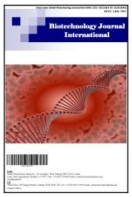

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Biochemical Characterization of Two Polygalacturonases Purified from the Digestive Juice of the Snail *Limicolaria flammea*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors BTS and DMK designed the study, wrote the protocol. Authors AKK and DMK anchored the field study, gathered the initial data and performed preliminary data analysis. While authors DMK, JOG and LPK managed the literature searches, interpreted the data and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Polygalacturonases constitute the major part of pectinase preparations for many bioprocess purposes. Investigation on the digestive juice of snail *Limicolaria flammea* led to purification of two polygalacturonases named PG1 and PG2. Properties of these enzymes were examined to explore their potential in biotechnology applications. A three steps procedure including size exclusion, anion and cation exchange and hydrophobic interaction chromatography were used for purification. The enzymes PG1 and PG2 had native molecular weights of approximately 46 and 86 kDa, respectively and functioned both as monomeric structures. The purified polygalacturonases PG1 and PG2 showed optimum hydrolysis activities at 50°C in sodium acetate buffer pH 5.6. The common inhibitor of the two purified polygalacturonases activity were Mn^{2+}, Ca^{2+}, Zn^{2+}, EDTA, SDS and L-cystein.

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NH3+ stimulate the polygalacturonase PG1 while Ba2+ was an activator for polygalacturonase PG2. Substrate specificity indicated that these enzymes hydrolyse a broad range of pectin from different sources. The highest activity of PG1 was observed with apple pectin and lemon pectin while PG2 showed its highest activity with orange pectin. The catalytic efficiency of PG1 was highest for lemon pectin (0.125 µmol/min/mL) and orange pectin (0.124 µmol/min/mL). PG2 displayed highest catalytic efficiency (0.325 µmol/min/mL) towards orange pectin. These results suggest that orange and lemon pectin would be the potential physiological substrates of the two purified enzymes.

Keywords: Polygalacturonase; digestive juice; snail Limicolaria flammea; purification; characterization.

1. INTRODUCTION

Pectinases or pectinolytic enzymes are hydrolases that specifically degrade pectic substances by various mechanisms. They were classified as de-esterifying enzymes and depolymering enzymes [1]. De-esterifying enzymes catalyze the de-esterification of pectin, releasing protons and methanol [2] while Depolymering enzymes consist of polygalacturonase (including endo-polygalacturonase (E.C. 3.2.1.15, EPG) and exo-polygalacturonase), pectin hydrolase (or pectin methylesterase), pectate lyase, and pectin lyase [3].

Polygalacturonases are widely distributed in many living organisms such as bacteria, fungi and plants and they constitute the major part of pectinase preparations for many bioprocess purposes. Indeed, these enzymes have a commercial application in the production of fruit juices and wines, mainly to clarify juice and to improve the pressing and extraction of juice from fruits and vegetables [4,5]. Pectin of certain fruits and vegetables remains in the pulp during the extraction step and causes a colloidal aspect in the juice.

The use of pectinolytic enzymes removes haze and cloudiness, which leads to reduction of viscosity and increases juice yields. Thus, in food industries, acidic endo-polygalacturonase (PG) from fungi are used during extraction and clarification of fruit juices [6]. Furthermore, these enzymes account for 25% of the world's industrial enzymes and about 75% of the world market share of enzymes for the food industry [7,8].

Many polygalacturonases have been purified and characterized from fungi, yeasts, and bacteria [9,10], among which fungal polygalacturonases are good candidates for the industrial production due to their stability at pH 4.0-6.0 [4,11]. Then, a variety of microorganisms including *Aspergillus niger*, *Aspergillus tamari*, *Aspergillus oryzae*, *Penicillium*, *Bacillus*, *Fusarium* and *Saccharomyces* species have been reported for its synthesis [12]. However, a robust polygalacturonase for bioprocesses involves not only a high catalytic activity but also acid- and thermo-stability against different physiochemical conditions. Moreover, very few studies have been devoted to polygalacturonases from animal and plant which could constitute good alternative in terms of stability and production cost. So, researchers are therefore focusing on the discovery of new sources for pectinolytic enzyme production and developing strategies to improve their catalytic characteristics for industrial biotransformation and synthesis [13,8].

In this study, we investigate the digestive juice of the snails *Limicolaria flammea*. Indeed, the digestive juice of snails constitutes a varied and very active enzymatic cocktail for the hydrolysis of many organic materials found in the plants which they consume [14,15]. In addition, *Limicolaria flammea* is found in different biotopes and is very abundant in gardens during the rainy season [16,17]. This paper describes the purification and characterization of two polygalacturonases from the digestive juice of snail *Limicolaria flammea* to explore their potential in biotechnology applications.

2. MATERIALS AND METHODS

2.1 Enzymatic Source and Crude Extract Preparation

Snail *Limicolaria flammea* were collected locally in Côte d'Ivoire at the National Floristic Center of the University Felix Houphouet-Boigny (5°21′ N and 3°54′ W). After isolation of the digestive tract, the digestive fluid was collected in a jar placed in ice, filtered through cotton wool, and then centrifuged at 10,000 × g for 30 min at

4°C. The supernatant constituted the crude extract.

2.2 Chemicals

Polygalacturonic acid (PGA), apple pectin, lemon pectin and orange pectin were purchased from Sigma-Aldrich. DEAE-Sepharose Fast Flow, CMSepharose CL-6B, Sephacryl S-200 HR and Phenyl-Sepharose 6 Fast Flow were provided from Pharmacia Biotech. Bovine serum albumin (BSA) was obtained from Fluka Biochemika. Standard molecular weights proteins were provided from BioRad. All the other reagents used were of analytical grade.

2.2.1 Purification procedure

All the purification procedure was carried out in a cold room. One (1) milliliter of the crude digestive juice of the snail *Limicolaria flammea* was loaded onto a Sephacryl S-200 HR column (capacity, 1.6 cm × 63 cm; flow rate, 20 mL/ h; fractions, 1 mL) previously equilibrated with 20 mM sodium acetate buffer pH 5.6 and, active fractions were pooled. Each pooled active fraction was loaded onto a DEAE 6 Fast Flow column (2.5 × 4cm) equilibrated with the same buffer. The column was washed at a flow rate of 2 mL/min with two bed volumes of equilibration buffer to remove unbound proteins. Bound proteins were then eluted with a stepwise salt gradient (0.2, 0.4 and 1 M) of NaCl in 20 mM sodium acetate buffer pH 5.6 and, fractions of 3 mL were collected. Two peaks of polygalacturonase activity were obtained. On the one hand, unbound polygalacturonase activity (Peak 1) and on the other hand, bound polygalacturonase activity (Peak 2). On the one hand, pooled polygalacturonase activity resulting from Peak 1 was loaded onto a CM-Sepharose column (2.5 × 4 cm) equilibrated with 20 mM sodium acetate buffer pH 5.6. The column was washed with the same buffer at a flow rate of 2 mL/min. Polygalacturonase activity was eluted with a stepwise salt gradient (0.2, 0.4 and 1 M) of NaCl in 20 mM sodium acetate buffer pH 5.6 and, fractions of 2 mL were collected. The second polygalacturonase activity (Peak 2) resulting from DEAE 6 Fast Flow chromatographies was saturated to 1.7 M final concentration of sodium thiosulphate and loaded onto the Phenyl-Sepharose 6 Fast Flow column (1.5 cm × 3.2 cm) previously equilibrated with 20 mM sodium acetate buffer pH 5.6 containing 1.7 M of sodium thiosulphate. The column was washed at a flow rate of 1 ml/min with a reverse stepwise salt-gradient of sodium thiosulphate (from 1.7 to 0 M) incorporated in the same sodium acetate buffer. 1 mL fractions were collected. Finally, the pooled active fractions were dialyzed overnight at 4°C against 20 mM sodium acetate buffer pH 5.6 and constituted the purified enzyme solution and stored at 4°C for assays.

2.2.2 Enzyme activity assay

The polygalacturonase activity was determining by using the 3.5-dinitrosalicylic acid (DNS) method [18] with D-(+)-galacturonic acid as the standards. All reactions contained 200 µL of 0.5% PGA, 200 µL of 100 mM sodium acetate buffer (pH 5.6) and 50 µL of enzyme solution. The reaction mixture was incubated at 37°C for 30 min. After incubation, 300 µL DNS was added to terminate the reaction, followed by 5-min incubation in boiling water bath. The absorption at 540 nm was measured at room temperature. All experiments were performed in triplicate. One unit (U) of polygalacturonase was defined as the amount of enzyme that released reducing sugars equivalent to 1 µmol of D-(+)-galacturonic acid per min under the standard conditions. The specific activity was expressed as unit of activity per mg of protein.

2.2.3 Proteins estimation

Protein concentrations and elution profiles from chromatographic columns were determined by the Folin method [19]. Bovine serum albumin (BSA) was used as the standard protein.

2.2.4 Polyacrylamide gel electrophoresis (page)

Electrophoresis was carried out by using Laemmli [20] method on 10% (w/v) acrylamide gels under denaturing and non-denaturing conditions. In denaturing conditions, samples were incubated for 5 min at 100°C with SDS-PAGE sample-buffer containing 2-mercaptoethanol. Concerning non-denaturing conditions, samples were mixed just before running in a sample-buffer without 2-mercaptoethanol and SDS. Silver staining was used to localize protein bands [21]. The standard molecular weights (Bio-Rad) comprising myosin (200 kDa), β-galactosidase (116.25 kDa), phosphorylase B (97.4 kDa), BSA (66.2 kDa) and ovalbumin (45.0 kDa) were used.

2.2.5 Native molecular weights determination

Purified enzymes were applied to gel filtration on a Sephacryl S-200 HR column (0.8 cm × 35 cm)

equilibrated with 20 mM sodium acetate buffer (pH 5.6) to estimate the native molecular weights. Elution was done at a flow rate of 0.2 mL/min and fractions of 0.5 mL were collected. Standard molecular weights (SIGMA) used for calibration were β-amylase from sweet potato (206 kDa), BSA (66 kDa), ovalbumin from egg white (45 kDa) and cellulase from Aspergillus niger (26 kDa).

2.3 ph and Temperature Optima

The effect of pH on the activity of the enzymes was determined by performing the hydrolysis of PGA in a series of buffers (100 mM) at various pH values (3.0–6.2). Buffers used were sodium acetate (pH 3.6–5.6) and sodium citrate (pH 3.0–6.2). pH values of each buffer were determined at 37°C. The effect of temperature on polygalacturonase activities was performed in 100 mM sodium acetate buffer (pH 5.6) over a temperature range of 30–70°C by using PGA (0.5%) as substrate under the enzyme assay conditions.

2.4 ph and Temperature Stabilities

The pH stability of each polygalacturonase was studied at a pH range of 3.0–5.6 with 100 mM acetate buffers. Buffer used were the same as in pH and temperature optima study. After 2 h preincubation at 37°C (room temperature), residual polygalacturonase activities were measured at 37°C for 30 min by adding substrate PGA. The thermal inactivation was determined at 37°C and at each enzyme optimum temperature (at pH 5.6). Enzymes in appropriate buffers (pHs) were exposed to each temperature for up to 120 min. Then, aliquots were withdrawn at intervals (15 min) and immediately cooled. Concerning thermal denaturation tests, aliquots of each enzyme solution were preheated at different temperatures ranging from 30 to 80°C for 30 min. Residual activity, determined at 37°C under the enzyme assay conditions, were expressed as percentage activity of zero-time control of untreated enzymes.

2.5 Effect of Some Chemical Agents

To determine the effect of various compounds (cations, detergents, and reducing agents) as possible activators or inhibitors of the purified polygalacturonase, each enzyme solution was preincubated at 37°C for 30 min with the compounds and the activity was assayed under the enzyme assay conditions. Residual activities were expressed as percentage referred to control without chemical agents.

2.5.1 Substrate specificity and kinetic parameters

The substrate specificities of PG1 and PG2 were determined by measuring the enzyme activities in acetate buffer (pH 5.6) containing polygalacturonic acid (PGA), apple pectin, lemon pectin and orange pectin (0.5%) under standard conditions.

Kinetic parameters of PG1 and PG2 were determined under standard conditions with 0.1-0.6 mg/ml PGA, apple pectin, lemon pectin and orange pectin as the substrates. The experiments were repeated three times, and each experiment included triplicate. The constants were calculated by fitting the date to a linear regression on Lineweaver-Burk plots.

3. RESULTS AND DISCUSSION

3.1 Protein Purification

The purification of the two polygalacturonases from the digestive juice of the snail *limicolaria flammea* required the involvement of four chromatographic steps including molecular exclusion, ion exchange chromatography and hydrophobic interaction chromatography. The molecular exclusion chromatography used as the first step in our purification procedure allowed us to obtain a single peak of polygalacturonase activity (Fig. 1). The active fractions after this step were loaded on anion exchange chromatography (DEAE-Sepharose 6 Fast Flow). This was our second chromatographic step and allowed us to detect two peaks of polygalacturonase activity named PG1 and PG2. The active proteins were eluted respectively at 0 and 0.2 M NaCl. (Fig. 2) These results are in agreement with the reports of Mohamed et al. [22] and Yuping et al. [23] who observed two polygalacturonases PGI and PGII separated respectively from *Trichoderma harzianum* and *Penicillium janthinellum sw09* by two chromatographic steps using DEAE-Sepharose and Sephacryl S-200.

After these first two chromatographic steps, the two polygalacturonases activities (PG1 and PG2) were purified using respectively cation exchange chromatography (CM-Sepharose) and hydrophobic interaction chromatography (phenyl-Sepharose 6 Fast-flow), (Fig. 3 and 4). The active proteins (PG1) were eluted at gradient change, between 0 and 0.2 M NaCl and PG2

were eluted with 0.8 M sodium thiosulphate (Fig. 3). The second and last chromatographies were crucial to purify the polygalacturonase activity of the digestive juice of the snail *Limicolaria flammea*. Indeed, anion exchange chromatography on DEAE-Sepharose 6 Fast Flow gel allowed the enzyme to be separated into two different isoforms (PG1 and PG2), while cation exchange chromatography (CM-Sepharose) and hydrophobic interaction on Phenyl-Sepharose 6 Fast Flow led to the separation of each polygalacturonase from other proteins and impurities. Finally, PG1 and PG2 were purified with overall yields of 7.05 and 10.58% and purity factors of about 6.44 and 6.49 times, respectively (Table 1). This low yield could be due to several fractionation steps used for purification of the two polygalacturonases PG1 and PG2. Generally, the use of several successive purification techniques results in a gradual decrease in yield [24]. Each isoenzyme showed a unique protein band by silver nitrate staining on native polyacrylamide gel electrophoresis (Fig. 5).

3.2 Molecular Mass of the Purifed Polygalacturonases

After SDS-PAGE analysis under reducing conditions, each polygalacturonase showed one bands.Their relative molecular weights were estimated to be 46.1 KDa for PG1 and 82.5 KDa for PG2 (Fig. 5). By gel filtration, the native enzymes had a molecular weight of 48.2 KDa for PG1 and 86.7 KDa for PG2. The results indicated that the native functional enzyme as a monomeric. Compared to other molecular weights of purified polygalacturonases, those from digestive juice of snail *Limicolaria flammea* are higher with regard to endopolygalacturonases PGI and PGII from *Aspergillus japonicas* (38 KDa and 65 KDa respectively) [25**]** and polygalacturonases PG1 and PG2 isolated from *Sclerotium rolfsii* (39.5 KDa; 38 KDa) [26]. Nagai et al. [27] reported 41 and 38 kDa molecular weight for Polygalacturonases isolated from *Aspergillus awomori* and *Sitophilus oryzae*,respectively**.**

The samples were loaded onto a 10% gel; Lane 1 and 2, crude enzyme extract; Lane 5, PG1; Lane 8, PG2; Lane 9, PG1; Lane 10, Markers; Lane 11, PG2. Numbers on the right indicate the molecular weights (kDa) of protein markers.

3.3 ph and Temperature

The purified polygalacturonases PG1 and PG2 showed optimum hydrolysis activities at 50°C in sodium acetate buffer pH 5.6 (Figs. 6, 8). They showed best stability over pH values ranging from 4.5 to 5.6 conserving at least about 80% of their total activities (Fig. 7). This zone of stability would be a good compromise for performing many fruit juice clarifications since these juices generally have an acidic pH [28]. The results of the thermal denaturation study showed that PG1 and PG2 were fairly stable at temperatures up to 50°C. Beyond this temperature, the activity decreases progressively. They lose about 80% of their activity at a temperature of 70°C (Fig. 9). The thermal inactivation study showed that at 37 and 50°C, in sodium acetate buffer (pH 5.6), the purified enzymes remained statistically stable for 120 min (Fig. 10). Indeed, the catalytic activity of the two purified polygalacturonases were weakly affected after a long-term (120 min) incubation at 50°C and they maintain more than 80% of their activity. In this context, running biotechnological processes for relatively long-term at their optimal temperature would be advantageous for application of these enzymes.

3.4 Effect of Metal Ions, Chelating, Sulfhydryl-Specific and Reducing Agents and Detergents

The results of the study of the influence of ions, reducing agent, detergents and EDTA on the activity of purified polygalacturonases PG1 and PG2 are summarized in Table 2 and 3.

The common inhibitor of the two purified polygalacturonases activity were Mn^{2+}, Ca^{2+}, Zn^{2+} and EDTA. The fact that EDTA (cations chelator) displayed an inhibitory effect on enzyme activities is tempting to speculate that PG1 and PG2 require metal ions which are essential at their active site to perform catalysis. Therefore, this inhibition could suggest that the purified polygalacturonase may be metalloenzymes. Famurewa et al. [29] and Sakamoto et al. [30] reported a inhibitory effect of ETDA on the polygalacturonase from *Aspergillus flavus*. It decreases the activity of the partially purified polygalacturonase (12-30%). Mg^{2+} and NH^{3+} have an inhibitory effect on the activity of the polygalacturonase PG2, while they stimulate the polygalacturonase PG1 as well as K^{+}. As concerned Ba^{2+}, it was an activator for polygalacturonase PG2.

SDS and L-cysteine inhibited the activity of the purified polygalacturonases PG1 and PG2. These results are similar to those obtained by Cheng et al. [31] for Endo-Polygalacturonase

CZ1028 from *Penicillium oxalicum*. The sensitivity of these enzymes in the presence of sulfhydryl-specific agent such as L-cysteine led us to assume that –SH groups participate probably in catalysis activities of the two enzymes.

The stimulatory effects shown by these detergents Tween 80 and Triton X-100 make them useful when purifying polygalacturonases from digestive juice of snail *Limicolaria flammea* since it is well known that detergents are used for protein solubilization before their purification. Li et al. [32] reported an activatory effect of Tween-80 on the activity of polygalacturonase from *Aspergillus repens*.

3.5 Substrate Specificity and Kinetics Parameters

A variety of pectin from different sources (PGA, orange pectin, apple pectin and lemon pectin) were tested for their suitability to serve as substrates. PG1 and PG2 hydrolyzed a broad range of these substrates (Fig. 11). The highest activity of PG1 was observed with apple pectin followed by lemon pectin and orange pectin. As regards PG2, its highest activity was obtained with orange pectin followed by apple pectin.

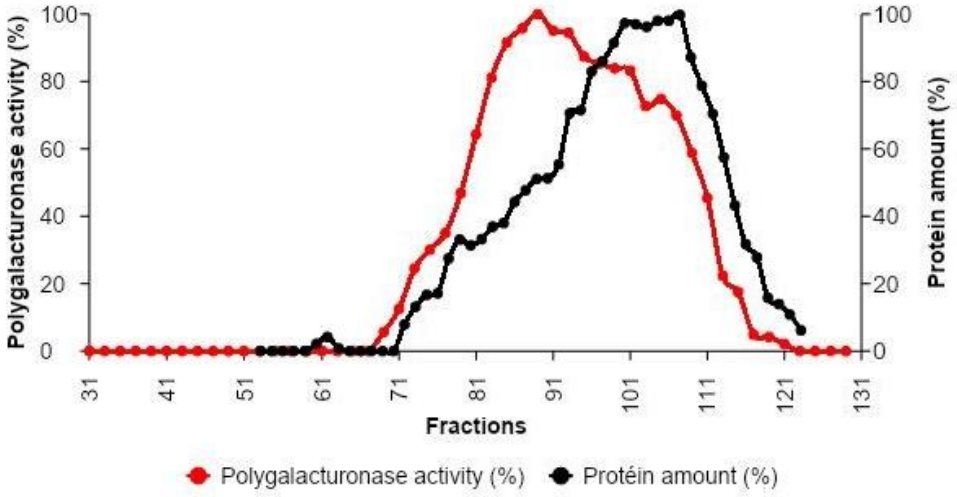

Fig. 1. Molecular exclusion chromatography profile on Sephacryl S-200 HR gel of polygalacturonase activity from the digestive juice of snail *Limicolaria flammea*

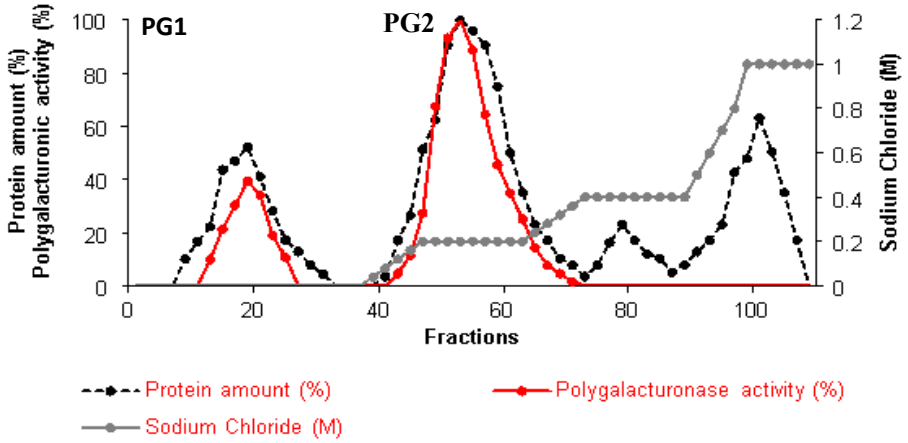

Fig. 2. Anion exchange chromatography on DEAE-6-Fast-flow gel of polygalacturonase activity from the digestive juice of snail *Limicolaria flammea*

Kinetic parameters of the two polygalacturonases were studied by using polygalacturonique acid (PGA), apple pectin, orange pectin and lemon pectin as substrates. With these substrates, enzyme activities were as predicted by the Michaelis-Menten equation. The KM and Vmax values, obtained from a Lineweaver-Burk plot in the range of concentrations from 0 to 5 mM, are reported in Table 4. Concerning polygalacturonase PG1, the catalytic efficiency given by the Vmax/KM ratio was highest for lemon pectin (0.125 µmol/min/mL) and orange pectin (0.124 µmol/min/mL).

Regarding PG2, it displayed highest catalytic efficiency (0.325 µmol/min/mL) towards orange pectin.

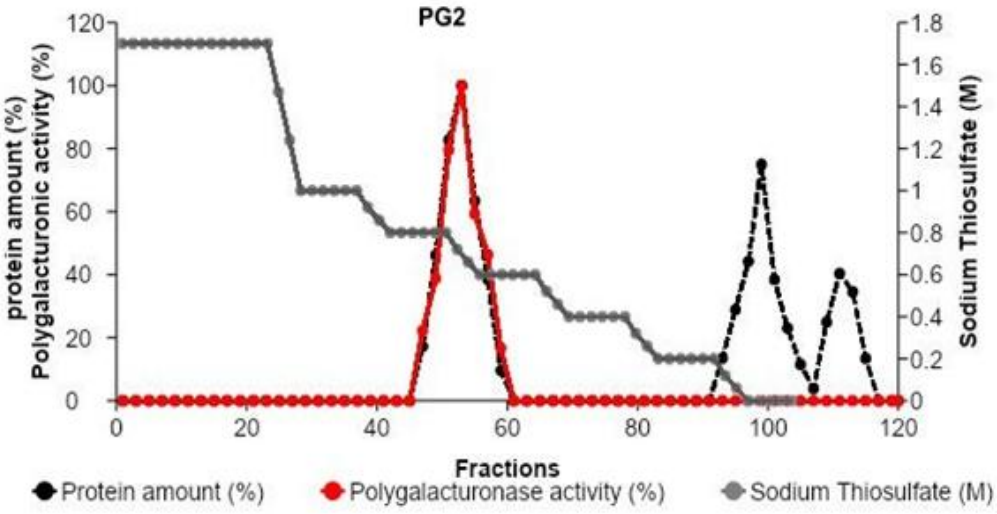

Fig. 3. Cations exchange chromatography on CM-Sepharose gel of polygalacturonase PG1 from the digestive juice of snail *Limicolaria flammea*

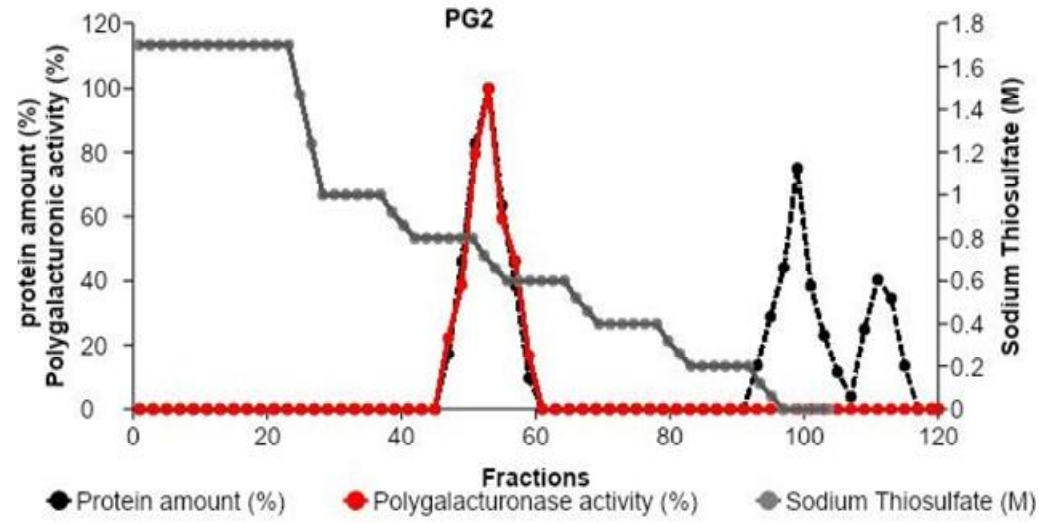

Fig. 4. Hydrophobic interaction chromatography on phenyl-Sepharose 6 Fast-flow gel of polygalacturonase PG2 from the digestive juice of snail *Limicolaria flammea*

Table 1. Purification procedure of polygalacturonases PG1 and PG2 from the digestive juice of snail *Limicolaria flammea*

Steps of purification	Total protein (mg)	Total activity (UI)	Specific activity (UI/mg)	Factor of purification	Yield (%)
Gross extract	32.54	0.849	0.026	1	100
Séphacryl S-200 Polygalacturonase	9.87	0.75	0.076	2.14	88.23
DEAE 6 Fast-flow Polygalacturonase PG1	3.80	0.43	0.115	4.44	50.58
Polygalacturonase PG2 CM-Sepharose	3.54	0.515	0.145	5.59	60
Polygalacturonase PG1 Phenyl-Sepharose 6 Fast flow	0.34	0.056	0.167	6.44	7.05
Polygalacturonase PG2	0.53	0.089	0.169	6.49	10.58

Table 2. Effect of some cations and chelating agent on the activity of polygalacturonases PG1 and PG2 purified from the digestive juice of snail *Limicolaria flammea*

Real estate	Concentration (mM)	Relative activity (%)	
		PG1	PG2
Control	0	100±0.01	100±0.00
Mn^{2+}	1	71.66±0.00	58.33±0.01
	5	48.33±0.02	55.55±0.02
K^{+}	1	160±0.01	102.77±0.00
	5	120±0.01	81.94±0.00
$NH3^{+}$	1	100±0.00	111.11±0.01
	5	170±0.00	86.11±0.00
Ba^{2+}	1	101.66±0.02	115.27±0.01
	5	101.66±0.01	133.33±0.01
Mg^{2+}	1	143.33±0.01	94.44±0.36
	5	195±0.01	88.88±0.01
Ca^{2+}	1	68.33±0.01	95.83±0.01
	5	86.66±0.02	59.72±0.00
Zn^{+}	1	48.33±0.01	41.66±0.00
	5	26.66±0.00	51.38±0.00
EDTA	1	88.33±0.00	80.55±0.00
	5	70±0.01	20.83±0.01

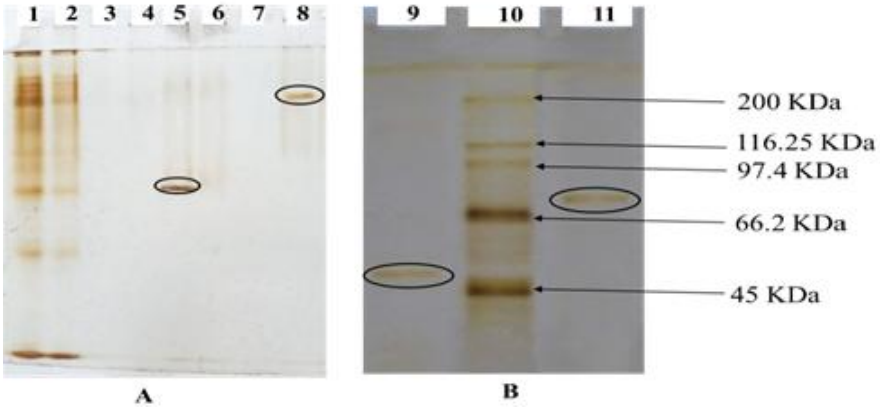

Fig. 5 . PAGE analys of purified polygalacturonases PG1 and PG2 from digestive juice of snail *Limicolaria flammea*: (A) purified enzymes PAGE. (B) purified enzymes SDS-PAGE

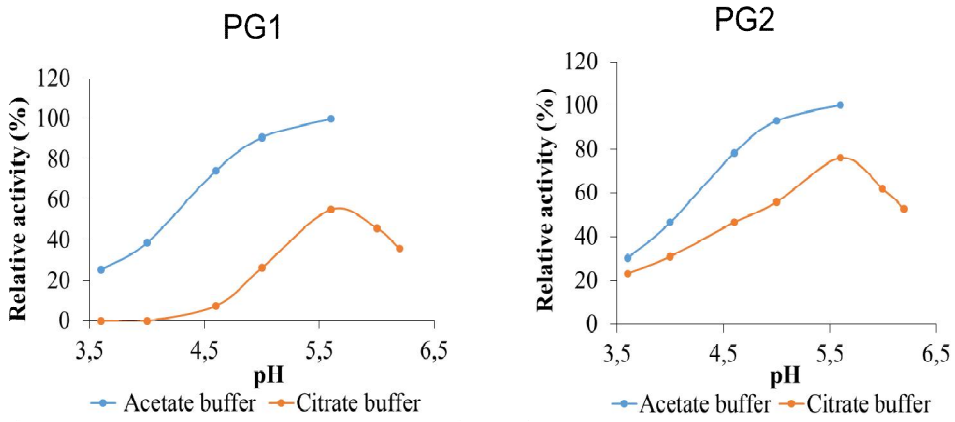

Fig. 6. Activity of purified polygalacturonases from the digestive juice of snail *Limicolaria flammea* at varying pH

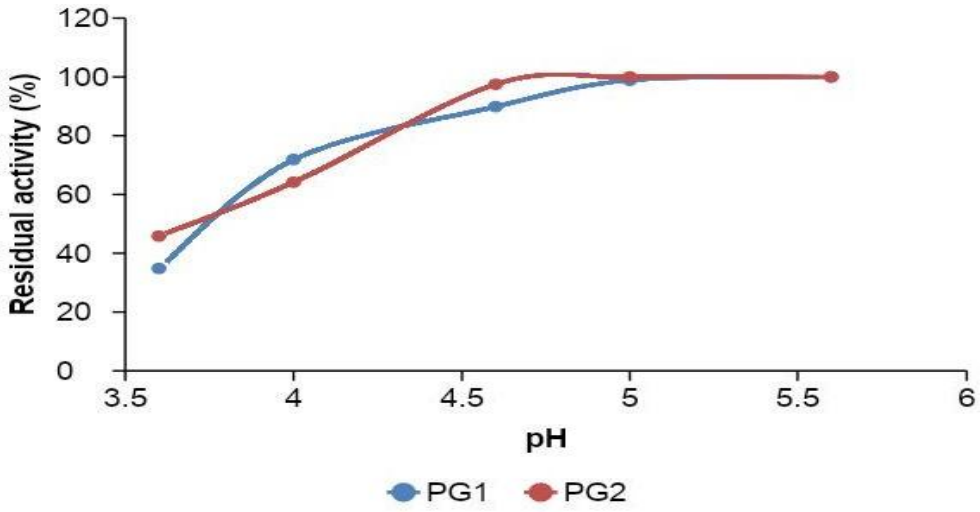

Fig. 7. pH stability of PG1 and PG2 polygalacturonases of digestive juice snail *Limicolaria flammea*

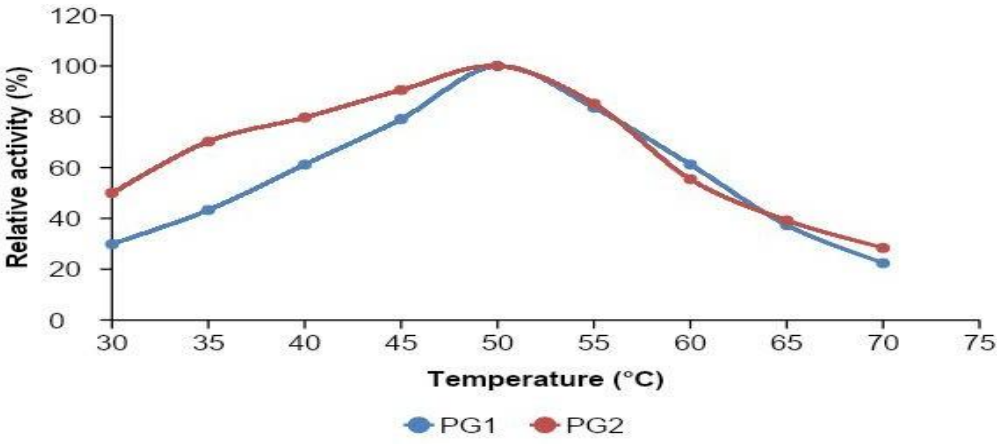

Fig. 8. Influence of temperature on the activity of polygalacturonases PG1 and PG2 purified from digestive juice of snail *Limicolaria flammea*

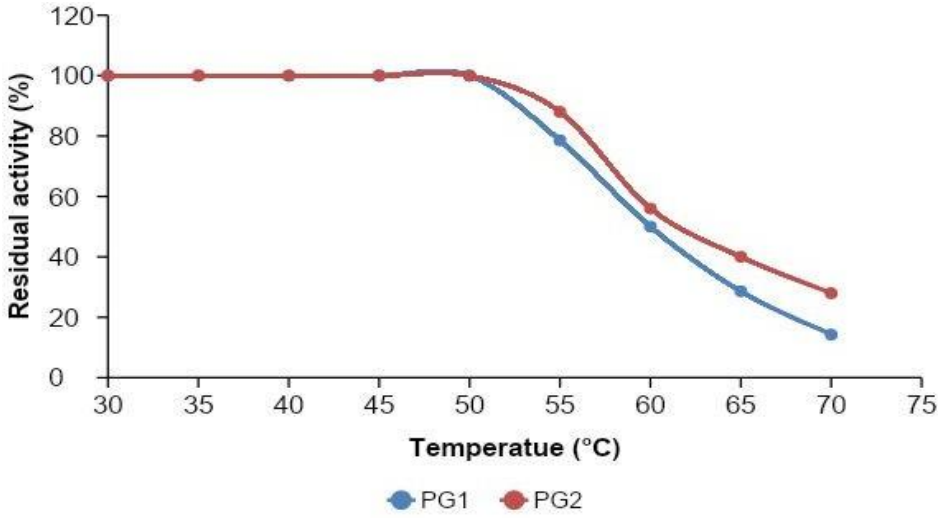

Fig. 9. Thermal denaturation of polygalacturonases PG1 and PG2 purified from digestive juice of snail *Limicolaria flammea*

Table 3. Effect of sulfhydryl specific, reducing agents and detergents on the activity of polygalacturonases PG1 and PG2 purified from the digestive juice of snail *Limicolaria flammea*

	Concentration	Relative activity (%)	
Real estate	**(%)**	**PG1**	**PG2**
Control	0	100±0	100±0
L-cystéine	0.1	90.56±0	63.49±0.01
	1	47.16±0.01	96.82±0
Urée	0.1	156.6±0.01	95.23±0
	1	101.88±0.04	84.12±0
SDS	0.1	37.73±0	13.2±0
	1	33.96±0	0±0
Triton X-100	0.1	109.43±0.01	52.38±0
	1	113.2±0.03	47.61±0
Tween 80	0.1	345.28±0.01	46.03±0
	1	169.81±0.01	42.85±0

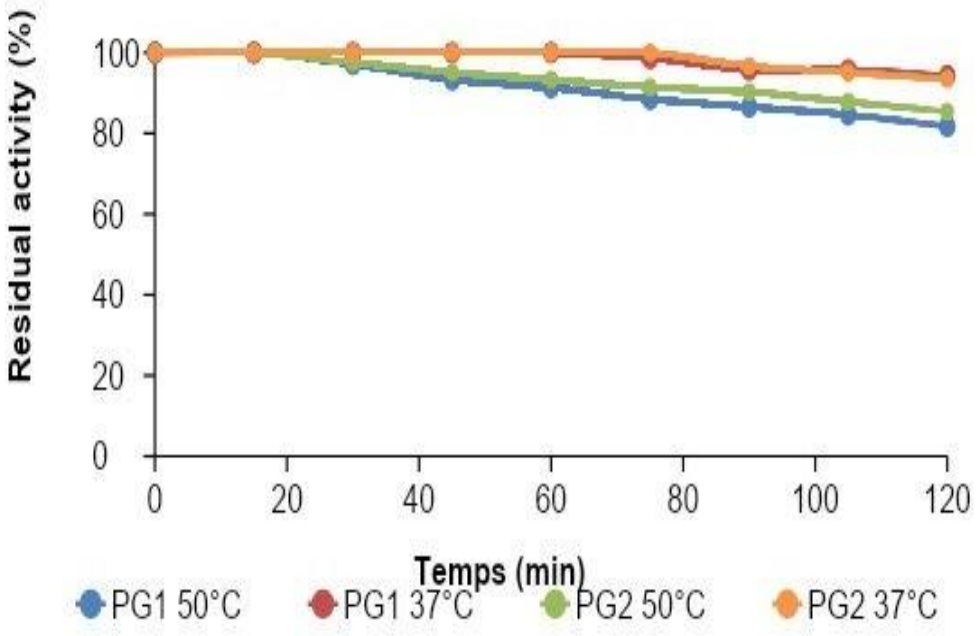

Fig. 10. Thermal inactivation of purified polygalacturonases from the digestive juice of snail *Limicolaria flammea*

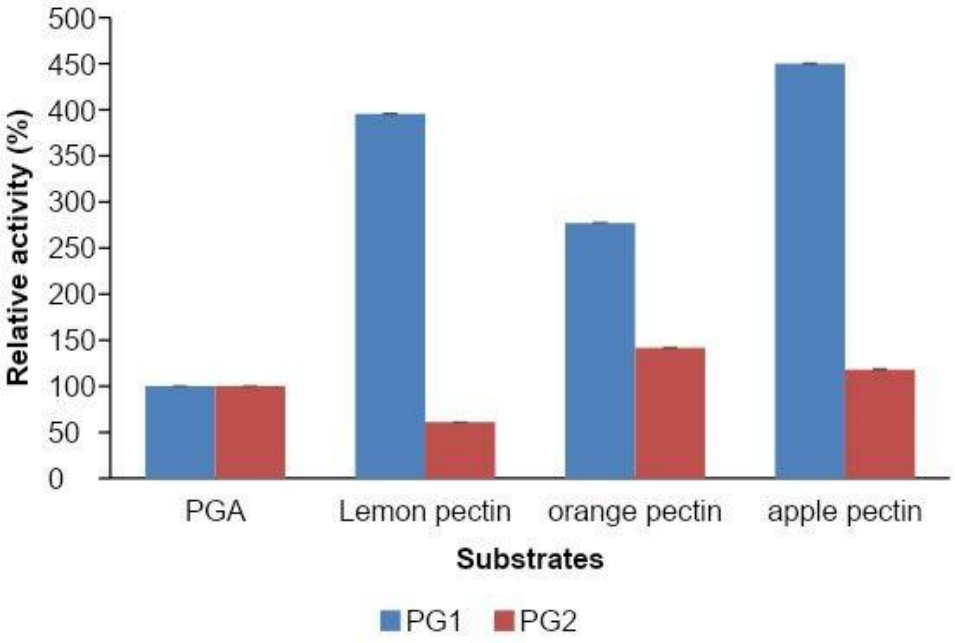

Fig. 11. Substrate hydrolysis specificity of polygalacturonases PG1 and PG2 purified from digestive juice of snail *Limicolaria flammea*

PGA : Polygalacturonic acid

Table 4. The kinetic parameters of PG1 and PG2 polygalacturonases of digestive juice snail *Limicolaria flammea*

	PG1			PG2		
	Km	Vmax	Vmax/Km	Km	Vmax	Vmax/Km
Substrats	(mg/mL)	(µmol/min/ mg)	(µmol/min/ mL)	(mg/mL)	(µmol/mi n/mg)	(µmol/min/ mL)
Apple Pectin	6.101	0.644	0.105	7.69	0.775	0.100
Lemon Pectin	2.45	0.308	0.125	4.35	0.230	0.052
Orange pectin	2.98	0.371	0.124	1.88	0.612	0,325
PGA	2.38	0.162	0.068	3.03	0.464	0,153

4. CONCLUSION

Two polygalacturonases named PG1 and PG2 were isolated from the digestive juice of snail *Limicolaria flammea* using four chromatographic techniques with a yield of 7.05 and 10.58%, a specific activity of 0.167 and 0.169 IU/mg protein and a purity factor of 6.44 and 6.49 respectively for PG1 and PG2. The two purified polygalacturonases PG1 and PG2 have a native molecular weight of 48.2 and 86.7 KDa and are acidic in nature with an optimum pH of 5.6. They have been shown to be mesophilic with an optimum temperature of 50°C. The substrate specificity and kinetic properties showed that orange and lemon pectin were the potential physiological substrates.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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