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Research article

INFLUENCE OF THE DRYING ON THE SCOPOLETIN INDUCTION OF CASSAVA CHIPS PRODUCED IN BENIN

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ABSTRACT: The production of cassava chips in Benin is a very common traditional activity. Previous studies on the conservation of these chips have shown the presence of scopoletin in the fresh roots and a significant increase of this substance after six days of sun drying. The objective of the present investigation was to determine the effect of drying on the induction of scopoletin of cassava chips. Four cassava varieties were dried using five different methods. Water content of chips obtained showed that sun drying on grid method at room temperature removed the maximum amount of water. The determination of scopoletin content by HPLC revealed the mean values ranging from 5.87 to 12.89 mg / kg for the fresh roots. After six days of drying, these contents increased significantly regardless of the drying method used. The study showed that the drying is a major factor promoting the accumulation of scopoletin. The method of sun drying on grid at room temperature indicates the highest scopoletin content and the lowest moisture content regardless of the cassava root variety studied.

Key words: Concentration, Hydroxycoumarin, HPLC, effect, Root.

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INTRODUCTION

In Benin, cassava is one of the most important plants grown on the entire national territory unlike shea and cashew, thus occupying a prominent place (Padonou, 2010). The storage of fresh cassava roots is very difficult because they are highly perishable products which are subjected to serious problems of contamination by fungi, bacteria and other germs due to the increase in metabolic activity (Ugochukwu et *al.* 1974). Beeching et *al.* (1998) reported that the deterioration process begins already between 24 and 48 hours after harvest. This process is known as post-harvest physiological deterioration by Bushman *et al.* (2000). Therefore, the transformation of cassava roots into chips is necessary for good storage. The results of Wareing *et al.* (2001) and Bassa *et al.* (2001) showed that the chips are most often attacked by fungi of the genus Aspergillus, Fusarium, Penicillium and Mucor. Their contamination by these fungi can cause the discoloration of the product, unpleasant taste and the production of musty odor (Gwinner *et al.*, 1996). Within this context, attention is increasingly paid to the problems of contamination of cassava chips by fungi and the production of mycotoxins. Thus, research carried out in Benin on cassava chips from all varieties revealed the absence of aflatoxins in cassava chips produced locally despite the presence of *Aspergillus flavus*, a fungus responsible for the production of these toxins (Gnonlonfin *et al.*, 2008).
In addition to these studies, Bushman *et al.* (2000) showed the presence of certain compounds such as esculincoumarinic, esculetinscopoletin and scopolin in cassava.

Among these, scopoletin is the compound whose concentration is the highest and having antifungal and antimicrobial properties (Rodriguez *et al.,* 2000; Giesemann *et al.,* 2008). Furthermore, the study on the storage of cassava chips showed the presence and the accumulation of scopoletin (Gnonlonfin *et al.,* 2011). According to Obidoa & Obasi (1991) scopoletin content varies from cassava roots to the products of its transformation. For 100g of cassava sample, these researchers found 57.6 ± 2 mg of scopoletin in gari and 78.8 ± 3.2 mg of scopoletin in cassava flour. The results obtained from the studies of Gnonlonfin *et al,* (2011), showed strong accumulation of scopoletin in the fresh roots varying on average between 4.1 and 11 mg/kg; after six days of sun drying and the content of scopoletin did significantly increase on average between 23.3 and 111.1 mg/kg in all the varieties. This is followed by a decrease in concentration after three months of storage. These authors did not determine the drying method giving the highest content of scopoletin. Therefore, the present study aims at determining the effect of drying on the induction of scopoletin of cassava chips produced in Benin.

MATERIELS AND METHODS

Plant Material

The plant materials used consists of Kpaki Kpika, Kpaki Soan and Logo gesse Kotorou varieties (Table 1). They were harvested on February 14th, 2012 after thirteen months of growth in the fields of Northern Agricultural Research Center - Ina (CRAN-Ina) where the conditions to grow cassava and produce chips are conducive in Benin.

Table 1: Characteristics of cassava varieties used

Name of varieties	Characteristics
Kpaki Soan (KS)	Bitter variety
	Red stem
	Inedible leaf
	High hydrocyanic acid content
Kpaki Kpika (KK)	Sweet variety
	White stem
	Edible leaf
	Low hydrocyanic acid levels
Logo gesse Kotorou (LK)	Sweet variety
	White stem
	Edible leaf
	Low hydrocyanic acid levels

Analytical Material

The determination of scopoletin was carried out on an HPLC system Agilent technologie 1120 Compact LC using a Lichrospher 5 µm RP-18 column (25 cm long; 4.1 mm internal diameter). This system is equipped with an isocratic pump (G4286A Isopump); a loop injector (Rheodyne P/N 5067-4102) and a UV-visible detector (G4286A).

Solvents

The solvents, supplied by Sigma Aldrich, were analytical grade for extractions and HPLC grade for HPLC analysis.

Fresh root processing methods into dried chips

The harvests were performed in the traditional way (uprooting and cut-slice of cassava roots) and they were taken to the laboratory. The various types of drying were done from 9am to 18pm for six days. Figure 1 shows the cassava chips production pattern.

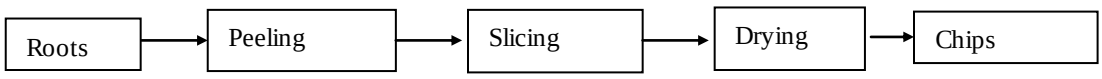

Figure 1: Diagram of traditional cassava chips production

Different types of drying

Sun drying on grid at room temperature

The pulps cut and washed were spread on raised grids lying at least 1m from the floor surface, to avoid contamination by impurities (sand grains, bird droppings etc.). Sun drying was carried out for six days at room temperature. The dryer were taken out in the morning at 9 am and was brought in each evening at 18 pm. Dryer were also brought in when rain threatens.

Traditional drying

The pulps cut but unwashed were spread on a PET bag, and spread out on the ground by the roadside at room temperature. They were dried for six days. During the six days of drying, the pulps were kept on ground even if there was rain. Thus, these roots were subjected to bad weather.

Shade drying

The pulps cut and washed were spread were on a bench in a clean room, closed and unventilated. These pulps were dried for six days.

Oven-drying

The pulps cut and washed were dried in an oven at a gradual temperature between 30-50 °C at 10 °C intervals. The variation of temperature was carried out in three stages. The oven was adjusted at 30 °C for 48 h, then at 40° for 48 h and finally at 50 °C for 48 h. The drying in the oven was also conducted for six days.

Storage of chips

The chips from different types of drying were distributed into small polyethylene (PET) bags of five kg. These bags were weighed using a weighing machine and then were immediately sewed with a needle and thread. The bags were labeled and put in a store already prepared for that purpose. The bags of chips obtained from different types of drying were placed on different pallets and at a distance of at least one meter from the walls of the store. These bags were stored for three months from February to May.

Sampling

Three kg of cut-slice and unwashed pulps of fresh roots were sampled. They were used for the determination of scopoletin content after peeling. Immediately, after obtaining the chips i.e. after the drying period, three kilograms of chips from different methods were sampled. Pending the dosage of scopoletin, these samples were distributed in batches and stored at – 4 °C. Batch 1 was the fresh cassava pulps, batch 2 was the chips obtained from the sun drying on grid at room temperature, batch 3 was the chips from traditional drying, and batch 4 represents the chips from shade drying while batch 5 was chips from the oven drying. After three months of storage of the batches, the content of scopoletin was quantified.

Water content determination

The water content and volatile matter content is defined as the sudden loss of mass in the measurement conditions. The method of Audigié *et al.* (1978) was applied to cassava chips samples obtained.

Analysis method of scopoletin by HPLC

Preparation of standards and samples

A stock solution of 1 mg/ml was prepared from scopoletin standards (Sigma Aldrich - TCH - 3471; purity ≥ 98%). We then made two dilutions (5 th and 10 th) of this stock solution. These three levels of concentrations were used for the determination of the response function. The extraction of scopoletin was performed following the method described by Bushman *et al.*, 2000. 5 g of each batch were macerated in 20 mL of absolute ethanol (Sigma Aldrich) for 48 h and filtered through a flutted filter paper Whatman No.1 (Qualitative cycle 150 mm cat No. 1001 150) in small sterile flasks.

Analytical Conditions

Scopoletin was separated and quantified on a high performance liquid chromatograph under the following analytical conditions:

- ✓ A column C18 (LiChrospher 100 (5 µm), 100 × 4.6 mm Merck Chemicals, Darmstadt, Germany) ;
- ✓ Mobile phase: aqueous solution of 0.5% orthophosphoric acid- acetonitrile (5/95, v / v);
- ✓ Flow rate: 1mL/min ;
- ✓ Pressure : 47.3 bars ;
- ✓ Running time: 10 min ;
- ✓ Detection wave length: 280 nm.

Quantification and validation procedure

Calibration curve

The calibration curve was established by injecting 20 µL of each dilution. The range of scopoletin contents was: 200, 400 and 2000 ng. The regression model was a linear function f (x) = ax + b.

Limit of detection and quantification

The limit of detection (LD) is the amount giving a peak of height equal to three times the background noise. The limit of quantitation (LQ) is the amount giving a peak of height equal to10 times the background noise. LD = 3 s/a; LQ = 10 s/a (Hubert, 2006) s = standard deviation of the response, a = slope of the calibration curve.

Repetitiveness

To validate the method developed by the accuracy criteria, we conducted an intra-day and inter-day repetition. Accuracy was determined for three levels of scopoletin, three times on the same day for intra-day accuracy and three times for four different days for inter-day accuracy. Three injection tests were performed for each dilution and the averages of the surfaces obtained under curves were calculated. These averages and their standard deviations were used to calculate the coefficients of variation

Recovery rate

The extraction yield was determined from cassava chips flour 5 g with addition of 4.6 mg of pure scopoletin. The recovery rate (RR) is given by the formula RR (%) = (A - B) / C x 100 with A: amount of scopoletin extracted from cassava chip flour mixed with pure scopoletin; B: amount of scopoletin in the cassava chip flour without addition of pure scopoletin; C: amount of added scopoletin.

Application of the method for the dosage of scopoletin from different batches

After calibration, the method was applied to the samples. The expression of the content (T) of scopoletin in µg in 100 g of sample is T = 20 q with the amount of scopoletin in 20 µL of extract injected.

Statistical Analysis

SPSS 16.0 software was used for statistical analysis. The test of Newman Skeuls (SNK) was applied to compare the averages of the water content, the content of scopoletin in the varieties tested and the average of scopoletin contents after three months of storage.

RESULT AND DISCUSSION

Water content

Table 2 summarizes the water content of cassava roots according to the variety and drying type.

Table 2: Water content of the different varieties of cassava roots

	Water content (%)			
Varieties Drying type	KK	KS	LG	Average
Sun on grid	44.5a	52.6b	45.8a	47.63
Traditionalsun	47.2d	56.7e	47.7f	50.53
Oven-drying	50.24g	53.20h	51.06i	51.5
Shade-drying	46.6a	54.2k	47.8l	49.53

The numbers in the same column followed by different letters are statistically different according to SNK test ($p < 0.05$).

The results of the water content show variability for all methods of drying performed (Table 2). The water content values obtained in all the drying methods were lower than that found in the fresh root which was 62.8% according to Bradbury & Holloway, (1988). In general, the variety KS contains more water after drying than other varieties for all drying methods. Moreover, the averages calculated allowed us to say that the method that removed the maximum water from the matrix was that of sun drying on grid at room temperature.

Validation of the identification method of scopoletin

Scopoletin was identified by HPLC method in ethanoic extracts by comparing the absorption chromatograms of the standard and that of the sample at the same retention time 2.49 min (Figure 2).

Sensitivity Test of the method

20 µL of each of these solutions (stock and dilutions), the following quantities 2000 ng, 400 ng and 200 ng were injected respectively. The chromatograms obtained are shown in Figure 2

The chromatograms of the three volumes (quantities) of injected standard show a single peak. The peaks were well superposed and appeared at the same time called retention time (RT) .This retention time was an average of 2.49 min. This result shows that the developed method was sensitive.

Specificity and selectivity

The developed method was used to separate and establish scopoletin in the matrix (cassava chips) represented by the extract. The quality of the chromatographic separation revealed the high degree of selectivity of the assay procedure

Function of response (calibration curve)

The calibration curve was established for a range of quantity from 200 ng to 2000 ng per molecule. The method showed a good linear relationship between the areas under curves of the peaks obtained and the set of quantities in scopoletin. The equation of the calibration curve is the regression line Y = 10.62X - 2.6164 with a correlation coefficient $R^2 = 0.999$.

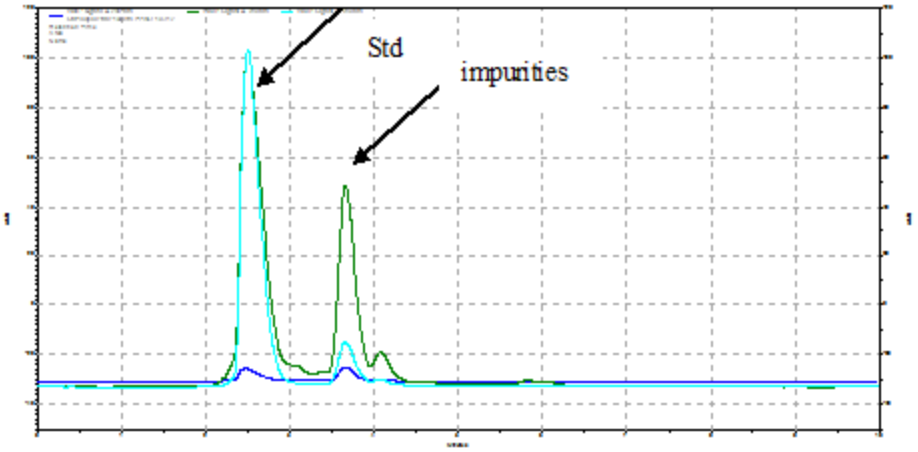

Figure 2: Chromatograms of standard quantities of scopoletin

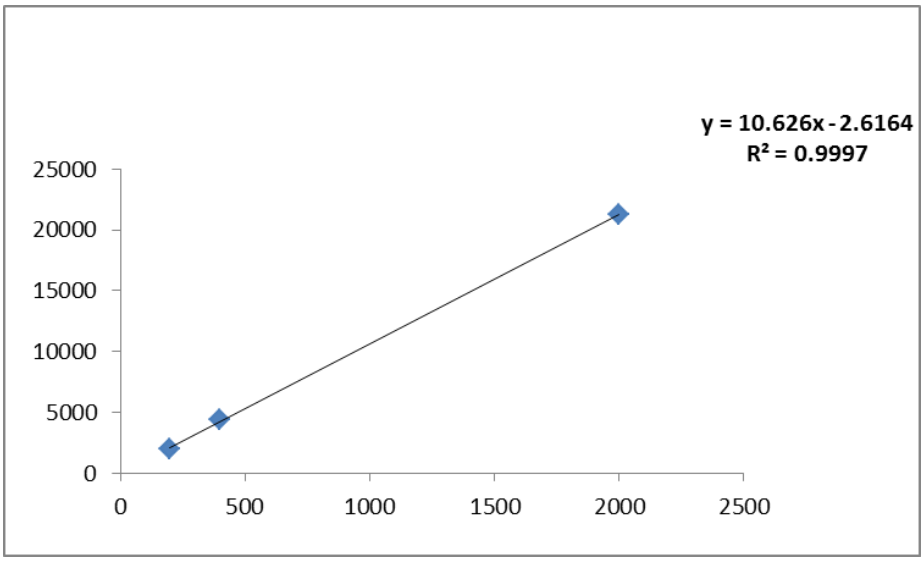

Figure 3: Regression line of scopoletin

The scopoletin assay by HPLC reveals the presence of scopoletin in the cassava roots. The intra-day variation coefficients (CV) are below 5% for the all the series of quantity analyzed, which is great in relation to the strategy used, which recommends 15% (SFSTP 2000). The injected quantities and the average areas under curves were used to plot the regression line (Figure 3). The equation of this regression line is: Y = 10.62X - 2.616 with a correlation coefficient R^2 = 0.999. This correlation coefficient obtained shows that there is a good correlation between the values of the areas under curve for the amount of scopoletininjected. These values therefore show a linear regression equation Y = 10.62X – 2.616 of the regression line which may be used to calculate the amount of scopoletin contained in the sample injected. Thus, the developed method was validated and could be therefore used for routine analyzes of scopoletin assay in cassava roots and chips.

Linearity

In the interval of dosage i.e. 200 ng - 2000 ng, the response (signal) was proportional to the amount of scopoletin in the sample. This indicates the linearity of the analytical procedure used.

Accuracy of the method

To estimate the accuracy of the developed method, the coefficients of variation (CV), repeatability (intra-day precision) and inter-day accuracy were calculated. The averages and their standard deviations were used to compute coefficients of variation (Table 3).

Tableau 3: Intra-day variation coefficient of scopoletin.

Quantity (ng)	Average	Standard Deviation	Coefficient of variation (CV) in %
2000	21229	312	1.47
400	4433	155	3.49
200	1958	58	2.96

Limits of detection and quantification

The values of these limits are presented in Table 4. These results confirm the sensitivity of the analytical system used.

Table 4: Limit of Detection (LD) and Limit of Quantification (LQ)

Standard quantity (ng)	LD and LQ (ng/µL)	
	LD	LQ
200	0.81	1.73
400	1.18	2.79
2000	2.13	7.68

Recovery rate

The recovery rates of scopoletin by the extraction method were on average 98% which is excellent given the complexity of the plant matrix. This result is similar to that of Ganse *et al.* (2013) who also found a recovery rate of 98%.

Application of the method to the samples

The comparison of the chromatogram of the sample to that of the standard indicates that the peaks appear at the same time and therefore have the same retention time. This allowed us to conclude that the injected sample contains scopoletin (Figure 4).

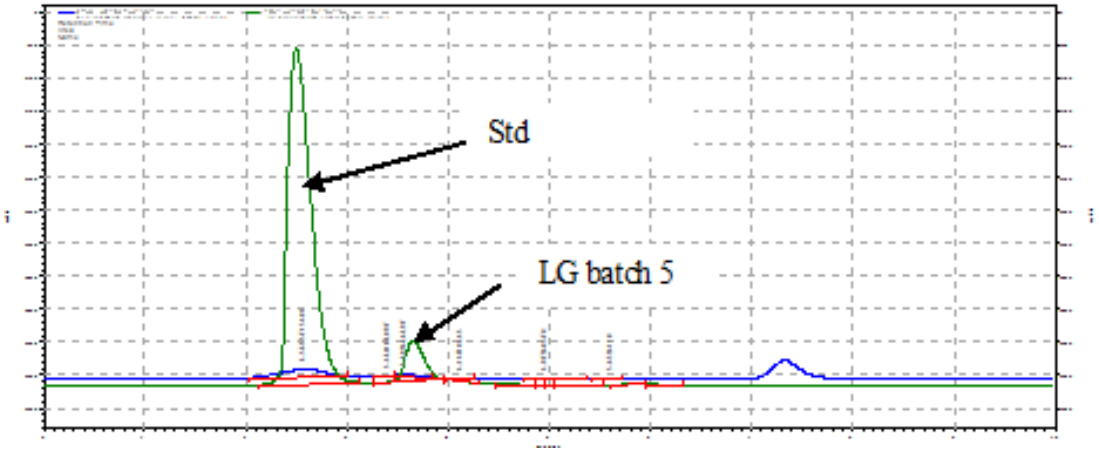

Figure 4: standard sample chromatogram

Tables 5 and 6 show scopoletin contents in fresh roots and cassava chips respectively

Table 5: Scopoletin content in fresh roots

Samples	Content (mg/Kg)
KK	12.89
KS	5.87
LG	9.11

The results in Table 5 show the presence of scopoletin in fresh cassava roots in all the varieties. These results corroborate those of Wheatley & Schwabe (1985); Bushman *et al.* (2000); Gnonlonfin *et al.* (2011). The values obtained were slightly greater than those found by Gnonlonfin *et al.* (2011).

Table 6 summarizes the content of scopoletin after the drying and the storage of batches 2, 3, 4 and 5.

Tableau 6: Scopoletin content in mg/Kg

	Batch 2			Batch 3			Batch 4			Batch 5		
Samples	KK	KS	LG	KK	KS	LG	KK	KS	LG	KK	KS	LG
Content (6 days)	198.93	47.65	88.90	110.64	27.03	44.62	16.22	14.11	17.93	39.34	16.40	22.7
Content (3 Months)	29.92a	14.91b	19.2c	0.22d	0.21e	0.12f	0.50g	0.7h	0.40g	0.14j	0.44k	0.22d

The numbers in the same row followed by different letters are statistically different according to SNK test ($p < 0.05$).

The analysis of table 4 indicates that the sweet or bitter characteristic of cassava roots did not affect the presence of scopoletin. Variety KK contained the highest scopoletin. This confirms the results obtained by Gnonlonfin *et al.* (2011). The traditional drying method also revealed the presence of scopoletin but lower than the sun drying on grid at room temperature. As for the drying in the shade, there was accumulation of scopoletin content whatever the variety, but is small compared to sun drying methods. From these results it is apparent that the drying is an essential parameter in the concentration of scopoletin. Furthermore, drying in an oven shows as in all other cases, an increase in the content of scopoletin compared to that found in the fresh roots. However these contents were lower than those of the sun-drying method on grid at room temperature, and this in all the varieties.

Moreover, regardless of the variety and the type of drying there is a decrease of scopoletin after three months of storage. This may be explained by further metabolic transformation. The studies of Gutierrez *et al.* (1995), Edwards *et al.* (1997) on the biosynthesis and metabolism of hydroxycoumarins in sunflower have shown that specific peroxidases can transform scopoletin in a blue-black precipitate. The results obtained are in line with those of Bushman *et al.* (2000) who showed that the content of scopoletin in cassava roots decreases after six days of storage. These results are also consistent with those of Gnonlonfin *et al.* (2011). In general, for all the methods of drying performed scopoletin contents found were lower than those obtained by Obidoa & Obasi (1991) in cassava flour and in gari. Sun drying on grid at room temperature concentrated more scopoletin after drying with a percent reduction of 76.74% on average while that of the other drying methods was around 99, 3%. The method of sun drying on grid at room temperature can be the optimal method for the production of cassava chips.

CONCLUSION

The results of this work indicate that the drying is a main factor promoting the accumulation of scopoletin. The method developed for the determination of scopoletin showed its acceptability according to the criteria of SFSTP strategy used. The method of drying in the sun on grid at room temperature reveals the highest content of scopoletin and the lowest water content irrespective of the variety of cassava root studied. This method may be the optimal method for the production of chips in Benin.

Conflict of Interests

The authors declare no conflict of interests.

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