

(RESEARCH ARTICLE)



## Screening of some catalytic activities of mature berries of *Solanum aethiopicum* cultivar "Klongbo"

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### Abstract

Aubergines of the *Solanum aethiopicum* cultivar "Klongbo" are widely cultivated and consumed by the Ivorian population. However, their post-harvest conservation is problematic. In order to prevent their post-harvest degradation, this study initiates the screening of enzymatic activities responsible for hydrolysis and oxidation activities in the berries of *Solanum aethiopicum* cultivar "Klongbo". Among the enzymatic activities tested, phosphatases, N'Acetylglucosaminidase, dopamineoxydase and pyrocatecholoxydase were the predominant enzymatic proteins. The optimum hydrolysis and oxidation conditions in terms of pH and temperature are listed as follows: phosphatases (pH 8.0; 50°C), N-acetylases (pH 5.6; 45°C), dopamineoxydase (pH 9.6; 10°C) and pyrocatecholoxydase (pH 9.0; 10°C). Cu<sup>2+</sup> ions inhibit all tested enzymatic activities and especially Zn<sup>2+</sup> ions for polyphenoloxidase activities. EDTA has an inhibitory effect on phosphatase and N'acetylase activities. SDS has an inhibitory effect on dopamineoxydasic, pyrocatecholoxydasic and phosphatasic activities. On the other hand, it has no effect on N'Acetylglucosaminidasic activities.

**Keywords:** *Solanum aethiopicum*; Polyphenoloxidase; Phosphatase; N'Acetylglucosaminidase

### 1. Introduction

The aubergine is a vegetable plant of the Solanaceae family, cultivated for its fruit and leaves consumed as a vegetable. It is a fruit-vegetable with economic importance in Mediterranean countries and Asia [1]. It is also found in America and Africa. It can be cultivated in a wide variety of climates (temperate, tropical dry or humid). Thus, it contains many types of cultivars that vary according to the colour, size and shape of the fruit [2]. Many local or introduced varieties, belonging to different species, such as *Solanum aethiopicum* gilo "N'drowa", *Solanum aethiopicum* "Klongbo" and *Solanum macrocarpon* "Gbokouman", are cultivated in small, generally urban and peri-urban market gardens in Côte d'Ivoire [3]. According to Lester et al., [4], *Solanum aethiopicum* anguivi is a bitter aubergine species much appreciated by the populations of the centre of the Ivory Coast, where it is commonly known in the Baule language as "Klongbo". This variety can reach a height of 96.3 cm to 106.3 cm with clustered inflorescences (3 to 9 flowers). *Solanum aethiopicum* "Klongbo" is the species that bears the largest number of fruits on the stem with very small diameters and elongated shapes, the average weight of the fruits varying from 16 to 20g [5]. Vegetables play an important role in human nutrition because of their content of vitamins, minerals and other nutritional compounds that contribute to better health. For example, the consumption of aubergine leaves and fruits provides the body with carotenes, various vitamins (B and C), folic acid, minerals and protein [6, 7]. With regard to its energy intake, aubergine provides about 18 kilocalories per 100 g, as do tomatoes, chicory and lettuce. It is rich in water (more than 92% on average). And its energy content remains

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limited. Despite its very high consumption, the marketing of aubergines remains informal and their post-harvest conservation remains a real problem. In Côte d'Ivoire, the north of the country has the highest percentage of production, but losses are estimated at 60%. In fact, the attack of micro-organisms and injuries caused during the harvest lead to many losses during harvesting, storage and conservation of these fruit vegetables. On a chemical level, these losses are caused by free radicals formed from quinones produced during oxidation reactions catalysed by polyphenoloxidases (PPO) and to a lesser degree by the peroxidases responsible for browning. Although sought after in certain food technologies such as coffee processing, enzymatic browning can lead to loss of nutritional value, discolouration and deterioration of the organoleptic quality of the food [8, 9, 10]. All the damage suffered is the result of mechanical processes (harvesting, storage, conservation) and enzymatic reactions that take place in the cells of *Solanum aethiopicum* "Klongbô" fruit-vegetable aubergines. This study aims to highlight some major enzymatic activities in this prized variety of aubergine in Ivory Coast and to determine the optimal pH and temperature conditions for hydrolysis of the predominant activities.

## 2. Material and methods

### 2.1. Material

#### 2.1.1. Biological material

The plant material consists of *Solanum aethiopicum* cultivar "Klongbo" (figure 1). These berries come from a market gardening culture in the town of Korhogo, located in the North of Ivory Coast (632 km from Abidjan, Ivory Coast). They are harvested at the mature stage and sent to the biocatalysis and bioprocessing laboratory of the Nangui Abrogoua University, for the preparation of the raw enzymatic extract.



**Figure 1** *Solanum aethiopicum* cultivar "Klongbo"

### 2.2. Methods

#### 2.2.1. Preparation of the enzymatic crude extract

Ten (10) grams of *Solanum aethiopicum* cultivar "Klongbo" were crushed in a blender in the presence of 400 ml of NaCl (0.9%, w/v). The obtained grinded material was centrifuged at 12500 rpm for 30 min at a temperature of 4°C in a centrifuge HERMECE Z 300K. The supernatant obtained is the raw enzymatic extract of the berries of *Solanum aethiopicum* cultivar "Klongbo".

#### 2.2.2. Techniques for determining enzymatic activities

##### Measurement of the osidic activities

The osidic activities were determined using the reaction medium consisting of 125  $\mu$ l NaCl (0.9%), 50  $\mu$ l crude enzyme extract. The mixture was brought to 37 in a water bath for 5 minutes. After pre-incubation, 75  $\mu$ l of substrate (xylane, starch, sucrose and carboxymethylcellulose) were added to each tube. This reaction medium was incubated in a water bath for 30 minutes at 37°C. Then 300  $\mu$ l of a solution of 3,5-dinitrosalicylic acid (DNS) were added to stop the enzymatic reaction [11]. After being heated in a boiling water bath for 5 min, the tubes were cooled to room temperature for 10 min and 2 ml of distilled water was added. The product was analysed by measuring the optical density at 540 nm.

### Measurement of esterase and heterosidasic activities

Esterase and heterosidasic activities were determined in a reaction medium containing 125  $\mu$ l of NaCl (0.9%), 1.5 mM of *p*-nitrophenyl (phosphate, N'Acetylglucosaminidase...) and 50  $\mu$ l of crude enzymatic extract. The mixture was incubated at 45°C for 10 min. The reaction was stopped by adding 2 ml of 2% Na<sub>2</sub>CO<sub>3</sub> (w/v). Absorbances were measured at 410 nm using a spectrophotometer. Para-nitrophenol (*p*NP) was used as standard

### Measurement of polyphenoloxidase activities

The reaction medium consists of 1.1 ml NaCl (0.9%), 0.8 ml substrate (dopamine, pyrocatechol, tyrosine, phenylalanine, pyrogallol) 8 mM; -0.1 ml enzymatic crude extract. This medium was the standard medium. The mixture was incubated at room temperature (25°C) for 10 min. The optical density reading was taken with a spectrophotometer at 480 nm against a control containing no crude enzyme extract [12].

A unit of activity has been defined as the amount of enzyme that hydrolyses 1  $\mu$ mol of substrate per minute according to the enzyme assay conditions. The specific activity was expressed as a unit of activity per mg of protein.

#### 2.2.3. Determination of optimum hydrolysis pH values

The buffers citrate phosphate 20 mM (pH 3.0 to 7.0), sodium acetate 20 mM (pH 3.6 to 5.6), potassium phosphate 20 mM (pH 5.6 to 8.0) and sodium glycine hydroxide 20 mM (pH 8.0 to 10.0) were used for the determination of the optimum hydrolysis pH of the enzymatic crude extract. The residual activities were determined under standard conditions.

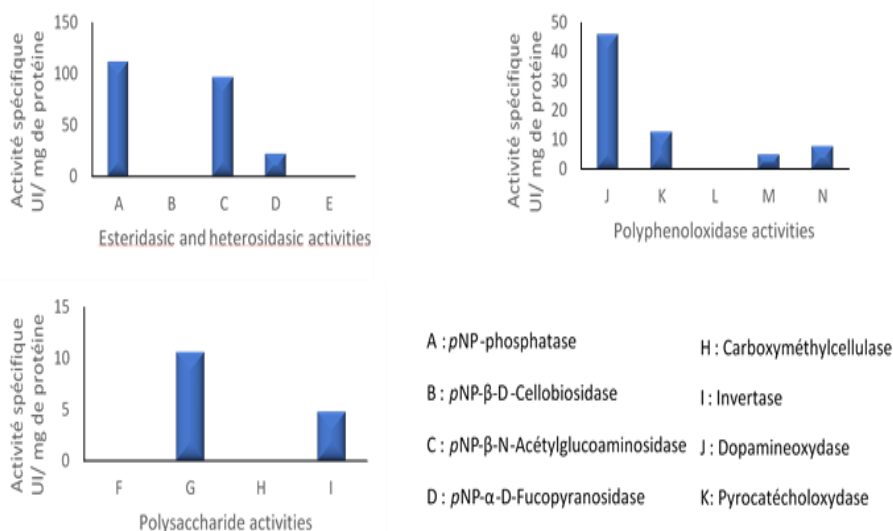
#### 2.2.4. Determination of optimum hydrolysis temperatures

The influence of temperature on the enzymatic crude extract was studied at temperatures between 30 and 80°C for osidic and *p*-NP (phosphate and N'Acetylglucosamine) activities and between 5 and 45°C for polyphenoloxidase activities. The enzymatic activity was determined under standard conditions. It was expressed as a percentage of the maximum activity.

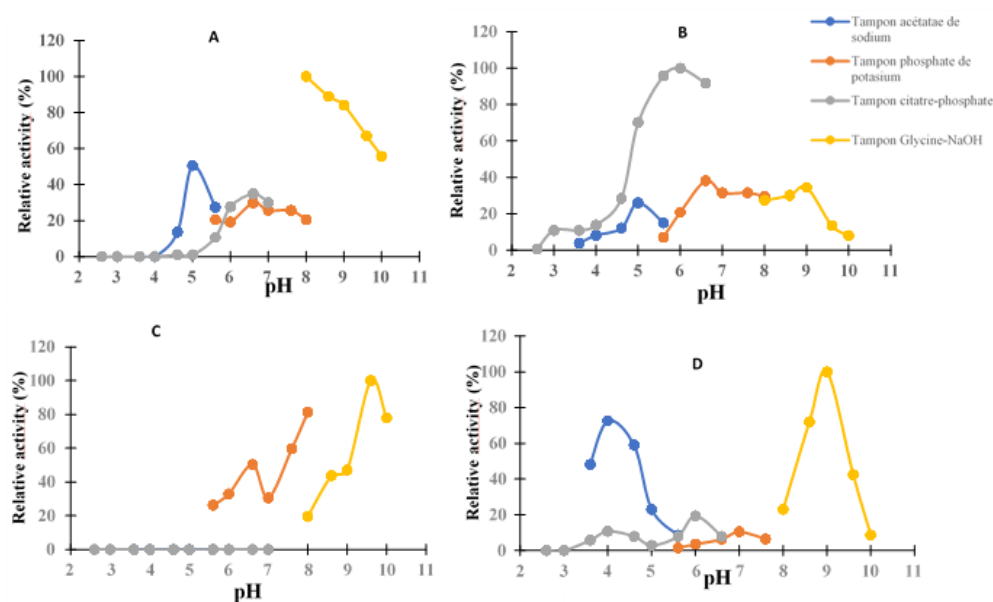
#### 2.2.5. Effect of certain chemical agents

To determine the effect of various compounds as possible activators or inhibitors, the enzyme crude extract was pre-incubated at 37°C for 1 hour with the compounds and the activity was determined under the conditions of the enzyme assay. Residual activities were expressed as a percentage of the control without chemical agents

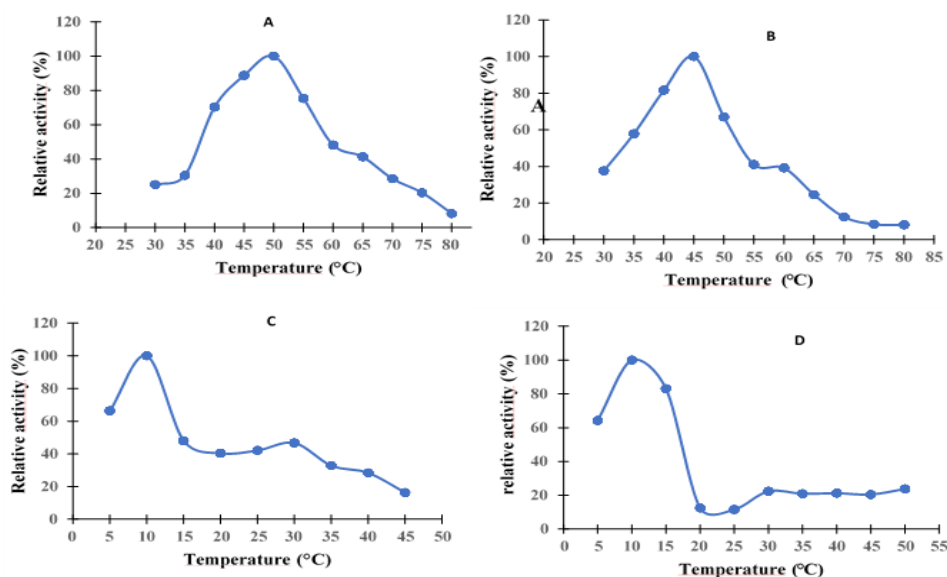
## 3. Results



**Figure 2** Determination of hydrolytic and oxidase activities of mature berries of *Solanum aethiopicum* cultivar "Klongbo"



**Figure 3** Determination of optimum pH for hydrolysis and oxidation of mature berries of *Solanum aethiopicum* cultivar "Klongbo". A: pNP-Phosphatase activity, B: pNP-N'acetylglucosaminidase activity, C: dopamineoxidase activity, D: pyrocatecholoxidase activity.



**Figure 4** Determination of optimal temperatures for hydrolysis and oxidation of mature berries of *Solanum aethiopicum* cultivar "Klongbo". A: pNP-Phosphatase activity, B: pNP-N'acetylglucosaminidase activity, C: dopamineoxidase activity, D: pyrocatecholoxidase activity.

**Table 1** Effect of chemical agents on the predominant enzymatic activities of the raw extract of the berry *Solanum aethiopicum* cultivar "Klongbo".

	Concentrations (mM)	pNP-Phosphatase (%)	pNP-N'acetylglucosaminidase (%)	Dopamineoxydase (%)	Pyrocatecholoxydase (%)
None	0	100	100	100	100
Ca <sup>2+</sup>	1	87.74±2.15	121.09±3.45	144.44±5.41	155.22±3.15
	5	88.44±0.43	108.84±2.08	129.73±2.08	201.49±3.37
K <sup>+</sup>	1	110.86±3.9	108.16±2.17	129.73±2.47	117.16±2.21
	5	101.11±1.31	101.36±1.65	92.79±2.01	119.16±2.08
Cu <sup>2+</sup>	1	44.84±0.06	29.93±0.23	34.68±0.05	30.6±2.04
	5	34.81±0.22	40.81±1.13	0	63.42±1.34
Mg <sup>2+</sup>	1	122.42±3.67	107.48±1.41	120.27±1.39	133.58±3.32
	5	130.22±2.18	104.08±2.16	145.05±2.27	158.21±3.45
Zn <sup>2+</sup>	1	89.41±2.81	132.65±3.25	81.99±1.17	19.4±0.37
	5	96.1±1.17	146.25±1.22	13.06±0.22	14.92±0.12
SDS	1	6.68±0.02	153.06±3.49	9.01±0.08	62.69±1.14
	5	0	251.06±3.18	13.06±1.17	140.3±2.42
EDTA	1	167.54±3.56	156.46±2.11	63.96±0.35	26.86±1.16
	5	135.93±2.16	129.93±2.04	39.4±0.43	34.33±1.09

#### 4. Discussion

Plant cells contain numerous substrates which are transformed by the action of endogenous enzymes. These enzymes are responsible for most of the ripening and senescence processes of fruit and vegetables, making their preservation problematic. The screening of enzyme activities and the determination of optimal pH and hydrolysis temperature conditions in the berries of *Solanum aethiopicum* cultivar "Klongbo" was the subject of this study. Thus, among the enzymes sought, phosphatase, acetylase, dopaminase and pyrocatecholase activities were found to be dominant (Figure 2). It has been shown that phosphatase is involved in the energy supply, phosphorylation and dephosphorylation of sugars during post-harvest metabolism in plants [13]. Indeed, the significant hydrolysis of pyrophosphate by phosphatase would provide phosphate groups which would participate in the degradation of starch by the phosphorylase route. This screening shows that amylase is the dominant enzymatic activity for the osidic ones. Among the polyphenoloxidase activities, dopamineoxydase and pyrocatecholoxydase proved to be dominant. Polyphenoloxidases catalyse the oxidation of both o-diphenolic substrates (dopamine, pyrocatechol) and triphenol (pyrogallol). Polyphenoloxidases extracted from *Solanum aethiopicum* berries have no affinity for monophenols (L-tyrosine and phenylalanine). Similar results have been obtained by several authors where ortho-diphenols and triphenols were the best substrates compared to monophenols [14, 15]. The presence of polyphenoloxidase activity is an indicator of cellular activity in fruits and vegetables [16]. Thus, like many vegetables, the berries of *Solanum aethiopicum* "Klongbo" contain polyphenoloxidase activities (PPO). The work of [17] Dan (2014) on the berries of *Solanum anguivi* Lam harvested in Côte d'Ivoire has shown that polyphenoloxidase activities are present in the plant cells of aubergines. According to Vamos-Vigyazo [18] these substances play a very important role in the polymerisation of quinones by creating insoluble polymers that act as a barrier against infections. The study of the optimal conditions of hydrolytic activity of the berries of *Solanum aethiopicum* cultivar "Klongbo" shows a diversity of reactivity according to the substrate used. The dopamineoxydase activities show their optimum pH in a basic environment. Two peaks are obtained, pH 9.6 in the glycine-hydroxide buffer and pH 8 (81.21% Relative Activity) in the potassium phosphate buffer. The pyrocatecholoxydase activities are also maximal in the glycine hydroxide buffer at pH 9.0. The work of Chuanjuna *et al*, [19], shows that the acidic medium inhibits the browning of Phalaenopsis Explants. This experiment proves that some PPOs react outside acidic pH and the change in hydrogen potential could inhibit their activity. The

value of the optimum oxidation pH determined is within the general range of the optimum pH values of polyphenoloxidases, which are between 4.0 and 9 [20, 21]. The pH is an important factor in the speed of reactions. Indeed, outside the area of PPO activity, the oxidation reaction is slower and can be inhibited by an acidification of the culture medium. This acidification of the pH outside the range of PPO activity can help to overcome the damage caused by browning. The pH of *p*-NP- N'Acetylglucosaminidase activities is acidic (pH 5.6-6.6, citrate-phosphate buffer) and that of *p*-NP-Phosphatase shows two peaks of activity with the glycine-hydroxide buffer pH 8.0 and the acetate buffer pH 5.0 (Figure 3). These two peaks of activity could be explained by the fact that the enzymatic crude extract has a heterogeneous medium expressing enzymatic protein isoforms. The berries of *Solanum aethiopicum* cultivar "Klongbo" have optimal mesophilic hydrolysis temperatures for phosphate and acetylase activity, 50 °C and 45 °C respectively (Figure 4). On the other hand, the optimal hydrolysis temperatures for dopamine oxidase and pyrocatechol oxidase activities are psychrophilic (10 °C). It should be noted that the control of storage temperatures can have significant effects on enzymatic activities.

Enzymes are known to be catalysts for various biochemical or biological reactions. It has been shown that the reactivity of some of them is induced or improved either by the presence of non-protein molecules or by that of metal ions. In chloroplast, many species of ions are present (H<sup>+</sup>, K<sup>+</sup>, Na<sup>2+</sup>, Cl<sup>-</sup>, Cu<sup>2+</sup>, Mg<sup>2+</sup>, Zn<sup>2+</sup>, Mn<sup>+</sup>...). The presence of ions in plant substances requires the testing of some monovalent and divalent ions on raw extracts of *Solanum aethiopicum* cultivar "Klongbo" berries. Cu<sup>2+</sup> ions inhibited all the enzymatic activities tested and more particularly Zn<sup>2+</sup> ions for polyphenoloxidase activities (Tableau 1). Like many copper metalloproteins [22, 23, 24], those of the berries of *Solanum aethiopicum* cultivar "Klongbo" do not require the action of Cu<sup>2+</sup> ions for their oxidase activity. EDTA (Ethylenediaminetetraacetate) is an ion chelator. This compound has an inhibitory effect on polyphenoloxidase activities (dopamine oxidase and pyrocatechol oxidase), which could possibly suggest metalloenzymes. On the other hand, EDTA has an inhibitory effect on phosphatase and N'Acetylglucosaminidase activities. As for Sodium Dodecyl Sulphate (SDS) which has an uncoiling effect on enzymatic proteins by cutting the thiol bridges (SH) has no effect on N'acetylase activities contrary to the enzymes investigated in this study. This result suggests that the N-acetylase enzyme protein of the berries of *Solanum aethiopicum* cultivar "Klongbo" could be monomeric and those of the polyphenoloxidase and phosphatase more than two monomers.

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## 5. Conclusion

The general objective of our study is the post-harvest conservation of berries of *Solanum aethiopicum* cultivar "Klongbo". Specifically, in this experiment, the screening of the hydrolytic and oxidative activities allowed us to highlight the phosphatase, N'Acetylglucosaminidase, dopamine oxidase, and pyrocatechol oxidase activities in the raw enzymatic extract. Phosphatase enzymatic proteins express their maximum activity in a basic and mesophilic environment. Also, the polyphenoloxidase activities have an optimum basic pH not against their optimum temperature is psychrophilic. The control of the activities of these biocatalysts could be an asset in the post-harvest conservation of berries of *Solanum aethiopicum* cultivar "Klongbo".

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## Compliance with ethical standards

### Disclosure of conflict of interest

All authors declare that they have no conflict of interest.

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