Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 101 (2007) 1606–1615
Food Chemistry
www.elsevier.com/locate/foodchem

Use of multivariate techniques in studying the flour making properties of some CMD resistant cassava clones

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Received 26 November 2005; received in revised form 1 April 2006; accepted 12 April 2006

Abstract

High quality cassava flour (HQCF) is one of the primary products of raw cassava root that has continued to find wider food application in Nigeria. In this study, some 43 newly developed cassava mosaic disease (CMD) resistant clones of cassava were screened based on some physical (flour yield, bulk density, and tri-stimulus colour characteristics (L^*, a^*, b^*, Chroma and Hue)), chemical (moisture, protein, ash, starch, amylose, sugar contents, TTA, pH, and cyanogenic potential), functional (water and oil absorption capacities, water solubility, swelling power, least gelation capacity, diastatic activity, percent damaged starch, and alkaline water retention), and pasting properties. One-way analysis of variance (ANOVA) showed that all properties measured varied significantly ($P < 0.001$). The flours had a wider range of starch content (65–88%), amylose content (13–23%), water absorption capacity (136–224%), diastatic activity (128–354 mg maltose), peak viscosity (77–328 RVU), final viscosity (56–217 RVU), and trough (32–152). Due to the peculiarity of the experimental data generated, two protocols of applying multivariate statistical techniques were evaluated for discriminating the cassava clones. By first applying principal component analysis (PCA), followed by cluster analysis (CA) and finally, discriminant function analysis (DFA) of the experimental data, it was possible to achieve about 87% correct classification of the cassava clones. The final viscosity and diastatic activity of the flours were found to be the most important variables for classifying the cassava clones.

Keywords: Cassava mosaic disease resistant clones; Cassava flour; Physical properties; Chemical properties; Functional properties; Multivariate data analysis

1. Introduction

Cassava is one of the world's most popular tropical root crops with Nigeria being the largest producer. Its production is currently at 33.8 million tonnes per year (FAO, 2002). Introduction of high yielding and disease resistant cultivars is one of the key factors for the over 300% increased production experienced in the last 20 years (Anon, 2004). In spite of these developments, efforts towards increasing novel uses, value addition and evolving improved processing methods are much less commensurate. Most of the roots produced have mainly been processed into traditional foods and consumed locally. Nweke, Spencer, and Lynam (2002) reported that per capita consumption of cassava increased from 88 to 120 kg per person per year between 1961–1965 and 1994–1998.

The outbreak of cassava mosaic disease (CMD), which led to a decline of cassava root production in several parts of Africa, has initiated research efforts to develop disease-resistant varieties of cassava. The overview of these efforts is presented elsewhere (Anon, 2004; FAO, 1999). Currently, the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, has developed about 43 CMD resistant varieties of cassava that are currently receiving multilocational field trials for full integration into the cropping system in Nigeria. We are aware that several hundreds of resistant cassava clones are currently being developed

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0308-8146/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2006.04.017

across the CMD prone areas in Africa. In addition to environmental effects, genetic variations existing in the new plant breeds may introduce some variations that could be important to the food and industrial applications of their roots. Therefore, screening the new crop breeds to determine their potential end uses is quite imperative. This can serve as a basis for selective cultivation and contract supply of appropriate varieties of fresh cassava roots for various industrial uses. Moreover, technical data on the influence of genotypic differences on the quality and industrial application of cassava products is scanty in the literature.

The Collaborative Study of Cassava in Africa (COSCA) indicated that cassava flour and dried chips make up to 45% cassava product identified (Nweke, 1994). Currently, the number of large-scale manufacturers of non-fermented cassava flour (NFCF) in Nigeria has increased tremendously as a result of growing demand for its industrial and food uses. For example, the renewed interest of the Federal government of Nigeria to promote cassava from a food security to an economic crop has had a lot of impact such as increased cassava cultivation, improved processing technology and uses. To indicate this growing interest, the export quantity of cassava flour by Nigeria was reported to have increased from about 20 metric tonnes in 1992 to about 2300 metric tonnes in 2002 (FAO, 2004). The uptake of the practical improvements in cassava processing technology has also had a major impact on the livelihoods of the poor Ghanian cassava farmers by increasing their income by 150–200% through sales of high-quality cassava flour. Rural bakers have improved their gross profit margins by 107% (NRI, 2002). Cassava flour is mainly used in baking and confectionery products to substitute wheat flour at varying levels. Other food uses include application in the manufacture of weaning foods and pasta, and the production of starch used by the food, pharmaceutical, and chemical industries. Some of these applications are now supported by legislation. In some of the cassava stakeholders' meetings held recently, flour millers reported that less than 15% of the high quality cassava flour (HQCF) supplies required by the flour mills in Nigeria to make composite flour have been realized. Apart from the inadequate quantity, flour mills are also contending with problem of how to determine specific flour properties to use in screening their supplies.

The physical, chemical and functional properties of flour from various biological origins have been studied widely. Examples of properties often reported in the literature include proximate analysis, pH, acidity, starch and amylose contents, cyanogenic composition and damaged starch, among others, while functional properties include water absorption, water solubility, swelling power (or index), gel consistency and pasting characteristics (Adebowale, Afolabi, & Lawal, 2002; Bhattacharya, Zee, & Corke, 1999; Lee, Baik, & Czuchajowska, 1998; Ruales, Valencia, & Nair, 1993; Tagodoe & Nip, 1994). Most of these properties have also been reported for both fermented or non-fermented cassava flours (Defloor, Leijskens, Bokanga, & Delcour, 1995; Idowu, Oni, & Amusa, 1996; Sanni, Oyewole, & Olowogbade, 1998; Shittu, Lasekan, Sanni, & Oladosu, 2001), and for a granular cassava product called gari (Almazan, 1992; Olorunda, Aworh, & Numfor, 1981). The variations observed in the measured properties of the cassava products were attributed to varietal differences, agronomic conditions, age at harvest and processing methods.

In this study, fresh roots from some newly developed CMD resistant varieties of cassava were evaluated for their potential use in producing high quality cassava flour. About thirty separate physical, chemical and functional parameters of the flour samples produced from 43 cassava genotypes were determined. We attempted to compare the ability of some multivariate statistical protocols to identify the similarities in the flour making properties among the genotypes for classification purposes groups and their differences to identify salient flour properties that could be used in future to screen cassava roots for flour making purposes.

2. Methodology

2.1. Cassava roots

Fresh roots from 43 CMD resistant clones of cassava were obtained from a multilocation trial plot of the International Institute for Tropical Agriculture (IITA), Mokwa, Niger State. Mokwa is situated in the Guinea savanna region of Nigeria (lat. 9.5°N, long. 5.1°E) with a mean annual rainfall of <1800 mm. The cassava varieties were planted during the rainy season (June 2003) in a randomized complete block design with two replications and two treatments (with and without fertilizer application). Only the crops without fertilizer application were harvested for use in this study. Harvesting was done at about 12 months after planting. The roots were processed within 4 h of harvesting.

2.2. Flour production

The fresh roots were peeled, washed thoroughly with water to remove adhering dirt and soil materials, and grated using a locally fabricated mechanical grater (Postharvest Unit, IITA, Ibadan). The grated wet cassava meal was then pressed using a mechanical screw press (Postharvest Unit, IITA, Ibadan) to reduce the moisture content to ease subsequent drying. The pressed cake was pulverized and spread thinly over a black high density polyethylene (HDPE) in the open air for drying under ambient conditions (30–34 °C, 72–85% RH). Drying of each sample took place within 48 h. The dried grits were milled using a locally fabricated hammer mill (IITA, Ibadan) fitted with a screen of 250 μm aperture size. The flour yield (FY) was determined in duplicate and calculated as:

$$\text{FY}\ (\%) = (100 * \text{Weight of flour})/\text{Weight of fresh root}$$

The flour samples produced were kept in the cold room (at about −10 °C) prior to use.

2.3. Chemical properties

The moisture content and diastatic activity of flour were determined using the AACC (1983) method. Protein was determined as Kjeldahl nitrogen × 5.7. Starch and sugar contents were determined using the phenol-sulfuric method of Dubois, Gilles, Hamilton, Rebers, and Smith (1956). Amylose content was determined according to Williams, Kuzina, and Hylinka, 1970. Cyanogenic potential (CNP) was determined using an automated enzymic assay described by Rao and Hahn (1984). The total titratable acidity (TTA) of flour was determined by soaking 10 g flour in 50 ml distilled water for 30 min with intermittent stirring. Ten milliliters aliquots of supernatant were titrated with 0.1 N NaOH. The TTA value was calculated from the titer value as follows:

$1\ \text{mL}\ NaOH \equiv 0.009\ \text{mg lactic acid}.$

2.4. Functional properties

The starch damage was determined using the extractability method of McDermott (1980). Diastatic activity of flours was determined as mg maltose (AACC, 1983). Water absorption capacity, water solubility, and swelling power were determined as described by Ruales et al. (1993). Alkaline water retention was determined as described by Adebowale et al. (2002). Oil absorption and least gelation capacity were determined according to Sathe and Salunkhe (1981). The pasting properties were determined using a Rapid Visco Analyzer (RVA) (Newport Scientific Company, Warriewood, NSW, Australia).

2.5. Data analyses

Descriptive analysis and one-way analysis of variance (ANOVA) were performed to explore the general trend of the experimental data. Principal component analysis (PCA) was done to examine the relative importance of each parameter on the variability between the cassava clones. Cluster analysis (CA) was done for the purpose of classifying the roots based on flour properties. Discriminant function analysis (DFA) was also used to determine which variables best discriminate groups of cassava clones from the others and to validate the classification obtained earlier. The combined features of SPSS Version 10.0 (SPSS Inc., Chicago, IL, USA) and S-PLUS Professional Release 1 (MathSoft Inc., Cambridge, MA, USA) statistical packages were employed in the analyses.

3. Results and discussion

3.1. General trends of properties

From the univariate statistical analyses, all the flour properties measured were shown to vary significantly ($P < 0.05$) among the cassava clones. Because of the large size of variables (32) and the cassava clones (43) studied, it is quite difficult to present the raw results here. However, some samples of the results are shown in Tables 1–4 while the summary has been displayed in the boxplots (Figs. 1a–1c). To avoid error of interpretation, it should be noted that certain variables such as bulk density, protein, sugar, ash contents, and total titratable acidity of the flour samples have smaller numerical values compared to other chemical components like the starch and amylose contents (Fig. 1a). This is directly reflected from the smaller thickness of the boxes and deviation from the means (depicted by the protruding bars) of the former compared to the latter. Similar comments apply to the physical and functional properties (Figs. 1b and 1c). Notably, the flours' functional properties showed higher variability than the chemical and physical properties.

3.2. Physical properties

The knowledge of flour yield is a very important physical and economic factor in screening the cassava clones for flour making properties. The flour yield ranged from 12.36% to 24.84% giving a mean of 19.47% on a fresh root basis. This is in agreement with a previous report (Rayong, 2003). Apart from the varietal influence, the inevitable processing loss and the subjective nature of the peeling process could have contributed to the observed variations in the flour yields. Based on the flour yield obtained in this study, the cassava clones have been grouped into high, medium, and low yielding ones (Table 1). It should also be pointed out that the final moisture content of the flour also varied from 11.00% to 16.75% mainly due to varying drying efficiency on the field. The bulk density is an important parameter that determines the ease of packaging and transportation of particulate foods. In a previous work involving just one variety of cassava (Shittu, Awonorin, Sanni, & Idowu, 2002), it was shown that bulk density of cassava

Table 1
The range of flour yields (fresh root basis) among the cassava clones

Categories	Flour yield (%)	Cassava clones
Low	<16.0	98/2101, 94/0561, 97/2205
Medium	16.0–21.0	97/4779, 30572, 98/2226, 82/00058, 97/0211, 99/3073, 98/0581, 97/4763, 96/0523, 4(2)1425, 99/2123, 96/1569, 96/1565, 92b/0006, 95/0379, 95/0166, 97/3200, 92/0067, 97/4769, 92/0057
High	21.0–25.0	96/1632, 98/0002, M98/0068, 96/1089a, 97/3200, 95/0289, 92/0326, 98/0510, 97/0162, 92/0325, TME 419, M98/0028, 98/0505

Table 2
Chemical composition of flours from selected cassava clones

Clone	Moisture (%)	Protein (%)	Starch (%)	Sugar (%)	Ash (%)	TTA (%)	CNP (mg/kg)	Amylose (%)
97/4779	13.03	1.41	79.66	1.57	1.68	0.36	3.13	18.58
30572	14.36	1.26	81.46	2.00	1.70	0.29	0.46	18.58
98/2226	13.98	1.12	81.86	2.17	1.43	0.36	0.00	18.91
82/00058	15.44	1.46	78.50	1.61	1.71	0.27	0.11	14.39
97/0211	14.14	1.02	83.07	2.24	1.60	0.25	0.00	18.36
96/1632	14.07	1.08	79.47	2.00	1.96	0.27	0.00	14.29
99/3073	16.75	1.11	87.46	1.61	1.64	0.27	0.00	17.38
98/2101	13.40	1.42	81.26	1.91	2.19	0.27	0.00	15.98
98/0581	15.03	0.98	83.87	2.33	1.77	0.32	0.00	16.31
97/4763	14.63	0.87	83.46	2.37	1.84	0.41	0.00	17.35
98/0002	13.09	0.77	84.65	2.00	1.66	0.32	0.11	19.22
96/0523	15.80	0.96	81.46	2.40	1.64	0.32	0.21	19.56
4(2)1425	13.82	1.44	76.30	2.89	1.78	0.34	0.21	18.49
99/2123	14.41	1.08	83.84	2.39	1.79	0.34	2.32	19.13
M98/0068	13.05	0.93	84.49	2.19	1.65	0.32	2.95	22.62
96/1569	16.40	0.99	82.65	1.76	1.70	0.34	0.04	22.85
96/1089a	14.15	0.99	78.24	2.42	1.47	0.38	0.00	16.43

TTA, total titratable acidity; CNP, cyanogenic potential.

Table 3
Functional properties of flours from some selected cassava clones

Clone	DS (%)	BD (g/cm^3)	WAC (%)	WS (%)	OAC (%)	LGC (%)	SWP	AWR	DA (mg maltose)
97/4779	0.43	0.39	211.00	13.79	112.77	7.00	15.40	1.52	210.00
30572	0.40	0.43	213.12	13.45	118.57	13.00	14.59	0.98	201.00
98/2226	0.48	0.44	136.07	13.54	115.12	8.00	14.68	1.25	260.50
82/00058	0.58	0.39	202.40	12.54	117.74	9.00	14.06	1.19	312.00
97/0211	0.53	0.49	163.41	16.37	105.75	6.00	15.22	1.26	288.50
96/1632	0.58	0.49	192.84	12.11	111.54	12.00	14.45	1.35	353.50
99/3073	0.60	0.42	176.44	13.23	131.99	8.00	13.25	1.13	276.00
98/2101	0.58	0.51	176.99	14.64	123.68	6.00	13.16	1.56	325.00
98/0581	0.50	0.45	197.18	13.52	115.45	5.00	14.63	1.11	344.00
97/4763	0.58	0.46	195.08	16.73	110.55	10.00	15.39	1.24	270.00
98/0002	0.48	0.55	169.80	17.96	93.83	10.00	16.17	1.18	234.00
96/0523	0.58	0.42	172.48	10.95	106.80	5.00	13.46	1.13	158.50
4(2)1425	0.53	0.47	176.99	19.46	113.78	13.00	13.56	1.26	204.00
99/2123	0.60	0.45	197.55	12.01	103.34	12.00	14.08	1.09	250.50
M98/0068	0.53	0.51	176.15	17.20	102.59	11.00	13.38	1.38	231.00
96/1569	0.60	0.43	197.42	19.51	110.00	11.00	13.41	1.24	279.50
96/1089a	0.53	0.45	179.13	13.57	150.15	12.00	13.62	1.29	140.00

DS, damaged starch; BD, bulk density; WAC, water absorption capacity; WS, water solubility; OAC, oil absorption capacity; LGC, least gelation capacity; SWP, swelling power; AWR, alkaline water retention; DA, diastatic activity.

flour was correlated with moisture content ($r = 0.986$, $P < 0.01$). Although the range of values obtained for bulk density were comparable, the correlation was not significant in the current study ($r = -0.153$, $P > 0.05$). This reflects the fact that apart from factors like particle size and moisture content, some other intrinsic factors may interact and dictate the bulking properties of the flour. The moisture content was also shown to have a significant effect on two tristimulus colour parameter (L^* and b^*). Lightness (L^*) was increased with a decrease in moisture content while the reverse was the case with greenness/yellowness (b^*). The typical colour characteristic of food is more conspicuous after reconstitution due to the higher level of moisture. A small number of cassava clones, currently under study in our laboratory, are known to have carotenoids. The observed increase in yellowness of the cassava flours is therefore expected. Since high quality cassava flour (HQCF) is customarily associated with a whitish colour, the influence of moisture on the technical and commercial values of the flour should be considered in subsequent product applications. The amount of HQCF added to food formulation and the processing treatment given will determine whether its colour will be a quality factor. For example, in a food product like noodles, the colour of flour is very important.

3.3. Chemical composition of flours

Products from pure cassava root have been shown to have very low protein and sugar content, but a high content of starch (Almazan, 1992; Eggleston, Omoaka, & Arowoshegbe, 1993). The result of chemical analyses from this study also showed a similar nutrient profile compared to the previous authors. The means and standard errors of means (SEM) in terms of the chemical composition of the flours from 43 CMD resistant clones of cassava are shown in Fig. 1a. The chemical composition of selected cassava clones are shown in Table 2. One-way analysis of variance (ANOVA) of the chemical composition showed that the starch, sugar, amylase, and the pH of the flour differed significantly ($P < 0.001$) among the cassava clones. The ash, sugar, and protein contents varied slightly between 1.5% and 3.6%, 1.2% and 2.2%, and 0.8% and 1.9% (dry basis), respectively. The cyanogenic potential (CNP) of the flours also ranged between 0 and 38 mg/kg (dry basis). Only one clone (92/0326) gave a CNP value above the maximum level of 10 mg/kg recommended for edible cassava flour (SON, 2004). The starch and amylose contents showed the highest variation ranging from 65% to 88% and 13% to 23% (dry basis), respectively. When compared to the values reported for flours from 10 different cassava clones by Eggleston et al. (1993), the sugar (2.4–5.5%) and protein contents (2.6–3.7%) were higher while the starch (60–68%) and amylose contents (12–14%) were remarkably lower than those observed in this study. It is particularly interesting to note that a cassava clone (4[2]1425) was commonly studied by both these groups of authors. The starch and amylose contents reported by the two authors for this same cassava clone are significantly different and it indicates that the breeding process undergone to confer resistance, and possibly some other agronomic factors, could have indirectly resulted into higher level of starch biosynthesis in the roots of CMD resistant clones of cassava currently being studied. The starch and amylose composition of staple food materials determines the processing and consumption characteristics of food products (Almazan, 1992; Olorunda et al., 1981; Pomeranz, 1991). Therefore, practical implications of the starch-based functional properties of these newly developed cassava clones needs to be properly established in future works.

Table 5
The eigenvalues of the discriminant functions used in the analysis

Function	Eigenvalue	% of variance explained	Cumulative % variance	Canonical correlation
Protocol 1				
1	53.478	76.1	76.1	0.991
2	13.391	19.1	95.2	0.965
3	2.525	3.6	98.8	0.846
4	0.847	1.2	100.0	0.677
Protocol 2				
1	7.168	88.7	88.7	0.973
2	0.912	11.3	100.0	0.691

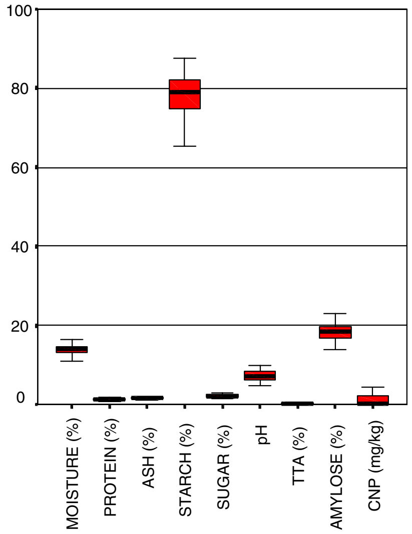

Fig. 1a. Box plot showing the variation in some chemical properties of flour of 43 CMD resistant clones of cassava.

Table 4
Pasting characteristics of flours from selected cassava clones

Clone	PV (RVU)	TRGH (RVU)	BKDV (RVU)	FV (RVU)	SBV (RVU)	PKTm (min)	PT (°C)	PTm (min)
97/4779	328.04	151.38	176.67	216.55	64.88	4.07	76.85	2.95
30572	182.59	76.29	105.50	120.46	44.17	3.93	79.15	2.93
98/2226	195.29	87.42	107.67	139.04	51.63	3.96	79.08	2.93
82/00058	215.08	82.42	131.13	134.79	52.38	3.86	78.33	2.93
97/0211	240.63	102.13	136.54	164.79	62.67	3.96	79.80	2.95
96/1632	150.29	41.88	89.84	70.04	28.17	3.62	78.25	2.90
99/3073	223.17	84.29	130.38	143.09	58.79	3.80	78.40	2.91
98/2101	270.71	133.88	136.84	205.29	71.42	4.11	80.90	3.07
98/0581	196.21	90.88	105.17	149.42	58.55	4.00	79.53	2.97
97/4763	135.50	48.71	78.08	83.63	35.17	3.80	78.33	2.93
98/0002	275.67	112.13	162.79	190.09	77.96	4.19	81.25	3.00
96/0523	191.59	81.25	108.92	137.00	55.75	3.96	80.38	3.03
4(2)1425	124.09	35.00	85.67	58.42	24.92	3.82	80.97	3.10
99/2123	202.88	81.29	109.13	110.00	28.71	3.75	79.40	3.03
M98/0068	175.46	67.75	104.88	111.04	43.29	3.93	78.83	2.93
96/1569	182.75	86.04	96.71	143.29	57.25	4.03	82.55	2.93
96/1089a	183.88	70.58	111.63	111.84	66.25	3.86	81.53	3.03

PV, peal viscosity; TRGH, trough; BKV, breakdown viscosity; FV, final viscosity; SBV, setback viscosity; PKtm, time to reach peak viscosity; PT, pasting temperature; PTm, pasting time.

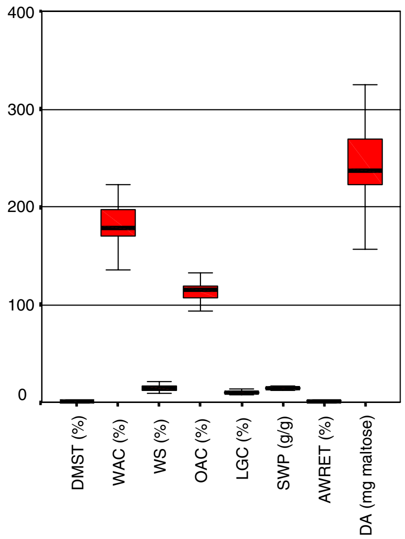

Fig. 1b. Box plot showing the variation in some functional properties of flour of 43 CMD resistant clones of cassava.

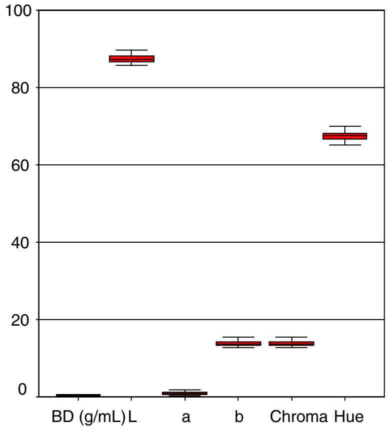

Fig. 1c. Box plot showing the variation in some physical properties of flour of 43 CMD resistant clones of cassava.

3.4. Functional properties of flours

The functional properties of flours are those that directly determine their end uses. The results of functional properties of flours from selected clones are shown in Table 3. The level of starch damaged in the flours ranged from 0.4% to 0.98%, showing a minimal destructive effect of processing on the starch granule integrity. This implies that the significant variations ($P < 0.001$) found with the flours' functional properties could have been due to some biological factors. This is reflected in the insignificant linear correlation values between starch damaged and all the functional properties (water, oil absorption and least gelation capacities, water solubility, swelling power, diastatic activity, and alkaline water retention) ($P > 0.05$, absolute values of r ranged between 0.067 and 0.263). It has been established that the composition and nature of macromolecules (proteins, fat, and carbohydrates) in food materials often affect their functionality (Ihekoronye & Ngoddy, 1985; Hung & Morita, 2003; Sopade & Okonmah, 1993). Only starch and sugar contents gave some significant correlations ($P < 0.05$) with damaged starch and water absorption capacity, respectively. It is also interesting to note that the ability of the flours to form gel as measured by the least gelation capacity reduced with increasing starch contents in cassava roots though the correlation is not significant ($r = -0.251$, $P > 0.05$). Lower gelation capacity will definitely have a favourable economic impact on use since this implies that less flour is required to make food gels. The gelation capacity is an important quality factor considered for flours used in pasta production. Another notable characteristic of the flours is that some of them have unusually higher diastatic activities than any one ever reported for cassava flour in literature. For example, the diastatic activities of the flours from this study ranged from 128 to 354 mg maltose and are significantly higher than the 115–208 mg maltose reported by Eggleston et al. (1993) for the 10 ancestral cassava clones compared to the current ones. This has an implication on the food and industrial uses of the flours from the clones. From the same authors' findings, it was concluded that flours with diastatic activities >145 mg maltose may not be good for bread making purposes. This may, however, be a favourable characteristic in fermentative uses of the flour such as in sour dough production. It was also found here that only the ash content and total titratable acidity significantly affected the diastatic activity (at $P < 0.05$, $r = 0.315$, and -0.298, respectively).

The commonest objective method of determining the cooking property of starch-based food products is through an amylograph pasting profile. Such information has been used to correlate the functionality of starchy food ingredients in processes like baking (Defloor, De Geest, Schellekens, Martens, & Delcour, 1994, 1995; Idowu et al., 1996; Rojas, Rosell, & Benedito de Barber, 1999) and extrusion cooking (Ruales et al., 1993). In this study, it was observed that the pasting properties (such as peak, trough, setback, breakdown, and final viscosities) of the cooked flours showed the widest variation among other properties measured. The peak viscosity is the maximum viscosity attainable during the heating cycle; the trough is an index of starch granule stability to heating; setback viscosity is an index of retrogradation of linear starch molecules during cooling. The maximum values of each obtained across the clones were about 3–10 times their minimum values. Cassava clone 92b/0006 had the least values for the peak, trough, and setback viscosities while clone 97/4779 had the largest amylograph viscosity values. Sample results are shown in Table 4.

It has been very difficult from past works to project the wheatless bread making potentials of cassava flour from its amylograph pasting properties. However, in agreement with previous works, it was concluded that attaining gelatinization at a lower temperature led to improved bread making quality (Defloor et al., 1994). High peak viscosity and stability (or low breakdown viscosity) were also associated with cassava starch which produces acceptable bread (Adeyemi & Omolayo, 1984; Olatunji & Akinrele, 1978). Further works are needed to really determine quantitatively how pasting properties of cassava flour relate to its food uses.

3.5. Multivariate statistical models for differentiating cassava clones

In the previous discussion it was mentioned that, based on ANOVA, all of the properties significantly varied among the cassava clones. Therefore, further analysis became necessary in order to identify specific grouping based on the measured properties. Statistically, clones that fall in the same group are believed to possess very similar characteristics. It has been demonstrated that multivariate statistical methods are very useful for this purpose (Baker & Tomlins, 1994; Resurreccion, 1988; Rouzaud & Martinez-Anaya, 1997). It should however be noted that different protocols were followed by these authors in applying the multivariate technique in their papers. For optimal exploration of the experimental data, we have decided to compare the results from two types of protocols combining PCA, CA, and DFA.

4. Protocol 1

Since the ANOVA results have shown that there were significant differences between the cassava clones in terms of all the properties measured, it means that each of them is a potential variable that could be used in classifying the clones into distinct groups. Therefore, hierarchical clustering was carried out to classify the clones based on all the measured properties using squared Euclidean distance as the measure of dissimilarity. The classification achieved is shown in the dendogram (Fig. 2). Cluster 1 is the largest consisting of 28 clones; Cluster 2 consists of 8 members and Cluster 3 consists of 5 members; while Clusters 4 and 5 consist of 1 clone each. It is interesting to note that clones 97/4779 and 92/0236 singular form two distinct clusters. These clones, respectively, have peak viscosity and cyanogenic potential that are distinctly higher than any other clone. Members of Cluster 3 are noted for their high diastatic activities. Stepwise DFA was then performed to combine the variables which have significant discriminating power for classification purposes. The best function should have the lowest number of variables (Resurreccion, 1988). The results also show that only four discriminant functions were sufficient for use in completely correcting the errors in the initial classification. The Eigenvalues and the proportion of variance explained by each function are shown in Table 5. Variables finally included in the standardized discriminant function are pH, a^*, b^*, hue, trough, CNP, and oil absorption (Table 6). Interestingly, it appears that at least one representative of the all the groups of properties studied (i.e., the physical, chemical, functional, and pasting properties) was "forced" into the final discriminant function. Also, it was shown that about 91% of the clones were correctly classified initially (Table 6). To validate the discrimination of the cassava clones, it was necessary to statistically test the robustness and classification ability of the discriminant functions. The approach often used is jack-

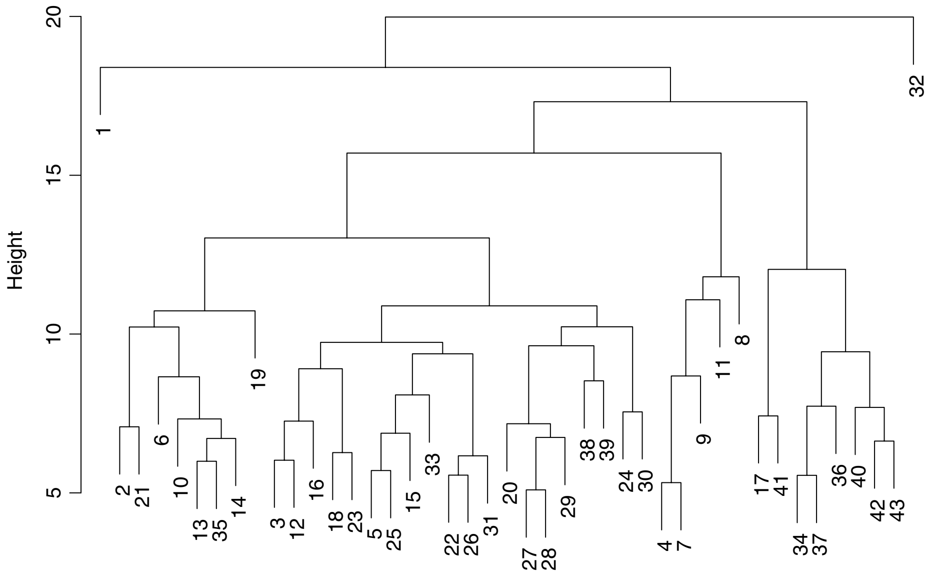

Fig. 2. Dendogram showing five hierarchical clusters of cassava clones based on all the flour properties of CMD cassava flours. Clustering method: complete linkage using Euclidean distance as measure of dissimilarity. Cluster 1: 30572 (2), 98/2226 (3), 97/0211 (5), 93/1632 (6), 97/4763 (10), 96/0523 (12), 4(2)1425 (13), 99/2123 (14), M98/0068 (15), 9/1569 (16), 96/1565 (18), 92b/0061 (19), 96/0603 (20), 94/0561 (21), 91/02324 (22), 94/0026 (23), 95/0379 (24), 97/2205 (25), 95/0166 (26), 97/3200 (27), 92/0067 (28), 96/1642 (29), 94/0039 (30), 95/0289 (31), 98/0510 (33), 97/0162 (35), M98/0028 (38), M98/0040 (39); Cluster 2: 96/1089a (17), 92/0325 (34), 99/6012 (36), TME 419 (37), 92B/0006 (40), 98/0505 (41), 97/4769 (42), 92/0057 (43); Cluster 3: 82/00058 (4), 99/3073 (7), 98/2101 (8), 98/0581 (9), 98/0002 (11); Cluster 4: 97/4779 (1); Cluster 5: 92/0326 (32).

knifing and cross-validation (Forveille, Vercauteren, & Rutledge, 1996; Resurreccion, 1988). After jackknifing and cross-validation, it was found that only about 72% of clones were correctly classified.

5. Protocol 2

The first step taken here was to perform PCA to identify measured variables that could be instrumental in differentiating the clones into distinct groups. The biplot in Fig. 3 shows the relationship between each variable and the cassava clones. The dominant variables found are the amylograph pasting viscosities and the diastatic activity of the flour in first and second principal components (PCs), respectively. All these variables had negative correlations with the two PCs. Notably, the physical and chemical variables were lumped up very close to the center of the axes, indicating their insignificant contributions to the genotypic differences. The first two PCs explained about 87.5% of the total variance (Table 8). In the first PC, which explained about 64.8%, peak viscosity had the greatest loading effect while setback viscosity had the least. Clones 1 (97/4779), 11 (98/0002), and 36 (99/6012) were distinctly separated from the rest based on the pasting characteristics of their flours whereas that of clones 4 (82/00058) and 9 (98/0581) had distinctive diastatic activity. Some functional properties namely, water and oil absorption capacities were included in the list of variables in PC3 and PC4 that collectively explained about 9% of the total variance. However, water absorption was heavier on these two PCs than oil absorption capacity (see Table 9).

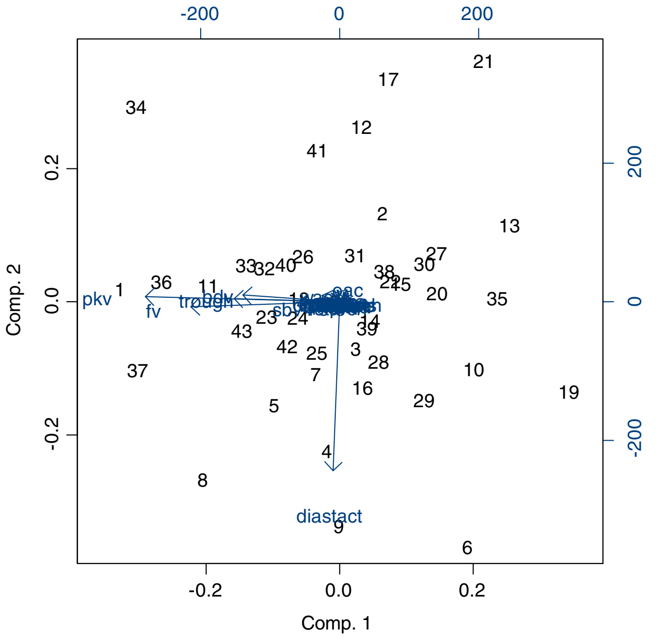

Fig. 3. The first two principal components showing the relationship between the chemical and functional properties of flours from the 43 CMD cassava clones. Scaling method used: covariance matrix.

The next step was to perform CA to identify distinctive groups and their members using the identified variables in the first two PCs as above. The dendogram (Fig. 4) shows the result of classification of the clones into five distinct groups. Clusters 1, 2, 3, 4, and 5 consist of 6, 6, 16, 12, and 3 members, respectively. Members of Cluster 1 are characterized by higher pasting viscosity profiles while members of Cluster 2 generally have higher diastatic activity than any other cluster. The validity of this classification was then confirmed through DFA using Wilk's lambda stepwise method. In the stepwise procedure only two variables, namely final viscosity and diastatic activity were found to give the most efficient combination for classifying the cassava clones (Table 7). The results of prior classification

Table 6
Prior, corrected, and validated clustering of cassava clones

	Prior count	Corrected group count					Validated group count				
		1	2	3	4	5	1	2	3	4	5
Protocol 1											
Group											
1	28	24	2	2	–	–	22	3	3	–	–
2	8	–	8	–	–	–	1	7	–	–	–
3	5	–	–	5	–	–	3	–	2	–	–
4	1	–	–	–	1	–	–	–	1	1	–
5	1	–	–	–	–	1	–	–	–	–	–
Total	43	24	10	7	1	1	26	10	6	1	0
Protocol 2											
Group											
1	6	6	–	–	–	–	6	–	–	–	–
2	6	–	5	–	1	–	–	5	–	1	–
3	16	–	–	15	1	–	–	–	15	1	–
4	12	–	1	–	10	1	–	1	–	10	1
5	3	–	–	–	–	3	–	–	–	1	2
Total	43	6	6	15	12	4	6	6	15	13	3

Table 7
Variable entered into the standardized discriminant functions

Step	Variable entered	*F* statistic	Sig. *P*
Protocol 1			
1	CNP	95.67	<0.001
2	b^*	40.79	<0.001
3	Trough	29.56	<0.001
4	pH	24.28	<0.001
5	a^*	21.06	<0.001
6	Hue	42.24	<0.001
7	Oil absorption capacity	39.12	<0.001
Protocol 2			
1	Final viscosity	64.32	<0.001
2	Diastatic activity	27.32	<0.001

Table 8
The relative importance of the first four principal components

	Principal component			
	1	2	3	4
Standard deviation	79.78	47.24	22.11	29.14
% of variance explained	64.78	22.70	5.00	3.88
Cumulative % variance	64.78	87.48	92.48	96.26

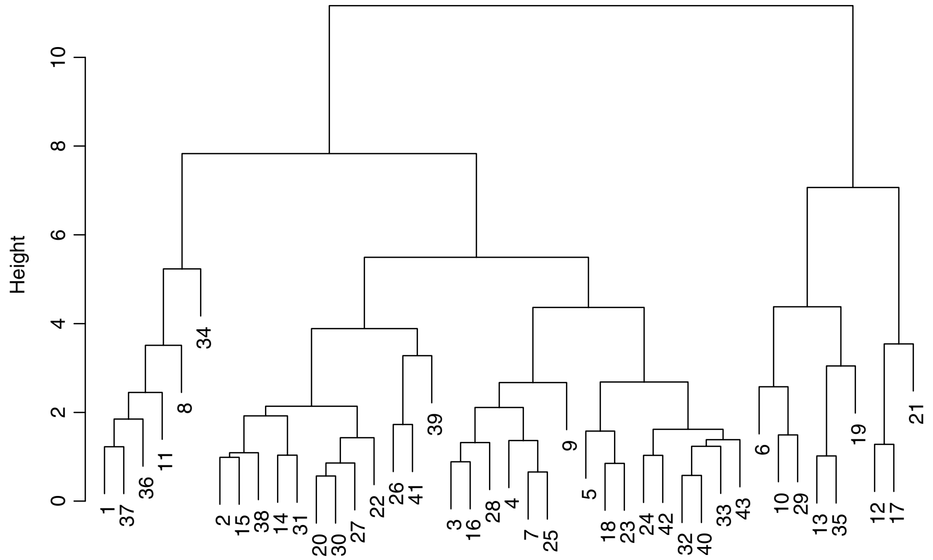

Fig. 4. Dendogram showing five hierarchical clusters of cassava clones based on pasting viscosity and diastatic properties of CMD cassava flours. Clustering method: complete linkage using Euclidean distance as measure of dissimilarity. Cluster 1: 97/4779 (1), 98/2101 (8), 98/0002 (11), 92/0325 (34), 99/6012 (36), TME 419 (37); Cluster 2: 93/1632 (6), 97/4763 (10), 4(2)1425 (13), 92b/0061 (19), 96/1642 (29), 97/0162 (35); Cluster 3: 98/2226 (3), 82/00058 (4), 97/0211 (5), 99/3073 (7), 98/0581 (9), 9/1569 (16)96/1565 (18), 94/0026 (23), 95/0379 (24), 97/2205 (25), 92/0067 (28), 92/0326 (32) 98/0510 (33), 92B/0006 (40), 97/4769 (42), 92/0057 (43); Cluster 4: 30572 (2), 99/2123 (14), M98/0068 (15), 96/0603 (20), 91/02324 (22), 95/0166 (26), 97/3200 (27), 94/0039 (30), 95/0289 (31), M98/0028 (38), M98/0040 (39), 98/0505 (41); Cluster 5: 96/0523 (12), 96/1089a (17), 94/0561 (21).

and cross-validated classification after jackknifing are shown in Table 6. In the latter, about 93% of the clones were correctly classified while after cross-validation 88.4% of the clones were found to have been correctly classified originally. It has become apparent following the second protocol, which involves applying a data reduction method (PCA), that the ability of a discriminant function to differentiate the cassava clone has been improved. The biplot (Fig. 5) of the PC regression scores using the two most discriminating variable (final viscosity and diastatic activity) clearly demonstrates this.

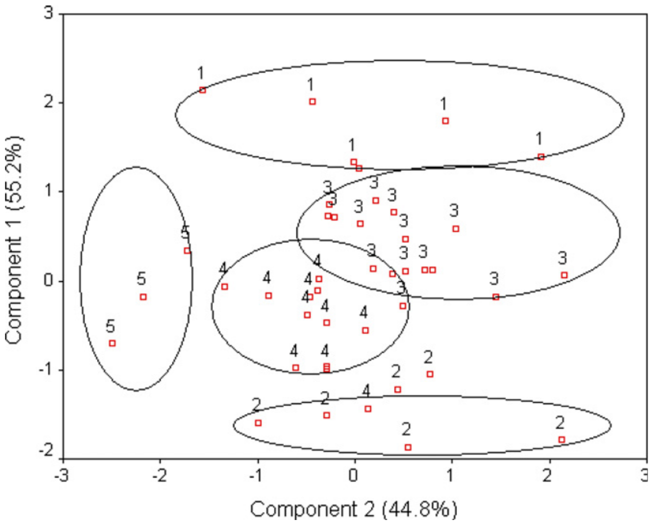

Fig. 5. Principal component plot using the regression coefficients of the two most discriminating flour properties.

Table 9
Factor loading of the principal components

Factor	Principal components			
	1	2	3	4
Diastatic activity	*	−0.994	−0.844	*
Peak viscosity	−0.676	*	0.211	0.357
Trough	−0.367	*	−0.172	−0.225
Breakdown viscosity	−0.337	*	0.288	0.424
Final viscosity	−0.517	*	−0.517	−0.455
Setback viscosity	−0.147	*	−0.147	−0.254
Water absorption capacity	*	*	0.787	−0.561
Oil absorption capacity	*	*	0.133	−0.215

[*] Loading value <0.1.

6. Conclusions

From the foregoing it can be concluded that flour from the 43 CMD clones of cassava have distinct physical, chemical, and functional properties. The flours have unusually wider values for starch, amylose, water absorption capacity, and diastatic activity. The most discriminating flour properties among the clones are diastatic activity and pasting viscosity parameters. Following the sequence of PCA, CA, and DFA, it was possible to achieve about 88.4% correct classification of the cassava clones for flour making purposes. The findings in this study call for further exploitation of flour making characteristics of the cassava clone for specific food and industrial uses.

Acknowledgements

The technical assistance provided by Messrs. G. Adenekan, K. Ogunsanya and I. Caminus of the Crop Utilization Unit are gratefully acknowledged. We also thank Dr. R. Okechukwu for his useful comment and corrections after reading through the manuscript.

References

AACC (1983). *Official methods of analysis*. St Paul, MN, USA: American Association of Cereal Chemists.

Adebowale, K. O., Afolabi, T. A., & Lawal, O. S. (2002). Isolation, chemical modification and physicochemical characterization of Bambara groundnut starch and flour. *Food Chemistry, 78*, 305–311.

Adeyemi, I. A., & Omolayo, O. (1984). Utilization of cocoyam flour and starch for biscuit and cake making. *Nigerian Journal of Science, 18*, 34–37.

Almazan, A. M. (1992). Influence of cassava variety and storage on *gari* quality. *Tropical Agriculture, 69*, 386–390.

Anon (2004). *Preemptive management of the virulent cassava mosaic disease through an integrated cassava development approach for enhanced rural sector economy in the south–south and south–east zones of Nigeria*. Ibadan, Niegria: Multiple Investor Development Project Document, IITA.

Baker, D. M., Gay, C., & Tomlins, K. I. (1994). Survey of Ghanaian cocoa farmer fermentation practices and their influence on cocoa flavour. *Food Chemistry, 51*, 425–431.

Bhattacharya, M., Zee, S. Y., & Corke, H. (1999). Physicochemical properties related to quality of rice noodles. *Cereal Chemistry, 76*, 861–867.

Defloor, I., De Geest, C., Schellekens, M., Martens, A., & Delcour, J. A. (1994). Impact of genotype and crop age on the breadmaking and physicochemical properties of flour produced from cassava (*Manihot esculenta* Crantz) planted in the dry season. *Journal of the Science of Food and Agriculture, 66*, 193–202.

Defloor, I., Leijskens, R., Bokanga, M., & Delcour, J. A. (1995). Impact of genotype, crop age and planting season on the breadmaking and gelatinization properties of flour produced from cassava (*Manihot esculenta* Crantz). *Journal of the Science of Food and Agriculture, 68*, 167–174.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for the determination of sugars and related substances. *Analytical Chemistry, 28*, 350.

Eggleston, G., Omoaka, P. E., & Arowoshegbe, A. C. (1993). Flour, starch and composite breadmaking quality of various cassava clones. *Journal of the Science of Food and Agriculture, 62*, 49–59.

FAO (1999). Food Outlook 22. November 1999.

Forveille, L., Vercauteren, J., & Rutledge, D. N. (1996). Multivariate statistical analysis of two-dimensional NMR data to differentiate grapevine cultivars and clones. *Food Chemistry, 57*, 441–450.

Hung, P. H., & Morita, N. (2003). Dough properties and bread quality of bread flours supplemented with cross-linked corn starch. *Food Research International, 37*, 461–467.

Idowu, M. A., Oni, A., & Amusa, B. M. (1996). Bread and biscuit making potential of some Nigerian cocoyam cultivars. *Nigerian Food Journal, 14*, 1–12.

Ihekoronye, A. I., & Ngoddy, P. O. (1985). *Integrated food science and technology for the tropics*. Oxford, UK: MacMillan Publishers.

Lee, L., Baik, B. K., & Czuchajowska, Z. (1998). Garbanzo bean flour use in Cantonese noodles. *Journal of Food Science, 63*, 552–558.

McDermott, E. E. (1980). The rapid non-enzymatic determination of damaged starch in flour. *Journal of the Science of Food and Agriculture, 31*, 405–413.

Nweke, F. I. (1994). Cassava processing in sub-Saharan Africa: the implications for expanding cassava production. *Outlook on Agriculture, 23*, 197–205.

Nweke, F. I., Spencer, D. F. I., & Lynam, J. K. (2002). *The cassava transformation: Africa's best kept secret*. East Lansing, MI, USA: Michigan State University.

Olatunji, O., & Akinrele, I. A. (1978). Comparative rheological properties of bread qualities of wheat flour diluted with tropical tubers and breadfruit flours. *Cereal Chemistry, 54*, 1096–1108.

Olorunda, A. O., Aworh, O. C., & Numfor, F. A. (1981). Technical note. Predicting the quality of cassava products with the aid of chemical and rheological properties. *Journal of Food Technology, 16*, 447–450.

Pomeranz, Y. (1991). *Functional properties of food components* (2nd ed.). USA: Academic Press, Inc, pp. 25–77.

Rao, P. V., & Hahn, S. K. (1984). An automated enzyme assay for determining the cyanide content of cassava (*Manihot esculenta* Crantz) and cassava products. *Journal of the Science of Food and Agriculture, 35*, 426–436.

Rayong, J. H. (2003). Processing cassava into flour for human food. Food Fertilizer Technology Center: An International Information center for Farmers in the Asia Pacific Region. Available from http://www.fftc.agnet.org/library/article/pt2003017.htm.

Resurreccion, A. V. A. (1988). Application of multivariate methods in food quality evaluation. *Food Technology, 42*, 128, 130,132–135,136.

Rojas, J. A., Rosell, C. M., & Benedito de Barber, C. (1999). Pasting properties of wheat flour-hydrocolloid systems. *Food Hydrocolloids, 13*, 27–33.

Rouzaud, O., & Martinez-Anaya, M. A. (1997). Relationships between biochemical and quality-related characteristics of breads, resulting from the interaction of flour, microbial starter and the type of process. *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung A-Food Research and Technology, 204*, 321–326.

Ruales, J., Valencia, S., & Nair, B. (1993). Effect of processing on the physico-chemical characteristics of quinoa flour. *Starch/Starke, 45*, 13–19.

Sanni, L. O., Oyewole, O. B., & Olowogbade, D. V. (1998). Effect of different drying methods on lafun (fermented cassava flour). *Tropical Science, 38*, 1–4.

Sathe, S. K., & Salunkhe, D. K. (1981). Functional properties of the Great Northern Bean (*Phaseolus vulgaris*) flour. *Journal of Food Science, 46*, 71–74.

Shittu, T. A., Lasekan, O. O., Sanni, L. O., & Oladosu, M. O. (2001). The effect of drying methods on the functional and sensory characteristics of pukuru – a fermented cassava product. *ASSET Series A, 1*, 9–16.

Shittu, T. A., Awonorin, S. O., Sanni, L. O., & Idowu, M. A. (2002). Dry milling characteristics of dried cassava chips as related to moisture content. *Journal of Applied Science and Technology, 2*, 13–18.

Standard Organization of Nigeria (SON) (2004). Nigerian standard for edible cassava flour. *NIS, 344*, 1997.

Sopade, P. A., & Okonmah, G. N. (1993). The influence of physicochemical properties of foods on their water absorption behaviour: a quantitative approach. *ASEAN Food Journal, 8*, 32–37.

Tagodoe, A., & Nip, W. K. (1994). Functional properties of raw and precooked taro (*Colocasia esculenta*) flours. *International Journal of Food Science and Technology, 29*, 457–462.

Williams, P. C., Kuzina, F. D., & Hylinka, I. (1970). A rapid colorimetric method for estimating amylose content of starches and flours. *Cereal Chemistry, 47*, 411–421.