Journal of Applied Biosciences 29: 1793 - 1808

ISSN 1997–5902

Biotechnological potentialities of three enzymatic activities from seeds of the neglected crop *Lagenaria siceraria*

Djary M. KOFFI[1], Jean T. GONNETY[2], Betty M. FAULET[2], Micaël E. BÉDIKOU[1], Irié A. ZORO BI[3] and Sébastien L. NIAMKÉ[1*]

[1]Laboratoire de Biotechnologies, Filière Biochimie-Microbiologie de l'Unité de Formation et de Recherche Biosciences de l'Université de Cocody, 22 BP 582 Abidjan, Côte d'Ivoire ; [2]Laboratoire de Biochimie et Technologie des Aliments de l'Unité de Formation et de Recherche des Sciences et Technologies des Aliments de l'Université d'Abobo-Adjamé, 02 BP 801 Abidjan, Côte d'Ivoire ; [3]Laboratoire de Génétique de l'Unité de Formation et de Recherche des Sciences de la Nature de l'Université d'Abobo-Adjamé, 02 BP 801 Abidjan, Côte d'Ivoire.

*Corresponding author e-mail: niamkes@yahoo.fr; Tel: (225) 07 84 64 09
Original submitted in 9th April 2010. Published online at www.biosciences.elewa.org on May 11, 2010.

ABSTRACT

Objective: To analyze the enzymatic potential of the seeds of *Lagenaria siceraria* (round-fruited cultivar), an indigenous cucurbit that is an orphan crop, though widely cultivated and distributed in Côte d'Ivoire.

Methodology and results: The seeds extract of *L. siceraria* round-fruited cultivar was screened using a variety of synthetic and natural substrates for hydrolytic enzymes activities. The best enzymatic hydrolysis mainly consisted of phosphatase (0.71 ± 0.02 UI/mg), β-galactosidase (0.31 ± 0.03 UI/mg) and α-mannosidase (0.21 ± 0.02 UI/mg) activities. Physicochemical characterization showed that the three enzymatic activities were acidic (pH 4.6 – 5.6) and mesophilic (55°C). Also, they appeared to be stable in the presence of most cationic, non-ionic and anionic detergents as well as in the presence of cations (Na^+, K^+, Ca^{2+}, Ba^{2+} and Mg^{2+}) assayed. Substrate specificity showed that the seeds extract hydrolyzed a broad range of natural substrates such as adenosine-5'-triphosphate, pyrophosphate, phosphorylated sugars (glucose-1-phosphate, glucose-6-phosphate, fructose-1-phosphate and fructose-6-phosphate), lactose and the three differently linked (α-1,2; α-1,3; α-1,6) mannobioses.

Conclusions and potential application of findings: The properties of the three enzymatic activities make them attractive for potential biotechnological applications. More investigations are proposed to characterise them further.

Key words: orphan crop; *Lagenaria siceraria*; phosphatase; β-galactosidase; α-mannosidase

INTRODUCTION

Cucurbits are among the most economically important crops worldwide and are grown in both temperate and tropical regions (Pitrat *et al*., 1999; Paris, 2001; Sanjur *et al*., 2002). In Sub-Saharan Africa, the indigenous species are prized for their oleaginous seeds that are consumed as thickeners of a traditional soup called *egusi* soup in Nigeria and Benin and *pistachio* soup in Côte d'Ivoire.

Cucurbits cultivated for seed consumption are reported to be rich in nutrients, namely protein (34.19 ± 0.85%) and fat (50.08 ± 1.23%) (De Mello *et al*., 2001; Enujiugha & Ayodele-Oni, 2003; Achu *et al*., 2005). There are also well adapted to extremely divergent agro-ecosystems and various cropping systems characterized by minimal inputs (IPGRI, 2002; El Tahir & Yousif, 2004).

Lagenaria siceraria (Molina) Standl belongs to this category of crop, and is one of the most widely distributed and consumed in both rural and urban areas in Sub-Saharan Africa. Research towards promotion of this orphan or minor crop have focused primarily on agronomic evaluation, estimation of the amount of genetic diversity and determination of the degree of genetic differentiation (Zoro Bi *et al*., 2003, 2006). In spite of the nutritional and agronomic potentials and genetic diversities of *L. siceraria*, in-depth basic investigations on the crop are scant. For example, to our knowledge, no study has been devoted to analysis of the crops enzymes. Due to their wide application, enzymes have been investigated in eukaryotes from yeast to man. These investigations have demonstrated that some of the most desired enzymes are abundant constituent of plant systems (Ali *et al*., 1995; Ahi *et al*., 2007; Konan *et al*., 2008).

Many roles have been ascribed to enzymes according to their substrate specificity. For example, acid phosphatases (orthophosphoric-monoester phosphohydrolase EC 3.1.3.2) catalyze the hydrolysis of a broad and overlapping range of phosphomonoester and have been implicated in the release, transport and recycling of inorganic phosphate (Yoneyama *et al*., 2004). Furthermore, plant acid phosphatases have recently been reported to play a role in defence against herbivorous insects (Liu *et al*., 2005). B-galactosidase (or lactase) is used to increase the sweetening properties of lactose, and thus, it is used for the treatment of milk and its derivatives for consumption by people who have lactose intolerance (Patel & Mckenzie, 1985; Furlan & Schneider, 2000).

In the past, most industrially used β-galactosidase preparations for lactose hydrolysis were either from bacterial or fungal sources. However, Dey (1984) suggested that widely distributed plant β-galactosidases could be good substituted for industrial lactose hydrolysis due to their wider availability and lesser cost. As regards α-mannosidases, they are enzymes of great importance because of their physiological role and wide application, e.g. in the pharmaceutical industry, they are currently used for treatment of mannosidosis, a congenital disorder of glycoside, by enzyme replacement therapy (Sun *et al*., 1999; Hirsch *et al*., 2003).

In search for new sources of enzymes, we have investigated the enzymes in seeds extract of a neglected crop, the round-fruited cultivar of *L. siceraria*. The seeds were previously described as an important staple food of high nutritional and economic values (Sanjur *et al*., 2002; Achu *et al*., 2006). This paper provides data on the enzymatic potential of this vegetable crop which could contribute to support in its promotion.

MATERIALS AND METHODS

Enzymatic source and enzymes extraction: Seeds of *Lagenaria siceraria* round-fruited cultivar were obtained from the collection of the University of Abobo-Adjamé (Abidjan, Côte d'Ivoire). To obtain sufficient number of seeds, *L. siceraria* round-fruited cultivar was grown during its appropriate cropping season (raining season from April to July) in 2008 at the experimental farm of the University (5°23N, 4°00 West, and 7 m above sea level). After harvesting, fruits were split using a stainless steel kitchen knife and the seeds removed for crude extract preparation.

Cucurbit seeds (10 g) were ground using a blender in 20 ml sodium chloride solution 0.9% (w/v). The homogenate was subjected to sonication using a TRANSSONIC T_{420} for 10 min and then centrifuged at 10,000 g for 30 min at 4°C. The supernatant filtered through cotton wool was used as the crude seed extract.

Chemicals: *para*-nitrophenyl- (*p*NP-) glycopyranoside substrates (*p*NP-α-D-mannopyranoside (*p*NP-α-D-Man), *p*NP-α-D-glucopyranoside (*p*NP-α-D-Glu), *p*NP-β-D-glucopyranoside (*p*NP-β-D-Glu), *p*NP-α-D-galactopyranoside (*p*NP-α-D-Gal), *p*NP-β-D-

galactopyranoside (*p*NP-β-D-Gal), *p*NP-α-L-fucopyranoside (*p*NP-α-L-Fuc), *p*NP-α-L-arabinopyranoside (*p*NP-α-L-Ara), *p*NP-β-D-xylopyranoside (*p*NP-β-D-Xyl)), *para*-nitrophenyl phosphate (*p*NPP), phenyl phosphate, sodium pyrophosphate, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, fructose-1-phosphate, α-nicotinamide adenosine dinucleotide (α-NAD), adenosine-2-3'-cyclomonophosphate, adenosine-5'-triphosphate (ATP), phytate, *para*-nitrophenol (*p*NP), sucrose, starch, carboxymethylcellulose (CMC), Inulin, xylan, lactose, 2-O-α-D-mannopyranosyl-D-mannopyranoside (α-1,2 Mannobiose), 3-O-α-D-mannopyranosyl-D-mannopyranoside (α-1,3 Mannobiose), 6-O-α-D-mannopyranosyl-D-mannopyranoside (α-1,6 Mannobiose), D-mannose and D-glucose were purchased from Sigma Aldrich. Bovine serum albumin (BSA) was from Fluka Biochemika.

Screening hydrolytic effects of seeds extract on synthetic and natural substrates: For synthetic *p*NP-glycoside and *p*NP-phosphate hydrolytic activities, the crude extract was mixed in a total volume of 250 µl composed of *para*-nitophenyl-glycoside substrate (5 mM) in 100 mM sodium acetate buffer [pH 5.0] and crude extract (50 µl which correspond to 0.25 mg of proteins). The reaction mixture was incubated with shaking at 37°C for 10 min. The liberated *para*-nitrophenol (*p*NP) was quantified spectrophotometrically at 410 nm under alkaline conditions (2% w/v, Na_2CO_3) and referenced to a standard *p*NP (absorbance as a function of concentration) curve obtained under similar conditions.

For natural substrates hydrolysis, the total volume was 300 µl, composed of 50 µl of the crude extract, 125 µl of substrate (0.25% w/v, final concentration) in 100 mM sodium acetate buffer, pH 5.0. The reaction mixture was incubated with shaking at 37°C for 30 min. The reaction was stopped by adding 150 µl of dinitro salicylic acid (DNS) and heating the resulting solution at 100°C for 5 min (Bernfeld, 1955). The liberated reducing sugars were quantified spectrophotometrically at 540 nm and referenced to standard glucose (absorbance as a function of concentration) curve obtained under similar conditions.

All values were determined in triplicate. One unit of enzymatic activities in the two cases (synthetic and natural substrates) released 1 µmol of liberated product (*p*NP or reducing sugar) per min under the above conditions, respectively. The specific activity was expressed as µmol per min (UI) per mg of proteins.

Estimation of protein concentration: The concentration of proteins was measured by the Folin ciocalteu method (Lowry *et al*., 1951) using BSA as the standard.

pH and temperature optima: The effect of pH on enzymatic activities was determined by performing hydrolysis of *para*-nitrophenylphosphate, *para*-nitrophenyl-β-D-galactopyranoside and *para*-nitrophenyl-α-D-Mannopyranoside in a series of buffers (100 mM) at pH values ranging from 3.0 to 6.2. Sodium acetate buffer from pH 3.6 to 5.5 and sodium citrate buffer from pH 3.0 to 6.2 were used. The pH values of each buffer were determined at 25°C.

The effect of temperature on phosphatase, β-galactosidase and α-mannosidase activities was performed in 100 mM acetate buffer (appropriate pHs) over a temperature range of 30 to 80°C using *p*NPP, *p*NP-β-D-Gal or *p*NP-α-D-Man (5 mM) as substrates under the enzyme assay conditions.

pH and temperature stabilities: The pH stability of each enzymatic activity was studied in a pH range of 3.0 to 6.2 with 100 mM sodium citrate buffer. The buffers were the same as in the pH and temperature optima study described above. After 2 h pre-incubation at 25°C, residual activities were measured at 37°C for 10 min by adding the substrate (*p*NPP, *p*NP-β-D-Gal or *p*NP-α-D-Man). Thermal inactivation was determined at 37°C and at each enzymatic activity's optimum temperature. Enzymes in appropriate buffers (pHs) were exposed to each temperature for 0 to 100 min. Aliquots were withdrawn at intervals and immediately cooled. For thermal denaturation tests, aliquots of the crude extract were preheated at different temperatures ranging from 30 to 80°C for 15 min. Residual activities determined in the three cases at 37°C under the enzyme assay conditions, were expressed as percentage activity of zero-time untreated enzymes (control).

Effect of selected chemical agents: To determine the effect of various compounds (cations, chelating, sulphidryl specific and reducing agents and detergents) as possible activators or inhibitors of the enzymatic activities, the crude extract was pre-incubated at 37°C for 30 min with each compound and then, the activity was assayed under the enzyme assay conditions. Residual activities were expressed as percentage based on results of the control treatment without chemical agents.

Characterization of hydrolytic specificity: Hydrolytic specificity was determined by separately incubating the seed extracts (50 µl which correspond to 0.25 mg of

proteins) with appropriate substrates for different times at 37°C in 100 mM sodium acetate buffer (each optimum pH).

For hydrolytic specificity of phosphorylated substrates, the inorganic phosphate (P_i) produced by the phosphatase activity was quantified by the method of Heinonen and Lahti (1981). Glucose and galactose released from the hydrolysis of lactose (1 mM) and mannose liberated from different linked mannobioses (1 mM) were visualized through TLC plates. Samples (3 µl) were spotted for each mixture. TLC plates were run with butanol-acetic acid-water (9:3.75:2.25, v/v/v) and then developed with naphto-resorcinol in ethanol and H_2SO_4 20% (v/v). The sugar spots were visualized at 110°C for 5 min.

RESULTS AND DISCUSSION

Enzymes are essential biocatalysts to metabolic processes of living organisms. Indeed, any loss of an enzymatic activity is irremediably followed by metabolic disorders, also called uninherited diseases (Noble & Bovey, 1997; Kranz *et al*., 2007). Interest in enzymes also lies in their application in food industry, agriculture and various biotechnological processes (Oehmig *et al*., 2007; Betancor *et al*., 2008). In this respect, research on new enzyme sources with improved properties remains of topical interest.

Screening of glycosidase and phosphatase hydrolytic activities: The seeds extract of *L. siceraria* round-fruited cultivar were screened over a variety of synthetic and natural substrates for hydrolytic enzymatic activities. The crude enzyme extract contained a number of hydrolases (Fig. 1) and the activities of phosphatase, α-D-galactosidase, β-D-galactosidase, α-D-mannosidase, β-D-glucosidase, α-L-arabinosidase, amylase, inulinase and xylanase were detectable. Among these activities, phosphatase activity appeared to be the predominant one with a specific activity of 0.71 ± 0.03 UI/mg followed by β-galactosidase (0.31 ± 0.02 UI/mg) and α-mannosidase (0.21 ± 0.02 UI/mg) activities (Fig. 1). Higher phosphatasic, β-galactosidasic and α-mannosidasic activities may be a good indicator of the present phosphorus, mannoside and galactoside-rich moieties such as phospho-, galacto- and manno- conjugates. Considering their specific activities, these hydrolasic activities were found to be higher than those already reported for plants and other sources, which were considered for purification nevertheless (Li *et al*., 2001; Ahi *et al*., 2007; Konan *et al*., 2008). Therefore, seeds of the round-fruited cultivar of *L. siceraria* appeared to be a promising alternative source of phosphatase, β-galactosidase and α-mannosidase.

Phosphatases are involved in the metabolic processes of germination and maturation of plants (Gonnety *et al*., 2006) while β-galactosidase is often associated with fruit ripening processes (Lazan *et al*., 2004; Balasubramaniam *et al*., 2005). Plant β-galactosidases have previously been reported to play important roles in the metabolism of galactosyl conjugates during carbohydrate reserve mobilization, cell wall expansion and degradation, and turnover of signalling molecules during ripening (Esteban *et al*., 2003; De Alcantara *et al*., 2006). α-mannosidases are key enzymes widespread in nature, and found in all eukaryotes from yeast to man (Tatara *et al*., 2003; Tremblay *et al*., 2007). They are involved in the processing of newly formed N-glycans by modifying oligosaccharide structures linked to appropriate asparagine residues of proteins, and thus influence their properties and bioactivity (Moremen *et al*., 1994; Akama *et al*., 2006).

pH and temperature dependences: The effect of pH and temperature on phosphatase, β-galactosidase and α-mannosidase activities is shown in Figure 2. The three enzymes were optimally active in acidic pH ranging from pH 4.6–5.6 and at 55°C (Figs. 2A and 3). This behaviour is in accordance with the majority of plant acid phosphatase, β-galactosidase and α-mannosidase activities reported previously (Ali *et al*., 1998; Gonnety *et al*., 2006; Ahi *et al*., 2007). At 37°C, these activities showed best stability over pH values ranging from 4.0 to 5.8 by conserving at least more than 80% of total activities (Fig. 2B). This stability is suitable and desirable as a good compromise for performing hydrolysis or synthesis reactions. Values of temperature coefficients (Q_{10}) calculated were around 1.8 for phosphatase and α-mannosidase activities and 1.5 for β-galactosidase activity. From Arrhenius plot (data not shown), values of 50.8, 35.1 and 34.4 kJ/mol were obtained for the activation energy of phosphatase, β-galactosidase and α-mannosidase activities, respectively.

The thermal denaturation test showed that phosphatase, β-galactosidase and α-mannosidase activities were fairly stable at temperatures up to 50°C.

At higher temperatures, hydrolytic activities decreased sharply (Fig. 4A). The thermal inactivation study indicated that at 37°C and at each optimum pH value, the three enzymatic activities remained fully stable for 100 min. However at 50°C (optima temperatures), they were less stable and retained about 80% of their activity after 40 min pre-incubation. Half-lives (50% of activity) of phosphatase and α-mannosidase activities were respectively obtained at 80 and 100 min pre-incubation while β-galactosidase retained about 60% of its activity after 100 min (Fig. 4B). These residual activities indicate a good potential for performing hydrolysis reactions using phosphatase, β-galactosidase and α-mannosidase from seeds of the round-fruited cultivar of *Lagenaria siceraria*.

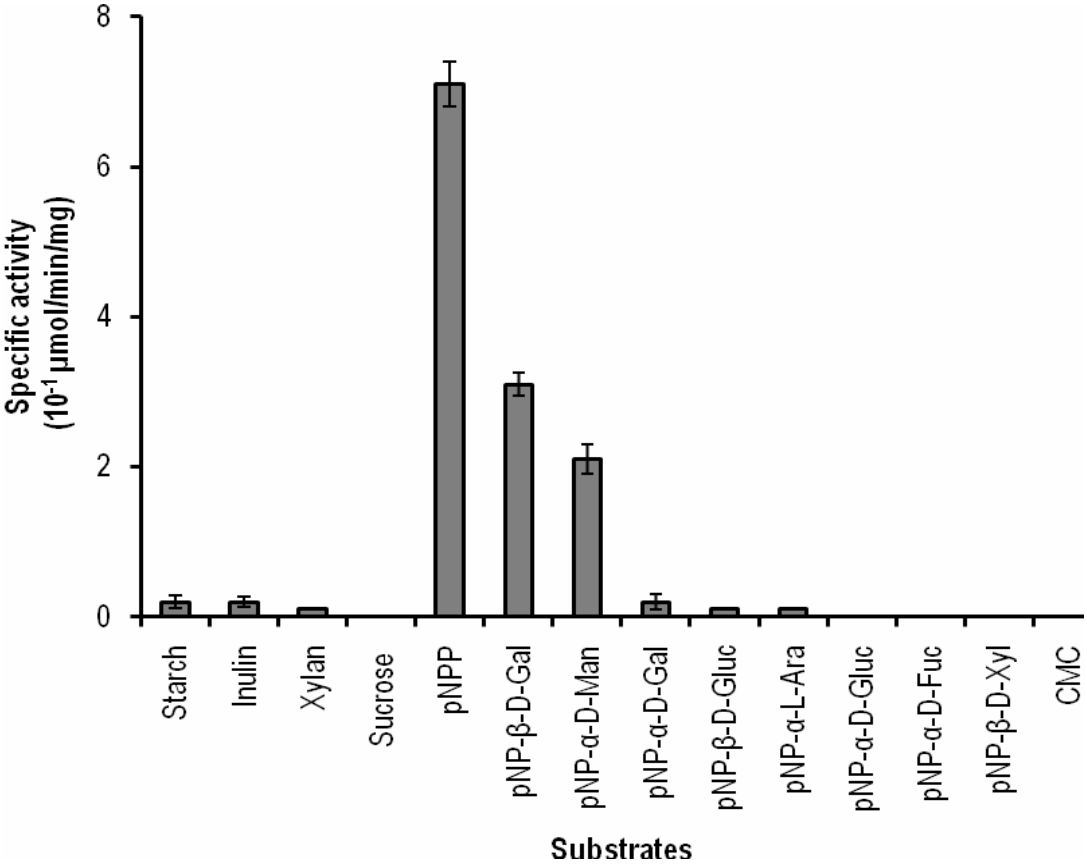

Figure 1: Screening of the seeds extract from the round-fruited cultivar of *Lagenaria siceraria* for glycosidase and phosphatase activities over synthetic and natural substrates.

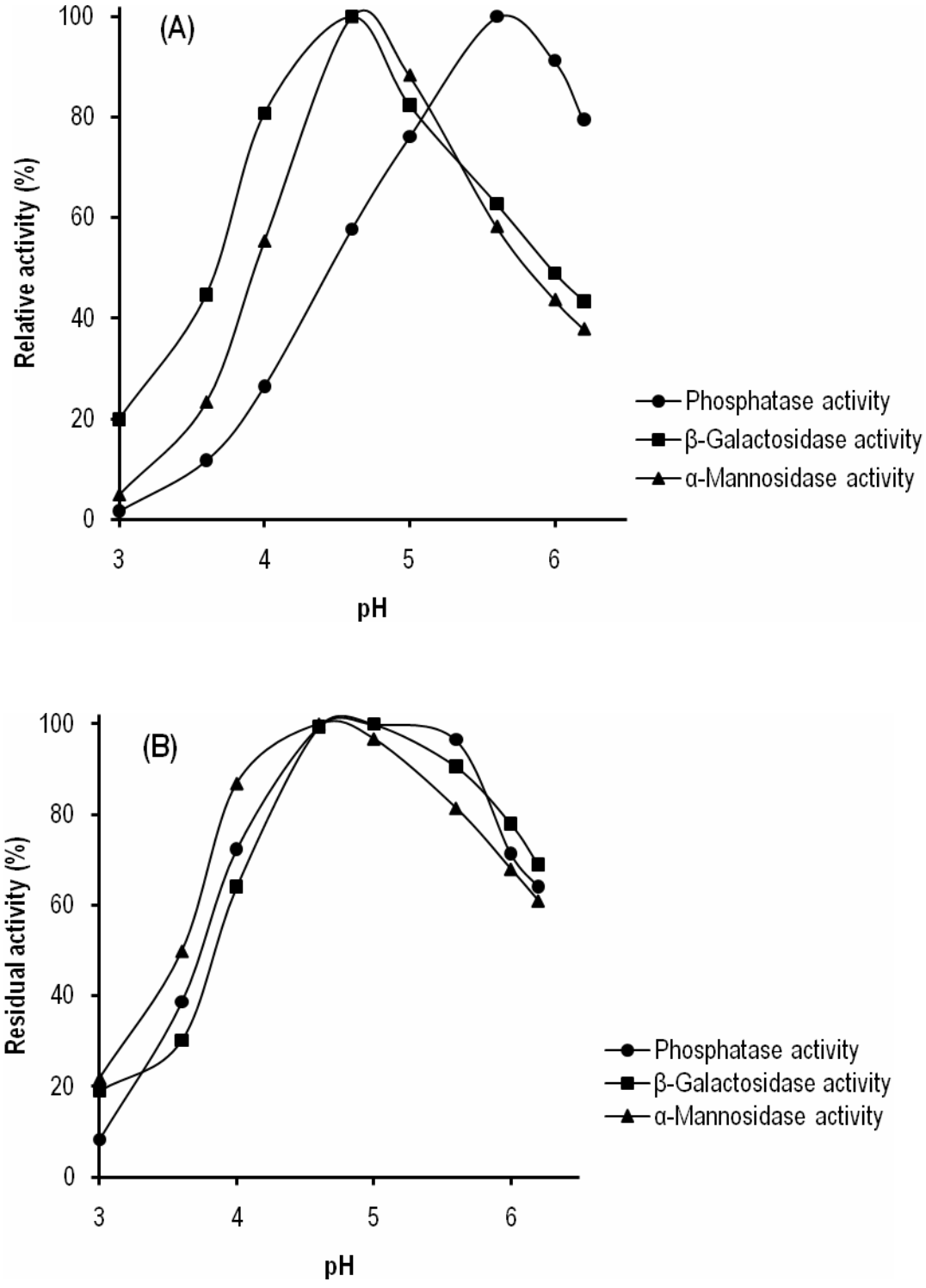

Figure 2: Effect of pH on phosphatase, β-galactosidase and α-mannosidase activities from the seed extracts of the round-fruited cultivar of *Lagenaria siceraria.* (A) Optimum pH. (B) pH stability.

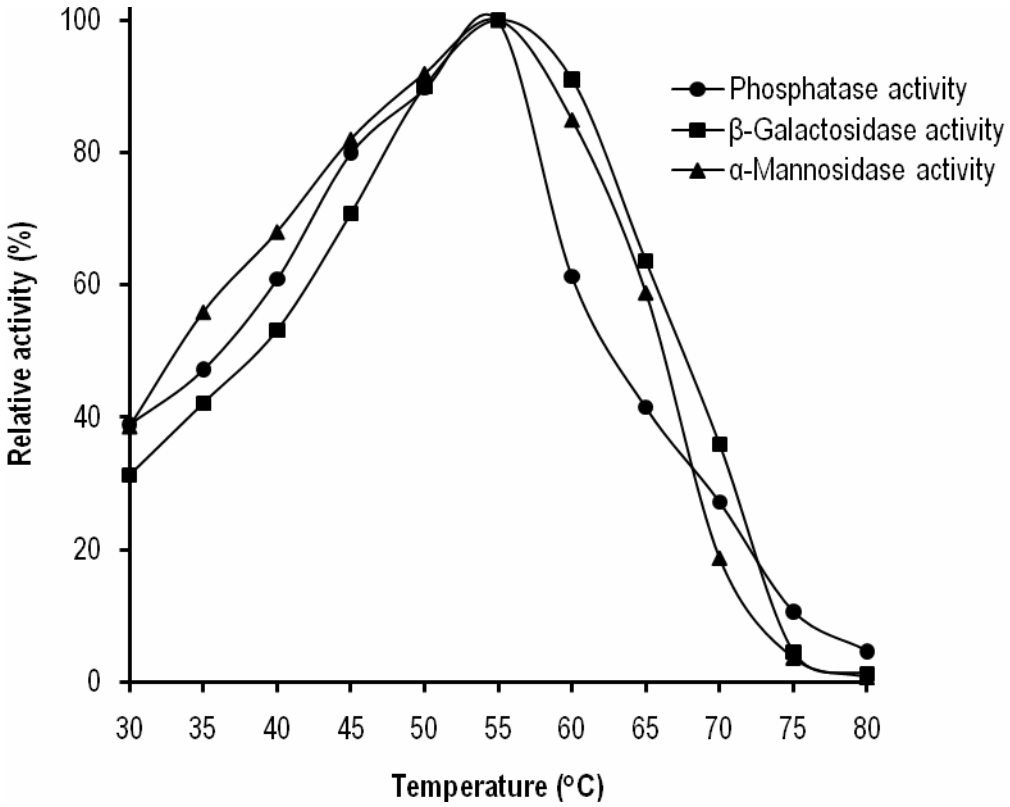

Figure 3: Effect of temperature on phosphatase, β-galactosidase and α-mannosidase activities from the seed extracts of the round-fruited cultivar of *Lagenaria siceraria.*

Effect of cations, chelating, sulphidryl specific and reducing agents: Phosphatase activity was slightly stimulated by Mg^{2+} (112.1 ± 4.0%) while β-mercaptoethanol (5 mM) inhibited by around 13% (Figs 5 & 6). The stimulatory effect displayed by Mg^{2+} has previously been reported for various plant acid phosphatases (Bozzo *et al*. 2002; Gonnety *et al*. 2006). As regards α-mannosidase activity, Ba^{2+}, EDTA and urea were found to be inhibitory in the range of 22 to 26% (Fig. 5). These results suggest that this enzyme requires divalent metal cations to be fully active. A-Mannosidase containing other binuclear metal centers has already been reported in *Drosophila melanogaster* (Van Den Elsen *et al*., 2001). B-galactosidase activity was inhibited by *p*CMB by around 23% (Fig. 6), which suggests that –SH groups participate in this enzymatic reaction. The other chemicals tested had little or no effect.

Effect of detergents: Except SDS that displayed a strong inhibitory effect (between 66 and 96%) on phosphatase, β-galactosidase and α-mannosidase activities, most of the detergents currently used for denaturing proteins showed, by and large, no pronounced effect on the three enzymatic activities (Table 1). However, non-ionic detergents (Tween 80, Lubrol Wx and Triton X-100) and anionic detergents (polyoxyethylene 9 lauryl ether and polyoxyethylene 10 oleyl ether) enhanced phosphatase hydrolytic activity up to 117.4 ± 4.9%. B-galactosidase activity was also activated by cationic detergents up to 112.9 ± 4.3%..

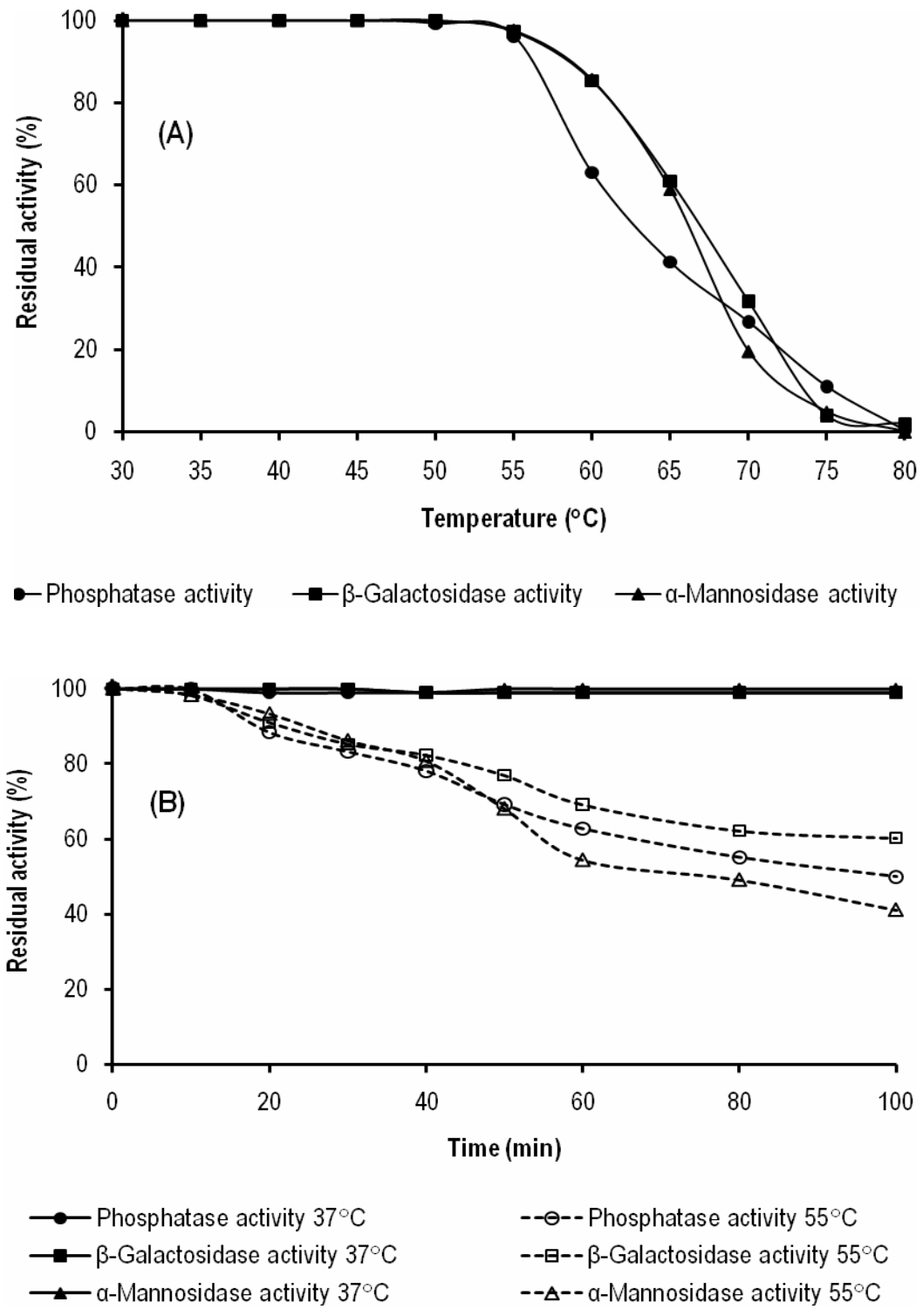

Figure 4: Thermal stability of phosphatase, β-galactosidase and α-mannosidase activities from the seed extracts of the round-fruited cultivar of *Lagenaria siceraria*. (A) Thermal denaturation. (B) Thermal inactivation.

Therefore, these detergents should be used in these enzymes preparations. Cationic detergents were found to be inhibitory to α-mannosidase activity by between 13 and 23% (Table 1). By and large, the stability of the three enzymatic activities in the presence of detergents constitutes an interesting characteristic for their potential industrial application. In addition, the identified detergents could be particularly useful when extracting these enzymes by improving their stability for further specific studies.

.

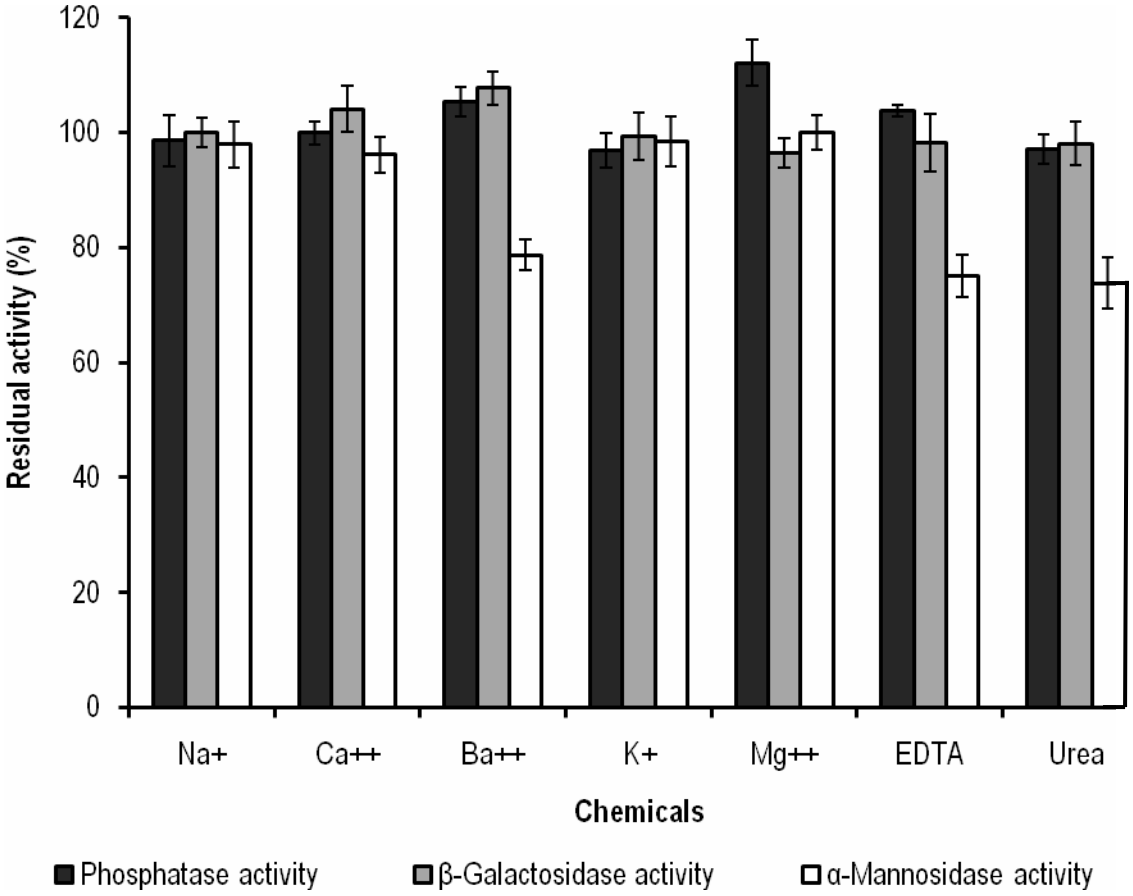

Figure 5: Effect of cations, chelating agent and urea on phosphatase, β-galactosidase and α-mannosidase activities from the seed extracts of the round-fruited cultivar of *Lagenaria siceraria*.

Substrates hydrolytic specificity: Phosphatase, β-galactosidase and α-mannosidase activities from the seeds extract of *Lagenaria siceraria* round-fruited cultivar were assayed for their capabilities to hydrolyze different specific substrates. Phosphatase activity hydrolyzed a broad range of phosphorylated substrates (Table 2). The highest activity (116.5 ± 2.1%) was observed with adenosine-5'-triphosphate (ATP) followed by *p*-nitrophenylphosphate (100.0 ± 1.6%), sodium pyrophosphate (77.8 ± 1.9%) and phenylphosphate (77.6 ± 2.4%). A higher rate of ATP and pyrophosphate hydrolysis by plant phosphatases was recently observed in sweet potato (Kusudo *et al*., 2003) and breadfruit (*Artocarpus communis*) seeds (Konan *et al*., 2008).

Also, phosphorylated sugars such as glucose-1 or 6-phosphate and fructose-1 or 6-phosphate were hydrolyzed in the range of 19.1 ± 2.3% to 44.7 ± 3.1% (Table 2). The hydrolysis of phosphate esters is an important process in energy metabolism and a wide variety of cellular signal transduction pathways of plant cells (Vincent *et al*., 1992). These observations showed that phosphatase activity from *L. siceraria* round-fruited cultivar seeds play an important role during seed

ripening. This acid phosphatase activity seemed to be involved in energy transfer, release of inorganic phosphate (Pi) and other reserve materials during fruit and seed ripening.

Hydrolysis of the two latter substrates remained interesting compared to that of sodium phytate (14.6 ± 2.8%) and α-nicotinamide adenine dinucleotide (12.9 ± 2.1%) which were less specific (Table 2). Clearly, sodium phytate hydrolysis showed proof of an efficient acid phosphatase activity from *L. siceraria* round-fruited cultivar seeds. Indeed, this enzyme could cleave phosphate moieties from phytic acid (myo-inositol-hexakisphosphate) present in cucurbits seeds, thereby generating myo-inositol, inorganic phosphate and some multivalent cations. It is well known that phytic acid (phytate) chelates multivalent cations and some proteins, rendering them biologically unavailable to animals (Harland & Morris, 1995). Furthermore, myo-inositol via this oxidation pathway, is directed to cell wall polysaccharide biosynthesis (Loewus & Murthy, 2000), thus is important for cell wall elongation and growth.

Table 1: Effect of detergents on phosphatase, β-galactosidase and α-mannosidase activities from the seed extracts of the round-fruited cultivar of *Lagenaria siceraria* (Cucurbitaceae).

Detergents*	Relative activity (%)		
	Phosphatase	β-Galactosidase	α-Mannosidase
Control	100	100	100
Cationic			
Tetradecyl Trimethyl Ammonium Bromide	104.2 ± 3.5	112.9 ± 4.3	87.5 ± 3.7
Hexadecyl Trimethyl Ammonium Bromide	102.9 ± 4.2	112.4 ± 3.6	77.4 ± 4.1
Non ionic			
Tween 80	114.5 ± 3.7	98.2 ± 2.5	95.9 ± 3.2
Lubrol Wx	117.4 ± 4.9	98.7 ± 3.1	97.7 ± 3.3
Triton X-100	108.6 ± 3.2	96.8 ± 4.2	92.7 ± 4.5
Anionic			
Polyoxyethylene 9 lauryl ether	111.6 ± 5.1	98.7 ± 3.3	95.5 ± 4.9
Polyxyethylene 10 tridecyl ether	100.0 ± 3.9	96.3 ± 4.7	97.2 ± 4.3
Polyxyethylene 10 oleyl ether	116.0 ± 5.4	98.4 ± 4.6	95.7 ± 5.1
Sodium dodecyl sulphate	3.8 ± 1.1	33.5 ± 3.4	15.5 ± 3.6

*Assays were performed at 37°C for 10 min with 1% (w/v) starting concentration of detergent

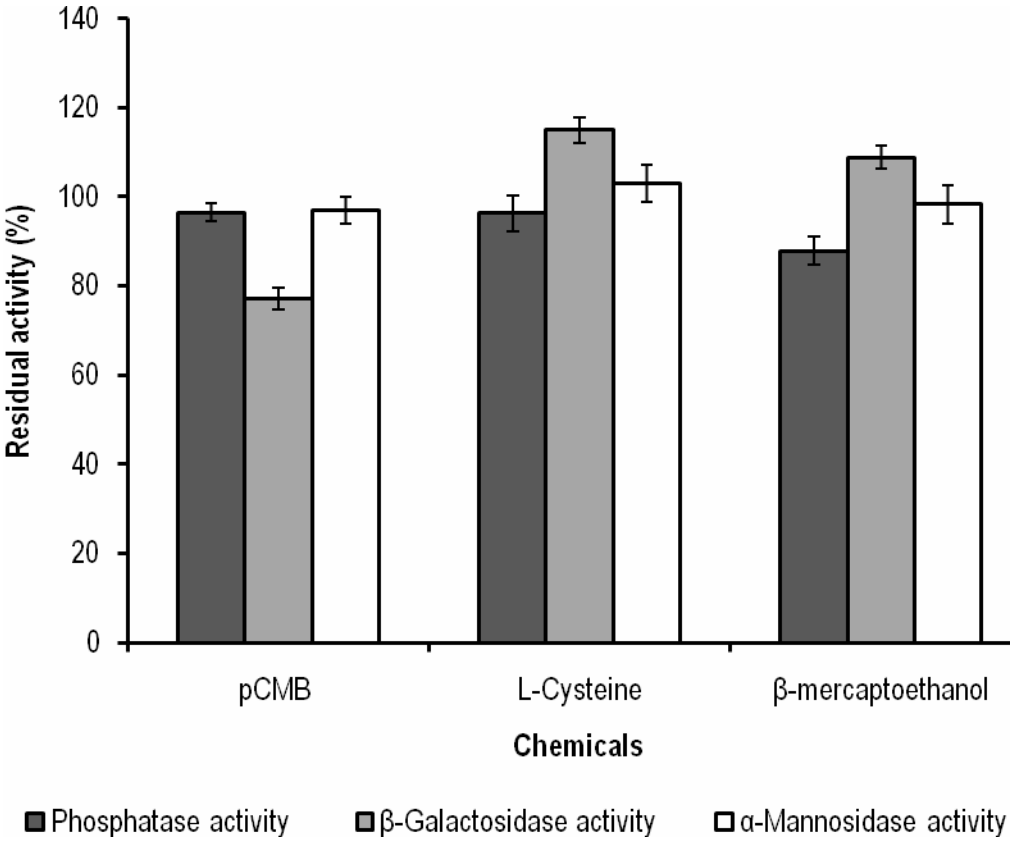

Figure 6: Effect of reducing agents on phosphatase, β-galactosidase and α-mannosidase activities from the seeds extract of the round-fruited cultivar of *Lagenaria siceraria*.

Figure 7A shows the ability of the seeds extract of *L. siceraria* round-fruited cultivar to hydrolyze lactose. The presence of glucose and galactose (products of lactose hydrolysis) were noted in the mixture and the remaining lactose concentration was very small. As a result, lactose is greatly hydrolyzed by β-galactosidase activity. This is an interesting property of a β-galactosidase activity for industrial and biotechnological applications. Indeed enzymatic hydrolysis of lactose by β-galactosidase has two main biotechnological applications; the utilization of whey, as glucose and galactose (the hydrolysates) having greater fermentation potential (Kosaric & Asher, 1985) and in the production of low lactose milk (and dairy products made from it) for consumption by lactose intolerant persons (Kretchmer, 1972). In the same way, β-galactosidase (or lactase) is used for the treatment of milk, for prevention of lactose crystallization in frozen and condensed milk products and also for the reduction of water pollution caused by whey (Patel & Mackenzie, 1985; Furlan & Schneider, 2000).

Thin layer chromatography analysis of the hydrolysis of natural substrates such as α-1,2; α-1,3 and α-1,6 mannobioses by α-mannosidase activity Showed that the three differently linked disaccharides were cleaved at different rates showing a broad specific α-mannosidase activity (Fig. 7B). However, this hydrolytic activity on α-1,2 mannobiose seemed to be greater than that on the two other linkages. Broad specific α-mannosidases are thought to be very important and are reported to be involved in cleaving the carbohydrate moieties of glycoproteins. Lysosomal α-mannosidases with these characteristics have previously been identified from several sources, e.g *Dictyostelium discoideum* (Schatzle *et al*., 1992) and a variety of mammalian tissues (Opheim & Touster, 1978; De Gasperi *et al*., 1991). Our results, together with the previous findings (Saint-Pol *et al*., 1999; Hirsch *et al*., 2003), suggests that the major potential application of this enzyme would be in treatment of the lysosomal storage disorder α-mannosidosis by enzyme replacement therapy. Indeed, α-mannosidosis results

from deficient activity of α-mannosidase. This disease is characterized by massive intracellular accumulation of mannose-rich oligosaccharide, that is oligosaccharides carrying α-1, 2; α-1,3 and α-1,6 mannosyl residues at the non-reducing termini. In this respect, the broad specific α-mannosidases become useful for application requiring removal of all α-mannosyl linkages.

CONCLUSION

To sum up this report, we can note that phosphatase, β-galactosidase and α-mannosidase activities are predominant in the seeds extract of *Lagenaria siceraria* round-fruited cultivar. These three enzymatic activities were acidic (pH 4.6 – 5.6) and mesophilic (55°C) and appeared to be stable in the presence of most detergents. Furthermore, they hydrolyzed a broad range of natural substrates such as ATP, sodium phytate, lactose and mannobioses. Based on the results of the present study, we conclude that seeds of *Lagenaria siceraria* round-fruited cultivar constitute an interesting source of phosphatase(s), β-galactosidase(s) and α-mannosidase(s) that deserve further investigation for potential industrial and biotechnological applications.

ACKNOWLEDGEMENTS

This work was supported by a PhD grant to the first author. The authors are grateful to Laboratoire de Génétique (UFR des Sciences de la Nature, Université d'Abobo-Adjamé, Côte d'Ivoire) for technical assistance.

Table 2: Hydrolytic activity of the seed extracts of the round-fruited cultivar of *Lagenaria siceraria* on a variety of phosphorylated substrates.

Substrates*	Hydrolytic activity (%)
p-Nitrophenylphosphate (Control)	100.0 ± 1.6
Phenylphosphate	77.6 ± 2.4
Adenosine-5'-triphosphate	116.5 ± 2.1
Adenosine-2'3'-cyclomonophosphate	24.4 ± 3.2
α-Nicotinamide adenine dinucleotide	12.9 ± 2.1
Sodium pyrophosphate	77.8 ± 1.9
Glucose -1-phosphate	19.1 ± 2.3
Glucose -6-phosphate	44.7 ± 3.1
Fructose-1-phosphate	24.5 ± 2.1
Fructose-6-phosphate	43.5 ± 2.2
Sodium phytate**	14.6 ± 3.8

*Assays were performed at 37°C for 30 min with 5 mM final concentration of substrate; **This reaction was performed at 50°C for 2 h

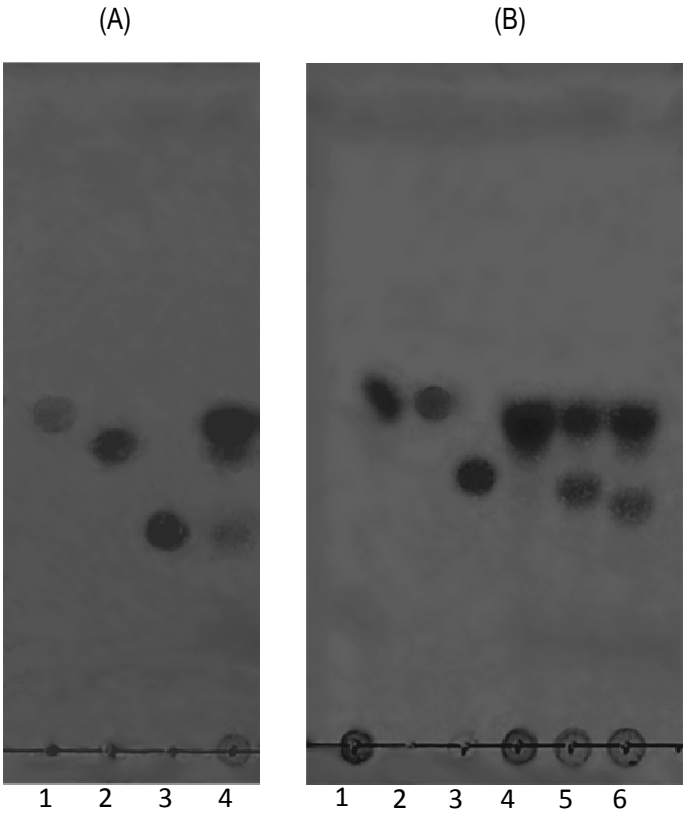

Figure 7: TLC plates showing β-galactosidase and α-mannosidase activities from the seed extracts of the round-fruited cultivar of *Lagenaria siceraria* towards differently linked disaccharides. (A) β-galactosidase activity on lactose: Lane 1, glucose; lane 2, galactose; lane 3, lactose, lane 4, reaction mixture with lactose. (B) α-mannosidase activity on α-1,2; α-1,3 and α-1,6-mannobioses: lane 1, crude extract; lane 2, mannose; lane 3, mannobiose, lanes 4-6, reaction mixtures with α-1,2; α-1,3 and α-1,6-mannobiose, respectively.

REFERENCES

Achu MB, Fokou E, Tchiegang C, Fotso M, Tchouanguep FM, 2005. Nutritive value of some Cucurbitaceae oilseeds from different regions in Cameroon. African Journal of Biotechnology 4: 1329–1334.

Achu MB, Fokou E, Tchiegang C, Fotso M, Tchouanguep FM, 2006. Chemical characteristics and fatty acid composition of *Cucurbitaceae* oils from Cameroon. IUFoST 4: 1249-1259.

Ahi AP, Gonnety TJ, Faulet BM, Kouamé LP, Niamké SL, 2007. Biochemical characterization of two α-mannosidases from breadfruit (*Artocarpus communis*) seeds. African Journal of Biochemistry Research 1: 106–116.

Akama TO, Nakagawa H, Wong NK, Sutton-Smith M, Dell A, Nakayama J, Nishimura S, Pai A, Moremen KW, Marth JD, Fukuda MN, 2006. Essential and mutually compensatory roles of α-mannosidase II and α-mannosidase IIx in *N*-glycan processing in vivo in mice. Proceedings of the National Academy of Sciences of the United States of America 103: 8983–8988.

Ali ZM, Armugam S, Lazan H, 1995. β-Galactosidase and its significance in ripening mango fruit. Phytochemistry 38: 1109–1114.

Ali ZM, Ng SY, Othman R, Goh LY, Lazan H, 1998. Isolation, characterization and significance of papaya β-galactanases to cell wall modification and fruit softening during ripening. Physiologia Plantarum 104: 105–115.

Balasubramaniam S, Lee HC, Lazan H, Othman R, Ali ZM, 2005. Purification and properties of a b-galactosidase from carambola fruit with significant activity towards cell wall

polysaccharides. Phytochemistry 66: 153–163.

Bernfeld P, 1955. Amylase and Proteases. Colswick SP and Kaplan NO (Editors), Methods in Enzymology. Academic Press, New-York, USA. pp 149–154.

Betancor L, Luckarift HR, Seo JH, Brand O, Spain JC, 2008. Three-dimensional immobilization of b-galactosidase on a silicon surface. Biotechnology and Bioengineering 99: 261–267.

Bozzo GG, Raghothama KG, Plaxton WC, 2002. Purification and characterization of two secreted purple acid phosphatase isozymes from phosphate-starved tomato (*Lycopersicon esculentum*) cell cultures. European Journal of Biochemistry 269: 6278-6286.

De Alcantara PHN., Martim L, Silva CO, Dietrich SMC, Buckeridge MS, 2006. Purification of a β-galactosidase from cotyledons of *Hymenaea courbaril* L. (Leguminosae). Enzyme properties and biological function. Plant Physiology and Biochemistry 44: 619–627.

De Gasperi R, Al-Daher S, Daniel PF, Winchester BG, Jeanloz RW, Warren CD, 1991. The substrate specificity of the bovine and feline lysosomal α-D-mannosidases in relation to mannosidosis. Journal of Biological Chemistry 266: 16556-16563.

De Mello MLS, Bora PS, Narain N, 2001. Fatty and amino acids composition of melon (*Cucumis melo* var. *saccharinus*) seeds. Journal of Food Composition and Analysis 14: 69-74.

Dey PM, 1984. Biochemistry of the multiple forms of glycosidases in plants. Meister A (Editor), Advances in Enzymology and Related Areas of Molecular Biology. John Wiley and Sons, New York, USA. pp. 180–186.

El Tahir IM and Yousif MT, 2004. Indigenous melons (*Cucumis melo* L.) in Sudan: a review of their genetic resources and prospects for use as sources of disease and insect resistance. Plant Genetic Research Newsletter 138: 36-42.

Enujiugha VN and Ayodele-Oni O, 2003. Evaluation of nutrients and some anti-nutrients in lesser-known, underutilized oilseeds. International Journal of Food Science and Technology 38: 525–528.

Esteban R, Dopico B, Munoz FJ, Romo S, Martin I, Labrador E, 2003. Cloning of a Cicer arietinum β-galactosidase with pectin-degrading function. Plant Cell Physiology 44: 718–725.

Furlan S and Schneider A, 2000. Formulation of a lactose-free, low-cost medium for the production of b-galactosidase by *Kluyveromyces marxianus*. Biotechnology Letters 22: 589–593.

Gonnety JT, Niamké S, Faulet BM, Kouadio EJPN, Kouamé LP, 2006. Purification and characterization of three low-molecular-weight acid phosphatases from peanut (*Arachis hypogaea*) seedlings. African Journal of Biotechnology 5: 35–44.

Harland FB and Morris ER, 1995. Phytate: A good or bad food component. Nutrition Research 15: 733-754.

Heinonen JK and Lahti RJ, 1981. A new and convenient colorimetric determination of inorganic orthophosphate and its application to the assay of inorganic pyrophosphate. Analytical Biochemistry 113: 313–317.

Hirsh C, Blom D, Ploegh HL, 2003. A role for N-glycanase in the cytosolic turnover of glycoproteins. EMBO Journal 22: 1036–1046.

IPGRI, 2002. Neglected and underutilized plant species: strategic action plan of the International Plant Genetic Resources Institute (IPGRI). Rome: IPGRI. 30 pp.

Konan KH, Ahi AP, Kouadio NEJP, Gonnety TJ, Kouamé LP, 2008. Characterisation of purple acid phosphatase from breadfruit (*Artocarpus communis*) seeds. Journal of Applied Biosciences 10: 449–460.

Kosaric N. and Asher YJ, 1985. The utilization of cheese whey and it components. Fiechter A (Editor), Advances in Biochemical Engineering. Springer, New York, USA. pp. 25–60.

Kranz C, Bobby GN, Sun L, Sharma V, Eklund EA, Miura Y, Ungar D, Lupashin VR, Winke D, Cipollo JF, Costello CE, Loh E, Hong W, Freeze HH, 2007. COG8 deficiency causes new congenital disorder of glycosylation type Iih. Human Molecular Genetics 16: 731–741

Kretchmer M, 1972. Lactose and lactase. Scientific American 227: 71–78.

Kusudo T, Sakaki T, Inouye K, 2003. Purification and characterization of purple acid phosphatase PAP1 from dry powder of sweet Potato.

Bioscience Biotechnology and Biochemistry 67:1609-1611.

Lazan H, Ng SY, Goh LY, Ali ZM, 2004. Papaya β-galactosidase/galactanase isoforms in differential cell wall hydrolysis and fruit softening during ripening. Plant Physiology and Biochemistry 42: 847–853.

Li SC, Han JW, Chen KC, Chen CS, 2001. Purification and characterization of isoforms of β-galactosidases in mung bean seedlings. Phytochemistry 57: 349–359.

Liu Y, Ahn JE, Datta S, Salzman RA, Moon J, Huyghues-Despointes B, Pittendrigh B, Murdock LL, Koiwa H, Zhu-Salzman K, 2005. Arabidopsis vegetative storage protein is an anti-insect acid phosphatase. Plant Physiology 139: 1545-1556.

Loewus FA and Murthy PPN, 2000. Myo inositol metalbolism in plants. Plant Science 150: 1-19.

Lowry OH, Rosebrough NJ, Farra L, Randall RJ, 1951. Protein measurement with Folin-phenol reagent. Journal of Biological Chemistry 193: 265–275.

Moremen KW, Trimble RB, Herscovics A, 1994. Glycosidases of the asparagine-linked oligosaccharide processing pathway. Glycobiology 4: 113-125.

Noble R and Bovey A, 1997. Therapeutic teat use for babies who breastfeed poorly. Breastfeeding Review (NMAA) 5: 2 37-42

Oehmig A, Cortes ML, Perry KF, Sena-Esteves M, Fraefel C, Breakefield XO, 2007. Integration of active human β-galactosidase gene (100 kb) into genome using HSV/AAV amplicon vector. Gene Therapy 14: 1078–1091.

Opheim DJ and Touster O, 1978. Lysosomal alpha-D-mannosidase of rat liver. Purification and comparison with the golgi and cytosolic alpha-D-mannosidases. Journal of Biological Chemistry 253: 1017-1023.

Paris HS, 2001. Characterization of the *Cucurbita pepo* collection at the newe Ya'ar research center, Israel. Plant Genetic Resources Newsletter 162: 41-46.

Patel GB and Mackenzie CR, 1985. Properties and potential advantages of β-galactosidase from Bacteroides polypragmatus. Applied Microbiology and Biotechnology 22: 114–120.

Pitrat M, Chauvet M, Foury C, 1999. Diversity, history and production of cultivated cucurbits. Acta Horticulturae 492: 21–28.

Saint-Pol A, Codogno P, Moore SEH, 1999. Cytosol to lysosome transfer of free polymannose-type oligosaccharides, kinetic and specificity studies using rat liver lysosomes. Journal of Biological Chemistry 274: 13547-13555.

Sanjur OI, Piperno DR, Andres TC, Wessel-Beaver L, 2002. Phylogenetic relationships among domesticated and wild species of *Cucurbita* (Cucurbitaceae) inferred from a mitochondrial gene: implications for crop plant evolution and areas of origin. Proceedings of the National academy of Sciences of the United States of America 99: 535–540.

Schatzle J, Bush J, Cardelli J, 1992. Molecular cloning and characterization of the structural gene coding for the developmentally regulated lysosomal enzyme, α-mannosidase, in *Dictyostelium discoideum*. Journal of Biological Chemistry 267: 4000-4007.

Sun H, Yang H, Askins ME, 1999. Retrovirus vector mediated correction and cross correlation of lysosomal α-mannosidase deficiency in human and feline fibroblasts. Human Gene Therapy 10: 1311-1319.

Tatara Y, Lee BR, Yoshida T, Takahashi K, Ichishima E, 2003. Identification of catalytic residues of Ca^{2+} independent 1,2-α-D-mannosidase from *Aspergillus saitoi* by site-directed mutagenesis. Journal of Biological Chemistry 278: 25289-25294.

Tremblay LO, Kovacs EN, Daniels E, Wong NK, Sutton-Smith M, Morris HR, Dell A, Marcinkiewicz E, Seidah NG, Mckerlie C, Herscovics A, 2007. Respiratory distress and neonatal lethality in mice lacking Golgi α-1,2-mannosidase IB involved in N-glycan maturation. Journal of Biological Chemistry 282: 2558-2566.

Van Den Elsen, JM, Kuntz DA, Rose DR, 2001. Structure of Golgi alpha-mannosidase II: a target for inhibition of growth and metastasis of cancer cells. EMBO Journal 20: 3008–3017.

Vincent JB, Crowder MW, Averil BA, 1992. Hydrolysis of phosphate monoester: a biological problem with multiple chemical solutions. Trends Biochemistry Science 17: 105-110.

Yoneyama T, Shiozawa M, Nakamura M, Suzuki T, Sagane Y, Katoh Y, Watanabe T, Ohyama T, 2004. Characterization of a novel acid phosphatase from embryonic axes of kidney bean exhibiting vanadate-dependent chloroperoxidase activity. Journal of Biological Chemistry 279: 37477-37484.

Zoro Bi I, Koffi KK, Djè Y, 2003. Caractérisation botanique et agronomique de trois espèces de cucurbites consommées en sauce en Afrique de l'ouest: *Citrullus sp., Cucumeropsis mannii* Naudin et *Lagenaria siceraria* (Molina) Standl. Biotechnologie, Agronomie, Societe et Environnement 7: 189–199.

Zoro Bi IA, Kouame KK, Djè Y, Malice M, Baudoin JP, 2006. Indigenous cucurbits of Côte d'Ivoire: a review of their genetic resources. Sciences & Nature 3: 1–9.