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## Control of enzymatic browning of aerial yam (*Dioscorea bulbifera*) cultivated in Côte d'Ivoire by six fruit juices

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### Abstract

The inhibitory effect of six fruit juices on polyphenol oxidase (PPO) and enzymatic browning of aerial yam (*Dioscorea bulbifera*) was investigated in order to evaluate the influence of these fruit juices on polyphenoloxidase activity, in a total purified preparation of aerial yam (*Dioscorea bulbifera*). All the six fruit juices used are inhibitors of the *Dioscorea bulbifera* yam polyphenol oxidase activity. At volumes of 400 µl fruit juices the activity of polyphenoloxidase was, totally inhibited. Ki values of 0.087, 0.163, 0.554, 1.077, 1.085 and 1.507 were found for the juices of papaw, orange, tangerine, grenadillo, lemon and grapefruit, respectively. The Ki values showed that papaw juice was the most effective inhibitor. The type inhibition was determined for each fruit. A competitive-type inhibition was obtained with all fruit juices used.

### Article Info

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### Keywords

Enzymatic browning; polyphénoloxidase; *Dioscorea bulbifera*; fruit juices; inhibition, control.

### Introduction

*Dioscorea bulbifera* is an aerial yam also known with common names as potato yam, cheeky yam, bulbils-bearing yam. It is cultivated in the Southeast Asia, West Africa, South America and Central America (Nwosu, 2013). Aerial yam is grown for its bulbis and eaten during famine season. Though it possess a distinctive flavour and comparable in nutritional content to most preferred yams, it does not have the same appeal compared to *D.alata* and *D.rotundata* and so it is less studied and has high rate of post-harvest losses (Sanful, 2013).

*D. bulbifera* is used as food and it provides substantial amount of calories and minerals such as iron, calcium and phosphorous (Tindall, 1983; Abara *et al.*, 2003).

*Dioscorea bulbifera* tuber is a rich source of starch that forms an important dietary supplement (Deb, 2002). Apart from starch, the root tubers of *Dioscorea* species contain protein, fat, fiber and some minerals such as Potassium, Sodium, Phosphorus, Calcium, Magnesium, Copper, Iron, Manganese, Zinc and Sulphur (Deb, 2002). Yam is unfortunately hampered by a phenomenon of enzymatic browning during postharvest storage or processing (Mohapatra *et al.*, 2010). These browning reactions have been linked to mechanical damage during handling and processing, abrasions, washing, senescence, and bacterial infestations. Basically, enzymatic browning can be defined as the initial enzymatic oxidation of phenols into slightly colored quinones (Nicolas *et al.*, 1994). These quinones are then subjected to further reactions leading to the formation of pigment (Ozoglu and Bavindirli, 2002). Enzymatic browning caused by

the oxidation of phenols by polyphenol oxidases and peroxidases is the most common phenomenon associated with the browning of yams during injury and processing at low temperatures (Teo *et al.*, 2016). The PPO are metalloproteins that contain three different types of copper ions attached to histidines (Espin *et al.*, 2000).

Different names have been associated with these enzymes including tyrosinase, cresolase, catecholase, diphenolase and phenolase. They are responsible for the hydroxylation of monophenols to diphenols and the oxidation of the latter to o-quinones (Nicolas *et al.*, 1994). Enzymatic and/or nonenzymatic browning reactions may adversely affect the quality, nutritional value and safety of foods (Laurila and Ahvenainen, 2002; Billaud *et al.*, 2003). Consequently, the control of enzymatic browning in order to maintain their quality, nutritional value and safety has aroused strong interest in the food industry (Krishnan *et al.*, 2010). One approach to the prevention of this phenomenon has been the use of anti-browning agents such as sulphite containing additives (Egwim *et al.*, 2013), ascorbic acid, acetic acid, citric acid etc. (Krishnan *et al.*, 2010). However, due to health concerns, sulphites use has been restricted (Anon, 1991).

The use of natural occurring materials as preservatives is a promising alternative to the use of chemicals (Howell, 1986). The potential sources of natural preservative are spices, herbs, fruits, seed, leaves, barks and roots (Pratt and Hudson, 1996).

According to Babarinde *et al.*, (2014), the use of organic products such as spices with antioxidant and antimicrobial properties can serve as alternative control methods. The polyphenol oxidase inhibitors occurring in natural resources have been studied in several plants (Jang *et al.*, 2002; Lee *et al.*, 2007; Adegokeet Odebade, 2017), but the development of natural and efficient polyphenol oxidase inhibitors is needed. The objective of this study was to evaluate the efficacy of six (6) fruit juices (papaw, orange, tangerine, grenadillo, lemon and grapefruit) in controlling browning of aerial yam (*Dioscorea bulbifera*) cultivated in Côte d'Ivoire.

## Materials and Methods

### Plant Material and polyphenol oxidase Preparation

The studied cultivar aerial yam (*Dioscorea bulbifera*) was grown during its appropriate cropping season in June 2008 at the experimental farm of the University

Nangui Abrogoua [Abidjan, Côte d'Ivoire] (5°23 latitude North, 4°00 longitude West, and 7 meters altitude). The ripened tubers were randomly harvested 6 months after planting (December 2008). After harvesting, bulbils were peeled using a stainless steel kitchen knife and the pulp was cut into slices. Then, 150 g were ground using a blender in 300mL of NaCl solution 0.9% (w/v). The homogenate was subjected to sonication (4°C) at 50-60 Hz frequency using a TRANSSONIC T420 for 10 min and then centrifuged at 10,000 x g for 30 min at 4°C. The supernatant filtered through cotton wool was kept refrigerated (4 °C) and used as the crude extract.

### Purification of enzymes

All the purification procedure was carried out in the cold room. The crude extract of aerial yam (20 mL) was loaded onto a DEAE-Sepharose CL-6B gel (2.4 cm × 6.5 cm) that had been equilibrated previously with 100 mM phosphate buffer pH 6.6. The unbound proteins were removed from the column by washing with two column volumes of the same buffer pH 6.6. Proteins were eluted using a stepwise gradient with 0 M, 0.3 M, 0.5 M and 1M NaCl in 100 mM phosphate buffer pH 6.6. Fractions (3 mL each) were collected at a flow rate of 180 mL h<sup>-1</sup> and assayed for enzyme activity. The active fractions were pooled and loaded directly into a CM-Sepharose CL-4B gel (2.5cm x 5.3 cm) which was pre-equilibrated with the same buffer pH 6.6. Proteins were eluted at a flow rate of 20 mL h<sup>-1</sup> using 100 mM phosphate buffer pH 6.6. Fractions of 3 mL were collected and active fractions were pooled together. The pooled fraction from the previous step was saturated to a final concentration of 1.7 M ammonium sulphate and applied on a Phenyl-Sepharose CL-6B column (1.4 cm x 7.5 cm) previously equilibrated with 100 mM phosphate buffer pH 6.6 containing 1.7 M ammonium sulphate. The column was washed with equilibration buffer and the proteins retained were then eluted using a stepwise gradient with 1.7 M, 1 M, 0.7M, 0.5 M, 0.3 M, 0.1 M and 0 M ammonium sulphate in 100 mM phosphate buffer pH 6.6. Fractions of 1 mL were collected at a flow rate of 15 mL h<sup>-1</sup> and active fractions were pooled together. The pooled fraction was dialysed against 100 mM phosphate buffer pH 6.6 overnight in a cold room.

### Fruit juices preparation

The six (6) fruits (papaw, orange, tangerine, grenadillo, lemon and grapefruit) used as natural anti-browning agents were purchased from a local market in Adjamé

[Abidjan, Côte d'Ivoire]. Each fruit was been pressed, and the homogenate was filtered through cheesecloth.

The filtrate was centrifuged at 10,000 x g for 20 min at 4°C and the supernatant, after centrifugation, was used for this experiment.

### Protein quantification

The protein content was measured according to the method of Lowry *et al.*, (1951) using bovine serum albumin as standard.

### Measurement of PPO activity

Under the standard test conditions, PPO activity was measured spectrophotometrically using a modification of the method of Lourenco *et al.*, (1990). The reaction mixture (2 mL) containing 0.8 mL of 8 mM pyrocatechol solution, 1.1 mL of a 100 mM sodium phosphate buffer (pH 6.6) and 0.1 mL of the enzyme solution was incubated at 25°C for 10 min.

After incubation, the activity was determined by measuring the absorbance of the reaction mixture at 420 nm. One unit of enzymatic activity was defined as an increase in absorbance of 0.001 per minute (Cong *et al.*, 2005). Experiments were performed in triplicate, and the results expressed as units of enzymatic activity per mg of protein.

### Effect of fruits juices on browning "In situ" of aerial yam (*Dioscorea bulbifera*)

The effect of fruits juices on browning of bulbils *Dioscorea bulbifera* yam was study according to the method of Lee *et al.*, (2007). Bulbils yam were cut into slices. Each slice placed in an individual Petri dish and was immersed for 2 min in 100 mM sodium phosphate buffer at pH 6.6 or fruit juices (grenadillo, lemon, orange, tangerine, grapefruit and papaw). Then, 1.0 ml of 5 mM pyrocatechol was spread over the whole surface of each slice. A lid was placed on each Petri dish to minimize evaporation. All the slices were then incubated at 25 °C for 60 min.

### Inhibition of natural reagent "In vitro" on PPO extract

Inhibition of aerial yam (*Dioscorea bulbifera*) PPO was made according to the method of Yoruk *et al.*, (2003). Briefly, the reaction mixture containing 0.3 mL of yam

polyphenoloxidase, 0.6 mL of sodium phosphate buffer (pH6.6, 100 mM) and various volumes (50; 100; 200; 300; 400; 500 and 600) µl of the fruits juices as inhibitor, was preincubated for 20min at 25°C.

To that reaction mixture was added to 0.8 mL of pyrocatechol solution (8mM) to initiate the enzyme reaction. As a control, 0.3 mL of PPO extract was added to 0.8 mL of the pyrocatechol solution to which 0.9 mL of 100 mM sodium phosphate buffer (pH6.6) had been added.

The PPO activity was measured spectrophotometrically (Shimadzu UV-12002, Kyoto, Japan). The absorbance at 420 nm was recorded continuously at 25 °C for 1 min (Zauberman *et al.*, 1991). The total volume of assay for inhibition of PPO activity was 2.0 mL.

The percentage of inhibition was expressed as:

$$\text{Inhibition (\%)} = [(A - A^*) / A] \times 100$$

Where, A and A\* indicate the variation of absorbance in absence and presence, respectively, of the inhibitor.

### Determining the type and kinetics of inhibition

The inhibition kinetics of the enzyme was analyzed using the Line weaver-Burk plots by taking the reciprocals of the initial velocity and the substrate concentration for (pyrocatechol) in presence of each inhibitor fruit juices (grenadillo, lemon, orange, tangerine, grapefruit and papaw).

## Results and Discussion

### Effect of fruits juices "In situ" and "In vitro" on polyphenol oxidase activity of aerial yam (*Dioscorea bulbifera*)

Figure 1 shows an enzymatic browning on the level of the witness T (pilot without fruit juice) marked by brown and red spots of color clear at the ends of the section. Those are visible and less intense on the level of the tests with the juices of lemon, grenadillo and orange (figure 1A, 1B and 1D). During these 10 min of incubation, figure 1 still shows small red spots on the tests having received the juices of tangerine, grapefruit and papaw (figure 1C, 1E and 1F).

After 20 min of incubation (Figure 2), red and brown colourings of the witness T (without fruit juice) are accentuated while those of the tests with the juices of tangerine, grapefruit and papaw are attenuated (figure

2T, 2C, 2E, 2F). On the tests with the lemon, grenadillo and orange juices, a red discolouration are visible on surfaces of the differences sections of the bulbils (figure 2A, 2B and 2D). After 40 and 60 min of incubation, one notes a stabilization of colouring in all the tests with the fruit juices and a colouring much darker i.e. a blackening partial on the level of the section of the witness 3T (4T)(without fruit juice). The results obtained with the juices of lemon, grenadillo and orange are identical to preceding the results (Figure 3A, 3B and 3D). On the level of the tests with the juices of tangerine, of grapefruit and papaw, it was noted a clear reduction in the red and brown tasks which strewed partially surface of the section with bulbils (Figure 3A, 3B and 3D).

After 60 min of incubation, the colouring of the various tests remained stable was marked by, the presence of a paste or crust on the surface of the various tests (Figure 3 and 4).

The experimental pH of fruit juices used varied between 2.02 and 5.14. Those are located in the interval defines by the literature (FAO, 2005). All these fruit juices are acid. The most acid pH is that of lemon (pH = 2.02). The pH of the juice of papaw is the least acid (pH=5.14) (Table 1). From a volume of 50 µl of juice added to the reactional medium, the inhibition of the polyphenoloxidase activity of the bulbils of *Dioscorea bulbifera yam* (cultivar yellow) is obtained for the 6 fruits used. This inhibition is marked for grapefruit and the lemon whose values are respectively 2.13 % and 9.02 %.

With 200 µl of juice used inhibition is pushed much with the juices of papaw, grenadillo, lemon, orange and tangerine or one has an inhibition of the enzyme to more than 90 %. As for the grapefruit juices, for 200 µl of juice added to the reactional medium one has more than 80 % of inhibition.

For all the juices, inhibition is quasi-total after the addition of more than 300 µl of juice. The more the quantity of juice increases, the more the percentage of inhibition increases. All the six fruit juices used are inhibitors of the bulbils of *Dioscorea bulbifera yam* (cultivar yellow) polyphenol oxidase activity (Table 1).

### Effect of reversible inhibitors

The representation of Lineweaver and Burk (1934) was used to obtain affine lines. Increase of inhibitors concentration resulted in a decrease of the slopes of the lines, showing that fruit juices have a reversible

inhibition on the polyphenol oxidase of aerial yam (*Dioscorea bulbifera*).

All the molecules of the fruit juices used responsible for the inhibition of the enzymatic browning exert a competitive inhibition on the polyphenol oxidase of the bulbils of *Dioscorea bulbifera yam* (cultivar yellow) (Figures 4 to 9). The constants of inhibition (K<sub>i</sub>) are 0.087, 0.163, 0.554, 1.077, 1.085 and 1.507 respectively for the juices of papaw, orange, tangerine, grenadillo, lemon and grapefruit (Table 2).

### Effect of fruits juices on polyphenol oxidase activity of aerial yam (*Dioscorea bulbifera*)

The enzymatic browning observed in the bulbils of *Dioscorea bulbiferayam* (cultivar yellow) without exogenic fruit juice contribution could be explained by the presence and the content of their phenolic compounds able to be oxidized by (or the) the polyphenol oxidase (s) or by (or the) the peroxidase (s) presents (s) in this biological environment (Weaver and Charley, 1974; Jayaraman *et al.*, 1982).

The presence of red and brown colors obtained during the enzymatic browning in fabric of the bulbil of yam could result on the one hand, of the variability and the concentration of the phenolic substrates present in this fabric (Aydemir, 2004; Dincer *et al.*, 2002) and in addition, of the presence of a possible peroxydase and polyphenol oxidase activity.

This situation shows that the phenolic compounds or the polyphenol oxidase and peroxydase activities unequally set out again (e) on the level of fabric of the bulbil.

The red and brown spots by place on surfaces of the sections of the bulbil show an unequal distribution of the contents of the substrates of the enzymes implied in the biosynthesis of the phenolic compounds.

Indeed, Gnangui *et al.*, (2009) found that the content of phenolic compounds in the proximal part is higher than in the other parts of the tuber of the *Dioscorea cayenensis-rotundatayam* (cultivar "Longbo") cultivated in the Côte d'Ivoire. These same results were already highlighted by Onayemi (1986) which found that the content of phenolic compounds in the proximal part is higher than in the other parts of the varieties of *Dioscorea aalata* and *Dioscorea cayenensis-rotundata* tubers cultivated in Nigeria. The fruits have different chemical compositions (FAO, 2008).

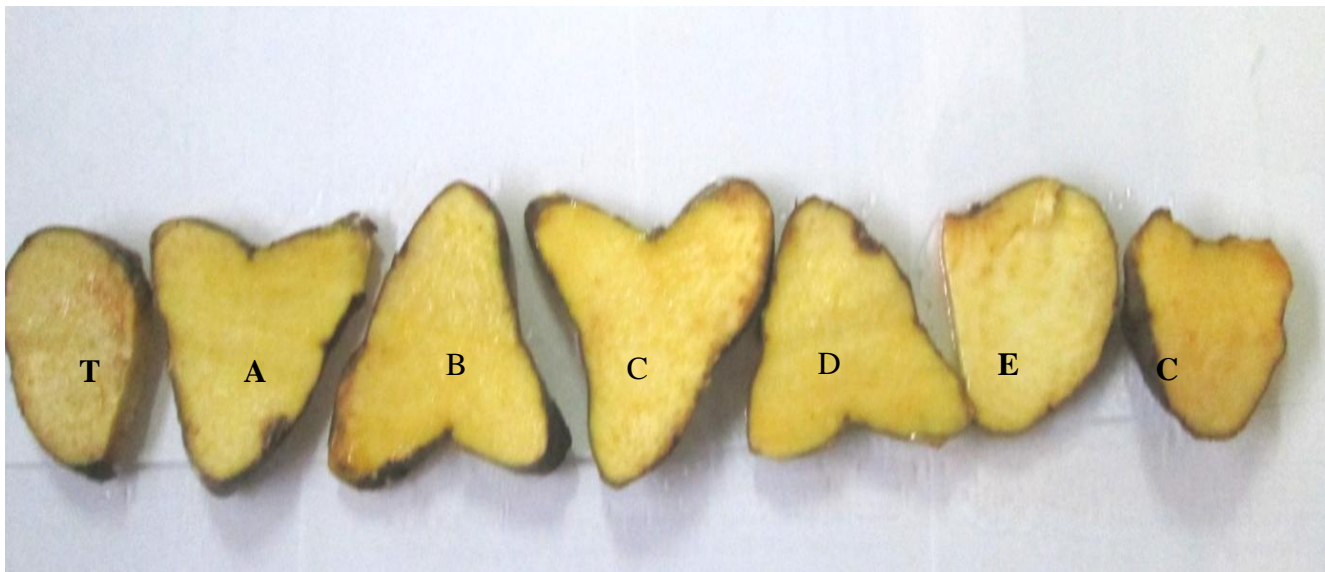
**Table.1** pH and residual activity of PPO of the bulbils of *Dioscorea bulbifera* yam incubated in varying fruit juices

Fruit juices	obtained pH	Interval of pH (FAO, 2005)	Percentage of inhibition (%) for each volume (µl) of fruit juices						
			50	100	200	300	400	500	600
Papaw	5.14 <sup>a</sup>	4.89-5.2	78.125	91.906	98.594	99.109	100	100	100
Orange	2.89 <sup>d</sup>	3.00-3.75	70.37	87.716	96.951	100	100	100	100
Tangerine	3.52 <sup>b</sup>	3.32-4.48	56.687	79.168	95.473	99.237	100	100	100
Grenadillo	2.65 <sup>e</sup>	2.09-2.70	39.056	84.12	99.241	100	100	100	100
Lemon	2.02 <sup>f</sup>	2.00-2.60	9.023	76.195	98.024	100	100	100	100
Grapefruit	3.06 <sup>c</sup>	3.00-3.75	2.139	14.765	86.706	99.096	100	100	100

**Table.2** Ki, inhibition order and inhibition modes of the bulbils of *Dioscorea bulbifera* yam PPO with different fruit juices

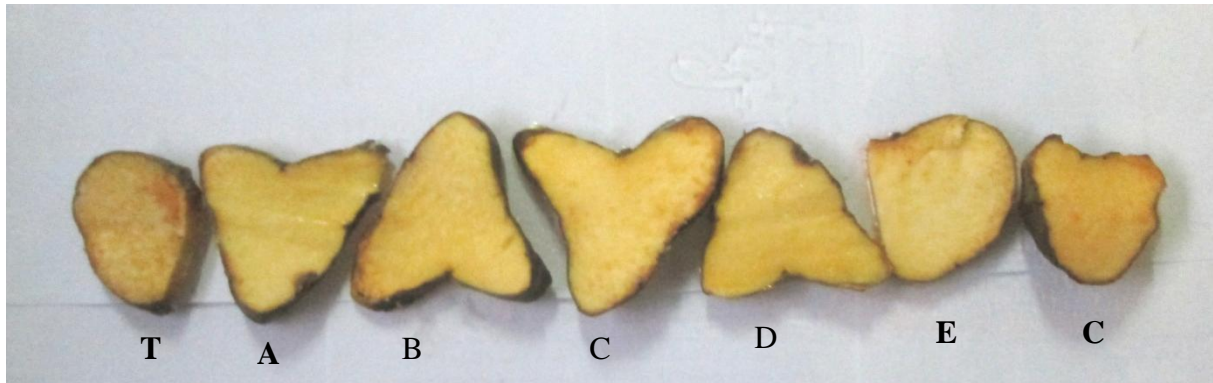
Fruits juices	Ki (µl)	Inhibition Order	Inhibition Type
Papaw	0.087	1	competitive
Orange	0.163	2	competitive
Tangerine	0.554	3	competitive
Grenadillo	1.077	4	competitive
Lemon	1.085	5	competitive
Grapefruit	1.507	6	competitive

**Fig.1** Effect of fruit juices on enzymatic browning of aerial yam (*Dioscorea bulbifera* ). All slices were observed after incubating at 25 °C for 10 min



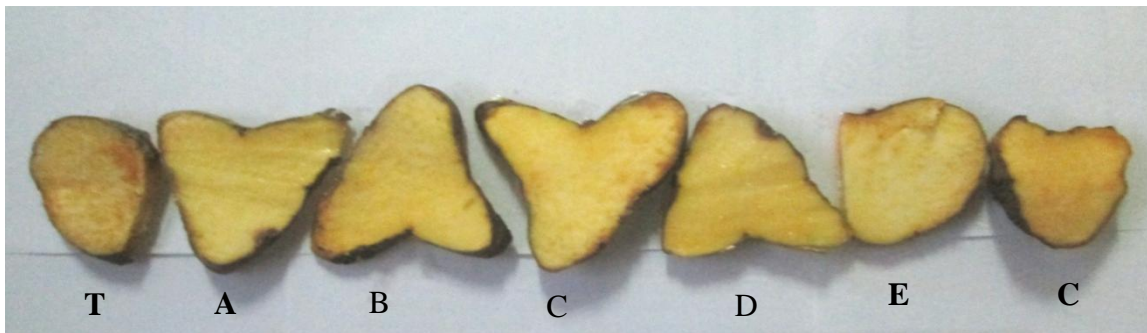
T: Witness without fruit juice, A: Test with lemon juice, B: Test with juice of grenadillo, C: Test with juice of tangerine, D: Test with orange juice, E: Test with juice of grapefruit, F: Test with papaw juice

**Fig.2** Effect of fruit juices on enzymatic browning of aerial yam (*Dioscorea bulbifera* ). All slices were observed after incubating at 25 °C for 20 min



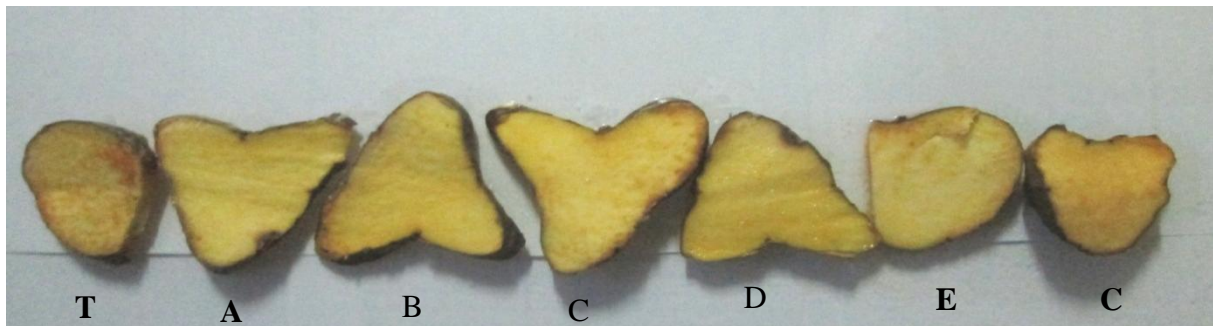
T: Witness without fruit juice, A: Test with lemon juice, B: Test with juice of grenadillo, C: Test with juice of tangerine, D: Test with orange juice, E: Test with juice of grapefruit, F: Test with papaw juice

**Fig.3** Effect of fruit juices on enzymatic browning of aerial yam (*Dioscorea bulbifera* ). All slices were observed after incubating at 25 °C for 40 min



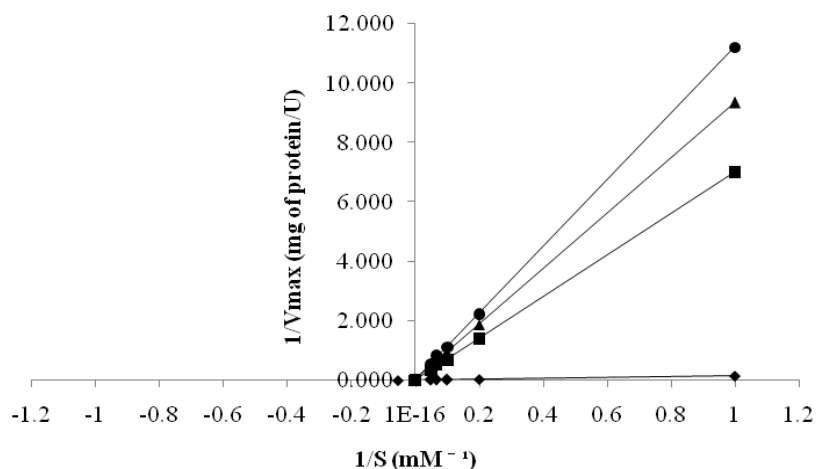
T: Witness without fruit juice, A: Test with lemon juice, B: Test with juice of grenadillo, C: Test with juice of tangerine, D: Test with orange juice, E: Test with juice of grapefruit, F: Test with papaw juice

**Fig.4** Effect of fruit juices on enzymatic browning of aerial yam (*Dioscorea bulbifera* ). All slices were observed after incubating at 25 °C for 60 min

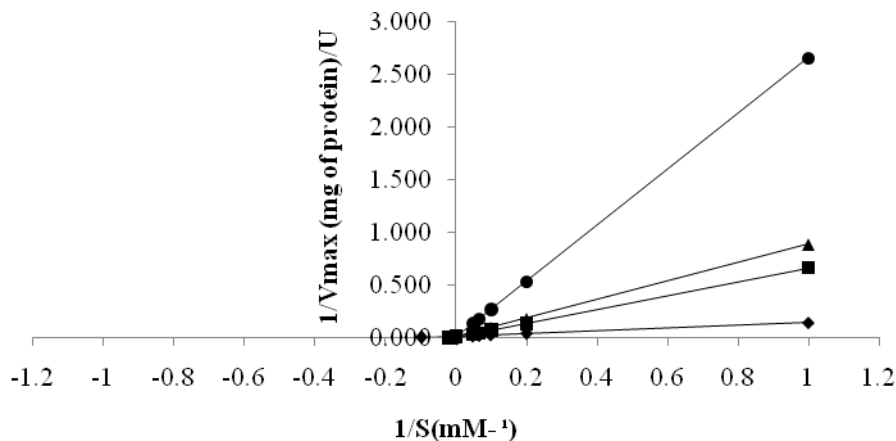


T: Witness without fruit juice, A: Test with lemon juice, B: Test with juice of grenadillo, C: Test with juice of tangerine, D: Test with orange juice, E: Test with juice of grapefruit, F: Test with papaw juice

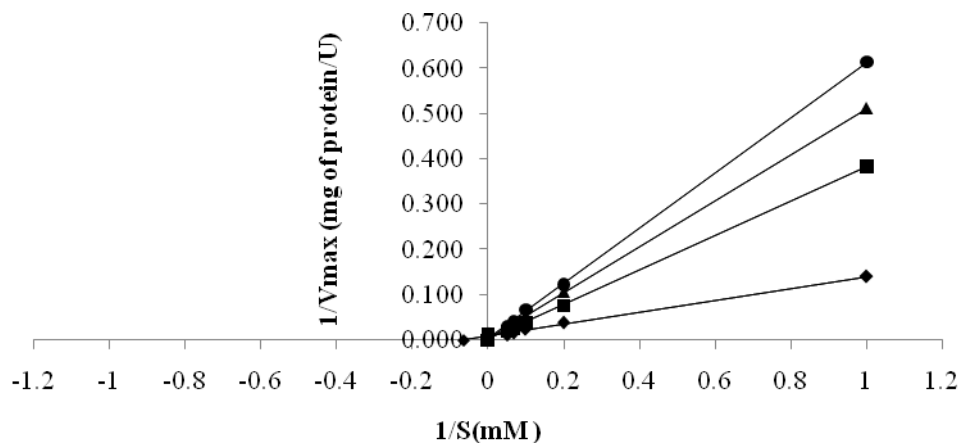
**Fig.5** Lineweaver-Burk plot of the inhibition of the oxidation of pyrocatechol by PPO from aerialyam (*Dioscorea bulbifera*) in the presence of fruit juice from papaw. ♦ 0  $\mu\text{l}$ , ■ 50  $\mu\text{l}$ , ▲ 60  $\mu\text{l}$ , ● 80  $\mu\text{l}$



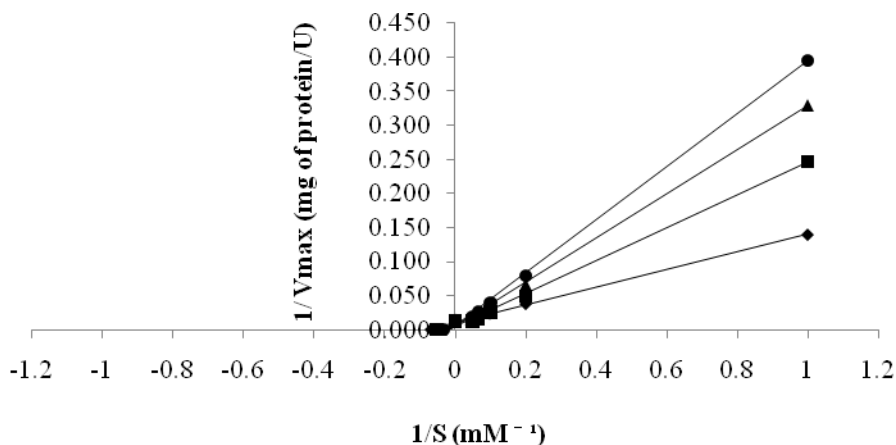
**Fig.6** Lineweaver-Burk plot of the inhibition of the oxidation of pyrocatechol by PPO from aerialyam (*Dioscorea bulbifera*) in the presence of fruit juice from orange. ♦ 0  $\mu\text{l}$ , ■ 50  $\mu\text{l}$ , ▲ 60  $\mu\text{l}$ , ● 80  $\mu\text{l}$



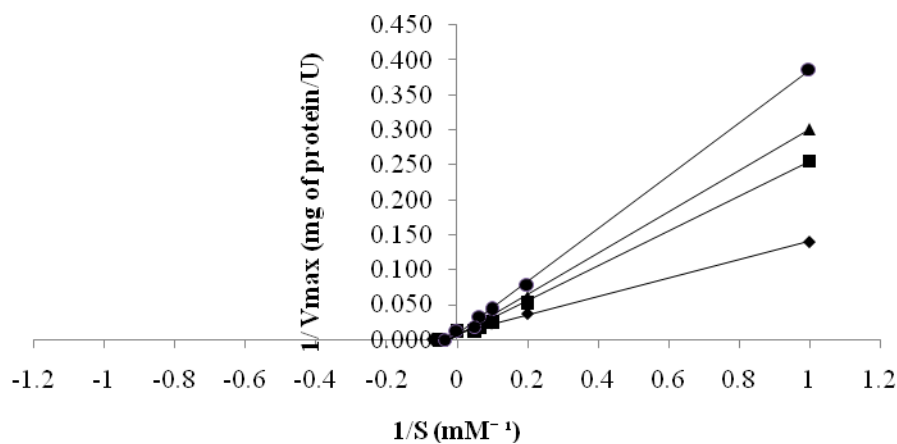
**Fig.7** Lineweaver-Burk plot of the inhibition of the oxidation of pyrocatechol by PPO from aerialyam (*Dioscorea bulbifera*) in the presence of fruit juice from tangerine. ♦ 0  $\mu\text{l}$ , ■ 50  $\mu\text{l}$ , ▲ 60  $\mu\text{l}$ , ● 80  $\mu\text{l}$



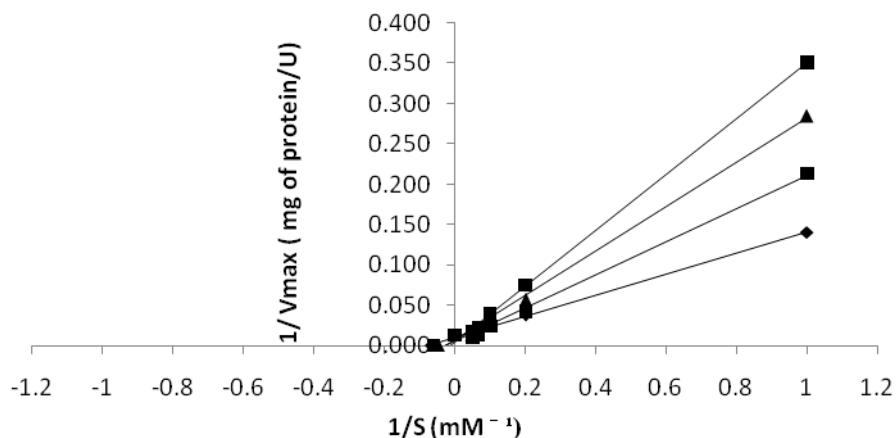
**Fig.8** Lineweaver-Burk plot of the inhibition of the oxidation of pyrocatechol by PPO from aerialyam (*Dioscorea bulbifera*) in the presence of fruit juice from grenadillo. ♦ 0  $\mu$ l, ■ 50  $\mu$ l, ▲ 60  $\mu$ l, ● 80  $\mu$ l



**Fig.9** Lineweaver-Burk plot of the inhibition of the oxidation of pyrocatechol by PPO from aerialyam (*Dioscorea bulbifera*) in the presence of fruit juice from lemon. ♦ 0  $\mu$ l, ■ 50  $\mu$ l, ▲ 60  $\mu$ l, ● 80  $\mu$ l



**Fig.10** Lineweaver-Burk plot of the inhibition of the oxidation of pyrocatechol by PPO from aerialyam (*Dioscorea bulbifera*) in the presence of fruit juice from grapefruit. ♦ 0  $\mu$ l, ■ 50  $\mu$ l, ▲ 60  $\mu$ l, ● 80  $\mu$ l





Those include/understand in great majority of the vitamin C, an antioxidant which plays a significant role in the inhibition of the polyphenol oxidases (Treche, 1997). Their content of vitamin C is likely to evolve/move according to and the degree shelf life of maturity of the fruit. This would explain why certain fruits are more inhibiting than others. The addition of the fruit juices on surfaces of the sections of the bulbil of yam made it possible to inhibit the enzymatic reaction of browning. After 20 min of incubation, the sections of the various tests have undergone a loss of the red colouring which streaked their surface thus showing a reduction with the reaction of the enzymatic browning. Indeed, the fruit juices contain salts like sodium chloride or calcium and the sugars frequently employed in combination with the ascorbic acid of which the use is limited by the modifications of the organoleptic properties of food (De poix *et al.*, 1998; De Rigal, 2001). After 60 min of incubation, the colouring of the various tests remained stable was marked by, the presence of a paste or crust on the surface of the various sections of the bulbil of yam. This action of crust can limit the oxygen content. Indeed, the enzymatic tanning requires oxygen. Thus the reduction of the tanning can be obtained by the maintenance of the foodstuffs in atmosphere deprived or strongly impoverished of oxygen. This is why, after the cutting or the peeling of food, this technique of crust uses the coating or the immersion of food by treating them, either by brine or by syrups of glucose or saccharose to slow down the enzymatic browning (Van den Broeck *et al.*, 1999). These substances will form a film on the surface of food thus decreasing the speed of diffusion of oxygen towards the interior of food and thus the reactivity of the polyphenol oxidases (Bouquetet, 2008). It should however be prevented that the vegetable fabric still physiologically active is not found in the presence of oxygen but under these conditions the processes of ferment would take the top. This is why, this experiment was carried out during a time of incubation around 60 min.

The orange and papaw juices would contain more vitamin C than the other juices used (FAO, 2008), but the juice of lemon and grenadillo have their pH more acid than those of the other juices used. This result is in agreement with that obtained by Janovitz-Klapp *et al.*, (1990) which showed that inhibition is much accentuated with acid pHs. Among these six fruits, the papaw has the pH more raised, but inhibition is total with party of the addition of more than 300 juice  $\mu\text{l}$ . This situation could be explained by the fact why, the papaw is one of the best sources of enzymes of vegetable origin. It is very

rich in vitamin C and carotenoids, two antioxidants powerful. Indeed, the papaw contains a very recognized proteolytic enzyme, papain able to inhibit the PPO (De Rigal, 2001). Moreover, the sensitivity of the vitamin C increases in the presence of enzymes proteolytic, of metals, but especially with ionization and the neutral or alkaline pH. In addition, these results also show that inhibition is not only dependent on the pH, but of other parameters like it underlined well Weemaes *et al.*, (1998b) and Van den Broeck *et al.*, (1999). These authors showed that the resistance of the enzymes is due to the environmental conditions such as the pH, the temperature, the presence of sugars, salts and food additives. Thus, this result makes it possible to conclude that the inhibition of the polyphenol oxidase activity of the bulbils of *Dioscorea bulbifera* yam (cultivar yellow) is dependent on volume used, the pH, the quantity of vitamin C, sugars, salts and certain not identified molecules contained in these various juices.

#### Effect of reversible inhibitors

The type of inhibition brought into play concerning the fruit juices is a competitive inhibition confirmed by maximum speeds of the reactions which do not vary while the constants of Michaelis-Menten change on the representations of Lineweaver and Burk (1934). This result is different from that of Gnanoui *et al.*, (2009) which found that the type of inhibition brought into play for the inhibition of the polyphenol oxidase of yam tuber of the *Dioscorea cayenensis- rotundata* (cultivar "Longbô") by the inhibitor of the rough onion extract is that of traditional noncompetitive inhibition. The inhibitors are fixed according to a mechanism randomly dependent or independent is, on a site of the enzyme other than the active center is, in this one thus preventing the substrate from fixing itself. Certain substances not identified present in the fruit juices which exert a competitive inhibition on the purified enzyme could be analogues of substrate. In comparison with the values of the constants of inhibition ( $K_i$ ) calculated, the papaw juice has it ( $K_i = 0.087$ ) weakest and is the most effective inhibitor since with weak concentration (50  $\mu\text{l}$ ), it thus has the highest percentage of inhibition (78.12 %) very strongly acts on the activity of the purified polyphenol oxidase.

Indeed, the affinity of an inhibitor for an enzyme is given by the constant of inhibition ( $K_i$ ), which represents the concentration in inhibitor for which the half of the enzymatic sites are occupied. Thus, the affinity of an inhibitor is all the more large as  $K_i$  is small. The most

effective inhibitor with the least effective inhibitor, the order of inhibition of these fruits is papaw ( $K_i = 0.087$ ), orange ( $K_i = 0.163$ ), tangerine ( $K_i = 0.554$ ), grenadillo ( $K_i = 1.077$ ), lemon ( $K_i = 1.085$ ) and grapefruit ( $K_i = 1.507$ ).

The fruit juices at different volume had significant effects in controlling browning of aerial yam (*Dioscorea bulbifera*). The fruit juice of papaw treatments were more effective than either others fruit juices treatments. Generally, the results obtained from screening of the different fruits juices confirmed the potency of these fruits and their use in food processing, storage and preservation. It can therefore be concluded that fruits juices can be used in controlling browning of aerial yam (*Dioscorea bulbifera*).

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