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Effect of Cross Contamination on Berries of *Solanum anguivi* Lam and Change of Some Antinutrients of Their Berries During Post-Harvest Storage

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Abstract: The aim of this study was to evaluate the effect of cross-contamination on *Solanum anguivi* Lam berries and the assessment of some antinutritional compounds of berries as affected by ripening stage (green, yellow, orange and red) during the post-harvest storage. Indeed, the rates of ripening and alteration with and without cross-contamination were determinated during ripening. The antinutritional factors such as total oxalate, phytate, tannin and alpha-amylase inhibitor contents were also investigated. Furthermore, the results showed that the rates of ripening and alteration of berries without cross-contamination varied during post-harvest storage. Indeed, the rates of ripening and alteration *S. anguivi* Lam berries increased much more when there was a cross-contamination. All berries were altered at the ninth day of post-harvest storage with cross-contamination while they were all altered at fourteenth day without cross-contamination. As for the antinutritional factors, the ANOVA showed that the ripening stage had significant effect ($p < 0.05$) on oxalate, phytate, tannin and alpha-amylase inhibitor contents. They all decreased meaningfully ($p < 0.05$) at different ripening stage during post- harvest storage. In fact, the oxalate, phytate, tannin and alpha-amylase inhibitor contents had respective rate of decrease of 33.31, 39.73, 23.70 and of 44.28%.

Key words: *S. anguivi* Lam, alteration, antinutrient, berries, cross-contamination, ripening

INTRODUCTION

Solanum anguivi Lam belongs to the *Solanaceae* family and can be found throughout the non-arid parts of Africa (Adanlawo and Akanji, 2003). It's one of the non-tuberous *Solanum* species which is widely distributed in non-arid areas of Africa notably in West Africa, Central Africa, East Africa as well as Southern Africa (Grubben and Denton, 2004). *S. anguivi* Lam grows mostly in the wild, but in Cote d'Ivoire, it is a semi-cultivated for their berries which are bitter and eaten raw as vegetable. They are also used as medicinal materials to treat malaria and fever. *S. anguivi* Lam is commonly known as "gnagnan". It's a kind of very consummate eggplant of Côte d'Ivoire because of their bitter and nutritional vertues (N'Dri *et al.*, 2010). As climacteric fruits or legumes, the berries of *S. anguivi* Lam assume different colors during ripening. During the harvest period, from green to red, berries of *S. anguivi* Lam are fresh and savoury, but after this period, they are not particularly appreciated because they become tasteless (N'Dri *et al.*, 2008). However, berries of *S. anguivi* Lam contain anti-nutritional compounds which prevent the absorption of minerals. Indeed, anti-nutrients are chemicals which have been evolved by plants for their own defense, among other biological functions. They reduce the

maximum utilization of nutrients (especially proteins, vitamins and minerals), thus preventing optimal exploitation of the nutrients present in a food and decreasing the nutritive value (Ugwu and Oranye, 2006). Furthermore, ripening may affect the anti-nutritional composition of *S. anguivi* Lam berries. It would be also affected the organoleptic, nutritional and commercial qualities of berries. During storage, the physiological evolution of vegetables is indicated by the progressive change of the color of the epicarp of vegetables (Andrews, 1995). The cells and the tissues of vegetables such as *S. anguivi* Lam contain natural substances that give them different colors. In addition, adulterated berries may negatively impact consumer's health. Therefore, it is crucial to consume fresh and healthy vegetables (Renard and Chevin, 2007). However, the dried berries of *S. anguivi* Lam are the more valued by consumers because they become insipid (N'Dri *et al.*, 2010). Although, reports abound on biological activities of *S. anguivi* Lam berries like, cholesterol lowering activity (Adanlawo and Akanji, 2008), hypolipidemic property (Elekofehinti *et al.*, 2012a), in vivo antioxidant activity (Elekofehinti *et al.*, 2012b), there are dearth of information on rate of ripening and alteration of "gnagnan" (*S. anguivi* Lam) berries as

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affected by cross-contamination during the post-harvest storage. There is also lack of information regarding *S. anguivi* Lam anti-nutrient contents. Therefore, there is a need to determine the effect of cross-contamination on *S. anguivi* Lam berries and ripening level impact on some antinutritional compounds during post-harvest storage for public and dietary awareness of nutritional status.

MATERIALS AND METHODS

Raw material: In this study, green, yellow, orange and red berries of *S. anguivi* Lam were used (Fig. 1). This plant was identified and authenticated at the Department of National Center of Floristic Research (Felix Houphouet-Boigny University, Cocody-Abidjan). Indeed, their berries were collected at different ripening stage during the post-harvest storage from rural zones of the central part of Ivory Coast. They were immediately transported raw and were stored at $27\pm3°C$

Sample preparation: *S. anguivi* Lam berries at different ripening stages during the post-harvest storage were air dried and then grounded into a powdery fine texture and stored at room temperature in air tight polyethylene bag prior to use.

Cross-contamination studies
Rate of ripening and alteration of berries without cross-contamination: Two batches of five hundred (500) green mast berries of "gnagnan" (*S. anguivi* Lam) were preserved on a laboratory bench at ambient temperature $(25\pm3°C)$ up to their alterations. The berries enumeration was carried out daily according to the colour corresponding to ripening stage and then altered berries were removed. The rates of ripening and alteration were expressed as follows:

$$\text{Rate of ripening} = 100 \times (W_2/W_1) \qquad (1)$$

$$\text{Rate of alteration} = 100 \times (W_3/W_1) \qquad (2)$$

Where:
W_1: Number of berries in the experiment
W_2: Number of berries for ripening stage
W_3: Number of altered berries

Determination of rate of ripening and alteration of berries with cross-contamination: Two batches of five hundred (500) of *S. anguivi* Lam green berries and have been preserved on a laboratory bench at ambient temperature $(25\pm3°C)$ up to their alteration. The enumeration berries was also performed daily according to each ripening stage without removing the altered berries. The rate of ripening and alteration were calculated in the same way as in the first experiment without cross-contamination.

Antinutrient analysis
Total oxalate: AOAC (1970) method was used for the total oxalate determination. 0.75 g of each sample was weighed into 100 mL Erlenmeyer flask containing 76 mL of distilled water and 4 mL of 6N HCl. The mixture was heated in a boiling water bath for 1 h and then cooled in an ice bath. The volume was adjusted with distilled water. This mixture was filtered and two aliquots of 40 mL were placed in two beakers and added to 6 N HCl. It was evaporated to half of its original volume and filtered through Whatman No. 1 filter paper. The precipitate on the filter paper was washed several times with warm double distilled water. To the filtrate, 3 drops of methyl red indicator were added to, followed by the addition of concentrated ammonia solution (1:4 w/v)until the solution turned faint yellow. The solution was then heated at $95\pm5°C$, cooled in an ice bath and filtered through Whatman No. 1 filter paper to remove precipitates containing ferrous ions. The filtrate was brought to boiling and 5 mL of $CaCl_2$ solution(1:20 w/v) was added with constant stirring and allowed to stand overnight. The precipitate was filtered, washed several times with distilled water and the filter paper containing the residue was transferred into a beaker and dissolved using H_2SO_4 (1:4v/v). To precipitate the heavy metals, 5 mL of tungsten phosphate reagent were added in the acidified extract and the mixture was centrifuged at 5000 rpm for 15 min. The beaker was then heated to near boiling in water bath and titrated against standard 0.05M $KMnO_4$ (aq) until first pink colour persisted for more than 30 s. Blank was treated in a similar manner. The oxalate contents were then calculated by taking 1 mL of 0.05M $KMnO_4$ as equivalent to 0.2 M oxalate acid solution.

Phytate: Phytate content was determined according to the method given by Mohammed *et al.* (1986). Five gram of each sample were weighed into 125 mL Erlenmeyer flask containing 25 mL of 3% trichloroacetic acid solution. The mixture was agitated with the help of magnetic agitator KS 10 mark at 25°C for 45 min. Eight milliliter aliquot of the mixture were transferred into a 40 mL conical centrifuge tube and were centrifuged at 20000 rpm for 15 min. Five milliliter of the gotten supernatant were added to 3 mL of 1% $FeCl_3$ and 6 mL of distilled water solution dissolved in 1N HCl. The mixture was heated in a boiling water bath for 45 min and cooled at the ambient temperature (25°C). The solution was then centrifuged at 20000 rpm at 4°C for 10 min. The remaining sediment in the tube was added to 1 mL of 0.5 N HCl and let cool at the ambient temperature (25°C) for 2 h. Then 7 mL of water and 3 mL of 1.5 N sodium carbonate was there added. The mixture was heated in a boiling water bath for 15 min. After cooling at ambient temperature (25°C), the mixture was centrifuged at 20000 rpm at 4°C for 10 min. After centrifugation, 0.2 mL of gotten supernatant was then

diluted in 4.6 mL of distilled water and 2 mL of chromogenic solution. The obtained mixture was heated at 95°C for 30 min. After cooling, the absorbance was measured at 830 nm against a reagent blank not containing phytate by using spectro-photometer. Phytic acid was used as a standard to draw the standard curve, from which the phytate content was estimated.

Tannin: The tannin content was determined according to the methods of Bainbridge *et al.* (1996). 0.5 g of each sample was weighed into a 500 mL beaker. 30 mL of methanol solvent (7:10 v/v) were there added with constant stirring to extract tannin. The filtrates were removed. The samples were filtered through a double layer filter paper to obtain the filtrate. A set of standard solution of Tannic acid (0.1 mg/mL) was prepared ranging from 0.01 to 0.05 mL in methanol (98%, v/v). Besides, 5 mL of vanillin reagent were added to each sample of 1 mL of extract into the tube. After 20 min of incubation to obscurity, the absorbance were read at 500 nm against reagent blank concentration of the same solution from a standard tannic acid curve by using spectrophotometer. The tannin content of the sample was determined from a standard curve of which the regression equation is:

$$Absorbance = 0.37C - 0.04 \qquad (3)$$

Where:
C: Tannin content in each tube

The percentage of tannin (T_C) in dry weight was expressed as follows:

$$T_C = \frac{C \times 100 \times}{w \times dw} \times 100 \qquad (4)$$

Where:
w : Flour weight (g)
F : Dilution facteur
dw : Sample dry weight

alpha-amylase inhibitor: The anti alpha-amylasic activity was carried out according to method described by Sidduraju *et al.* (1990). One gram of sample at each ripening stage was dissolved in 10 mL acetate buffer (100 mM; pH 5.4). The different mixtures were homogenized and allowed to stand at 4°C for 12 h. They were then centrifuged at 5000 rpm for 20 min. A 0.25 mL of aliquot of these extracts was diluted in 25 mL of alpha-amylase solution and 0.5 mL of acetate buffer (100 mM; pH 5.4). The mixtures were pre-incubated at 37°C for 15 min. A 0.5 mL of aliquot of starch (1%, w/v) was there added. After 30min of incubation, the reaction was stopped by addition of 2 mL of dinitro salicylic acid (DNS) and heated in a boiling water bath for 5 min. The

mixtures were cooled for 10 min and then 3mL of distilled water were there added. The absorbance was determinated by using JASCO V-530 spectrophotometer at 540 nm against a blank not containing enzymatic extract. A blank not containing inhibitor was also carried out in a similar manner.

Statistical analysis: All analyses were carried out in triplicates. Results were expressed by means±SD. Statistical significance was established using one-way analysis of Variance (ANOVA) models to estimate the effect of ripening stage on some anti-nutritional compounds levels of flour from berries of *S. anguivi* Lam at 5% level. Means were separated according to Duncan's multiple range analysis (p<0.05), with the help of the software STATISTICA 7 (StatsoftInc, Tulsa-USA Headquarters) and XLSTAT-Pro 7.5.2 (Addinsoft Sarl, Paris-France).

RESULTS AND DISCUSSION
Rates of ripening and alteration of berries with and without cross-contamination: The results showed that the rates of ripening and alteration of berries with and without cross-contamination varied during post-harvest storage (Fig. 2 and 3). They showed also that ripening of *S. anguivi.* Lam berries was observed by successive changes of their green, yellow, orange and red colours at the different ripening stages during post-harvest storage. Our results were not in agreement with that reported on the berries of *S. anguivi* Lam by N'Dri *et al.* (2010) who noted three ripening stages that are green, orange and red colours. Besides, the harvested freshly berries at mast stage [first day (D1)] had all a green colour (first repining stage). This coloration can be attributed to the presence of chlorophyll which had an essential role in photosynthesis (Hounsome *et al.*, 2008). It was observed until the sixth day (D6) and the fourth day (D4) of *S. anguivi* berries post-harvest storage with and without cross-contamination respectively. Moreover, the rate of green berries decreased progressively until sixth day (D6) and the fourth day (D4) of post-harvest storage to reach a rate of 12% with and without cross-contamination. The reduction in the rate of green berries may be due to gradual spoilage of chlorophyll by the chlorophyllase (Hounsome *et al.*, 2008). This result agreed with the findings of Martinoia *et al.* (1982) and Blackbourn *et al.* (1989). These authors showed that the colour changes of banana epicarp were attributed to the degradation of chlorophyll by oxidases set which includes the chlorophyll-oxidase. Otherwise, after these respective storage periods, there were not anymore green berries.
At the second day (D2) of post-harvest storage with and without cross-contamination, it appeared yellow berries (second repining stage) with respective rate of 21 sand
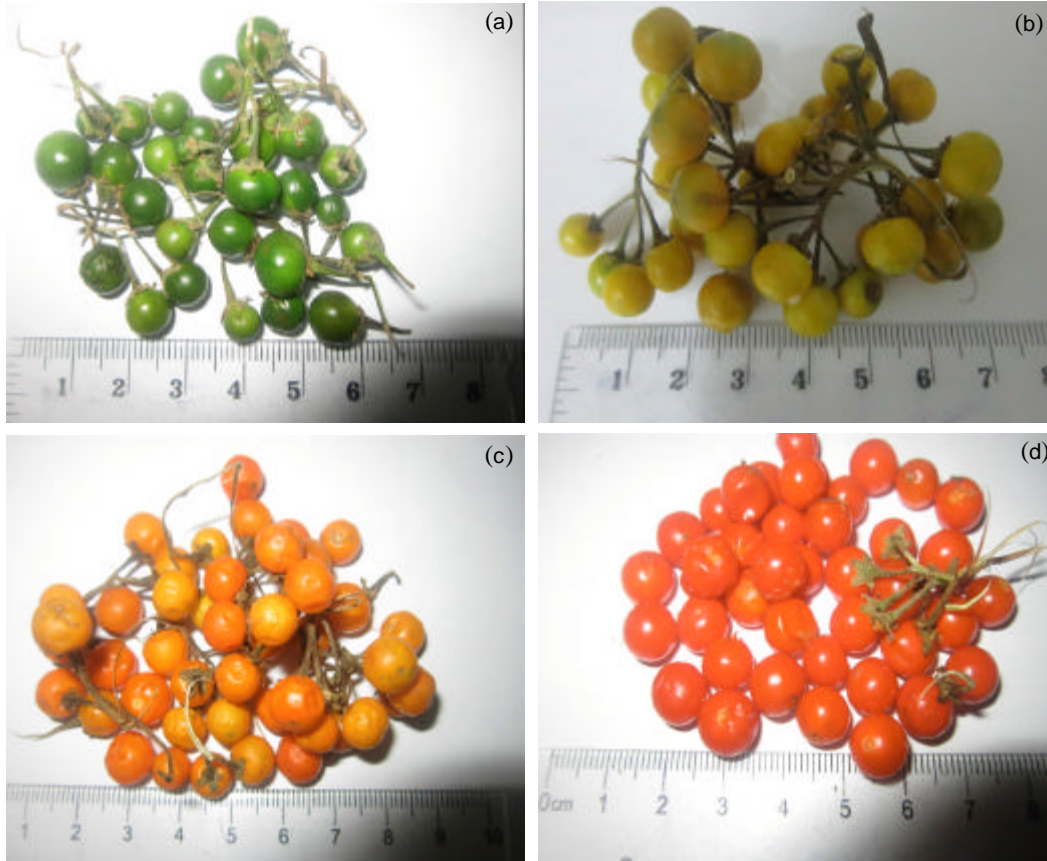
Fig. 1(a-d): *Solanum anguivi* Lam Berries at different ripening stages (Green: (a), Yellow: (b), Orange © and Red (d)) during post-harvest storage
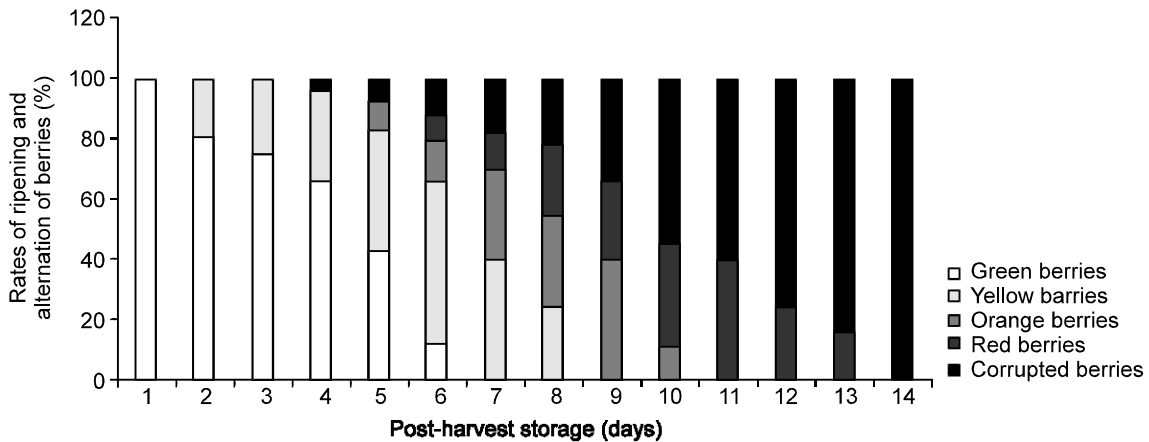

Fig. 2: Bar chart showing the variation of rate of ripening and alteration of *S. anguivi* Lam berries during post-harvest storage without cross-contamination

19%. The appearance of this colour could be resulted from the synthesis of carotenoid pigments such as xanthophyll (Heber and Bowerman, 2001). The main xanthophylls could be the lutein, the viola xanthin and zeaxanthin. Besides, the rate of yellow berries increased to reach a maximum rate of 54% at the sixth day (J6) of post-harvest storage and then decreased until at the seventh day (D7) with a rate of 25% for berries without cross-contamination. As for the berries with cross-contamination, the rate of this berries colour increased
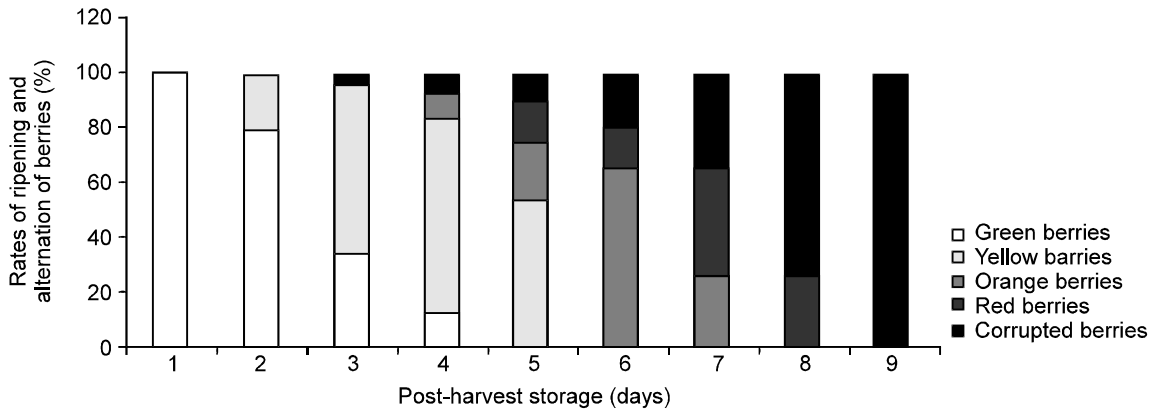
Fig. 3: Bar chart showing the variation of rate of ripening and alteration of *S. anguivi* Lam berries during post-harvest storage with cross-contamination

also to reach a rate of 72% at the fourth day (D4). Then, it decreased to reach a rate of 54% at the fifth day (D5). The decrease of the rate of yellow berries was explained by the gradual disappearance of flavonoids.

Furthermore, the third ripening stage corresponding to the orange colour of berries was observed at the fourth day (D4) and the fifth day (D5) of post-harvest storage with a of 10% rate for berries with and without cross-contamination. The change in orange colour of *S. anguivi* Lam berries would be due to important synthesis of alpha and beta-carotenes, precursors of vitamin A (Hounsome *et al.*, 2008). Besides, the orange colour of berries increased progressively until the ninth day (D9) to reach a 40 rate% for berries without cross-contamination. After this storage time, it decreased to reach a 12% rate at the tenth day (D10). Concerning the berries with cross-contamination, the rate of orange berries also increased progressively until to reach a 60% rate at the sixth day (D6) of post-harvest storage and it then decreased until to a rate of 23% at the seventh day (D7). Indeed, the increase of orange berries rate could be also due to the increase in rate of beta-carotene. Similar result was recorded by Hornero-Mendez *et al.* (2000) who showed that the rate of beta-carotene increased in chili during the ripening.

Otherwise, the red colour of *S. anguivi* Lam berries corresponding the fourth repining stage (last repining stage) appeared at the fifth day (5) and sixth day (D6) of post-harvest storage for berries with and without cross-contamination. The red coloring of *S. anguivi* Lam berries appearing at the fifth day (D5) and sixth day (D6) of post-harvest storage could be attributed to the presence the lycopene. This substance provided important antioxidant properties and played an essential role in the intracellular communication (Heber and Bowerman, 2001). Concerning the berries with cross-contamination, the rate of red berries increased progressively to reach a rate of 75% at the eighth day (D8) of post-harvest storage. There was not a red colour

of berries beyond this post-harvest storage. On the other hand, the rate of red berries was 10% at the sixth day (D6) of post-harvest storage and then it increased until to reach a 40% rate at the eleventh day (J11) for berries without cross-contamination. This rate decreased until the thirteenth day (D13) of post-harvest storage with a 16% rate. At the fourteenth day (D14) of post-harvest storage, the red colour of *S. anguivi* Lam berries did not anymore appear.

The altered berries appeared at the third day (D3) and the fourth day of post-harvest storage for berries with and without respectively with a rate of 4%. This rate increased until the fourteenth day (D14) to reach a maximum rate of 100% for berries without cross-contamination while it ranged from 4% (third day (D3)) to 100% (ninth day (D9) of post-harvest storage for berries with cross-contamination. Indeed, the rate of altered berries of *S. anguivi* Lam increased much more when there was a cross-contamination. These altered berries of *S. anguivi* Lam had brown colour.

Total oxalate content: The total oxalate content of flour from "gnagnan" (*S. anguivi* Lam) berries ranged from 688.32±32.02 mg/100 g dw (dry weight) to 459.03±32.21 mg/100 g dw for first ripening stage and fourth repining stage respectively, representing 33.31% of decrease (Fig. 4). It appeared slight differences between the total oxalate contents of flour from "gnagnan" (*S. anguivi* Lam) berries at the last three repining stages (yellow, orange and red colour). The analysis of variance showed that the ripening stage had significant effect ($p < 0.05$) on oxalate content (Table 1). However, the total oxalate contents at the last three ripening stages did not differ meaningfully ($p > 0.05$) during post-harvest storage. Otherwise, they differed significantly ($p < 0.05$) from the total oxalate content of flour from "gnagnan" (*S. anguivi* Lam) berries at first ripening stage (green colour). Indeed, the reduction of total oxalate content could be due to the ripening and germination which are the

Table 1: ANOVA table for one-way of main effect of ripening stages on antinutritional composition during ripening

Parameters	Effect	df	Sum of squares	Mean square	F-ratio
	Ripening stage	3	66660.603	22220.201	15.821
Total oxalate	Error	4	5617.857	1404.464	-
	Total	7	72278.460	-	-
	Ripening stage	3	3.799	1.266	20.674
Phytate	Error	4	0.245	0.061	-
	Total	7	4.044	-	-
	Ripening stage	3	0.001	0.000	25.459
Tannin	Error	4	0.000	0,000	-
	Total	7	0.001	-	-
	Ripening stage	3	1.628	0.543	160.008
Inhibitor unit	Error	4	0.014	0.003	-
	Total	7	1.642	-	-

Symbol of *state shows significant difference at 5% level   Df: Degrees of freedom

factors reducing the total oxalate content following the increase of the water content in the food product (Ghavidel and Prakash, 2007). During post-harvest storage, the obtained values were higher than those recorded by Agoreyo *et al.* (2012) who reported the respective values of 41.72 and 23.97 mg/100 g dw for round and oval purple eggplants. Otherwise, the reduced oxalate content on berries could have positive impact on the consumers' health. The reduction of oxalate levels during ripening is expected to enhance the bioavailability of essential dietary minerals of the berries and reduce the risk of kidney stones occurring among consumers.

Phytate content: The phytate content of flour from "gnagnan" (*S. anguivi* Lam) berries varied from 3.75±0.28 to 2.26±0.14 mg/100 g dw for first repining stage and fourth repining stage respectively, representing 39.73% of decrease (Fig. 5). The flour from the green berries (first ripening stage) had the highest phytate content whereas the lowest value was obtained with the flour from the red berries (fourth repining stage). Furthermore, the analysis of variance indicated that the ripening stage had meaningful effect (p = 0.05) on phytate content (Table 1). Indeed, the phytate contents decreased meaningfully (p<0.05) during post-harvest storage. This decrease may be attributed to the phytase activation at the time of the biochemical and physiological modifications in the plant during ripening (Chavan and Kadam, 1989). In the flour, the phytates formed the complex with the proteins and some minerals such as calcium, iron, zinc, the manganese and copper (Amarowicz *et al.*, 2010). Otherwise, the results were lower than those published for round and oval varieties of the purple eggplant (*Solanum melongena*) by Agoreyo *et al.* (2012) who noted respective values of 28.19 and 18.67 mg/100 g dw. Moreover, there was no significant difference (p>0.05) between the phytate contents at first and second ripening stage and also between the phytate contents at third and fourth ripening stage.
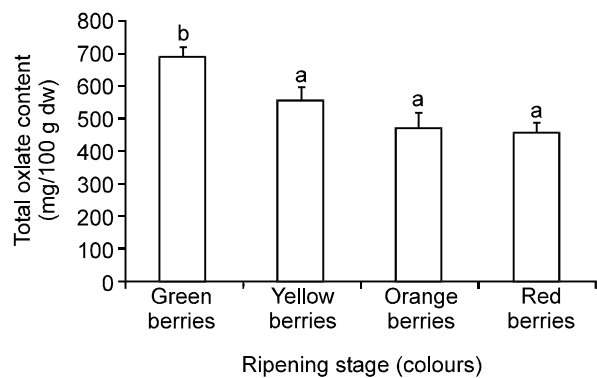

Fig. 4: Bar chart showing the variation in mean total oxalate content of flour from the berries of *Solanum anguivi* Lam during ripening
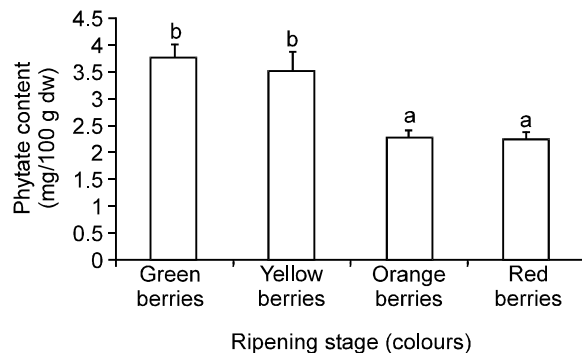

Fig. 5: Bar chart showing the variation in mean phytate content of flour from the berries of *Solanum anguivi* Lam during ripening

Tannin content: The tannin content of flour from "gnagnan" (*S. anguivi* Lam) berries ranged from 0.135±0.006 to 0.103±0.003 mg/100 g dw for first ripening stage and fourth ripening stage, respectively, representing 23.70% of loss (Fig. 6). The flour from green berries (first repining stage) had the highest tannin content whereas the flour from red berries possessed the lowest tannin content during
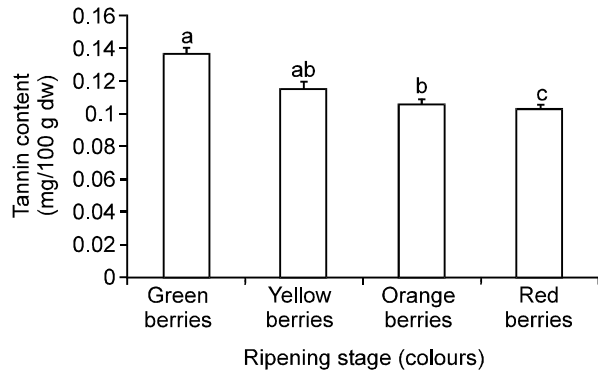
Fig. 6: Bar chart showing the variation in mean tannin content of flour from the berries of *Solanum anguivi* Lam during ripening
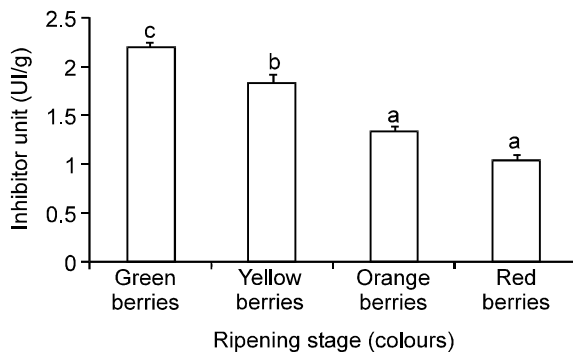

Fig. 7: Bar chart showing the variation in mean alpha-amylase inhibitor content of flour from the berries of *Solanum anguivi* Lam during ripening

post-harvest storage. Otherwise, the analysis of variance showed that the ripening stage had significant effect (p = 0.05) on tannin content of flour from "gnagnan" (*S. anguivi* Lam) berries at different ripening stage (Table 1). Indeed, the tannin contents decreased meaningfully (p<0.05) during post-harvest storage. The reduction of tannin content during the ripening would be caused by the damage of cellular partitions. During the ripening, the gotten values were lower than 1 mg/100 g dw. This result showed that tannin did not affect the *in vivo* bioavailability of the proteins (Li *et al.*, 2003). Our obtained values were lower than those recorded for round and oval varieties of purple eggplants which were 12.82 mg/100 g dw and 11.34 mg/100 g dw respectively (Agoreyo *et al.*, 2012). These results were also lower than the 26.45 mg/100g dw recorded by Ugwu and Oranye (2006) on African breadfruit. However, there was not meaningful differences (p>0.05) between the tannin content at the first and second repining stage and also between the tannin content at the second and third repining stage.

Alpha-amylase inhibitor content: The alpha-amylase inhibitor content of flour from "gnagnan" (*S. anguivi* Lam)

berries varied from 22.02±0.42 to 12.27±0.41 UI/g dw for first repining stage and fourth repining stage, respectively representing 44.28% of decrease (Fig. 7). The flour from the green berries (first repining stage) had the highest alpha-amylase inhibitor content whereas the lowest value was obtained with the flour from the red berries (fourth repining stage). Otherwise, the analysis of variance indicated that the repining stage had meaningful effect (p<0.05) on alpha-amylase inhibitor content of flour from "gnagnan" (*S. anguivi* Lam) berries during post-harvest storage (Table 1). Indeed, the alpha-amylase inhibitor content decreased meaningfully (p<0.05) during post-harvest storage. This decrease would be bound to the decrease of phenolic compounds and phytate contents (Deshpande *et al.*, 1982; Weselake *et al.*, 1983). Indeed, the phenolic compounds and the phytates played the role of inhibitor of alpha-amylasic activity and their rates decreased during ripening. Besides, the reduction of the proportion of maltose free units of following the deterioration of the starch would also contribute to decrease the rate of inhibitor of the alpha-amylase (Weselake *et al.*, 1983). Nevertheless, the rate of inhibitor of alpha-amylasic activity remained persevering for the last two ripening stages. However, it did not appear significant differences between alpha-amylase inhibitor contents at the third and fourth repining stage.

Conclusion: The study revealed that the ripening of *S. anguivi* berries was observed by their successive change of coloring green, yellow, orange and red at different ripening stages. It showed also that the ripening was accelerated when there was a cross-contamination. Otherwise, the results showed that ripening could also be a way of reducing antinutrients in food samples. The determination of the amount of antinutrients in the samples is also necessary because they can reduce essential nutrients bioavailability

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