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Evaluation of Eight Orange Fleshed Sweetpotato (OFSP) Varieties for Their Total Antioxidant, Total Carotenoid and Polyphenolic Contents

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Abstract

Eight varieties of OFSP whose yields ranged from 6 t/ha (Caromex) to 15.22 t/ha (BF82xCIP-18) have a water content less than the current value observed for OFSP (71%).These varieties have been studied for their total antioxidant contents (TAC), total polyphenolic contents (TPC) and total carotenoid contents (TCC) using Ferric Reducing Antioxidant Power (FRAP), Folin-Ciocalteu reagent (FCR) and a method described by McMurry respectively. TAC differed significantly ($p < 0.05$) depending on the variety. Among the varieties obtained by crossing, BF92xResisto-14 and BF92xCIP-6 with the highest levels of total antioxidants (respectively 4.12 and 3.04 mg of Trolox equivalent/g of dw), had also the highest levels of total polyphenols (respectively 2.432 and 2.269 mg of Gallic acid equivalents/g of dw) and total carotenoids (respectively 0.180 and 0.132 mg of Beta-carotene equivalents/g of dw). There was a good correlation first, between the TAC and the TPC ($r^2 = 0.957$) and between the TAC and the TCC ($r^2 = 0.867$) on the other. Antioxidant activities observed may be due to the effect of synergy between polyphenols and carotenoids.

Keywords: Yield, OFSP extracts, total antioxidant content, total polyphenolic content, total carotenoid content, FRAP, FCR

1. Introduction

The sweetpotato (*Ipomoea batatas* [L.] *Lam.*) of the Convolvulaceae family is a tuberous plant that grows in tropical and subtropical areas. Native from Latin America (Mark et *al.,* 2009;Davidson, 1999), it occupies an important place in the agricultural production of Sub-Saharan Africa countries, covering about 3.2 million hectares with a production estimated at 13.4 million tons of tubers in 2005 (Faostat, 2005). It grows easily, quickly matures (4-5 months), produces abundant food with respect to the space used for the plant and stores more or less well. In many countries, its culture is essential, because it contributes to reduce food shortages in times of crisis (natural disasters or wars).

In addition, several studies showed that orange-fleshed sweetpotato, the less common variety in sub-Saharan Africa, particularly Burkina Faso, is a potential source of vitamin A (Mark et *al,.* 2009; Haskell *et al.,* 2004), minerals (Fe, Zn, Mn), and other micronutrients such as polyphenols and carotenoids (Haskell *et al.,* 2004). These micronutrients have biological properties of interest for humans, and pharmacological or nutritional properties. Carotenoids in particular, likewise vitamin C, vitamin E or polyphenols, have antioxidant properties (Maruf *et al.,* 2009; Gaston *et al.,*2009;Yuan *et al.,* 2004) and are able of trapping free radicals generated constantly by our organism. Ingested with food, these compounds strengthen our natural defense against oxidative stress and thus prevent various chronic diseases such as cancer, cardiovascular diseases (Huang *et al.,* 2004; Philpott *et al.,* 2004).

Promoting micronutrient-rich orange-fleshed sweetpotato varieties is needed as a strategy to fight malnutrition in sub-Saharan Africa where more than 43 million children under five are at risk of vitamin A deficiency with the precursor β-carotene in orange-fleshed sweetpotato. Through collaborative studies INERA and University of Ouagadougou are working to develop OFSP hybrid varieties better adapted to the agro-ecological context of Burkina Faso. This work aims to determine the antioxidant, the phenolic and carotenoid contents of these hybrids obtained through crossing as well as their agronomic performance.

2. Material and methods

Chemicals and reagents: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Gallic acid, $Fe(III)(TPTZ)_2Cl_3$ (TPTZ = 2,4,6-tripyridyl-s-triazine), Ferric chloride were obtained from Sigma chemical Inc, USA. All other reagents and chemicals used were of analytical grade.

2.1 *Plant material*:

Eight varieties of OFSP studied come from two sources. Two varieties (TIB-440060 and Caromex) were introduced from CIP (International Centre of Potato) of East Africa as a source of beta carotene (provitamin A), but not adapted to the agro-ecological context of Burkina Faso. The other six varieties: BF82xCIP-13; BF82xCIP-18; BF82xTIB-4; BF82xTIB-6; BF92xResisto-14; BF92xCIP-6 were developed through hybridization at the of Environmental and Agricultural Research Institute (INERA) by the crosses of two locally adapted parents BF82 and BF92 onto three introduced beta-carotene-rich parents (TIB-440060, Resisto, CIP-199260-1) not adapted to the local environmental. These six varieties were developed in order to have beta carotene-rich orange-fleshed sweetpotato adapted to the agro-ecological context of Burkina Faso that can be used in the fight against recurrent malnutrition especially in the mothers and their children. These eight varieties were evaluated in three ago-ecological zones in Burkina for their performance and stability at: Farakoba in the western region, in a soudanian climatic zone characterized by an average rainfall ranged from 900 to 1200 mm; Loumbila belonging to the central region in the sudano-sahelian climatic zone with an average rainfall ranging from 700 to 800 mm; and Kouaré, located in a sudano-savannah zone in the Eastern Region with an average rainfall ranging from 850 mm to 1050 mm.

2.2 Sampling and sample preparation

Drying of OFSP tubers: the sweetpotatoes storage root was peeled and left to dry at the laboratory for twenty days. To ensure that the samples were dried, the weight of each variety was measured every day until a constant weight was reached. The samples were then sealed in plastic soda-bag.

Extraction of plant material: 3 g of powder obtained by crushing the plant material were extracted three times by maceration with 10 mL of with the following solvent systems: acetone-water-acetic acid (70: 29.5: 0.5 v/v) (Asami *et al.,* 2003) for TAC and TPC assays and acetone-hexane (50: 50, v/v) (Kowalski *et al.,* 2000) for TCC assay. Extracts were vigorously stirred for 30 minutes and then carefully filtered using filter paper Wathman N ° 1 and kept at 4 ° C protected from light in the refrigerator until the determination.

2.3 Determination of TAC using FRAP (Ferric Reducing Antioxidant Power) assay

In this method, a ferric salt, Fe (III) $(TPTZ)_2Cl_3$ (TPTZ = 2,4,6-tripyridyl-s-triazine) is used as an oxidant (Benzie *et al.,* 1996; Pulido *et al.,* 2000). It is prepared by mixing 1 mL (1 mL, 10 mM in 40 mM) TPTZ, 10 mL of a solution of sodium acetate buffer (pH = 3.6) and 1 mL of solution of Fe (III), H_2O (Pulido *et al.,* 2000). About 30 µL of distilled water was mixed to20 µL of appropriately diluted extract and then, 200 µL of solution FRAP were added. Absorbances of the intense blue discoloration were measured at 593 nm using a microplate (spectrophotometer SAFAS, MP96) drive about 10 minutes after. A calibration curve was established using Trolox as reference of antioxidant. Results, determined from the calibration curve equation ($y = 28.67\ x + 0.066$; $R^2 = 0.999$), were expressed in mg of Trolox Equivalent (TE)/g dried weight. All measurements were performed in triplicate.

2.4 Determination of TPC using Folin-Ciocalteu assay

Total phenolic phytochemical concentration was measured using the Folin-Ciocalteu method (Nihal *et al.,* 2007). Briefly, 60 µL of appropriate diluted samples and a standard solution of gallic acid (3,4,5-trihydroxybenzoic acid)were mixed to60 µL of Folin-Ciocalteu's Reagent (FCR-1: 10 dilution) and left to stand for 8 min at room temperature to allow for the FCR to react completely with the oxidible substances or phenolates. 120 µL of Na_2CO_3 (7.5 % in water) were added to destroy the residual reagent. Absorbances were measured at 760 nm using a 96-well (glass vials 250 µL) microplate spectrophotometer (Microplate Autoreader MP96; SAFAS Instruments) after incubation for 30 min at 37 °C against distilled water as a blank.Total phenolic contents of the samples determined from the calibration curve equation ($y = 46.41\ x + 0.063$, $R^2 = 0.998$) were expressed in mg of gallic acid equivalents (GAE) per gram of dried weight. All measurements were performed in three replications.

2.5 Determination of total carotenoid contents (TCC)

Total carotenoid contents of OFSP extracts were evaluated according to the method described by McMurry (Jun *et al.,* 2011; McMurry, 2008) slightly modified. A direct measurement of absorbance was made. After suitable dilution, the absorbances of extracts kept at room temperature and protected from light, were red at 455 nm. Total carotenoid contents were obtained by reporting absorbances of suitable diluted extracts on a curve ($y = 25.56\ x + 0.016$; $R^2 = 0.999$) established using β-carotene as standard. TCC were expressed in mg of β-carotene Equivalents (BCE)/g of dried weight. All measurements were performed in three replications.

2.6 Statistical analyses

All experiments were conducted in three replications. The results are expressed in mean ± SD. Analysis of variance (ANOVA) allowed to appreciate the differences between varieties for their antioxidant, polyphenolic and beta carotene contents using the statistical software Genstat, 14th Edition. Multivariate analysis was performed to generate a dendrogram to appreciate the relationship between the varieties for their total antioxidant, polyphenolic and carotenoid contents. The $p< 0.05$ values were considered statistically significant.

3. Results and discussion

Field evaluation during 2010 and 2011 cropping season in three different agro-ecological zones of Burkina Faso allowed recording the agronomic performance of these eight varieties. Varieties BF82xCIP-18, BF82xTIB-4, BF82xTIB-6, BF92xCIP-6 and the introduced variety TIB-440060 had average yields of 15.22 t/ha, 13.50 t/ha; 11.56 t/ha, 17.11 t/ha and 11.11 t/ha, respectively, higher than the national mean yield of sweetpotato around 10 t/ha (table 1). However, BF82xCIP-13, BF92xResisto-4 and Caromex presented respectively a potential of 9.89 t/ha, 7.50 t/ha and 6.78 t/ha under the national mean performance, but showed tubers of regular form in accordance with the preferences of farmers.

Table 1 presents the results obtained from measurement of the moisture content of the eight OFSP varieties studied. Water contents determined were lower than the average water content in OFSP 70% to 80% encountered in the literature (Vimala et al., 2011). The introduced varieties Caromex and TIB-440060 have the lowest water contents (respectively 27 and 56.06%). Except for BF92XCIP-6 (71.39%), all the varieties had an average water content around 62%; which represents 38% of dry matter content greater than the average value of 29% of the literature. In general, high level of water content is associated to high loss in storage root of OFSP. These crosses have provided less perishable hybrid varieties. The results suggested that the hybrids presented much lower water content (38% of dry matter) than the two introduced varieties (Caromex and TIB-440060) (respectively 73 and 44% of dry matter). However, the water content recorded might have affected by the duration (two days) between the harvesting and the water quantification.

The levels of antioxidants, polyphenols and total carotenoids presented in table 2 were analyzed statistically using Genstat 14th Edition. Significant differences were observed ($P < 0.05$) between the eight varieties of OFSP studied for these three parameters. Indeed, total antioxidant levels varied between 1.07 (BF82xTIB-4) and 4.12 (BF92xResisto-14) mg of TE/g of dry weight; which represents a variation of about 4-fold. The difference between the two introduced varieties (TIB440060 and Caromex) and BF82xCIP-18 was not significant ($P > 0.05$) for total antioxidants (TAC); they had the same TAC of about 1.75 mg of TE/g dry weight more than BF82xCIP-13 (1.62 mg of TE/g dry weight). BF82xTIB-6, BF92xCIP-6 and BF92xResisto-14 had the best total antioxidants contents. However, BF92xResisto-14 which recorded the lowest yields has the higher total antioxidant content.

The study of the TAC in relation with TPC showed that there was a good correlation ($r^2 = 0.957$) between TAC and TPC. Varieties presenting the highest TPC had the highest TAC in agreement with previous studies which showed that polyphenolic compounds are mainly responsible for the antioxidative activities of plant extracts (Ou *et al.*, 2005). In this work, they contributed for more than 91 % in the antioxidant activities of OFSP extracts of varieties studied (table 4). Significant differences ($P < 0.05$) were also observed between the these varieties for their TPC content ranging from 1.06 (BF82xTIB-4) to 2.43 mg equivalents of Gallic acid (GAE) /g dry weight (BF92xResisto-14); which represents a variation of about 2-fold (table 3). In addition, the introduced varieties TIB440060 and Caromex had an average of 1.37 and 1.45 mg of GAE per gram of dry weight respectively compared to the highest contents (between 2.30 and 2.43 mg of GAE) and to the lowest (of 1.06 and 1.1843 mg of GAE) observed on the newly developed hybrids varieties.

Polyphenols are not solely responsible of antioxidant activities of plant extracts. Carotenoids are also powerful natural antioxidants. OFSP are a source of vitamin A available for poor families in Sub-Saharan Africa. Indeed, they contained between 300 and 4620 equivalents of beta carotene (provitamin A) per 100 g of OFSP (Tumwegamire *et al.*, 2004). OFSP, rich in beta carotene is accepted by children and can contribute to increase retinol concentrations in the serum of young consumers (Jan *et al.,* 2007).

Total carotenoid contents assessed in these varieties of OFSP certainly contributed to their TAC. High correlation was noticed between TCC and TAC, confirming the positive impact of TCC on TAC (table 4). TCC (table 3) varied from 0.016 to 0.18 mg of Beta-carotene equivalent (BCE)/g of dried weight (respectively for BF82xTIB-4 and BF92xResisto-14), which represents an increase of more than 11 times and nutritional improvement compared to the introducedvarietiesTIB-440060 and Caromex.

A multivariate analysis combining different levels (total antioxidants, polyphenols and carotenoids) evaluated and providing an overall assessment of results had been translated using a dendrogram (figure 1). The dendrogram showed an important influence of the parental line on TAC of varieties developed by cross-breeding and the effect of the parents TAC in the descendants. Indeed, both BF82xCIP-13 and BF82xCIP-18 sharing the same parent (BF82 and CIP-199062-1) had similar antioxidant levels. It was the case of BF82-TIB-4 and BF82-TIB-6 (obtained by crossing of BF82 and TIB-440060) which are close to both above (BF82xCIP-13 and BF82xCIP-18) varieties with whom they share the female parent (BF82). The varieties involving BF92 as female parent in crosses are nearby. Genetic analyses have shown that the choice of parents is very important in the development of offspring that accumulates good beta-carotene content; notwithstanding their initial beta carotene content, some parents have a good ability to develop offspring with a high TCC. This could be the case for the levels of antioxidants.

4. Conclusion

This research work fruit of an important collaboration between researchers of INERA and university of Ouagadougou was based on locally adapted OFSP varieties developed in Burkina Faso. The results revealed that the varieties BF92xResisto-14 and BF92xCIP-6 had the highest total antioxidant, polyphenolic and carotenoid contents. Except for BF92XResisto-14, the other OFSP varieties had yields higher than the average yield (9t/ha) recorded in the country. These findings on the antioxidant, polyphenolic and total carotenoid contents obtained from these locally developed OFSP will contribute in the fight against the malnutrition that is endemic in Burkina Faso.

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Table 1: yields and water contents of OFSP

Varieties	yields (t/ha)	water contents (%)
BF82xCIP-18	15,22	64,36
BF82xTIB-4	13,50	62,83
BF82xTIB-6	11,56	62,975
BF92xCIP-6	17,11	71,39
TIB-440060	11,11	56,06
BF82xCIP-13	9,89	55,74
BF92xResisto-14	7,50	63,02
Caromex	6,78	27,15

Table 2 : Calibration curves equations for TAC, TPC and TCC determination

Calibration curve	Standard	equations	Correlation coefficients
TAC	Trolox	y = 28.67 x + 0.066	$R^2 = 0.999$
TPC	Gallic Acid	y = 46.41 x + 0.063	$R^2 = 0.998$
TCC	Beta-carotene	y = 25.56 x + 0.016	$R^2 = 0.999$

Table 3: Total antioxidant, polyphenolic and carotenoid contents

Variétés	TAC (mg TE/g of dw)	TPC (mg GAE/ of dw)	CT (mg BCE/g of dw)
TIB	1,78±0,041^{bc}	1,374±0,089$^{bc'}$	0,072±0,0006$^{b''}$
BF92xResisto-14	4,12±0,045^{e}	2,432±0,009$^{d'}$	0,180±0,0004$^{f''}$
BF92xCIP-6	3,04±0,079^{d}	2,269±0,018$^{d'}$	0,132±0,0038$^{e''}$
BF82xTIB-6	1,85±0,072^{c}	1,187±0,005$^{ab'}$	0,075±0,0024$^{b''}$
BF82xCIP-13	1,62±0,011^{b}	1,251±0,067$^{b'}$	0,115±0,0005$^{c''}$
BF82xTIB-4	1,07±0,049^{a}	1,062±0,033$^{a'}$	0,016±0,0007$^{d''}$
BF82xCIP-18	1,77±0,029^{bc}	1,431±0,035$^{c'}$	0,024±0,0002$^{a''}$
Caromex	1,71±0,040^{bc}	1,459±0,013$^{c'}$	0,068±0,0004$^{b''}$

Data are expressed as means ± SE of triplicate experiments. Means in a column not having a common letter are different (P<0.05).

Table 4: TAC, TPC and TCC correlation of OFSP extracts

correlation	R	R^2%
TAC Vs TPC	0,957	91.58
TAC Vs TCC	0,867	75.16
TCC Vs TPC	0,803	64.48

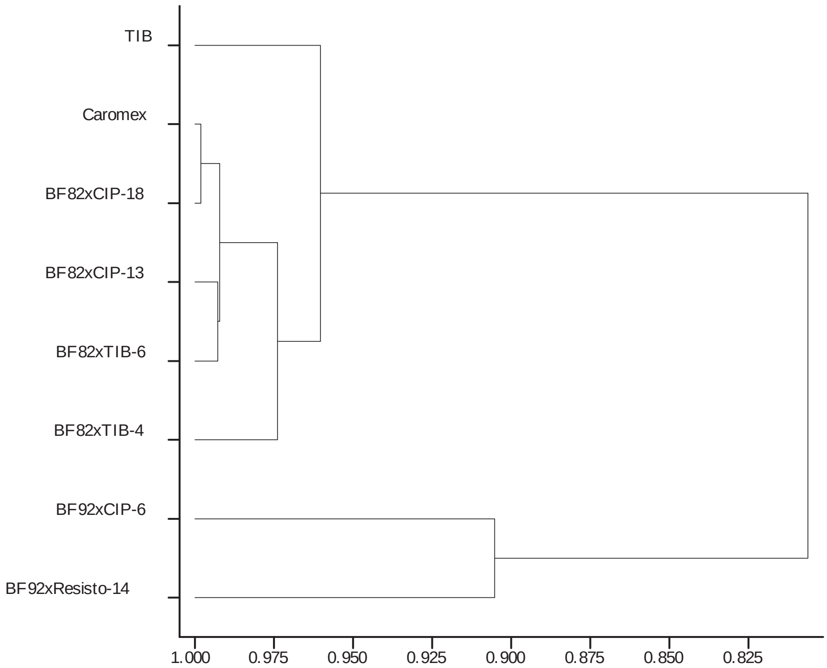

Figure 1 : Dendrogram showing the relationship between the eight varieties for their TAC, TPC and TCC contents