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Studies on the Nutrient Composition, Antioxidant Activities, Functional Properties and Microbial Load of the Flours of 10 Elite Cassava (*Manihot esculenta*) Varieties

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ABSTRACT

Flours of 10 cassava varieties: TME778, TMS/1646, TMS00/0355, 01/0103, TMS94/0330, TMS91934, TMS/0338, TMS91/1730, NR87/184 and TMS92/0057 were screened for their nutritional composition, antioxidant activities, functional properties and microbial loads. TMS91934 had the highest starch composition among the 10 varieties while TMS94/0330 had the least (17.81 ± 1.38). The loose bulk density of TMS01/0103 was higher than that of other varieties (0.308 ± 0.03) ($p < 0.05$) with TMS91934 having the least (0.147 ± 0.025) while TMS001/0355 had higher packed bulk density (0.449 ± 0.06) and moisture content (68.77 ± 0.84) among other varieties studied with TME778 having the least packed bulk density (0.324 ± 0.02) and moisture content (57.38 ± 0.59). The percentage dry matter contents of the cassava varieties ranged from 30.4 to 46.23 with TME778 having the highest dry matter content (42.57 ± 0.66) and TMS001/0355 having the least (31.24 ± 0.84). The cassava varieties were found to possess antioxidant activities as evaluated by the phenolic composition of methanolic extracts of the flours and reducing power tests. Phytochemical analysis of the flours indicated that they contained significant quantities of alkaloids, flavonoids, tannins with their cyanogenic glucoside content lower than LD_{50} values for man. All flours of the 10 cassava varieties had good viable and fungal counts in addition. The results obtained indicate the biosafety in the consumption of these cassava varieties with antioxidant potentials whose shelf life could be extended if packaged well and stored. In addition, they're quite suitable as drug binder and disintegrant in pharmaceuticals.

Key words: Nutritional composition, functional properties, phytochemicals, cassava varieties, microbial load

INTRODUCTION

Cassava, a cheap and reliable source of food for more than 700 million people in the developing world is Africa's second most important staple food after maize in terms of calories consumed (FAO, 2003). Its estimated that 250 million people in Sub-Saharan Africa derive half of their daily calories from cassava (FAO, 2003) with Nigeria being the largest producing country in the world.

In most developing tropical countries, the food situation is worsening owing to increasing population, shortage of fertile land and high prices of available foods (Sadik, 1991). This has resulted in a high incidence of hunger and malnutrition, a situation in which children and women, especially pregnant and lactating women are most vulnerable (Weaver, 1994).

Predictions of future food needs based on the current rates of population increase and food production emphasize the seriousness of this problem (Masek, 1966). There can be no immediate single solution to the problem of food sufficiency; an interdisciplinary approach is necessary (Avery, 1991).

Food processing is probably the most important source of income and employment in Africa, Asia and Latin America. The Food and Agriculture Organization of the United Nations has stated that value added through marketing and processing raw materials can be much greater than the value of primary production (Anonymous, 1995).

Some micro-organisms produce chemicals that can color, flavor and stabilize foods, thereby increasing their storage lives (Ogunjobi *et al.*, 2005). These types of food are important because of their increased nutritional values as well as improved aroma and flavor characteristics.

The projected shortages of food throughout the world as a result of global recession prompted the screening of 10 cassava varieties developed at the molecular biology laboratory of National Root Crops Research Institute, Umudike for their starch, dry matter, Functional properties, phytochemicals, phenols, cyanogenic glucoside contents, microbial loads and reducing power and the results are reported in this study.

MATERIALS AND METHODS

The study was carried out in 2010 in the Biochemistry Department of National Root Crops Research Institute, Umudike, Umuahia, Abia State, Nigeria. Ten elite cassava varieties: TME778, TMS/1646, TMS00/0355, 01/0103, TMS94/0330, TMS91934, TMS/0338, TMS91/1730, NR87/184 and TMS92/0057 were used.

Preparation of plant materials: The cassava tubers were thoroughly washed, peeled, weighed and fractionated into little pieces and dried at 40°C in a hot air oven to a constant weight. The dried samples were then ground to fine powder by using an electrical grinder and biochemical analysis were carried out on the methanolic extracts of the flours.

The method of Hsu *et al.* (2003) was used in the preparation of cassava extracts for reducing power test. The methanolic cassava extract (40 mg mL⁻¹) was diluted to suitable concentrations: 10, 20, 30 and 40 mg mL⁻¹ for the reducing power test.

For starch analysis, starch was extracted by the method of Moorthy and Nair (1989) with modifications. One hundred gram of freshly harvested tubers were homogenized with 1% sodium-metabisulfite (900 mL) solution using a warring commercial blender. The mixture was filtered through a triple-layered cheese cloth and starch washed thoroughly using distilled water. The granules were allowed to settle for 3 h and the water decanted off. The wet cakes of the starch were dried in an oven at 40°C, ground into powder and analyzed.

Nutritional composition of the flours: The AOAC (1990) method was used for the analysis of the total starch, dry matter, moisture and phytochemical composition of the flours while the alkaline picrate method (Onwuka, 2005) was used in cyanide determinations.

Total phenolic assay: The Total phenolic content of the flours was measured using the modified Folin - Ciocalteu method (Singleton *et al.*, 1999). The hydrophilic extract (0.5 mL) was diluted with distilled water to 5.0 mL, to which 0.5 mL of Folin-Ciocalteu reagent was added and allowed to react at room temperature for 3 min. One milliliter of 1N sodium carbonate was added and the

mixture was incubated at room temperature for 1 h. The absorbance was taken at 725 nm distilled water as blank. Chlorogenic and gallic acids were used as standards. Total phenolic content was reported as milligrams of chlorogenic acid equivalents per gram of fresh sample weight or dry weight which can be converted to milligrams of gallic acid by multiplying by a factor of 0.445.

Test of reducing power: The method of Hsu *et al.* (2003) was adopted in the analysis of the reducing power. Absorbance was taken at 700 nm with a spectrophotometer. Increased absorbance of the reaction mixture indicated increased reducing power.

Water Solubility Index (WSI): The WSI of the cassava flours were analyzed using the method of Anderson and Sefa-Dedeh, 2001 2.5 g each of the cassava flours and water were vigorously mixed in a 100 mL centrifuge tube, incubated in a 37°C water bath for 30 min and then centrifuged (4000x g for 10 min). The supernatant was collected in a pre- weighed beaker and the residue was weighed after the water was evaporated below 105°C. The percentage of residue with respect to the amount of cassava flour used in the test was taken as the water solubility index.

Loose bulk density and packed bulk density: The method of Wang and Kinsella (1976) was used for bulk density determination.

Microbial analysis: The method of Ogunjobi *et al.* (2005) was used in the microbial analysis and characterization. The microbial identification was according to the taxonomic tools of Sneath *et al.* (1986). All experiments were carried out in triplicates.

RESULTS AND DISCUSSION

The percentage starch composition of TMS91934 was higher ($p < 0.05$) than that of other varieties studied on a wet basis (29.885 ± 0.26) while TMS 94/0330 had the least starch content (17.81 ± 1.38) (Table 1). The yield obtained could be an indication of the starch composition in the fresh tubers. Nwokoro *et al.* (2005) has reported the percentage starch composition of cassava to be up to 30%. The low starch contents of these cassava varieties could be as a result of biofortification.

Table 1: Percentage moisture, dry matter and starch content of 10 cassava varieties

Variety	Moisture	Dry matter	Starch
	------(%)-----		
TMS 92/0057	59.67±0.25	40.33±0.25	28.33±0.99
TMS 1646	63.26±4.88	36.74±4.88	26.86±0.27
TMS 0338	66.99±0.29	33.02±0.29	25.74±1.24
01/0103	62.65±1.88	37.35±1.88	23.95±0.27
NR87184	59.75±0.82	40.25±0.82	27.12±2.32
TMS 94/0330	67.58±6.15	32.42±6.15	17.81±1.38
TMS 91/1730	65.09±0.59	34.86±0.66	20.12±0.98
TMS 001/0355	68.77±0.84	31.24±0.84	19.31±0.30
TME 778	57.38±0.59	42.57±0.66	26.45±1.10
TMS 91934	60.89±1.40	37.90±3.11	29.89±0.26

The values in the table were calculated from the Mean±SD of 3 experiments. N = 10

Table 2: Functional properties of 10 cassava varieties

Variety	LBD ------(g mL ⁻¹)-----	PBD	Water solubility index (%)
TMS 92/0057	0.28±0.0010	0.390±0.00	82.44±1.30
TMS 1646	0.283±0.006	0.359±0.00	82.76±1.53
TMS 0338	0.268±0.002	0.355±0.01	83.20±0.34
01/0103	0.308±0.030	0.355±0.02	84.64±2.60
NR87184	0.268±0.004	0.402±0.04	82.96±3.06
TMS 94/0330	0.275±0.013	0.354±0.012	87.20±0.91
TMS 91/1730	0.287±0.009	0.355±0.013	63.72±19.52
TMS 00/0355	0.268±0.020	0.449±0.06	87.48±1.53
TME 778	0.271±0.002	0.324±0.02	82.92±1.30
TMS 91934	0.147±0.025	0.369±0.03	80.72±1.36

Values in the table are the Means±SD of triplicate experiments. LBD: Loose bulk density. PBD: Packed bulk density. N = 10

The percentage moisture contents of the 10 cassava varieties as shown in Table 1 indicated that TMS 001/0355 had the highest moisture content (68.77±0.84) while TME778 had the least moisture content (57.38±0.59).

The percentage dry matter contents of the 10 cassava varieties as shown in Table 1 ranged from 30.40 to 40.33 with TME 778 having the highest dry matter content (42.57±0.66) while the dry matter content of TMS 001/0355 was the least (31.24±0.84). Present results are consistent with reports of FAO (2003).

Bulk density is a function of particle size while particle size is inversely proportional to bulk density. The relatively high value of the bulk density of these cassava varieties is a significant finding in this study as it suggests their suitability as drug binder and disintegrant in pharmaceuticals (Table 2).

Water solubility index reflects the extent of starch degradation (Diosady *et al.*, 1985). TMS00/0355 had the highest starch degradation (87.48±1.53) while TMS 91/1730 showed the least starch degradation (63.72±19.52) (Table 2).

In recent years, phenolic compounds have attracted the interest of researchers because they show promise of being powerful antioxidants that can protect the human body from free radicals, the formation of which is associated with the normal metabolism of aerobic cells (Halliwell, 1996; Oboh and Rocha, 2007). In addition, Phenolic compounds existing in plants are also important for their contribution to colour and sensory attributes of food (Maga, 1978). All 10 varieties of cassava flour studied contained significant quantities of phenols with the phenolic content of TME 778 higher than that of other varieties investigated (0.174±0.008 mg g⁻¹ CAE) while TMS 91934 had the least phenolic content(0.046±0.026) (Table 3).

Reducing power is a measure of the ability of the methanolic extracts to reduce Fe³⁺ to Fe²⁺. Reducing power has been one of the antioxidant capability indicators of medicinal herbs (Duh and Yen, 1997). This is because, antioxidants are strong reducing agents and this is principally because of the redox properties of their hydroxyl groups and the structural relationships of any parts of their chemical structure (Oboh and Rocha, 2007). All cassava varieties studied had good antioxidant activity as seen by their increased reducing power at higher concentrations (Table 4).

The high content of flavonoids, alkaloids and tannins observed in the 10 varieties of cassava flours is striking in this study (Table 5). Flavonoids, alkaloids and tannins are polyphenolic

Table 3: Total phenolic composition of 10 cassava varieties

Variety	Phenolic content
TMS 92/0057	0.149±0.00
TMS 1646	0.075±0.023
TMS 0338	0.102±0.020
01/0103	0.105±0.003
NR87184	0.139±0.019
TMS 94/0330	0.122±0.038
TMS 91/1730	0.086±0.013
TMS 00/0355	0.171±0.008
TME 778	0.174±0.008
TMS 91934	0.046±0.026

Reported values are the Means±SD of 3 experiments. CAE: Chlorogenic acid equivalent. N = 10

Table 4: Reducing power of 10 cassava varieties

Variety	Conc. (mg mL ⁻¹)	OD (700 nm)	Variety	Conc. (mg mL ⁻¹)	OD (700 nm)
TMS 92/0057	10	0.058 ^a	TMS 94/0330	10	0.063
	20	0.063 ^{ab}		20	0.069
	30	0.0746 ^b		30	0.075
	40	0.062 ^a		40	0.085
TMS 1646	10	0.063	TMS 91/1730	10	0.063
	20	0.061		20	0.053
	30	0.070		30	0.075
	40	0.074		40	0.083
TMS 0338	10	0.076	TMS 00/0355	10	0.058 ^a
	20	0.052		20	0.076 ^a
	30	0.070		30	0.069 ^{ab}
	40	0.075		40	0.068 ^{ab}
01/0103	10	0.058	TME 778	10	0.044 ^a
	20	0.059		20	0.061 ^b
	30	0.056		30	0.073 ^c
	40	0.062		40	0.069 ^{bc}
NR87184	10	0.057 ^a	TMS 91934	10	0.075 ^{ab}
	20	0.063 ^a		20	0.082 ^b
	30	0.080 ^b		30	0.054 ^a
	40	0.083 ^b		40	0.073 ^{ab}

Reported values were derived from the means of triplicate experiments. The values in the table are significant at p<0.05. N = 10. OD = Absorbance

Table 5: Phytochemical composition of flours of 10 cassava varieties

Variety	Alkaloid	HCN (mg kg ⁻¹)	Flavonoid (%)	Tannin (%)
TMS 92/0057	4.35±0.006	9.56±0.002	3.82±0.001	0.13±0.002
TMS 1646	3.50±0.001	12.72±0.003	5.28±0.002	0.15±0.001
TMS 0338	2.62±0.00	8.14±0.001	3.70±0.001	0.14±0.00
01/0103	4.18±0.003	10.38±0.002	4.26±0.00	0.07±0.00
NR87184	2.90±0.004	12.30±0.000	4.75±0.00	0.12±0.00
TMS 94/0330	3.82±0.001	9.70±0.01	4.19±0.00	0.08±0.00
TMS 91/1730	1.80±0.002	11.30±0.002	4.32±0.00	0.08±0.001
TMS 00/0355	4.23±0.00	10.65±0.003	3.64±0.00	0.10±0.00
TME 778	3.24±0.016	10.35±0.00	3.42±0.002	0.90±0.00

Values in the table are the means of triplicate Means±SD. HCN: Cyanide. N = 10

Table 6: Total viable and fungal counts of 10 cassava varieties

Variety	Total viable counts	Total fungal counts
TMS 001/0355	2.5×10^3	2.5×10^3
01/0103	8.0×10^3	1.0×10^3
91/1730	4.5×10^5	1.5×10^3
TME 778	6.5×10^3	0.5×10^3
TMS 91934	3.5×10^3	1.0×10^3
92/0057	4.5×10^4	1.0×10^3
NR87184	2.5×10^3	1.0×10^3
TMS/0338	3.0×10^3	0.5×10^3
TMS/1646	3.0×10^3	1.5×10^3
TMS 94/0330	4.5×10^3	1.0×10^3

Reported values are the average of triplicate experiments and are in coliform units (cfu). Values are significant at $p < 0.05$. N = 10

compounds with antioxidant properties and phenolics as stated have also been associated with antioxidant properties of food in addition (Robbins, 2003). Kirakosyan *et al.* (2003) reported that phenolic compounds in plants possess antioxidant activity and may help protect cells against the oxidative damage caused by free radicals. The implication is that all cassava varieties studied could have some antioxidant properties.

The low cyanide contents of the cassava varieties is another significant finding in this study. Cyanide being an effective cytochrome oxidase inhibitor in the electron transport chain, interferes with aerobic respiration (Onwuka, 2005). Bolhuis (1954) has reported that the lethal dose of cyanogenic glucoside for an adult man is 50-60 mg kg⁻¹ b.wt. Since, the values obtained were from fresh tubers, the cyanogenic glucoside content of the 10 varieties of cassava flours studied are quite too low to cause any deleterious effects (Table 5).

The Total Viable and Fungal Counts as shown in Table 6 indicated that they ranged from 0.5×10^3 to 4.5×10^5 cfu g⁻¹ with TMS 91/1730 having the highest viable count (4.5×10^5) and TMS 0338 and 1646 having the least viable count (3.0×10^3) while TMS 001/0355 had the highest fungal count (2.5×10^3) and TMS 0338 and TME 778 had the least fungal count (0.5×10^3). Our results obtained are consistent with FAO (2003) and this indicates that all the flours analyzed were safe for consumption and their shelf life could be extended if packaged well and stored.

CONCLUSION

All flours of the 10 cassava varieties analyzed were found to have good nutritional and functional properties making them suitable as drug binder and disintegrants in pharmaceuticals. With low microbial load and cyanide contents, their shelf life could be extended if stored well and they're quite safe for consumption in addition. However, investigation into other post-harvest characteristics of these cassava varieties is recommended

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