate (olive oil + gum Arabic 1:3) concentration.

Effect of NaCl concentration on Lipase Activity and Stability: Enzyme activity assay was repeated with varying NaCl concentration (1-10%).

RESULTS AND DISCUSSION

Fig 1 illustrates that as the fermentation time increases, the activity of lipase in all the oil seeds, also increased until an optimum enzyme activity was attained. Odibo *et al.*, (2008) reported that lipase activity was obtained to be very high and increasing throughout the duration of fermentation.

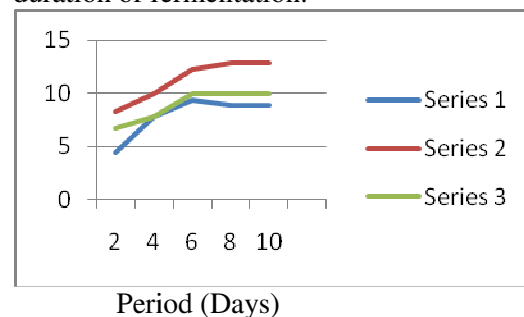


Fig 1: Enzyme activity against period of fermentation for African locust bean, Castor bean seed and African oil bean seeds.

It was illustrated from fig 2 and 4, that increase in temperature & pH lead to a rise in lipase activity in all the oil seeds. The optimum temperature & pH are 30⁰C & 7 respectively, at which there is the highest lipase activity for all the oil seeds. This is consistent with the report of Victor *et al.*, (2004) that optimum temperature for the activity of the oil bean lipase was 30⁰C and optimum pH being neutral.

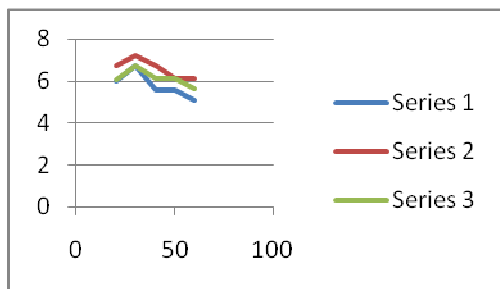


Fig 2: Enzyme activity-Temperature profile of lipase from African locust bean, Castor Bean and African oil bean seeds.

It was detected that the lipase activity increases with increase in substrate concentration for oil seeds (Fig 3) until an optimum substrate concentration. Further increase in substrate concentration neither increases nor decreases the rate of lipase activity, which agrees with the report of Martinek (1969).

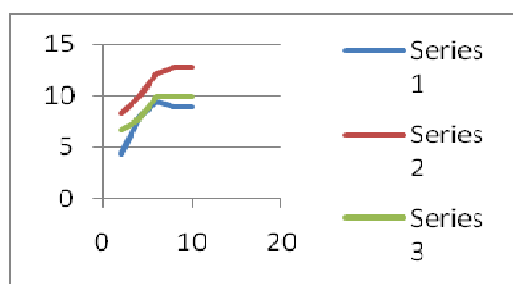


Fig 3: Enzyme activity-Substrateconcentration profile of lipase from African locust bean, Castor oil bean seed and African oil bean seed

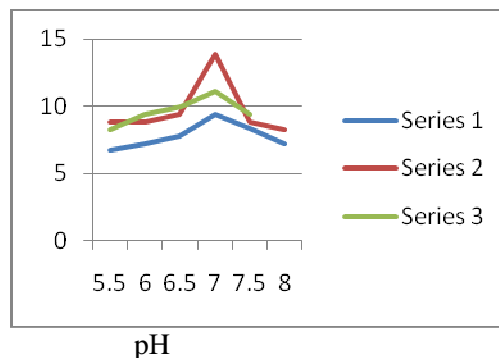


Fig 4: Activity-pH profile of lipase from African locust bean, Castor bean and African oil bean seeds.

The activity of lipase decreases with increase in concentration of NaCl (Fig 5). The activity of lipase in fermented African oil bean is $6.7 \times 10^3 \mu\text{mol}/\text{sec}$ when 1% NaCl is added during the fermentation process. $5.0 \times 10^3 \mu\text{mol}/\text{sec}$ lipase activity was recorded when 5% NaCl was added to the fermentation process of castor bean. 10% NaCl further reduces the activity of lipase to $5.0 \times 10^3 \mu\text{mol}/\text{sec}$ in fermented African locust bean to produce daddawa (Achi, 2005). This inhibition in lipase activity is desirable especially during the production local condiments. The inhibition in the activity of in some fermented foods has been considered desirable because of the problem of objectionable taste and development of rancidity (Odufa, 1983, 1985). However there has been a report of beneficial effects of lipase in the development of characteristic flavour and aroma (Whitaker, 1998; Ouoba *et al.*, 2005).

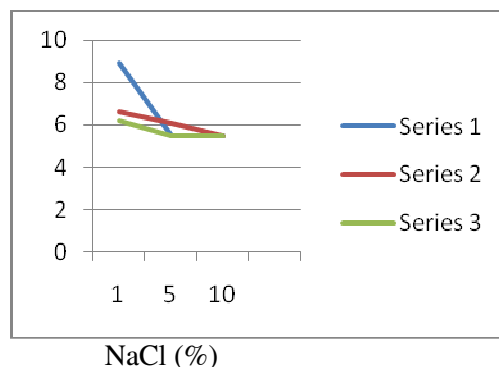


Fig. 5: Effect of NaCl concentration on lipase activity.

Series 1 = Castor oil bean
 Series 2 = African locust seed
 Series 3 = African oil seed

NaCl is an inhibitor of lipase activity in fermented African locust bean, castor bean seeds and African oil bean. This was also reported by Victor *et al.*, (2004) who stated that the presence Ca²⁺ during the fermentation of African oil bean increased lipase activity by 64% while sodium chloride (NaCl) and mercuric chloride inhibited the activity by 36% and 28.5% respectively.

Conclusion:The activity of lipase is affected or influenced by temperature, pH, substrate concentration and period of fermentation with each parameter having its optimum value at which lipase activity is maximum. Fermented leguminous seeds (e.g. African locust bean, castor seed and African oil bean) can be used as good sources of lipase. Further research could be focused on production techniques.

REFERENCES

- Achi, O.K. (2005). The upgrading of fermented foods through biotechnology. *African Journal of Biotechnology*, **4**:375-380.
- Achinewhu, S. R. and Barbar, C. O. (1992). Microbiological and Toxicological aspect of fermentation of castor oil seeds for ogiri production. *Journal Food Science*, **50**: 758-760.
- Adewakun, A.E., (1988). Technological improvement and shelf-life studies of "Iru" production from African locust bean (*Parkia biglobosa*). Unpublished thesis, Department of Food Science, University of Ibadan pp. 1-3.
- Adewusi' O.A., Shashidi, F.O and Niyi, W.C. (1992). Biochemical changes in fermentation during production of ogiri, a fermented melon (*Citrullus vulgaris* schrad) product. *Plant Foods Hum. Nutrition*, **32**: 11-18.
- Azopkpota, O.C., Cerryld, D., Wiyeye, O. and Delley. C. (2005). Quality attributes of fermented yam flour supplemented with processed soy flour. *Plant Foods Hum. Nutrition*, **54**:151-158.
- Berkow, S.O. (1997). Dihydrofolate reductase from *Escherichia coli*: The Kinetic Mechanism with NADPH and reduced acetylpyridine adenine dinucleotide phosphate as substrates. *Biochemistry*, **26**:493-499.
- Dupriez, H. (1984). African Garden and Orchard growing vegetable and fruits. *Journal of Agriculture*, **2**: 10-19.
- Gitlesen T, Bauer M and Adlercreutz P (1997). Adsorption of Lipase on Polypropylene powder, *Biochimica et Biophysical Acta*. **1345**: 188-196.
- Gombert AK, Pinto al, Castilho LR, Freirc DMG (1999). Lipase by *Penicillium restrictum* in solid state fermentation using Babassu oil cake as substrate, *Process Biochem*. **35**, Pp.85-90
- Gulati R., ISAR Jasmine, Kumar V., Parsad A. K, Parmar V.S, Saxena R. K. (2005). Production of a novel alkaline lipase by *Fusarium globulosum* using Neem oil and its applications. *Pure and Appl Chem*, **77(1)**: 251-262.
- Gunstone, F. D. (1999). Enzymes as biocatalysts in the modification of natural lipids. *J. Sci. Food and Agric*. **79(12)**: 1535-1549.
- Macedo, G.A., Park, A.k. and Pastore, G.M. (1997). Partial purification and Characterization of an extracellular lipase from newly isolated strain of *Geotrichum sp.* *Rev. Microbiol*. **28(2)**: 90-95.
- Martinek, G.H. (1969). Microbiology and amino acid composition of ogiri a food condiment from fermented melon seeds. *Die Nahrung*, **25**:811-816.
- Muralidhar RV, Marchant R, Nigam P. (2001). Lipases in racemic resolutions. *J. Chemical Technol & Biotechnol*. **76(1)**: 3-8.
- Odibo, F., Carkhou, V., Taore, A.S. and Jackoosen, M. (2008). Glutamic Acid Production by *Bacillus* isolates from fermented vegetable proteins. *World Journal of Microbiological Biotechnology*, **6**: 377-383.
- Odunfa, S.A. (1983). Carbohydrate changes in fermenting locust bean during Iru preparation. *Plant Foods Hum. Nutrition*, **32**: 1-10.
- Odunfa, S.A. (1985). Biochemical changes in fermenting African locust bean (*Parkia biglobosa*) during Iru fermentation. *Journal of Food Technology*, **20**: 295-303.
- Odunfa, S.A. (1986). Daddawa. In: *Legume-based fermented Foods*. (Eds). Rrddy, N.R., Pierson, M.D. and Salunkhe, D.K. CRS Press Boca Raton, Pp.173-189.
- Omafuvwe, B.O., Abiose, S.H. and Shonukan, O.O. (2000). Fermentation of soybean (*Glycine max*) of soy dawadawa

- production by starter culture *Bacillus*.
Food
- Ouoba, L.H., Cantor, M.D., Diawara, B., Traore, A.S. and Jakobsen, M. (2003). Degradation of African locust bean oil by *Bacillus subtilis* and *Bacillus pumillus* isolated from soumbala, a fermented African locust bean condiment. *Journal Applied Microbiology*, **95**: 868-873.
- Roger, J. and Gadfer, I. (1999). In: Steinkraus, K.H. (Eds). Indigenous fermented foods involving an alkaline fermentation. *Food Research international*, **54**: 234-237.
- Smith, A.D. (2004). Processing and fermentation of African locust bean (*Parkia filicoides* welw) seeds for the production of dawadawa. *Plant Foods Hum. Nutrition*, **36**: 179-184.
- Steinkraus, K.H. (1995). In: Steinkraus, K.H. (Eds). Indigenous fermented foods involving and alkaline fermentation. *Food research International*, **54**: 234-237.
- Victor, K., Cuoory, I., Eayolur, C.V. and Torrgy, J. (2004). The microbial ecology of soyabean soaking for tempe production. *Int. Journal of Food Microbiology*, **8**: 35-46.
- Whitaker, V. (1998). The animal fatty acid synthase: One gene, one polypeptide, seven enzymes. *Fasels Journal*, **8(15)**: 1248-1259.