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Anti-nutritional properties and organic acids composition of seven varieties cassava (*Manihot esculenta* Crantz) consumed in Côte d'Ivoire

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Abstract

In Côte d'Ivoire, new varieties cassava (*Manihot esculenta* Crantz) are produced and consumed by populations. The aim of this research work was to study the anti-nutritional and antioxidant properties, organic acids of seven varieties cassava (*Agbablé* 3, *Bondoukou* 4, *Bonoua* 2, *Boufouh* 3, *Boufouh* 4, *Soclopouopo* 3 and *Totoba* 2) to better value them. Thus, phytate contents varied from 50.34±0.03 (*Bondoukou* 4) to 60.36±0.04 mg/100g (*Soclopouopo* 3), those of tannins contents from 0.046±0.014 (*Agbablé* 3) to 0.054 ± 0.014 (*Bondoukou* 4). The flavonoid contents range from 0.62 ± 0.02 (*Agbablé* 3) to 3.01 ± 0.01 mg / 100 g (*Boufouh* 3). Total polyphenols ranged from 51.24±1.79 (*Totoba* 2) to 81.12±1.97 mg/100 g (*Bondoukou* 4). Oxalates contents varied from 5.1±1 (*Agbablé* 3) to 5.6±1.5 (*Bondoukou* 4). Anti-oxidant power varied from 0.06 (*Agbablé* 3, *Bondoukou* 4, *Boufouh* 3) to 0.08 (*Boufouh* 4). Most abundant organic acids are carboxylic acids ranging from 122.5±2.04 (*Boufouh* 3) to 147.65±0.61 µg/100g (*Bonoua* 2) and benzoic acids from 80.11±0.17 (*Boufouh* 3) to 126.15±0.98 (*Bondoukou* 4). *Agbablé* 3 variety has low contents of tannins, flavonoids, total phenols and oxidizing power. *Bondoukou* 4 and *Bonoua* 2 varieties respectively had low contents of phytates and total polyphenols.

Keywords: Cassava, flours, variety, organic acids, anti-nutritional, *Manihot esculenta*

Introduction

Root crops constitute a dominant portion of a standard diet for people of the tropics and subtropics. Roots and tubers have always been critical components in the diet during the early evolution of mankind and they were the most important food crops of very ancient origin, always associated with human existence and survival, and allowing human socio-economic history (Asha and Nair, 2002) [2]. Cassava originated in South America and subsequently was distributed to tropical and subtropical regions of Africa and Asia (Blagbrough *et al.*, 2010) [7]. Cassava is the most widely cultivated root crop in the tropics and because of long growth season (8–24 months), its production is limited to the tropical and subtropical regions in the world. Cassava is a perennial shrub belonging to the family Euphorbiaceae. The genus *Manihot* comprises 98 species and *M. esculenta* is the most widely cultivated member (Nassar *et al.*, 2008) [24]. Cassava plays an important role as staple for more than 500 million people in the world due to its high carbohydrate content (Blagbrough *et al.*, 2010) [7]. Cassava (*Manihot esculenta*) roots are largely cultivated in tropical countries. It has been earmarked as the crop that can spur rural industrial development and raise income for producers, processors, and traders (Echebiri and Edaba, 2008) [15]. Cassava is the chief source of dietary food energy for the majority of people living in the lowland tropics, and much of the subhumid tropics of West and Central Africa. Cassava has been viewed as a means of attaining household food security and increasing food availability (Lebot, 2009) [21]. A number of bioactive compounds, namely, cyanogenic glucosides such as linamarin and lotaustralin, noncyanogenic glucosides, hydroxycoumarins such as scopoletin, terpenoids, and flavonoids, are reported in cassava roots (Blagbrough *et al.*, 2010; Reilly *et al.*, 2004) [7, 29]. In recent years, cassava has received more attention as a root crop not only for its resistance to abiotic stresses (Chavez *et al.*, 2005; Baguma, 2004) [11, 4], but also its high productivity with considerable starch yield (up to 30% of the fresh root or 80% of root dry matter) and purity (Ceballos *et al.*, 2006; Benesi, 2005) [10, 6]. In Côte d'Ivoire, new varieties cassava (*Agbablé* 3, *Bondoukou* 4, *Bonoua* 2, *Boufouh* 3, *Boufouh* 4, *Soclopouopo* 3, *Totoba* 2) were obtained by CNRA (National Center for Agricultural Research) after several crosses between two original varieties V4 (IAC, white)

and V23 (Anango agba, yellow) (N'zué *et al.*, 2001) [27]. However, these new varieties although many produced and consumed do not have enough biochemical, technological, etc. (Sodjinou, 2006) [34]. This study of traditional varieties cassava consumed in Côte d'Ivoire aims to determine some parameters (anti-nutrients, anti-oxidants, organic acids) in order to better value and promote them in various fields.

Material and Method

Plant material

The roots of seven new improved cassava (*Agbablé 3, Bonoua 2, Boufouh 3, Boufouh 4, Bondoukou 4, Soclopouopo 3, Totoba 2*) of twelve months old were harvested from CNRA (National Center for Agronomic Research) experimental plot (Bouake, Côte d'Ivoire). Roots were put in coolers to preserve their fresh state, they were transported to the Laboratory of Biochemistry and Food Technology of University of Nangui Abrogoua (Abidjan, Côte d'Ivoire) where study was conducted.

Flour sample preparation

Fresh roots were peeled manually and cut into small pieces with a inox knife. The pieces obtained were washed and dried in an oven at 45 °C for 48 hours. Dry pieces were crushed and sieved to obtain the raw cassava flour that has been used for various analyzes.

Methods

Phytates determination

The method described by Wheeler and Ferrel (1991) [39] was used for determination of phytates content. A quantity (0.5 g) of dried powdered sample was mixed with 25 mL of trichloroacetic acid (3%, w/v) and centrifuged at 3500 rpm for 15 min. The supernatant obtained was treated with FeCl₃ solution and the iron content of the precipitate was determined using spectrophotometric method at 470 nm. A 4 :6 Fe/P atomic ratio was used to calculate the phytic acid content.

$$\text{Phytates (mg/100g)} = \frac{DO_{490} \times 4}{0,033 \times m_e}$$

Where,

Calibration line: DO₄₉₀ = 0.033. Mass (µg) sodium phytate; R² = 0.99. m_e: mass (g) of the sample.

Tannins determination

Tannins of samples were quantified according to Bainbridge *et al.* (1996) [5]. For this, 1 mL of the methanolic extract was mixed with 5 mL of vanillin reagent and the mixture was allowed to incubate at ambient temperature for 30 min. Thereafter, the absorbance was read at 500 nm by using a spectrophotometer (PG Instruments, England). Tannins content of samples was estimated using a calibration curve of tannic acid (2 mg/mL) as standard.

$$\text{Tannins (mg/100g)} = \frac{DO_{500} \times 10^3}{3,11 \times m_e}$$

where,

Calibration line: DO₅₀₀ = 3.11. Mass (mg) tannic acid; R² = 0.99 m_e: mass (g) of the sample

Flavonoids determination

The total flavonoids content was evaluated using the method

reported by Meda *et al.* (2005) [22]. Briefly, 0.5 mL of the methanolic extract was mixed with 0.5 mL methanol, 0.5 mL of AlCl₃ (10%, w/v), 0.5 mL of potassium acetate (1 M) and 2 mL of distilled water. The mixture was allowed to incubate at ambient temperature for 30 min. Thereafter, the absorbance was measured at 415 nm by using a spectrophotometer (PG Instruments, England). The total flavonoids were determined using a calibration curve of quercetin (0.1 mg/mL) as standard.

$$\text{Flavonoids (mg/100g)} = \frac{DO_{415} \times 2 \times 10^3}{18.12 \times m_e}$$

Where,

Calibration line: DO₄₁₅ = 18.12. Mass (mg) quercetin ; R² = 0.99; m_e: mass (g) of the sample.

Polyphenols determination

Polyphenols content was determined using the method reported by Singleton *et al.* (1999) [32]. A quantity (1 g) of dried powdered sample was soaked in 10 mL of methanol 70% (w/v) and centrifuged at 1000 rpm for 10 min. An aliquot (1 mL) of supernatant was oxidized with 1 mL of Folin-Ciocalteu's reagent and neutralized by 1 mL of 20% (w/v) sodium carbonate. The reaction mixture was incubated for 30 min at ambient temperature and absorbance was measured at 745 nm by using a spectrophotometer (PG Instruments, England). The polyphenols content was obtained using a calibration curve of gallic acid (1 mg/mL) as standard.

$$\text{Polyphenols (mg/100g)} = \frac{DO_{725} \times 5 \times 10^3}{5.04 \times m_e}$$

Where,

Calibration line: DO₇₂₅ = 5.04. Mass (mg) gallic acid; R² = 0.992 m_e: mass (g) of the sample.

Oxalates determination

The titration method as described by Day et Underwood (1986) [14] was performed. One (1) g of dried powdered sample was weighed into 100 mL conical flask. A quantity of 75 mL of sulphuric acid (3 M) was added and stirred for 1 h with a magnetic stirrer. The mixture was filtered and 25 mL of the filtrate was titrated while hot against KMnO₄ solution (0.05 M) to the end point.

$$\text{Oxalats (mg/100g)} = \frac{2.2 \times V_{eq} \times 100}{m_e}$$

V_{eq}: volume (mL) of KMnO₄ equivalence. m_e: mass (g) of sample.

Antioxydant Activity

Antioxidant activity is determined according to Choi *et al.* (2002) [12] method. One (1) g flour is homogenized in 10 ml of 70% methanol. Solution obtained is centrifuged at 1000 rpm for 10 min. 2.5 ml of supernatant are removed and 1 ml of DPPH solution (3 mM in methanol) is added thereto. The tube is allowed to stand for 30 minutes in the dark and absorbance is read spectrophotometrically at 415 nm against the blank.

$$AA(\%) = \frac{(DO_c - (DO_e - DO_b))}{DO_c} \times 100$$

DOc: absorbance of control tube (1 ml of DPPH + 2.5 ml of methanol).

ODE: absorbance of test tube (1 ml of DPPH + 2.5 ml of methanolic extract).

ODb: absorbance of blank tube or control (1 ml of methanol + 2.5 ml of methanolic extract).

Organic acids

Method of Ho *et al.* (1999) [16] coupled with high performance liquid chromatography (HPLC) was used to identify and quantify organic acids. Fifty (50) mg of powder was dissolved in 75 ml of distilled water. The mixture, homogenized by manual stirring for 2 min at room temperature (28 °C), was centrifuged at 4000 rpm for 30 min at 4 °C in a centrifuge (Sigma Aldrich 2-PK, France). Collected supernatant was filtered on Wattman #4 paper and then through a 0.45 µm millipore filter. Twenty (20) µl of filtered solution (clear) was analyzed by an HPLC system (Shimadzu Corporation, Japan) equipped with a binary pump (LC-6A) coupled to a UV-VIS detector (SPD-6A). Chromatographic separation of organic acids was carried out on an ICsep ICE ORH-801 column (30 cm, Interchom, France) at a temperature set at 35 °C. The eluent was sulfuric acid (0.004 N). The elution rate was 0.6 ml / min. The chromatograms obtained at 280nm were compared with those of organic acids standards. Peak areas have made it possible to quantify identified organic acids.

Statistical analysis

The data were brought to one-way analysis of variance (ANOVA), and the significance of the difference between means was determined by Duncan's multiple-range test using SPSS (Version 21.0, SPSS Inc., Wacker Drive, Chicago, USA). Values expressed are means of triplicate determination ± Standard deviation.

Results and Discussion

Anti-nutritional and anti-oxidant properties of flours from varieties cassava are presented in table 1. Phytate content ranged from 50.34±0.03 mg/100g (*Bondoukou* 4) to 60.36±0.04 mg/100 g (*Soclopouopo* 3), respectively. Phytate contents of cassava varieties are not toxic because they are respectively less than 250 mg/100g (Bushway *et al.*, 1998) [9], 60 mg/100g (Safety Officer in Physical Chemistry, 2005) [30]. Phytic acid has long been known as an anti-nutritional factor since it reduces bioavailability of several minerals due to its ability to chelate them (Sandberg *et al.*, 1989) [31]. At present, growing concern about phytic acid and their hydrolysis products has arisen from the finding that it might have beneficial effects such as antioxidant function, protecting against cancer risk (Vucenik and Shamsuddin, 2006) [38]. In addition, they have been considered antinutritional components because they can react with certain essential amino acids, limiting their availability (Crépon *et al.*, 2010) [13].

Tannin contents of seven cassava varieties ranged from 0.046±0.014 mg/100 g (*Agbablé* 3) to 0.054±0.014 mg/100 g (*Bondoukou* 4). Tannin contents of cassava varieties are

statistically different ($p < 0.05$), are lower than tubercle powder of *Tacca leontopetaloides* (L.) Kuntze (12.21±0.64 mg/100g of dry matter) (Ndouyang *et al.*, 2009) [25], yam *Dioscorea alata* cv *Bete-Bete* (136 mg/100 g of dry matter) (Assa *et al.*, 2014) [3]. Tannins are major groups of antioxidant polyphenols, have attracted lot of interest for the reason that, their multifunctional properties advantageous to human well-being. They have been well thoughtout as cardioprotective, anti-inflammatory and antimutagenic agents (Kumari and Jain, 2012) [20]. Tannins were compounded with organic compounds such as proteins, starches and digestive enzymes thus reduce the dietary importance of foods (Serrano *et al.*, 1994) [30]. They inhibit protein absorption and reduce iron availability (Bravo, 1994) [8]. So, the minimum level of tannins in the diet is recommended.

Flavonoid content of cassava varieties ranged from 0.62±0.02 mg/100g (*Agbablé* 3) to 3.01±0.01 mg/100g (*Boufouh* 3). The flour richest in flavonoids is that of the variety *Boufouh* 3 and the least rich is that of the variety *Agbablé* 3. Flavonoid content (3.35-76.88 mg/100g) of yams/soy/ vegetables infant meals reported by Soro *et al.* (2014) [36] is higher compared to flavonoid contents of seven varieties cassava. Flavonoids are described for their protective activity against UV radiation. They can prevent or reduce skin damage induced by oxidative stress, especially that caused by UV radiation by modulating the cellular response and trapping oxygenated radical species (Zhai and Maibach, 2002) [42]. Flavonoids comprise the most common group of plant polyphenols and provide flavour and colour.

Total phenol content of cassava varieties ranged from 51.24±1.79 mg/100g (*Totoba* 2) to 81.12±1.97 mg/100g (*Bondoukou* 4). Total phenol contents in cassava varieties are statistically similar ($p > 0.05$). Quantity of phenolic compounds present in a given species of plant material varies with a number of factors such as cultivar, environmental conditions, cultural practices, postharvest practices, processing conditions, and storage (Naczka and Shahidi, 2006) [23]. Polyphenols have been traditionally considered undesirable components in food products because they may cause darkening due to oxidation of phenols, leading to formation of dark pigments. In addition, they have been considered antinutritional components because they can react with certain essential amino acids, limiting their availability (Crépon *et al.*, 2010) [13].

Oxalate contents of cassava varieties ranged from 4.7±1 mg/100g (*Boufouh* 3) to 5.6±1.5 mg/100g (*Bondoukou* 4). Oxalates contents of seven cassava varieties are statistically identical ($p > 0.05$). Oxalate contents of seven cassava varieties are higher than yam *Dioscorea bulbifera* (2±0.045 mg/100g dry matter) (Afuikwa and Igwe, 2015) [1], yam *Dioscorea dumetorium* (2.32 mg/100g dry matter) (Ogbuagu, 2008) [28] but lower than tubers taro (*Colocasia esculenta*) (19 to 156 mg/100g of dry matter) (Huang *et al.*, 2006) [17]. Oxalates appear as end-metabolism products in many vegetable tissues. When they are consumed, oxalates can link calcium and other minerals (Noonan and Savage, 1999) [26].

Table 1: Anti-nutritional and anti-oxidant properties of flours from varieties cassava

Composition (mg/100g)	Cassava varieties							
	<i>Agbablé 3</i>	<i>Bondou-kou 4</i>	<i>Bonoua 2</i>	<i>Soclo-pouopo 3</i>	<i>Bou-fouh 4</i>	<i>Bou-fouh 3</i>	<i>Totoba 2</i>	
Phytates	53.45±0.04 ^a	50.34±0.03 ^b	54.49±0.01 ^d	60.36±0.04 ^f	52.31±0.02 ^c	53.45±0.02 ^a	57.97±0.01 ^e	
Tannins	0.046±0.014 ^a	0.054±0.014 ^b	0.048±0.008 ^c	0.047±0.008 ^a	0.048±0.007 ^a	0.052±0.008 ^b	0.049±0.008 ^a	
Flavonoids	0.62±0.02 ^b	2.32±0.02 ^a	1.98±0.01 ^c	1.50±0.01 ^c	2.20±0.04 ^a	3.01±0.01 ^f	1.83±0.01 ^d	
Total Polyphenols	70.55±2.35 ^b	81.12±1.97 ^a	58.79±3.17 ^d	79.93±1.5 ^a	72.24±2.18 ^b	80.58±2.79 ^a	51.24±1.79 ^c	
Oxalates (µg/100g)	5.1±1 ^a	5.6±1.5 ^a	5.3±1 ^a	5.4±2 ^a	5.4±2 ^a	4.7±1 ^a	5.2±1 ^a	
Antioxidant power	0.06±0.01 ^a	0.06±0.00 ^a	0.07±0.01 ^a	0.07±0.01 ^a	0.08±0.00 ^b	0.06±0.00 ^a	0.08±0.00 ^b	

Mean ± standard deviation: Online data followed by the same letter are not significantly different ($P < 0.05$); online data followed by different letters are significantly different ($p < 0.05$).

Some organic acids of cassava varieties flours are presented in table 2. Major organic acids in varieties cassava flours are carboxylic and benzoic acids with respective contents of 129.01±0.37 (*Boufouh 3*) to 147.65±0.61 µg/100g (*Bonoua 2*) and 80.11±0.17 (*Boufouh 3*) at 126.15±0.98 µg/100g (*Bondoukou 4*). Organic acids distributed in foods are digestible and provide a source of energy, improve the bioavailability of minerals by forming complexes, stimulate the secretion of endogenous enzymes through acidification, prevent the proliferation of microorganisms in foods (Smulders and Greer, 1998) [34]. Organic acids have the capacity to complex the metal ions in the solution which degree depends on the particular organic acid, the concentration and the type of metal and pH (Jones, 1998) [19].

Organic acids lower pH in the stomach thus reducing the growth of some pathogenic bacteria (Suryanarayana *et al.*, 2012, Huyghebaert *et al.*, 2011) [37, 18]. Organic acids distributed in the food are digestible and constitute a source of energy, they improve the bioavailability of the minerals by forming complexes; stimulate the secretion of endogenous enzymes through acidification (Vondruskova *et al.*, 2010) [38]. Acute toxicity of benzoic acid as a food additive is low. However, it was observed that in sensitive persons, intake of benzoic acid lower than 5 mg/kg of body weight per day, can cause non-immunological contact reactions (pseudoallergy) (World Health Organization, 2000) [41]. Some studies suggested that very high intake of benzoic acid can cause adverse health effects such as metabolic acidosis, hyperpnoea and convulsions (World Health Organization, 2000) [41].

Table 2: Some organic acids of flours from cassava varieties

Organics acids (µg/100g)	Cassava varieties							
	<i>Agbablé 3</i>	<i>Bondou-kou 4</i>	<i>Bonoua 2</i>	<i>Soclo-pouopo 3</i>	<i>Bou-fouh 4</i>	<i>Bou-fouh 3</i>	<i>Totoba 2</i>	
Lactic acid	2.69±0.03 ^a	2.95±0.04 ^b	3.93±0.02 ^d	5.01±0.01 ^e	3.012±0.00 ^c	2.74±0.033 ^a	2.95±0.03 ^b	
Formic acid	4.74±0.01 ^a	62.02±0.02 ^g	21.31±0.03 ^c	10.12±0.02 ^c	14.95±0.08 ^d	5.18±0.05 ^b	40.01±0.07 ^f	
Carboxylic acid	129.01±0.37 ^c	138.3±1.46 ^d	147.65±0.61 ^b	125.02±1.65 ^a	146.52±0.05 ^b	122.5±2.04 ^a	122.84± 2.2 ^a	
Propionic acid	4.88±0.21 ^c	27.52±0.06 ^g	21.41±0.03 ^f	5.24±0.02 ^d	14.34±0.32 ^e	4.41±0.012 ^b	2.2±0.04 ^a	
Butric acid	19.48±0.56 ^f	9.52±0.05 ^b	12.38±0.58 ^a	14.12±0.11 ^d	15.84±0.13 ^e	12.32±0.02 ^a	11.41±0.03 ^c	
Ascorbic acid	12.30±0.12 ^c	19.11±0.01 ^d	20.45±0.08 ^e	29.25±0.36 ^g	9.78±0.1 ^b	27.4±0.24 ^f	4.43±0.06 ^a	
Citric acid	49.43±0.11 ^a	57.27±0.68 ^a	54.32±0.1 ^a	47.78±0.09 ^a	51.55±0.61 ^a	55.81±1.48 ^a	7.44±0.016 ^b	
Sulfuric acid	0.12±0.02 ^a	0.42±0.06 ^b	-	0.38±0.02 ^b	-	0.2±0.08 ^a	0.58±0.02 ^c	
Benzoic acid	100.49±0.28 ^c	126.15±0.98 ^g	92.36±1.03 ^b	112.37±0.34 ^e	118.82±0.83 ^f	80.11±0.17 ^a	106.33±0.1 ^d	

Mean ± standard deviation: online data followed by the same letter are not significantly different ($P < 0.5$); online data followed by different letters are significantly different ($p < 0.5$).

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