Research Article

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Changes in scopoletin concentration in cassava chips from four varieties during storage

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Abstract

BACKGROUND: The use of the root crop cassava (*Manihot esculenta* Crantz) is constrained by its rapid deterioration after harvesting. Chemical and spectroscopic examination earlier revealed the accumulation of the four hydroxycoumarins esculetin, esculin, scopolin and scopoletin derived from the phenylpropanoid pathway, during the time course of postharvest deterioration. In this investigation the scopoletin level in parenchymal samples of four cassava cultivars used in Benin, i.e. Kpaki kpika, Kpaki soan, Logoguesse kotorou and BEN 86052, was investigated by high-performance liquid chromatography (HPLC).

RESULTS: Presence was shown in all four varieties with a mean in fresh roots between 4.1 and 11.1 mg kg^{-1} dry weight. A strong increase in the content of scopoletin was noticed after a peeling and drying process (6 days) for chip production, the mean content reaching 242.5 mg kg^{-1} dry weight in the cultivar BEN 86052. After 3 months of storage this had decreased to 0.7 mg kg^{-1} dry weight.

CONCLUSION: Strong accumulation of scopoletin in cassava roots used for chip production in Benin is followed by a decrease in its concentration.

Keywords: cassava roots; chips; wound response; scopoletin; Benin

INTRODUCTION

Cassava or manioc (*Manihot esculenta* Crantz, Euphorbiaceae) is widely grown as a staple food and animal feed in countries of tropical and subtropical Africa, Asia and Latin America between 30°N and 30°S, with a total cultivated area of over 13 million ha, more than 70% of it being in Africa and Asia.[1] In comparison to others crops cassava counts as one of the most important sources of food. Hence, concerning carbohydrate this commodity, after rice, sugarcane and maize, is of major importance nourishing over 500 million people in the developing countries of the tropic and subtropics. Its main economic value is in its storage roots with a dry matter containing more than 80% of starch.[1]

Despite its economic importance, this crop suffers from two major disadvantages. All parts of the plant contain cyanogenic glycosides that can release toxic cyanide. Thorough processing of the root significantly reduces or eliminates this toxicity prior to consumption.[2] However, the major constraint for the use of cassava is that farmers, processors and consumers suffer substantial losses during storage of the roots. This is due to rapid physiological deterioration processes that initiate within 24–48 h after harvest,[3] followed by a second stage (after 5–7 days) involving microbial decay. The biochemical processes and histological changes of the first stage are to be classified as postharvest physiological deterioration (PPD) or vascular streaking.[2] A discoloration of the vascular tissue to blue-black is the first visible sign of PPD, followed by a browning of the parenchymatic tissues. Microscopically, observations of the early processes reveal occlusions in the xylem vessels, as well as the occurrence of tyloses: a thickening of the horny layer of the skin.[4] This is followed by a rapid accumulation of fluorescent compounds in the parenchyma that have been described by different authors and identified as hydroxycoumarins, scopolin, esculin and scopoletin.[3,5,6] Scopoletin is a phenolic coumarin and an important member of the group of phytoalexins isolated from many plants.[7,8] Phytoalexins are low molecular weight compounds which are biosynthesized by plants *de novo* following exposure to microorganisms.[9]

This study investigates cassava chips produced from four different varieties. Results include the level of moisture and scopoletin respectively over a period of 3 months.

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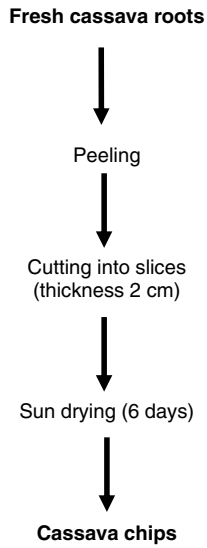

Figure 1. Flow diagram outlying the production of cassava chips.

MATERIALS AND METHODS

Plant material

Manihot esculenta Crantz stalks from the cultivars named Logoguesse kotorou, Kpaki Soan, Kpaki kpika and BEN 86 052 were grown at the North Agricultural Research Center located at Ina, Institute of Agricultural Research, Ministry of Agriculture, Livestock and Fisheries, Benin. Developed cassava roots were harvested after 6 months. Apart from Kpaki Soan, which is typically high in content of cyanogenic glucosides (bitter cultivars), all others are typically low (sweet cultivars). These cassava cultivars are those mostly used by farmers for cassava chip production in Benin.

Plant material processing, sampling, storage and chemicals

The processing method of fresh cassava tubers roots into chips is shown in Fig. 1. Cassava chips were made in January 2010 and dried for 6 days (common practice in Benin) on a cemented floor following the various steps in Fig. 1. Immediately after this drying period, dried cassava chips (four lots of 12 kg, each lot corresponding to one cultivar) were stored in woven polyethylene bags, each holding 4 kg, for 3 months (January–April 2010) in a storage room on a pallet.

Approximately 3 kg of cassava roots as fresh-cut slices were sampled from each lot (cassava cultivar) at harvest (fresh), after 6 days of sun drying (chips) and after 3 months of storage (chips). For the stored chips the 3 kg was a mixture from the three bags holding the cultivar. Cassava roots (fresh-cut-slices) were analyzed for scopoletin content upon arrival in the laboratory, while dried roots (chips) were stored at 4 °C until scopoletin analysis was performed within a week from sampling. The temperature and relative humidity at the time of drying and during storage were recorded using a data logger (EL-USB, Lascar Electronics Ltd, Salisbury, UK), which was placed on the top of the sample piles during drying and under the roof (in the shade) of the store room both inside and outside. The data were recorded at 1 h intervals.

Pure scopoletin (95%) was purchased from Sigma Aldrich (St Louis, MO, USA). All other chemicals used were analytical or HPLC grade.

Moisture content determination

Moisture content of samples (in triplicate) was taken upon arrival in the laboratory. This parameter was evaluated by heating at 105 °C for 2 h to constant weight.[10]

Scopoletin extraction and analysis

Scopoletin extraction procedure was based on that of Buschmann *et al*. (2000).[2] Fresh roots (1000 g (one to two roots) of each cultivar) were sampled, peeled and cut into slices (~2 cm). 3000 g of chips were sampled from each lot (cultivar) of 12 kg. From this, a subsample of 1500 g was taken and ground using a laboratory mortar. Extract was obtained from the fresh sliced root as well as from the dried roots (chips) in triplicate using the method of Buschmann *et al*.[2] Thus 1g in triplicate of fresh peeled (parenchyma) cassava tissue or chip material was homogenized in 10 mL ethanol (Sigma Aldrich) by means of a blender. The extract was filtered through a fluted filter paper (Whatman No. 1), evaporated at room temperature (~25 °C) to an end volume of 3 mL and stored until use at −18 °C.

The compound was analyzed with an autosampler HPLC (model L-2200, Waters, Milford, MA, USA) using a modified method of Bushmann *et al*.[2] The main modifications were therefore as follows:

- column C18 (LiChrospher® 100 C18 (5 µm), 100 × 4.6 mm Merck Chemicals, Darmstadt, Germany);
- mobile phase: aqueous solution 0.5% H_3PO_4 with acetonitrile in a gradient of 2–98%;
- running time: 45 min;
- flow rate: 0.5 mL min^{-1};
- detection wavelength: 280 nm;
- detector: ultraviolet HPLC-UV (Waters, model L-2400).

The method was investigated for recovery by spiking true samples with pure scopoletin.

Statistical analysis

SPSS for Windows version 12.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Analysis of variance (ANOVA) and Student–Newman–Keuls (SNK) test were used to compare the means of percentages of moisture content throughout the storage period and across the cassava varieties. This test was also used to compare the mean levels of scopoletin between cassava varieties. Independent variables included cassava varieties and storage time.

RESULTS

Environmental parameters at the time of drying and during the storage period

During the drying period temperature and relative humidity ranged from 17.5 to 44.5 °C and from 7.0 to 85.5%, respectively. During the storage period, for the outside of the storage room the temperature ranged from 19.5 to 46.5 °C and the relative humidity from 7.5% to 87.5%. In contrast, inside the storage room, these parameters ranged from 22.5 to 45.0 °C and from 9.0% to 70.0%, respectively.

Moisture content of fresh cassava roots and of cassava chips

Results from the present study show that the moisture content varied significantly between varieties and throughout the storage period (Table 1).

Table 1. Moisture content of cassava roots and chips throughout the storage period

Cassava variety[a]	Mean moisture content (%)[b] Fresh roots	After 6 days of drying	After 3 months of storage
Kpaki Soan	82.1 ± 1.5b	15.5 ± 0.5d	4.6 ± 0.2a
Kpaki Kpika	82.0 ± 0.9b	10.8 ± 0.8a	4.1 ± 0.2a
Logoguesse kotorou (local)	80.2 ± 0.2ab	13.1 ± 0.7bc	4.2 ± 0.2a
BEN 86052	79.1 ± 0.9a	12.7 ± 0.9b	4.6 ± 0.2a

Values in the same column followed by the same letter are not significantly different. Least significant difference at $P = 0.05$.
[a] Local names.
[b] Mean moisture content; mean ± SD; $n = 3$.

Table 2. Scopoletin level in cassava roots and chips throughout the storage period

Cassava variety[a]	Mean scopoletin level[b] (mg kg^{-1} cassava tissue) Fresh roots	After 6 days of drying (chips)	After 3 months of storage (chips)
Kpaki soan	4.1 ± 0.2a	23.3 ± 0.2a	0.2 ± 0.06b
Kpaki kpika	4.2 ± 0.2a	111.1 ± 0.1c	0.2 ± 0.06b
Logoguesse kotorou (local)	11.1 ± 0.2c	45.5 ± 0.4b	0.1 ± 0.01a
BEN 86052	4.9 ± 0.5b	242.5 ± 0.2d	0.7 ± 0.00c

Values in the same column followed by the same letter are not significantly different. Least significant difference at $P = 0.05$.
[a] Local names.
[b] Scopoletin level on dry basis; mean ± SD; $n = 3$.

Scopoletin identification and quantification

Scopoletin was identified and quantified in the samples of fresh cassava roots and flours made from the processed chips by HPLC, an example of a chromatogram showing the scopoletin peak being shown in Fig. 2. Scopoletin was found in the roots of all four cassava cultivars at harvest, after processing into chips, including sun drying and after 3 months of storage as chips. An increase of scopoletin was observed after 6 days of sun drying and a decrease of this concentration was observed after 3 months of storage in all cassava cultivars (Table 2). The concentrations in the unstored chips (6 days of drying) were (mg kg^{-1} dry weight/corresponding to mg kg^{-1} original fresh weight) Kpaki soan (23.3/4.4), Kpaki kpika (111.1/14.6), Logoguesse kotorou (45.5/7.4) and BEN 86 052 (242.5/38.9). The ratio between the highest and the lowest mean level of scopoletin found in the fresh roots was around 3 : 1. However, for the chips after 6 days of storage the ratio was seen to be close to a factor 10 : 1 (Table 2). Analytical recovery of the method used was showed to be 95%, with a linearity of $R^2 = 0.9864$.

No significant correlations were observed between scopoletin levels and moisture content.

DISCUSSION

Data showed the presence of scopoletin in all four cassava cultivars in Benin, confirming the results described by other authors.[2,5] The early increase (after 6 days of drying) (Table 2) is due to its accumulation in the cell walls of xylem vessels and in parenchymatic cells.[2] The increase can be interpreted as a direct response to wounding that occurred during processing into chips. It is interesting to observe that while the levels found in fresh intact root tissues differed relatively little between cultivars, one cultivar (BEN 86 052) showed a much higher capacity of *de novo* formation upon damage (chip processing). The results obtained in this study corroborate the obervations of Buschmann *et al.*[2] in

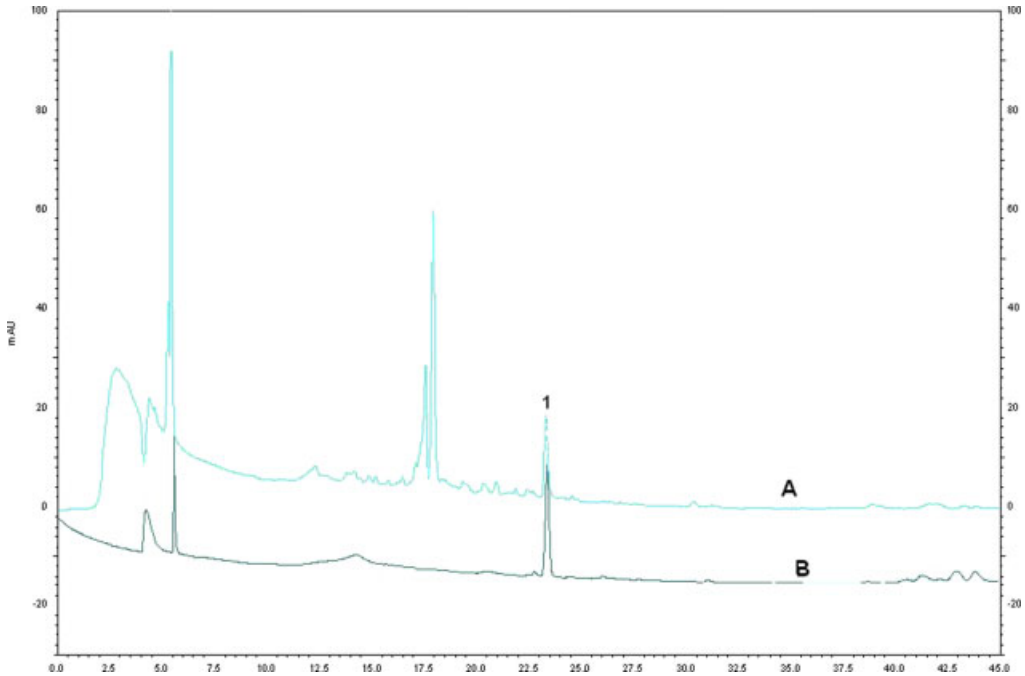

Figure 2. Liquid chromatograms obtained during analysis: (A) cassava flour; (B) pure standard; 1, scopoletin peak.

some Colombian cassava cultivars that were stored as fresh cutting roots slices under controlled conditions (dark, 29 °C and 80–90% relative humidity) for 6 days (highest content of scopoletin reached 123.94 nmol g^{-1} fresh weight).

This is the first report on processed cassava chips that were sun dried for 6 days under environmental conditions (mean temperature of 33 °C and relative humidity 34.6%). This processing method helps to prolonge the shelf life of the product, which is a staple food for many people, especially in the developing world including Benin.

The levels of scopoletin had declined significantly after a storage period of 3 months (Table 2). This can be explained by further metabolic transformations. Early research on the biosynthesis and metabolism of hydroxycoumarins in sunflower showed that specific peroxidases can metabolize scopoletin into an insoluble blue-black precipitate *in vitro* and *in vivo*.[9,11]

In general, the biosynthesis of hydroxycoumarins is still under debate. For scopoletin, a separate synthesis from ferrulic acid was suggested by Strack (1997).[12] In contrast, Cabello-Hurtado *et al*.[13] suggest a synthesis starting from caffeic acid to esculetin which is then modified to scopoletin. It seems that there are different possible pathways and that plants may have developed convergent pathways leading to scopoletin.[14]

The toxic mechanism of scopoletin against microorganisms is still unclear. This raises intriguing questions as to the potential role of scopoletin in plant wound response. If scopoletin is toxic, how does the plant cell cope with it? Strack (1997)[12] suggests that coumarins are glycosylated during their synthesis into a non-toxic glycoside, and then stored in the vacuole. In response to stress, this glycoside is then transported into the apoplast, where it is deglycosylated by a beta-glycosidase into the active compound. This would explain the blue fluoresence due to scopoletin that was observed using fluorescence microscopy (excitation 366 nm) in the cell walls of the xylem tissue and inside the parenchymatic cells of deteriorated cassava roots by Buschmann *et al*.[2]

The results presented here demonstrate that there is a strong accumulation of scopoletin in cassava varieties used for chip production in Benin as followed by a decrease in its concentration. Investigations are needed to fully understand the biosynthesis mechanism and regulation of this compound.

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REFERENCES

1 El-Sharkawy MA, Cassava biology and physiology. *Plant Mol Biol* **53**:621–641 (2003).
2 Buschmann H, Rodriguez MX, Tohme J and Beeching JR, Accumulation of hydroxycoumarins during post-harvest deterioration of tuberous roots of cassava (*Manihot esculenta* Crantz). *Ann Bot* **86**:1153–1160 (2000).
3 Beeching JR, Han Y, Gomez-Vasquez R, Day RC and Cooper RM, Wound and defense responses in cassava as related to post-harvest physiological deterioration. *Recent Adv Phytochem* **32**:231–248 (1998).
4 Rickard JE and Gahan PB, The development of occlusions in cassava (*Manihot esculenta* Crantz) root xylem vessels. *Ann Bot* **52**:811–821 (1983).
5 Tanaka Y, Data ES, Hirose S, Taniguchi T and Uritani I, Biochemical changes in secondary metabolites in wounded and deteriorated cassava roots. *Agric Biol Chem* **47**:693–700 (1983).
6 Uritani I, Biochemistry on postharvest metabolism and deterioration of some tropical tuberous crops. *Bot Bull Acad Sin* **40**:177–183 (1999).
7 Murray RDH, Mendez J and Brown SA, *The Natural Coumarins: Occurrence, Chemistry and Biochemistry*. Wiley, Chichester (1982).
8 Tal B and Robeson DJ, The induction, by fungal inoculation, of ayapin and scopoletin biosynthesis in *Helianthus annuus*. *Phytochemistry* **25**:77–79 (1986).
9 Edwards R, Stones SM, Mellado MCG and Jorrin J, Characterization and inducibility of a scopoletin degrading enzyme from sunflower. *Phytochemistry* **45**:1109–1114 (1997).
10 Association of Official Analytical Chemists, *Official Methods of Analysis* (14th edn). AOAC, Arlington, VA (1984).
11 Gutierrez MC, Parry AD, Tena M, Jorrin J and Edwards R, Abiotic elicitation of coumarin phytoalexins in sunflower. *Phytochemistry* **38**:1185–1191 (1995).
12 Strack D, Phenolic metabolism, in *Plant Biochemistry*, ed. by Dey PM and Harbone JB. Academic Press, London, pp. 387–416 (1997).
13 Cabello-Hurtado F, Durst F, Jorrin JV and Werck-Reichhart D, Coumarins in *Helianthus tuberosus*: characterization, induced accumulation and biosynthesis. *Phytochemistry* **49**:1029–1036 (1998).
14 Kai K, Shimizu B, Mizutani M, Watanabe K and Sakata K, Accumulation of coumarins in *Arabidopsis thaliana*. *Phytochemistry* **67**:379–386 (2006).

