PURIFICATION AND PHYSICOCHEMICAL CHARACTERIZATION OF THE α-GLUCOSIDASE OF THE DIGESTIVE JUICE OF THE SNAIL LIMICOLRIA FLAMMEA (Müller 1774).

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ABSTRACT: In this study we are interested by the α-glucosidase, a protein biocatalyst from the digestive juice of a snail *Limicolaria flammea* which we purified by the chromatographic methods. Then the physico-chemical characteristics of this enzyme were determined. With a specific activity of the crude extract of 4.31 U/mg, purification have been done on Sephacryl S-200 HR gel then, specific activity has passed to 34.17 U/mg with purification factor of 7,93.With Anx-sepharose 4 fast flow gel, specific activity increased to 92.58U/mg with purification factor of 21.48 and 147 U/mg on phenyl-Sepharose CL 6B with purification factor of 34.11. The apparent molecular weights of the α-glucosidase purified on gel filtration (64,000 Da) and by electrophoresis on polyacrylamide gel (68,200 Da) were nearly identical. With a stability zone of pH between 4 and 7.5, the α-glucosidase had its maximum activity at pH 6.5. Optimum temperature of hydrolysis was obtained at 45°C and was stable at 37 to 40°C. The study of substrate specificity showed that para-nitrophenyl-α-D glucopyranoside and sucrose are hydrolyzed by the enzyme. It was inhibited by Cu^{2+}, Ni^{2+}, Hg^{2+} and activated by Mn^{2+}. Ideal conditions for activity of this enzyme were known therefore it could be used to achieve synthesis.

Key-words: α-glucosidase, physico chemical characterization, digestive juice, *Limicolaria flammea,* purification.

INTRODUCTION

Enzymes are protein biocatalysts with functional groups in which the substrates are transformed to give products.

Knowledge of the latter, reaction conditions, structural stability make that they are used in biotechnological processes [27]. Applications include pharmaceutical, chemical, food. Microorganisms have been studied most often to find enzymes that could meet the new demands of their applications [11, 26, 32]. To lower energy costs of converting starch compounds, it appears important to use enzymes capable to acte effectively in mild reaction conditions that do not allow pre- gelatinization of starch [7]. It is therefore rightly the priority is given to the search of new sources of enzymes capable of converting raw starch or achieve amylolytic at low temperatures [34, 37]. Enzymes that have always been used are those of recombinant microorganisms or not [5. 9, 12,15]. Our objective in this study is to investigate these types of enzymes in the digestive juice of the snail *Limicolaria flammea*. To do this, we will purify an enzyme of the digestive juice of the snail: the α-glucosidase through chromatographic methods. Then we do the physicochemical characterization on gel filtration of the enzyme to determine the conditions for maximum activity of the latter.

MATERIALS AND METHODS
Purification of the alpha glucosidase
Emzymatic source
The enzyme source is the digestive juice of the snail *Limicolaria flammea*. Snails are made fasting for three days to complete their digestion and they are freed from their shells.

The digestive tract is isolated from the visceral mass by holding it between the thumb and forefinger. By successive presses of the fingers on the digestive tract, the juice flowing is collected in a jar placed in an ice bath.

The digestive juice is centrifuged at 10,000 revolutions / min for 30 minutes in a centrifuge refrigerated between 0 and 5°C to remove debris. The resulting supernatant is the crude extract enzyme.

The enzymes were purified by a combination of various chromatographies low pressures that are gel filtration chromatography, anions exchange and cations, and hydrophobic interaction.

Dosage of para-nitrophenylglucosidasic activities
The reaction mixture is composed of 50 µL of enzyme solution, 2 mM para- nitrophenyl-D-glycopyranoside in a final volume of 150 µL of 50 mM of acetate buffer pH 5. The reaction mixture was incubated at 37°C during10 minutes and then the reaction was stopped by adition of 2 mL of 1M of sodium carbonate. The intensity of staining of para- nitrophenol released was measured in a spectrophotometer (Spectra-physic UV visible) at 420 nm against a control containing all reagents except enzyme solution. The quantity of para-nitrophenol released is determined by using a calibration curve made under standard enzyme assays.

Dosage of proteins
The protein content of the crude extract and different fractions collected during successive chromatographies were assayed according to the method of Lowry [22], using the Folin-Ciocalteus.

Stages of purification
Molecular exclusion chromatography on Sephacryl S-200 HR gel.
One (01) milliliter of digestive juice of the snail *Limicolaria flammea* is deposited on the gel column of Sephacryl S-200 HR (1.6 x 63 cm) previously equilibrated with 20 mM acetate buffer pH 5.

The molecular exclusion chromatography separates molecules according to their hydrodynamic volume and molecular weight. The Sephacryl S-200 -HR gel separates globular macromolecules having molecular weights of between 5 and 250 KDa. The proteins were eluted with the same acetate buffer at a flow rate of 15 mL / h. Fractions of 1 mL were collected by a collector (Gilson TDC 80) which is connected to a Gilson peristaltic pump connected to the column. Active fractions of each enzyme are collected to be divided again by ion exchange chromatography.

Anion exchange chromatography on Anx-Sepharose 4 Fast Flow gel
Active protein fractions from gel filtration chromatography were pooled and deposited on the Anx-sepharose gel (3.14 x 7 cm) previously equilibrated with 20 mM acetate buffer pH 5. The proteins retained on the column were eluted by applying a stepwise gradient of NaCl in 20 mM acetate buffer pH 5. The rate of elution was 2 mL/ minute

Hydrophobic interaction chromatography on Phenyl-Sepharose CL-6B gel
Enzyme fractions from chromatographies above were dialyzed, concentrated and saturated with ammonium sulphate 1.7 M. They are then deposited on the phenyl-Sepharose CL-6B gel (1.5 x 4.5 cm) previously equilibrated with 20 mM acetate buffer pH 5 containing ammonium sulphate 1.7 M.

The proteins retained on the column were eluted by applying a stepwise gradient of decreasing ammonium sulphate (1.7, 1.5, 1, 0.75, 0.5, 0.25, 0 M) in 20 mM acetate buffer pH

5. The rate of elution was 0.5 mL / minute.

Polyacrylamide gel electrophoresis in native conditions
The purity of α-glucosidase purified was determined by electrophoresis under native conditions   on olyacrylamidegel without of SDS (sodium dodecyl sulfate). Polyacylamide gel resulting from the polymerization of acrylamide in presence of a small amount of crosslinking agent: N, N'-methylene bisacrylamide. This polymerization is initiated by free radicals resulting from the chemical composition of ammonium persulfate ($(NH_4)_2 S_2O_8$) and accelerated by the addition of TEMED (N, N, N ', N'-tetramethylethylenediamine). Crosslinking depends on the amounts of acrylamide and bisacrylamide and their quantity. In this type of gel, proteins migrate according to their charge and size. The revelation was made with silver nitrate according to the method of Blum [2]. In this method, the carrier composed of the gel and protein undergoes incubation in a solution of silver nitrate in which Ag+ ions are deposited on the gel and the proteins.

After a brief wash in order to remove excess reagent, the disclosure is made by means of a solution of sodium carbonate and formaldehyde. Ag+ ions complexed with proteins then are reduced to metallic silver, which precipitates to form black-brown stripes.

Physico-chemical characterization of the purified α-glucosidase

Influence of pH on enzyme activity

pH changes have an effect on either the enzyme or the substrate.

On the enzyme, there may be changes of the degree of ionization of certain functional groups whose charge is necessary, or to the formation of the enzyme-substrate, either maintaining the native three-dimensional conformation of the enzyme protein.

As the substrate, there may be a change in the degree of ionization may prevent the formation of enzyme-substrate complex. There is therefore for each enzyme, a pH range of stability of the native conformation and an optimum pH for the enzymatic reaction of a substrate in a medium of given composition.

pH optimum of hydrolysis

Several types of buffers at various pH are used to determine the influence of pH on α- glucosidase activities. These are, 100 mM acetate buffer pH 3.6 to 5.5, 100 mM citrate buffer pH 3 to 7, and 100 mM phosphate buffer pH 5.7 to 8. Determining the activities was performed as described above.

pH of stability

pH of stability was studied by pre-incubating the enzyme at the temperature of 37 ° C for two hours in 20 mM acetate buffer with pH ranging from 4 to 8. Activities are determined as before.

Effect of temperature

The catalytic activity of enzymes depends upon the integrity of the structure as a protein. Exposure of these proteins on inappropriate temperatures leads to their denaturation and the loss of their catalytic activity resulting from the rupture of non-covalent bonds that causes the breakdown of secondary structure, tertiary and quaternary enzyme. It is therefore necessary to know the optimum temperature at which each enzyme has a maximum activity.

Optimal temperature of hydrolysis

The α-glucosidase activities are determined at temperatures ranging from 30 to 80 ° C in 100 mM acetate buffer pH 5 and 100 mM phosphate buffer pH 6. The residual enzyme activities were determined as above.

Thermal denaturation

Aliquots of 100 µl enzyme preparations were preincubated for 80 minutes at temperatures ranging from 40 to 70 ° C and then cooled in an ice bath. The residual activities were determined as above.

Thermal inactivation

The enzyme α-Glucosidase purified was preincubated at temperatures of 45°C, 50°C and 55°C in sodium acetate buffer 100 mM pH 5.0 for various times. At regular time intervals (15 min), an aliquot (100 µl) of the reaction medium is removed and cooled in an ice bath and the residual activities were determined at 37° C.

Thermodynamic analysis

The enzyme activity over time at a given temperature is determined by the Arrhenius equation Ln [At / A○] = kt
Where At is the enzyme activity at time t, A0 is the initial activity of the enzyme and the constant k of the reaction rate (min^{-1}). The values of k are obtained with the regression line of Ln [At / A0] versus time, where k is the slope.

Thermodynamic parameters

The values of the activation energy (Ea) and Arrhenius constant (A) allow us to determine thermodynamic parameters [24]. These are the enthalpy changes ΔH #, entropy

ΔS # and free energy ΔG #. ΔH # = Ea –
RT

ΔS # = R (lnA - ln (K_B / H_P) - ln T) ΔG = ΔH # #
- T ΔS #

K_B, H_P and T are the Boltzmann constant (1.38 x 10^{-23} J / K), Planck's constant (6.626 x 10^{-34} Js) and the absolute temperature.

Substrate specificity

This study was conducted with various natural and synthetic substrates. The activity of the purified enzymes was determined for each substrate after incubation at 37°C in 15 minutes or 30 minutes as before.

Influence of effectors

Effector is a compound capable of reducing (inhibitor) or increase (activator) enzyme activity, α-glucosidase purified snail *Limicolaria flammea* has been subjected to the action of some effectors. The purified enzyme was pre-incubated with effectors in 30 minutes at 37°C. Then the residual activity was determined.

Kinetic parameters

Kinetic parameters (Vmax and Km) were determined using the representation of Lineweaver and Burk (1934) using different concentrations of substrate. Activities are determined as above.

Determination of the molecular weight of α-glucosidase

Two methods were used to determine the molecular weight of the purified enzyme of *Limicolaria flammea*. It is the electrophoretic method in denaturing conditions according to the method of Laemmli, U.K. [20] and the method of gel filtration on Sephacryl S-200 HR. *By SDS-PAGE* in denaturing conditions. Electrophoresis on polyacrylamide gel in the presence of SDS (SDS-PAGE) was performed according to the method described by Laemmli U. K. (1970). To determine the molecular weights of the purified enzymes a sample of enzyme is added to 125 mM Tris-HCl buffer pH 6.8 containing 4% (w/v) SDS, 1% (v/v) β-mercaptoethanol, 20% (w/v) glycerol and 0.025% (w/v) bromophenol blue. This mixture is heated to a boiling water bath for 5 minutes. In these conditions, proteins dissociate in their subunits by fixing large amounts of detergent (about 1.4 g/g of protein) which completely hides the natural charge of the protein subunit and gives a net charge negative. More molecules are large, the more charge is important in SDS, which means that the electrophoretic mobility of a molecule depends on its size (molecular weight). However, in a gel, the friction forces predominate and large molecules despite their heavy load with low mobility. The polyacrylamide gel electrophoresis in denaturing conditions (SDS-PAGE) of the purified enzymes of *Limicolaria flammea* is performed on acrylamide gel plates (7 x 8 cm) and 1.5 mm thick. The gel contains 12% acrylamide, Tris- HCl 375 mM (pH = 8.8) and 10% SDS (w / v). Once cured, it is transferred to a device Hoeffer Scientific Instrument (Mighty Small II Unit) and cooled by circulating water.

The electrophoresis is performed with a current of 15 mA in the running buffer Tris (25 mM) / glycine (192 mM) containing 0.1% (w / v) SDS. The revelation was also performed with silver nitrate according to the method of Blum et al. (1987). The molecular weight of the α-glucosidase of *Limicolaria flammea* is estimated by comparing its relative mobility to that of the reference proteins which molecular weights are known. The reference proteins used are phosphorylase B (97,000 Da), bovine serum albumin (66,200 Da), ovalbumin (45,000 Da), carbonic anhydrase (35,100 Da) and lysozyme (20,800 Da) (photography 2). The graphical representation of the logarithm of the molecular weight of the reference proteins according to their relative mobility is used to deduce the molecular weight of the purified enzyme.

By gel filtration on Sephacryl S-200 HR.

The determination of the molecular weight of the purified α-glucosidase of *Limicolaria flammea* was made by gel filtration on a column of Sephacryl S-200 HR (0.6 x48). It was equilibrated with 20 mM acetate buffer pH 5.5. Final and total volumes of the column were determined respectively by the blue dextran and para-nitrophenol.

Protein markers used were β-amylase (204,100Da), bovine serum albumin (67,000), amyloglucosidase (62,900 Da), ovalbumin (45,000 Da), lysozyme (20,800) and cytochrome C (12,400 Da).

Knowing the elution time and the molecular weights of the reference protein, the straight line of the molecular weights of these proteins as a function of their elution time. The molecular weights of α-glucosidases are deduced on the line drawn knowing their elution time.

RESULTS AND DISCUSSION

Molecular exclusion chromatography on Sephacryl gel S-200 HR.

The digestive juice of the snail *Limicolaria flammea* is deposited on the gel molecular sieve Sephacryl S-200 HR. A single protein peak of α-glucosidase activity was obtained (Figure 1). The digestive juice of the snail *Limicolaria flammea* constitutes the crude enzyme solution to be purified contains an α-glucosidase whith specific activity (SA) of 4.31 U / mg of protein. After chromatography of the digestive juice on Sephacryl S-200 HR gel, the fraction which contains the α-glucosidase has a specific activity (SA) of 34.17 U / mg protein and a purification yield of 91.1%. This enzyme was purified 7.93 fold (Table 1).

Table 1: Summary of the purification of α-glucosidase.

Steps of purification	Protein (mg)	Total Activity (U)	Specific Activity (U/mg)	Factor of purification	Yield (%)
Crude extract	218	937,63	4,31	1	100
Sephacryl S-200	25	854,25	34,17	7,93	91,1
HR Anx-Sepharose 4	0,7	64,8	92,58	21,48	7
fast flow Phenyl-Sepharose CL 6B	0,027	3,97	147	34,11	0,42

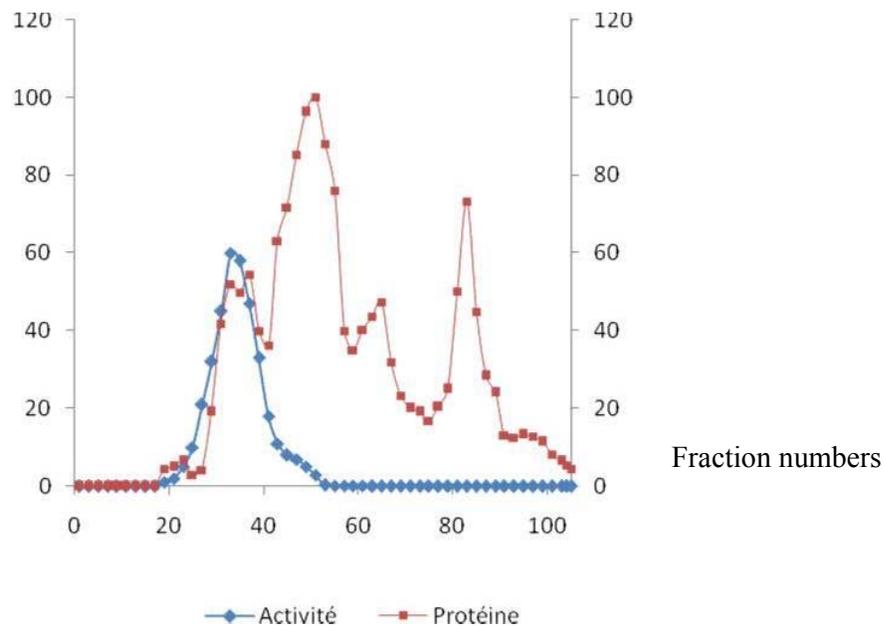Proteins at 280 nm   (%) Activity (%)

Figure 1: Molecular exclusion chromatography on Sephacryl S-200 HR gel of the digestive juice of the snail *Limicolaria flammea*.

Anion exchange chromatography of α-glucosidase on Anx-Sepharose 4 fast flow gel.

The α-glucosidase fraction chromatographed on freezing Anx-sepharose 4 fast flow gave a single peak of activity obtained with a NaCl 0.025 M (Figure 2).

At this stage of purification, α-glucosidase has a SA of 92.58 U / mg with a purification factor of 21.48 and a yield of 7% (Table 1).

Proteins (%) at 280 nm   Activity (%)
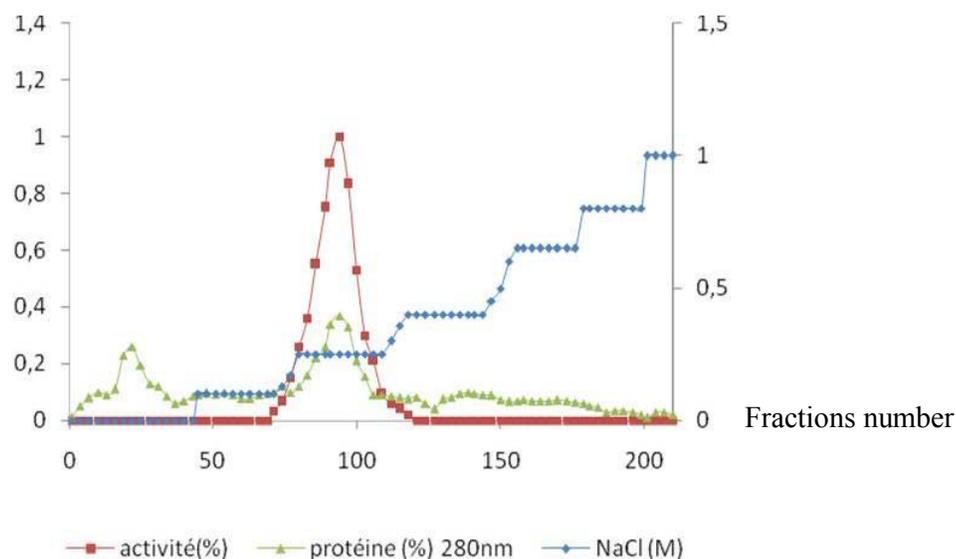
Figure 2: Anion exchange chromatography on Anx-Sepharose 4 fast flow gel of α-glucosidase.

Hydrophobic interaction chromatography on Phenyl-Sepharose CL-6B gel
The α-glucosidase eluted at a concentration of 0.25 M thiosulphate (Figure 3) has a specific activity of 147 U/mg, a purification factor of 34.11 and a yield of 0.42% (Table I).
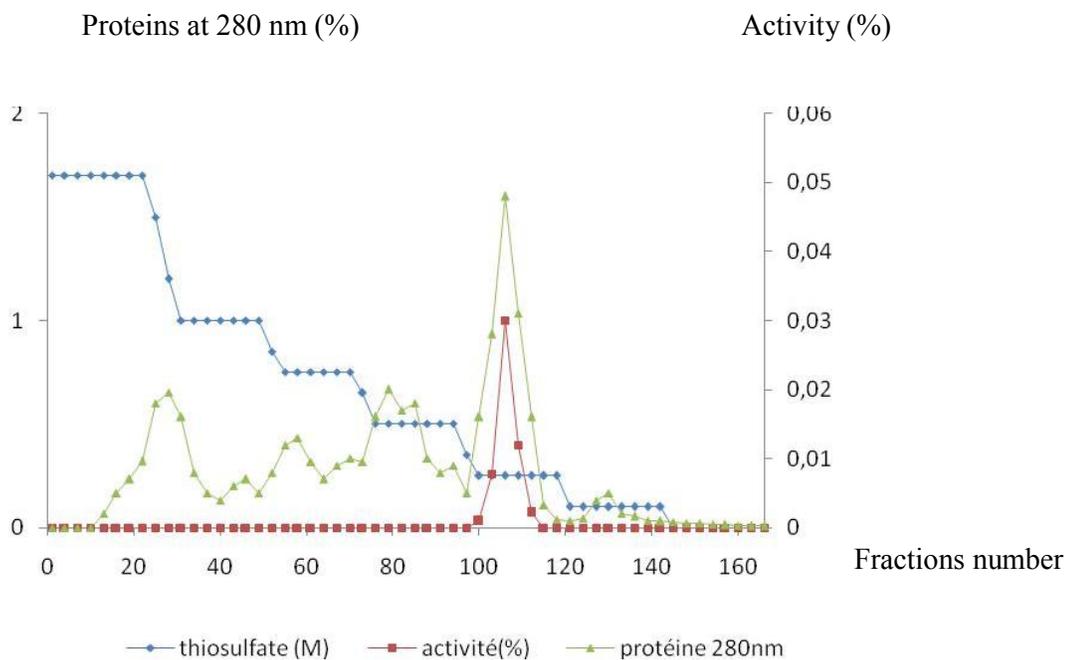

Figure 3: Hydrophobic interaction chromatography on Phenyl-Sepharose CL 6B gel of the α-glucosidase.

The SA of α-glucosidase of *Limicolaria flammea* is much lower than those of α- glucosidase A (63525 U/mg) and B (5120 U/mg) of the termite *Macrotermes subhyalinus* [17] of *Mortierella alliacea* (3200 U/mg) [30] and bee *Apis Mellifera* (11300 U/mg) [18] but higher than α-glucosidase (19.7 U/mg) of the snail *Archachatina ventricosa* [28].
The polyacrylamide gel electrophoresis under denaturing conditions of the purified α- glucosidase showed a single protein band (photography1).
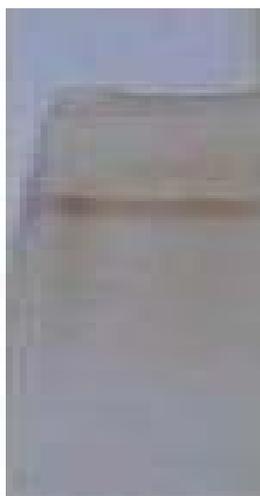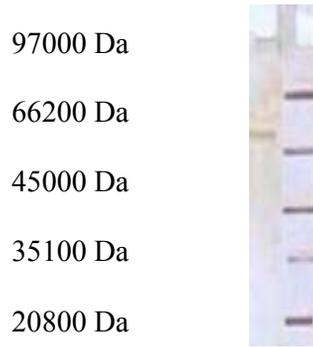

Photography 1: Electrophoresis on polyacrylamide gel of the α-glucosidase in native conditions
This is not the case of the purified α-glucosidase (dimeric) of the digestive juice of the snail *Archachatina ventricosa* [28] *Aplysia fasciata* (tetrameric) [1].

Determination of molecular weights

The apparent molecular weights of purified α-glucosidase estimated by gel filtration (64,000 Da) (Figure 4) and by polyacrylamide gel electrophoresis (68,200 Da) (Figure 5) under denaturing conditions (photography 2) are almost identics.

97000 Da

66200 Da

45000 Da

35100 Da

20800 Da

Photography 2: Electrophoresis on polyacrylamide gel of the α-glucosidase in denaturing conditions

Log (MW) Gluc

$y = -0,034x + 6,162$
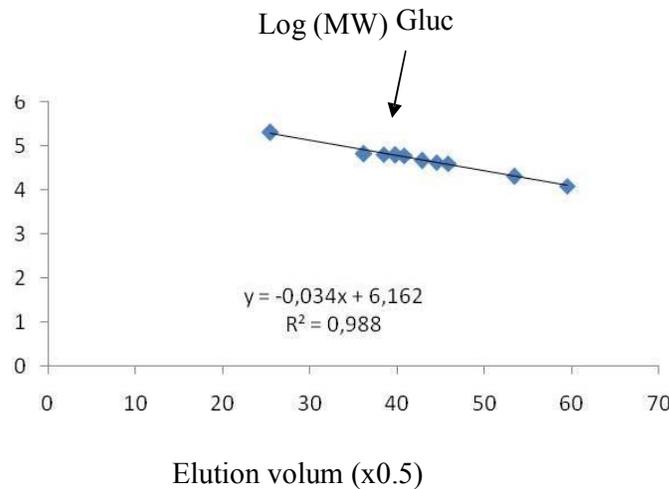$R^2 = 0,988$

Elution volum (x0.5)
Figure 4: Determination of Molecular Weight of purified α-glucosidase by gel filtration.

Markers: A: β-amylase (204,200 Da), B: bovine serum albumin (66,200 Da), C: ovalbumin (45,000   Da), D:lysozyme (20,800 Da), E:cytochrome C (12,400 Da). Gluc: α-glucosidase (64000Da)Marqueurs :A : β-amylase (204200 Da), B : sérum albumine bovine (66200 Da), C : ovalbumine (45000 Da), D : lysozyme (20800 Da), E : cytochrome C (12400 Da).Gluc : α-glucosidase (64000Da)

Log (MW) Gluc

$y = -1,810x + 5,332$
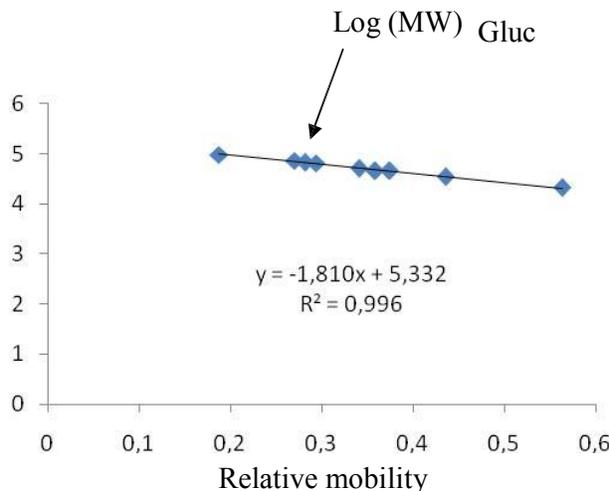$R^2 = 0,996$

Relative mobility
Figure 5: Determination of Molecular Weight of α-glucosidase purified by electrophoresis.

Markers: A: phosphorylase B (97,000 Da), B: bovine albumin serum (66,200 Da), C: ovalbumin (45,000 Da), D: carbonic anhydrase (35,100 Da), E: lysozyme (20,800 Da). α-glucosidase (68,200 Da). This shows that the enzyme molecul of α-glucosidase is in monomeric form as revealed by electrophoresis under denaturing conditions (photography 2). The molecular weight of the purified α-glucosidase of the digestive juice of the snail *Limicolaria flammea* is lower than that of *Geobacillus* sp HTA-462 (130 KDa) (Hung, V.S. et al., 2005). The α-glucosidase of *Limicolaria flammea* has a molecular weight which is between the range of molecular weights (42-115 KDa) of most of glucosidases [13].

Effect of pH and temperature

The **α-**glucosidase of *Limicolaria flammea* has its maximum activity at pH 6.5 (Figure 6) which is near of the neutrality.
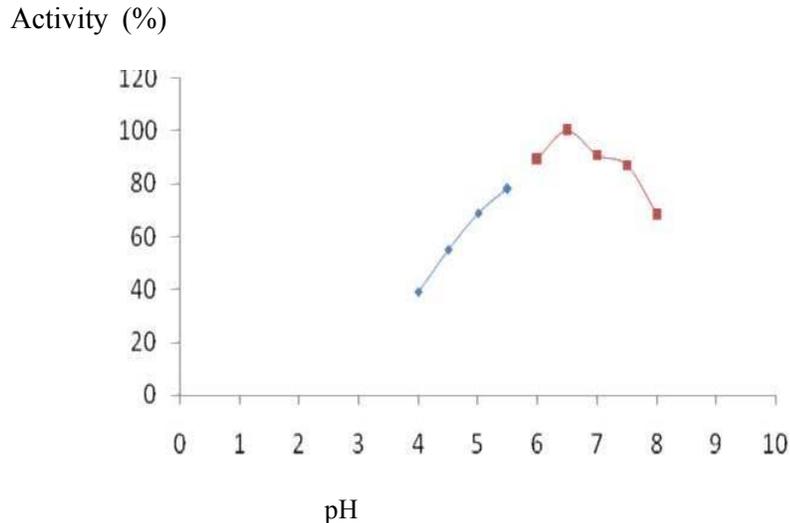
Activity (%)

pH
Figure 6: Effect of pH on the activity of α-glucosidase
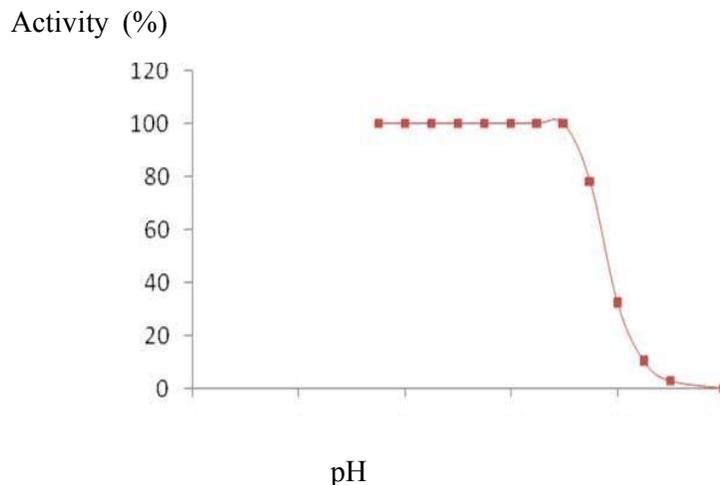
Activity (%)

pH
Figure 7: pH stability of α-glucosidase

This enzyme has the same optimum pH with that of *Entamoeba histolytica* [4]. However, the optimum pH of α-glucosidase of *Limicolaria flammea* is higher than those of α-glucosidases of the insect *Apis mellifera* (pH 5.5) [25] and *Trichomonas vaginalis* (pH 4.5 and 5.5) [31]. The optimum pH for hydrolysis of the α-glucosidase is in the overall margin of pH optimum of hydrolysis of these biocatalysts which is 6 to 7 [19, 23].

The zone of stability of the pH of the α-glucosidase is between pH 4 and 7.5 (Figure 7). The pH stability margin is wide, it might be beneficial to synthesis reactions or hydrolysis which uses purified α-glucosidase in biotechnology processes. The purified α-glucosidase of the digestive juice of the snail *Limicolaria flammea* has an optimum temperature of hydrolysis at 45°C (Figure 8).
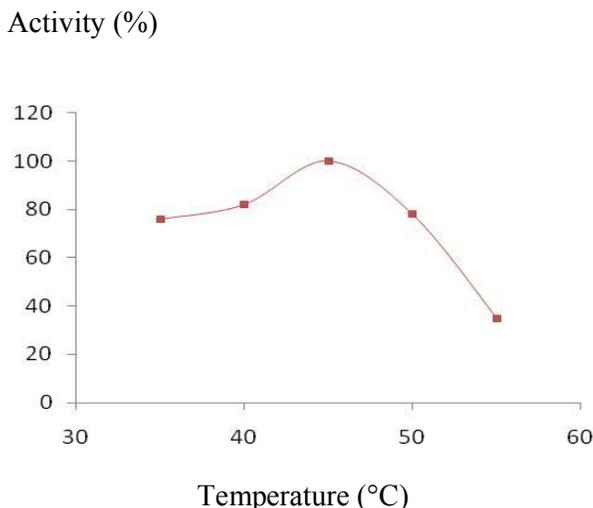
Activity (%)

Figure 8: Influence of temperature on the activity of α-glucosidase.

This temperature is less than (81°C) this of the α-glucosidase of *Bacillus thermoamylolique faciens* KP1071 [29]. The purified α-glucosidase is stable at temperatures of 37°C to 40°C and the activity begins to decrease with the rise of the temperature (Figure 9).
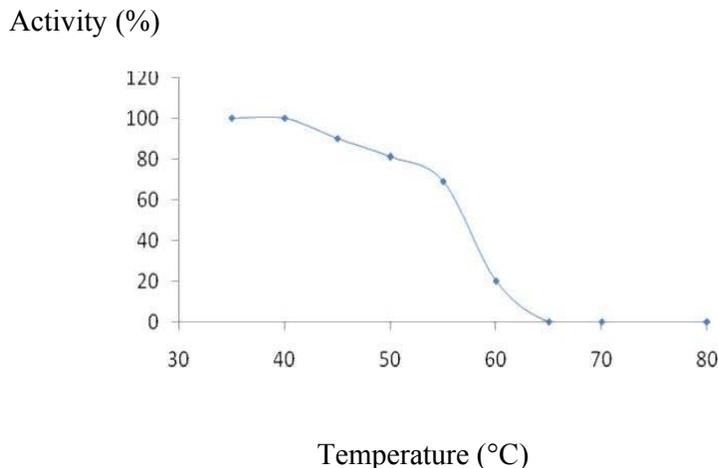
Activity (%)

Temperature (°C)

Figure 9: Thermic stability of α-glucosidase

This phenomenon is due to the proteic nature of the enzyme. Indeed, the increase of temperature causes the breaking of certain bonds such as ionic bonds, hydrogen or van der Vaals [6]. This has resulted in the modification of the native structure of the enzyme molecule inducing denaturation and loss of activity.

The influence of temperature on the activity and conformation of the enzyme can be shown by the determination and interpretation of thermodynamic parameters. Thus, the values of the enthalpy vary slightly (from 57.98 to 57.89 kJ/mol) in this study between 45°C and 55°C (Table 2).

Table 2: Inactivation thermodynamic parameters of the purified α-glucosidase.

Temperature 45°C			Temperature 50°C			Temperature 55°C		
ΔH kj/mol	ΔS j/mol/k	ΔG kj/mol	ΔH kj/mol	ΔS j/mol/k	ΔG kj/mol	ΔH kj/mol	ΔS j/mol/k	ΔG kj/mol
57.98	-104.51	91.21	57.94	-104.65	91.74	57.89	-104.7	92.26

The observed variation is the energy (heat) provided to the α-glucosidase to move from its native state to its transitory conformation at 55°C. This conformational change has reduced the activity of α-glucosidase at 65% (Table 3).

Table 3: Residual Activity of purified α-glucosidase after thermic inactivation

Temperature (°C)	Residual activity all over time (%)					
	15 min	30 min	45 min	60 min	75 min	90 min
45	97.02	95	90.3	85	82.11	78.12
50	93	90.6	85	80.7	76.3	72.5
55	86	81.6	77	72.3	68.9	65

The residual activity of the α-glucosidase is influenced by the temperature increase. Thus, at temperatures of 45 °C, 50°C and 55 ° C, the residual activities of the α-glucosidase are respectively 78.12%, 72% and 65% after a period of 90 minutes.

Thermal inactivation and kinetic parameters

Thermal inactivation of the α-glucosidase is a reaction which receives heat from the external environment, the Gibbs free energy (ΔG) is positive (Table 2). The conformational change that could produce heat on the enzyme is measured by the change of entropy. The entropy of the purified α-glucosidase of *Limicolaria flammea* calculated is negative (-104.7 j / mol / °K) (Table 2), it has been shown in the literature [8]. The negative value of the entropy indicates that the temperature caused a change in the structure of the α-glucosidase. Thus, the α-glucosidase is not a thermostable enzyme.

The Michaelis and Menten constant (Km) and maximum speed (Vmax) are determined from the representation of Lineweaver and Burk [21] (Figure 10).
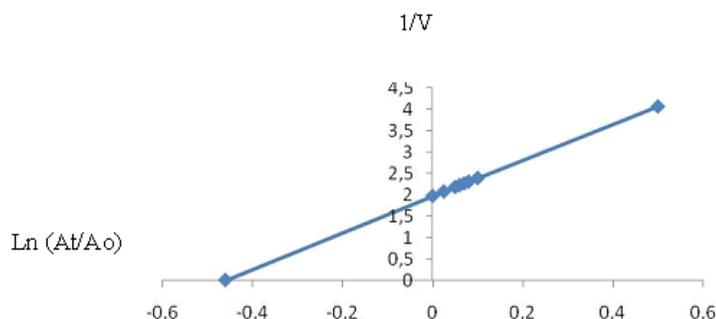
Figure 10: Determination of kinetic parameters of the α-glucosidase.

Parameters Km and Vmax are respectively 2.17 mg/mL and 0.51 mg/min for the purified α- glucosidase of the digestive juice of the snail *Limicolaria flammea*.

Substrate specificity and effects of chemical agents on α-glucosidase

The study of the specificity of the α-glucosidase showed that this enzyme hydrolyzes the para- nitrophenyl-α-D glucopyranoside and sucrose but not acting on the para-nitrophenyl β-D- glucopyranoside (Table 4).

Table 4 : Substrate specificity of the purified α-glucosidase.

Substrates	Hydrolysis results
p-nitrophényl-α-D glucopyranosiside	+
p-nitrophényl-α-D galactopyranoside	-
p-nitrophényl-α-D xylopyranoside	-
p-nitrophényl-α-D mannopyranoside	-
p-nitrophényl-β-D glucopyranoside	-
p-nitrophényl-β-D galactopyranoside	-
p-nitrophényl-β-D mannopyranoside	-
Starch	+
Sucrose	-

(+): hydrolysed   (-): none hydrolysed

This strict specificity of anomeric and a group was also observed at the level of α- glucosidases of *Candida albicans* [3] *Apis mellifera* [18] and the fungus *Paccilomyces lilacimus* [16]. However, the strict specificity observed face to the α-glucosyl residue is not always respected for some α-glucosidases. Thus, α-glucosidases of *Pyrococcus furiosus Archeobactérium* [10] of the endosperm of kernels of beetroot and edible beet *Alliumfistulum* [35, 35],do not hydrolyze sucrose in which we are an α-glucosyl group.The α-glucosidase purified from the snail *Limicolaria flammea* does not hydrolyze starch. This result was also observed for α-glucosidase of *Trichomonas vaginalis* [31] and *Thermotoga maritima* . The study of the influence of effectors on purified α-glucosidase showed a significant activation of this enzyme by Mn^{2+} ion and its almost complete inhibition by SDS. SDS is an ionic detergent which destroys some essential links in the structure of the enzyme causes the loss of the catalytic ability of the enzyme (Table 5). The α-glucosidase is also inhibited by Cu^{2+}, Ni^{2+}, Hg^{2+} and chemical agents such as pCMB, DTNB and EDTA (Ethylène Diamino Tetra Acétique). The action of EDTA could be explained by its interaction with a metal ion housed in the structure of α-glucosidase. This result indicates that the α-glucosidase is a metalloenzyme. This enzyme also contains thiol groups in its structure because it is inhibited by agents such as pCMB. The presence of thiol groups in the essential conformation of the enzyme is shown also by the inhibitory action of the Hg^{2+} ion. Indeed, the reduction of enzyme activity by Hg^{2+} ion indicates that thiol groups are not only located in the active center of the enzyme but these thiol groups participate in the catalytic act [33]. The study of effectors is always useful in biotechnology processes where the use of these effectors is often necessary for optimal use of enzymes.

CONCLUSION

The α-glucosidase of digestive juice of the snail *Limicolaria flammea* was purified in three chromatographic steps low pressure. Molecular sieve chromatography on Sephacryl S-200 HR gel was the beginning of the purification followed by separation on Anx-sepharose 4 fast flow gel and the last step is the hydrophobic interaction chromatography on phenyl sepharose CL-6B gel. The α-glucosidase has a specific activity of 147 U/mg, with an optimum pH of 6.5 and an optimum temperature of 45°C. It remains stable until the optimum temperature and thermostability begins to decrease gradually when the temperature is raised and cancel at 70°C. The α-glucosidase is active on the paranitrophenyl-α-glucopyranoside and sucrose but does not hydrolyze paranitrophenyl-β-D-glucopyranoside and the paranitrophenyl-α- glucopyranoside. It is activated by Mn^{2+} and Sr^{2+}, while Na^+ has no effect and, Cu^{2+} and Hg^{2+} are potent inhibitors.

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