African Crop Science Journal, Vol. 16, No. 3, pp. 191 - 202
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ISSN 1021-9730/2008 $4.00

QUANTIFICATION OF STARCH PHYSICOCHEMICAL CHARACTERISTICS IN A CASSAVA SEGREGATING POPULATION

E. NUWAMANYA, Y. BAGUMA[1], R.S. KAWUKI[1] and P.R. RUBAIHAYO
Department of Crop Science, Makerere University, P. O. Box 7062, Kampala, Uganda
[1]National Crops Resources Research Institute (NaCRRI), P. O. Box 7084, Kampala, Uganda

(Received 13 March, 2009; accepted 18 July, 2009)

ABSTRACT

Culinary cassava (*Manihot esculenta* L.) qualities that make a variety popular are a function of starch physicochemical properties. Hence, in response to inferior root qualities in some released cassava varieties in Uganda compared to the local germplasm, a study was undertaken to examine: (i) starch physicochemical characteristics in both introduced and local varieties; and (ii) the variation in the starch properties of their F_1 progenies. The local varieties included Bao, Nyaraboke, Kakwale, and Bamunanika; and the introduced genotypes SE/95/00036, NASE 10, NASE 12, TME 5, and TME 14. Of the generated 7000 F_1 seedlings, 1077 seedlings were selected, cloned (6-8 plants per genotype) and established in a single-row trial. Root samples were collected per clone and examined for starch physicochemical properties. Considerable variations were observed in the F_1 families compared to the parental lines with weak correlations in most starch properties (r < 0.25). Amylose content ranged from 10 to 25%, with the amylose: amylopectin ratio between 1:3 and 1:9. Among the F_1 families, solubility and swelling power ranged from 1-15g100g^{-1} and 40-140g100 g^{-1} starch at 60^{o}C, respectively. In the parents, it ranged between 1.3-8.6 and 50-67g100g^{-1} starch at 60^{o}C, respectively. Fresh root starch yield ranged from 18 to 34%, with dry matter content varying from 19-47% in both the F_1 families and the parents. Ash and lipid content varied among the F_1 families and parents with ranges 0.05-0.29% for ash and 0.1-0.32% for lipids. In both the parents and the F_1 families, the reducing sugar and protein content ranged between 0.7-1.7 and 0.23-0.43%, respectively. These findings demonstrate: (i) existence of considerable genetic variations in starch physicochemical properties in both local and introduced cassava genotypes and their progenies, and (ii) potential utilisation of cassava starches for various applications based on the inherent differences in physicochemical characteristics.

Key Words: Amylose, cassava starch, *Manihot esculenta,* protein, reducing sugar

RÉSUMÉ

Les qualités qui rendent le manioc culinaire (*Manihot esculenta* L.) une variété populaire sont fonction des propriétés physico-chimiques de l'amidon. Ainsi, en réponse aux qualités inférieures des racines dans certaines variétés de manioc diffusées en Ouganda en comparaison avec les matériels génétiques locaux, une étude avait était menée dans le but d'examiner: (i) les caractéristiques physico-chimiques de l'amidon aussi bien dans les variétés locales que dans les variétés exotiques introduites; en plus (ii) la variation dans les propriétés de l'amidon des progenies F_1. Les variétés locales sont Bao, Nyaraboke, Kakwale, Bamunanika et les génotypes exotiques SE/95/00036, NASE 10, NASE 12, TME 5 et TME 14. De 7000 F_1plantules générées, 1077 plantules ont été sélectionnées, clonées (6-8 plants par génotype) et établies dans un essai en ligne unique. Les échantillons des racines avaient été collectées par clone et examinées pour les propriétés physico-chimiques de l'amidon. Les variations considérables avaient été observées dans les familles F1 comparativement aux lignés parentales avec faibles corrélations dans la plupart de propriétés de l'amidon (r < 0.25). Le contenu en amylose était de 10 à 25%, pour l'amylose : rapport d'amylopectin entre 1: 3 et 1: 9. Parmi les familles F_1, la solubilité et le pouvoir de gonflement variaient respectivement de 1-15g100g^{-1} et 40-140 g 100 g^{-1} d'amidon à 60^{0}C; tandis que dans les parents ils variaient respectivement entre 1,3-8.6 et l'amidon 50-67g100g^{-1} à 60^{o}C. Le rendement en amidon des racines fraîches était de 18 à 34 %, la teneur en matière sèche allant de 19-47 % au sein des familles F_1 et les parents. Le contenu en cendres et lipides variait dans les familles F_1 et les parents dans un intrvalle de 0,05- 0,29 % pour les cendres et de 0,32-0,1 % pour les lipides. Dans aussi bien les parents que les familles F_1, le contenu en sucre reduit et en protéines variait respectivement entre 0,7- 1,7 et 0,23-0,43%. Ces conclusions demontrent: (i) l'existence de variations génétiques considérables dans les propriétés physicochimiques de l'amidon aussi bien pour les génotypes exotiques de manioc que traditionnels, ainsi que leurs progenies, et (ii) l'utilisation potentielle des amidons du manioc pour diverses applications basées sur les différences inhérentes aux caractéristiques physico-chimiques.

Mots Clés: Amylose, amidon de manioc, *Manihot esculnta,* protéines, sucre reduit

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the source of energy for more than 500 million people world-wide (Ceballos *et al*., 2006). It has vast uses as food (Ceballos, 2002) and industrial applications (Niba *et al*., 2007). Cassava starch is equitable to other tuber and cereal starches on top of being easily extracted (FAO, 2000). However, it has limitations given deficiency in some of its quality characteristics such as solubility, swelling power and root quality parameters of dry matter and starch yield (Benesi, 2005). There are other starch associated biomolecules such as lipids; proteins and ash that make starch from different sources to have significant uses in the dietary sector (Ceballos *et al*., 2006).

Given this potential, various breeding programmes have been initiated in Uganda aimed at producing varieties with different starch physico-chemical and functional characteristics to suit various uses. More effort has been devoted to producing cultivars that are improved in terms of starch quantity and quality properties. However, the outcomes of different breeding programmes in Uganda have not been evaluated fully. This study involved comparative analysis of cassava root starch properties from both local and introduced varieties and the F_1 families derived from them in one of the breeding programmes.

MATERIALS AND METHODS

The F_1 progenies were generated in field experiments set up at National Crops Resources Research Institute (NaCRRI), in Central Uganda, using four landraces namely, Bao, Bamunanika, Kakwale, Nyaraboke; three IITA introductions namely Nase10, Nase12, 95/SE-00036 and two Nigerian landraces namely TME5 and TME14 in a poly-cross nursery block. Clonal evaluation consisted of nine half-sib families comprising of 1,077 clones that were planted using family replication procedure (Ceballos *et al*., 2005).

Progenies were harvested at 12 months after planting. Two roots were randomly selected per progeny and prepared for starch extraction by peeling and cleaning with distilled water.

For each parental line, roots were subdivided into three equal portions, namely, (i) distal end, (ii) proximal end and (iii) middle portion. Starch was extracted from each portion separately and the amount of starch produced recorded as starch yield. In case of the F1 progenies the middle portion of the root which registered the highest average starch yield in the parents was used for starch extraction.

Native cassava starch extraction was carried out using a modified method as described by Riley *et al*. (2006). The clean middle portion of the fresh tuberous roots (100 g) were homogenised with 1M NaCl solution (10 g of tuber in 20 ml of solution) using a Waring blender. The pulp (mixture) was stirred for 2 min and filtered using a triple cheese cloth. The filtrate was allowed to stand until the starch sedimented and the top liquid was decanted and discarded. The starch sediment was again washed with distilled water, followed by centrifugation at 3,000 g for 10 min and the supernatant solution was discarded.

The starch produced was air-dried on aluminium pans at room temperature (25^0C) for 18-24 hr and stored in dry plastic air tight containers at room temperature until when needed for further analysis. The starch content and hydrolysis of starch were achieved using a-amylase (Riley *et al*., 2006). Glucose released was quantified (Dubois *et al*., 1956) and total starch was obtained using a factor of 0.93 (FAO, 2000).

Storage root dry matter content was determined according to Benesi (2005).

The amylose content of starch was obtained by defatting starch using ethanol (AOAC, 1996) and applying the Riley *et al*. (2006) method.

Starch solubility at 60^0C was determined using the Benesi (2005) method. Cold water solubility was determined according to Benesi (2005) method. Changes in swelling power were determined according to Leach *et al*. (1959) at temperatures ranging from 30 to 80^0C at an interval of 10^0C. The moisture content of native starch was determined according to International Starch Institute (ISI, 1997) method.

For determination of reducing sugars, 500 mg of starch was mixed with 1 ml 95% ethanol in a centrifuge tube and 2 ml distilled water was added and mixed. Then, 10 ml of hot 95% ethanol was added followed by vortexing for 5 min and centrifugation for 10 min. The supernatant was decanted into a volumetric flask and made up 100 ml with distilled water and 10 ml from this solution was used for determination of glucose according to Dubois *et al*. (1956).

Protein content was determined by Dumas Combustion in a nitrogen protein analyzer Leco FP-528, using EDTA as a standard and a factor of 6.25 to convert nitrogen to protein.

Crude fat and ash contents were determined according to Helrich (1990) methods.

RESULTS AND DISCUSSION

Results for cassava root starch yield from the different parts of the root in the parental lines and the total yield of starch from the root are presented in Table 1. Among the different parents, the starch yield ranged from 17.82 to 34.43%, with the middle portion of the root having significantly higher ($P<0.05$) starch yield. The only exception was TME 5 and TME 14 where no difference was observed among the different portions. Basing on these results, the middle part of the root was used for further starch extraction in the study.

Cassava root starch quantity parameters for the parents and their respective F_1 families are presented in Table 2. Starch content, starch yield, and dry matter content have been a major focus in the improvement of cassava in a bid to ensure maximum output of the total utilisable solid matter within the crop. In this case, cassava varieties with high dry matter contents and high starch yield are sought for. These traits translate into high starch content which qualifies cassava for use in the starch producing industries. These parameters are also important in the acceptability of cassava for food and dietary uses (Mariscal *et al*., 2002).

Among the parental lines, starch content ranged from 70-90%, while it ranged from 50-80%

TABLE 1. Comparative yield of starch from the different parts of the cassava root in the parental lines in Uganda

Parent	Starch extracted (%)			
	Proximal end	Middle	Distal end	LSD (5%)
Bamunanika	30.25	35.00	22.30	2.66
Bao	34.22	36.41	32.66	2.81
Kakwale	28.44	31.27	30.67	3.14
Nyaraboke	25.47	22.67	17.82	NS
Nase 10	20.35	24.34	21.42	3.68
Nase 12	12.50	17.82	23.13	4.59
TME 5	22.41	22.89	21.57	3.04
TME 14	21.57	18.81	20.16	NS
95/SE/00036	25.00	25.32	28.28	NS
LSD	5.04	NS	NS	

Results are means from 3 roots obtained from 3 different plants at different locations in the field for each variety, [a]The high value of the middle allowed its use for starch extraction for other analyses*LSDs significant at 5% among the different parental lines

TABLE 2. Cassava root starch parameters for the parents and the generated clones in a study in Uganda

	S C[1] (%)	S C[2] (%)	G C[1] (%)	G C[2] (%)	D M[1] (%)	D M[2] (%)	S Y[1] (%)	S Y[2] (%)
Bamunanika	78.84	58.12	88.30	64.58	41.99	37.45	29.20	24.43
Bao	78.49	80.19	87.91	89.1	40.83	39.39	34.20	24.32
Kakwale	81.79	65.63	91.61	72.92	38.79	37.27	30.13	24.18
Nyaraboke	81.76	74.63	91.57	82.92	36.66	38.46	21.99	24.22
Nase 10	85.38	74.64	95.63	82.93	43.33	37.97	22.04	24.13
Nase 12	76.12	67.16	85.25	74.62	46.25	38.17	17.82	23.78
TME 5	77.27	60.57	86.54	67.30	45.28	38.45	20.18	24.03
TME 14	70.36	66.15	78.80	73.5	44.67	39.02	26.20	23.24
95/SE/00036	89.90	53.44	100.68	59.38	44.17	39.37	22.29	24.16
LSD $_{(5\%)}$	8.63	17.36	0.34	2.67	9.17	NS	15.81	NS

Results presented are family averages of three replicates for a particular clone in each of the families [a] Means followed by the same superscript in each column are not different at P<0.05. SC = Starch Content, GC= Glucose Content, DM= Dry Matter, SY= Starch Yield, [1]Parental results, [2]Clone results, All LSDs not significant at P< 0.05

within the progenies. Most of the parents showed no significant variation in the amount of pure starch in the crude extract (starch content) except TME 14 and 95/SE/00036 which had significantly (P<0.05) higher starch content. The F_1 families showed more variation (> 90% starch), while others had as low as 47%. Besides the F_1 families, TME 5 and Nyaraboke as female parents showed the highest variation suggesting that families with high starch contents could be selected in these crosses.

High starch content normally suggests the relatively high digestibility of cassava starch (Riley *et al.*, 2006). The low starch contents observed in some progenies, especially in the TME series, may be due to the high dry matter displayed by these progenies and, hence, low digestibility and creation of side products during hydrolysis such as isomaltose and maltose (Van der Veen *et al.*, 2005) which are not detected by glucose specific tests used in this analysis. Starch content affects other starch properties such as the swelling power (Tester *et al.*, 1993) since the extent of starch hydrolysis depends on the ability of starch to take up water, allowing hydrolases to attack starch granules (Moore and Amante, 2005). It is important in commercial applications of starches such as in brewing and in the use of starch as an adjunct (Tester *et al.*, 1993). Industrial and dietary applications of starch require cassava varieties with high starch content. Thus, in the selection of the best performing parents in terms of starch content, Nyaraboke, Bao and NASE 10 would be used given their high starch content. Progenies from these parents also showed high starch content justifying their importance in terms of starch content and, thus overall starch yield.

Results for hydrolysis of starch were expressed through the glucose content released. This ranged from 55.5% to about 100% gramme of total carbohydrate including starch in both the parents and progenies. Among the parental lines, high values were observed in the local landraces compared to the introduced varieties. A similar phenomenon was observed in the F_1 families. Prolonged hydrolysis of starch with amylases results into breakdown of the starch polymer to glucose as suggested by Riley *et al.* (2006) and sometimes maltose (Karkalas, 1985).

High glucose contents may be obtained after hydrolysis, indicating the presence of other organic compounds in starch such as already existing sugars and break down products of other plant carbohydrates, which on hydrolysis lead to the production of reducing sugars and maltodextrins. Like glucose, the products above are also detectable by the reducing sugar test used (Van der Veen *et al*., 2005), hence, very high reducing sugar contents may not necessarily imply high glucose contents.

Root dry matter among the parental lines ranged from 36.6 to 46.3%, and from 37 to 40.0% for the F_1 families (Table 2). Among most of the F_1 families, dry matter values were 33 - 38%, although some registered values as low as 19% and others as high as 49%, giving a much wider range of variation than in the parents. Since selections for dry matter are based on the high dry matter producing varieties, F_1 families with relatively high dry matter are important in this case. F_1 families with low dry matter are also important in production of glucose syrups given their supposedly high sugar concentrations (Cardosso *et al*., 2006) and are, thus, important for production of sugar syrups (Table 2).

There were no significant differences in the dry matter among the progenies. Differences in dry matter were observed among the parental lines with NASE 12 registering high dry matter comparable to the TME series (Table 2). There was a significant ($p< 0.05$) relationship between dry matter and starch yield since starch contributes significantly on the overall dry matter of the root (Wholey and Booth, 2006). Starch constitutes about 80% dry matter in cassava, making it a significant portion of the total dry matter (Benesi, 2005). This explains the high starch contents displayed by cassava among root, tuber and other starch crops (Moorthy 2002). From Table 3, negative correlations were observed between root dry matter and the sugar content explaining the reductive effect of accumulation of sugar on the overall dry matter of the cassava root (Cardosso *et al*., 2006).

Fresh root starch yield among the parental lines ranged from 17.8 to 34%, while for the progenies, it was between 19 and 26%, with no significant differences among different parents and the progeny families (Table 2). The starch

TABLE 3. Correlation among different starch chemical parameters in the F_1 families

	Amy.	Ash.	D M.	G C.	Lipid.	MC.	Protein.	R. Sg.	SOL.	SP.	SC.	SY.	WSM.
Amy%	1												
Ash%	0.252	1											
D_M%	0.202	0.284	1										
G_C%	-0.647*	0.164	0.116	1									
Lipid	0.198	0.456*	-0.662*	-0.200	1								
MC%	0.648*	-0.123	0.106	-0.311	-0.047	1							
Protein	-0.055	-0.726*	-0.120	-0.370	-0.379	0.112	1						
R_Sugar	-0.168	-0.096	-0.723*	-0.066	0.427*	-0.399*	-0.099	1					
SOL	-0.097	-0.105	0.201	-0.041	-0.298	0.114	0.689	*-0.401*	1				
SP	-0.195	0.057	-0.163	0.286	0.243	0.093	0.121	-0.341	0.317	1			
S_C%	-0.647*	0.164	0.116	1*	-0.199	-0.311	-0.370	-0.066	-0.041	0.286	1		
S_Y%	-0.186	-0.423*	-0.274	0.049	0.057	-0.121	0.073	-0.035	-0.343	-0.072	0.049	1	
WSM	-0.536*	0.429*	-0.192	0.487*	0.252	-0.565*	-0.476*	0.454*	-0.105	-0.283	0.487*	-0.103	1

*Significant correlations at P<0.05. Amy = Amylose content, Ash = Ash Content, D_M = Dry matter Content, G_C = Glucose Content, Lipid = Lipid Content, MC = Moisture Content, Protein = Protein Content, R- Sg. = Reducing sugar content, SOL= Solubility, SP = Swelling Power, S_C = Starrch Content, S_Y = Starch Yield, WSM= Cold water soluble Materials yield for the different progenies was lower than that of the parents, except for NASE 12, where it was low in the parent compared to its progenies. The yield was low among elite introductions despite having high dry matter . This may be due to the high fiber content associated with them especially the TME series (Niba *et al*., 2007).

Among the introduced parents, TME 14 and while Nyaraboke were different from other local landraces (Table 2). Starch yield obtained in both the progenies and their progenitors was comparable to results obtained by Benesi (2005) from Malawian landraces. Starch yield is important in determining the yield potential of the cassava crop since it influences the dry matter and starch content of the roots (Wholey and Booth, 2006). In the use of cassava as an industrial crop, high starch yields are sought for. This makes the local varieties especially Bamunanika, important in this respect. F_1 families from these varieties were also important since they showed an overall high starch yield. However, the absence of variations in terms of starch yield among the F_1 families would make selections from these families impossible.

Results for the different starch functional parameters investigated are presented in Table 4. Swelling power, solubility, cold water solubility and moisture content are important in the various applications of starch. They influence the functional properties of starch and are hence important in the modification of starches to suit various uses. These properties define the characteristics of starch based on their effect on starch functionality (Tester *et al*., 1993). They can also be used to characterise starch from different biological/ botanical origins.

The swelling power at 60 ^{0}C among the different parental lines ranged from 2.79 to 6.67 g 10 g^{-1}, and from 6 to 7.5 g 10 g^{-1} in the F_1 families. High swelling power was observed in F_1 families among the families of TME 14 and NASE 10 with low values in Bamunanika and TME 5, although there were no significant (P>0.05) differences among the varieties when swelling power was compared at different temperatures (Fig. 1).

F_1 families from introduced varieties had considerably high swelling power compared to local landraces (Table 4). Among the parental

TABLE 4. Starch functional properties from the nine parental lines and F_1 families in a study in Uganda

Property	SP[1]	SP	SOL[1]	SOL	WSM[1]	WSM	MC[1]	MC
Bamunanika	5.63	6.23	5.57	7.01	1.68	1.99	16.49	15.88
Bao	5.08	6.33	1.63	6.00	1.37	2.40	16.47	15.50
Kakwale	5.27	6.83	4.84	5.22	1.38	2.24	14.62	15.18
Nyaraboke	5.79	6.46	5.29	6.95	1.31	2.58	16.66	14.80
NASE 10	6.17	7.51	1.45	7.66	0.80	2.35	14.77	15.75
NASE 12	6.54	6.12	2.81	6.12	1.37	2.68	14.79	16.11
TME 5	6.01	6.05	2.97	6.79	0.99	2.91	14.09	14.90
TME 14	6.67	6.72	5.61	7.67	1.38	2.14	14.97	15.60
95/SE/00036	5.41	6.57	1.33	6.70	1.31	1.48	16.34	16.09
$LSD_{(5\%)}$	0.34	NS	3.21	5.46	NS	0.63	NS	NS

[1]Results for the parental lines, used to generate the clones, SP= Swelling power (g/10g), Sol = Solubility (g/100g), WSM = cold water soluble materials (g/100g); MC = Moisture content (%). Values are triplicate averages of the analysed samples. [a]Means with the same letters in the column are not significantly different at 5% .

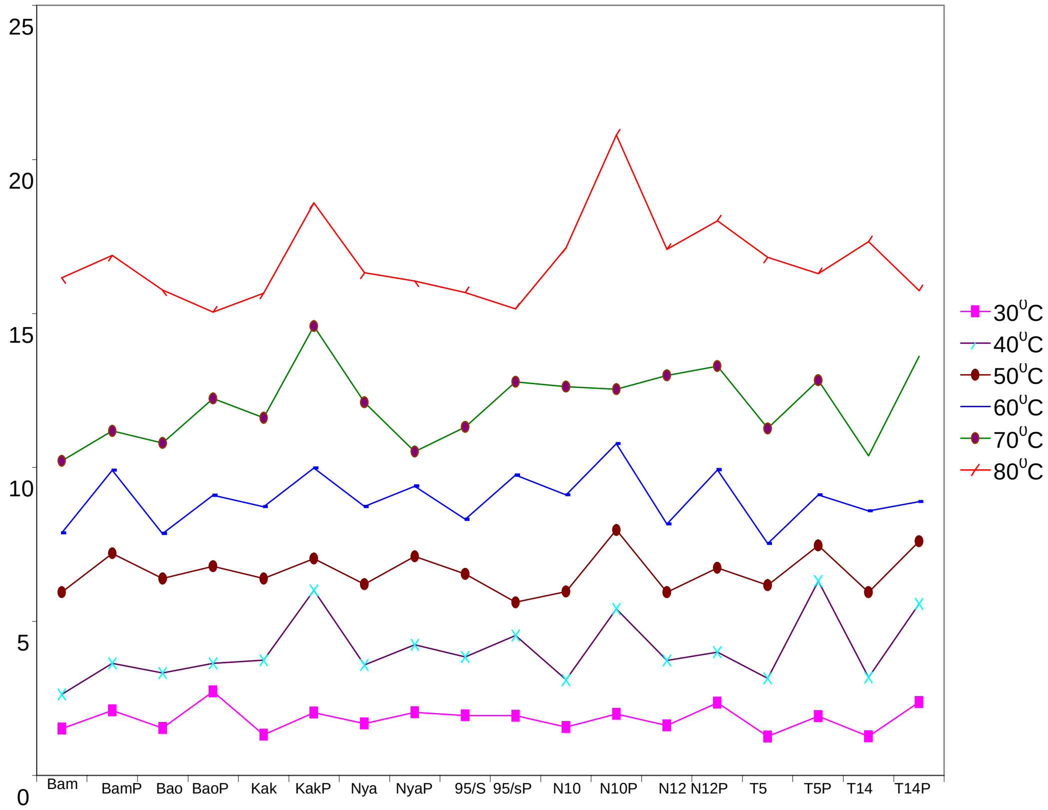

Figure 1. Graph showing variation in swelling power with increasing temperature for starch from nine parental varieties used and their progenies. Parental lines are: Bam = Bamunanika, Bao= Bao, Kak= Kakwale, Nya= Nyaraboke, 95/S = 95/SE/00036, N= NASE, T= TME. P= Progenies from a particular parent; SP = Swelling power in g10g^{-1}.

lines, variations occurred in swelling power though there was no clear trend on comparison. Parental lines TME 14 and NASE 12 registered high swelling power comparable to progenies. Although the F_1 families did not show considerable significant variations in terms of swelling power, the relatively high swelling power they presented makes selection among the F_1 families appropriate for industrial uses of starch where relatively high swelling power is required.

The swelling power of starch depends on the ability of certain components of starch, especially amylose to solubilise in water, hence, allowing water to attack starch molecules. Thus, increases in swelling power are a function of increased solubility (Moorthy, 2007). In the use of starch for baking and other dietary applications, especially in solution, starch with high swelling power is required given its high digestibility and the advantages that come with the increases in size attained in this case. Thus, starch from introduced varieties, especially TME 14, and some of its F_1 families is important in this case. However, the swelling power of starch is affected by the presence of reducing sugars in starch which lead to unavailability of total starch for water absorption, hence the negative correlation between the two (Moore and Amante, 2005). The starch content also affects the swelling power since increase in total starch leads to increase in swelling power (Rampersad *et al.*, 2003).

The solubility at 60 ^{0}C for parental lines ranged between 1.33-5.61 g 100 g^{-1}, and was lower than that obtained in F_1 families which ranged between 5.22-7.67 g 100 g^{-1} (Table 4). Significant ($P<0.05$) variations were observed in the F_1 families compared to the parents, which in addition to the high solubility presented by some of the F_1

families, gives more room for selection of the best performing F_1 families for use in a number of applications where starch is used in solution and at high solubility (Murkejea *et al.*, 2007). Improved solubility among the F_1 families implies an improvement in the industrial application of this starch, especially where starch is used in solution like in pharmaceuticals (Benesi, 2005). Cold water solubility ranged from 0.80 to 1.68 g 100 g^{-1} starch among the parental lines; while it ranged from 1.48 to 2.91 g 100 g^{-1} starch among the progenies. There were no significant variations in cold water solubility among the parents and the F_1 families, although the relatively high cold water solubility presented by the F_1 families (Table 4) can be used to facilitate selections among the F_1 families.

Low solubility at room temperature explains the effect of increased temperature on the overall solubility of starch (Moorthy, 2001). Like solubility, cold water solubility was high in introduced varieties compared to local varieties among the progenies. Among the parental lines, the cold water solubility was lower than the progenies and with no significant differences among them. Like solubility, selections for F_1 families with better traits for cold water would be done among the progenies but especially those produced from introduced varieties in this case TME 5 and NASE 12. Compared to solubility values at 60^0C at an average of 5.9%, cold water solubility of 2.53% showed that native starch is relatively insoluble at low temperatures (Riley *et al.*, 2006). However, this was higher than the results reported by Benesi (2005). Cassava generally has higher solubility comparable to corn starch and higher than other starches (Mukerjea *et al.*, 2007) making it a suitable substitute for corn starch in dietary uses.

Moisture content among the parental lines ranged between 14.09 and 16.49 %, and 14.80 to 16.11% in the progenies. Significant ($P<0.05$) variations were observed among the F_1 families, except for the parents Nyaraboke and NASE 10. These variations are important in the selection of low moisture content F_1 families since they are important, especially in the commercial use of starch where low moisture content is required. The moisture content for both the parental lines and progenies (Table 4) was consistent with results reported by Benesi (2005). Compared to other starches, the results showed that cassava has high moisture contents. This is not desirable since it promotes the growth of micro-organisms like fungi that degrade starch (Nanda *et al.*, 2006). This has implications on the storage and shelf-life of cassava starch, especially in commercial applications. It also has implication on the pasting properties of cassava starch (Moorthy, 2001) and has been found to affect the tensile properties and the overall granular structure of starch (Willet and Doane, 2002).

There were no significant differences in the moisture content of starch from introduced varieties and the local landraces (Table 4), revealing that moisture content does not vary among cassava varieties. The lack of significant differences in moisture makes selections based on moisture content futile. However, the F_1 families produced with low moisture content can be improved by lowering their moisture content and aiming at increase in the over-all dry matter content of the crop. Moisture content may be affected by the environment and the method of storage used for the starch. Thus, evaluation of the different F_1 families produced in the different agro-ecological zones of Uganda is necessary. This also puts into consideration the storage method used for this starch.

Results on biomolecules associated with starch are presented in Table 5. In the improvement of cassava for human consumption, the ash, lipid and protein content have been targeted for their effect on starch physicochemical properties and their importance in the nutritional aspects of cassava (FAO, 2000). The reducing sugar content is important in the use of cassava for production of glucose syrups and it is also important due to its effects on the overall starch content and the dry matter content of the crop (Cardosso *et al.*, 2006). On the other hand, the amylose content is one of the major factors considered in the application of starch. Determination of the amylose content elucidates the ratio of amylose to amylopectin which is very important in starch applicability (Moorthy, 2001).

On average, the ash content ranged from 0.10-0.20% in both the parental lines and their respective F_1 families. Generally, the ash content was low among the parental lines compared to

TABLE 5. Cassava root chemical composition in F_1 families and the female parents

	Ash[1]	Ash[2]	Lipid[1]	Lipid[2]	R.Sugar[1]	R.Sugar[2]	Protein[1]	Protein[2]	Amylose[1]	Amylose[2]
Bamunanika	0.10	0.19	0.32	0.22	0.94	1.74	0.26	0.30	17.64	20.79
Bao	0.10	0.20	0.18	0.16	1.15	1.43	0.43	0.25	21.62	18.94
Kakwale	0.20	0.22	0.10	0.32	0.94	1.87	0.24	0.25	24.22	20.43
Nyaraboke	0.10	0.24	0.32	0.24	1.26	1.72	0.27	0.27	22.42	20.09
Nase 10	0.20	0.24	0.12	0.29	0.74	1.41	0.27	0.27	21.69	19.44
Nase 12	0.20	0.29	0.22	0.30	0.75	1.75	0.25	0.24	23.71	23.34
TME 5	0.10	0.26	0.32	0.27	1.08	1.67	0.26	0.26	21.38	19.65
TME 14	0.15	0.22	0.14	0.19	0.86	1.59	0.30	0.28	24.43	20.81
95/SE/00036	0.05	0.24	0.14	0.24	0.96	1.31	0.23	0.27	21.26	24.81
$LSD_{(5\%)}$	0.08	NS	0.17	0.09	0.23	NS	0.16	NS	2.31	NS

Values for a particular parameter followed by the same superscript in a column for each parent/clone are not significantly different at p=0.05. [1]Results for parental lines, [2]Results for the F_1 famileis. R .Sugar = Reducing sugars

the derived F_1 families. In addition, the F_1 families showed significant ($P< 0.05$) variations allowing selections to be made among these families. Although F_1 families from introduced varieties registered high percentages of mineral matter, there were no differences in ash contents among F_1 families derived from local landraces and those derived from elite varieties. Presence of minerals in starch makes it more amenable for use in dietary regimes. F_1 families with high mineral matter would, thus be selected for use in such a case. Since materials soluble in water at room temperature include mineral matter, they also affected the amount of cold water soluble materials. Mineral matter influence a number of starch characteristics such as paste viscosity (Moorthy, 2001). However, ash content could not explain the solubility at 60 ^{0}C since other materials in starch are soluble at increased temperature (Vigneshwaran *et al*., 2006).

A few studies have been conducted on the effect of ash on starch properties. However, phosphorus, which is among the main component of ash or mineral matter in starch, has been found to affect the swelling power, solubility, and the pasting properties of starch (Karim *et al.*, 2007). Thus, selection for high ash content among the F_1 families should be done without compromising the other parameters of starch important in its applicability.

The lipid content for the parental lines ranged between 0.10-0.32%, and in the progenies 0.16-0.30%. Similar results were reported by Benesi (2005). Significant variations were observed in the lipid content of the F_1 families (Table 5) allowing for selections to be made for different purposes. High lipid content starches would be selected from families such as those in TME 5 and Kakwale. While low lipid content starches for use in industrial purposes where starch with a low percentage of impurities like lipids is required would also utilise F_1 families from TME 14 and TME 5. However, some parents such as NASE 10, Kakwale, TME 14 and 95/SE/00036 registered low lipid contents which can qualify F_1 families from them for use in industrial uses of starch.

There were no significant differences in the lipid contents of introduced varieties and the local landraces. A weak negative correlation existed between lipid content and solubility (r = -0.05) with no significant relationship between the two (p = 0.226), suggesting negative effects of lipids on the overall solubility of cassava starch. There was no significant relationship between swelling power and lipid content (p =0.518) since high lipid contents are associated with low swelling powers and solubility (Rampersad *et al.*, 2003). The lipid content of F_1 families from elite varieties was slightly higher than the other F_1 families and even the parental lines, though no significant differences were observed among them. The slight increase in the lipid content is important in the enhancement of these F_1 families for nutritional purposes, where high lipid content is required. In the use of cassava for nutritional purposes especially among the local farmers, F_1 families with higher lipid contents are important. However, since lipids affect solution properties of starch, the use of starch for industrial uses requires F_1 families with very low lipid contents.

The reducing sugars in starch ranged from 0.74-1.26 % among the parental lines, and 1.31-1.87 % in the progenies (Table 5). Individual F_1 family results ranged from 0 to about 6% gramme starch, suggesting wider variations within the F_1 families, hence ,opportunities for selection among them. Significant (P< 0.05) variations were also observed among the F_1 families compared to the parental lines. These variations, on top of the high reducing sugars displayed by the F_1 families, are important in the selection of high reducing content F_1 families in cases where high reducing sugar contents are required. Generally, local landraces had higher values compared to elite varieties though no significant differences were observed among the two.

A similar observation was made among the F_1 families. High reducing sugar content among landraces could partly explain why they are preferred by farmers given their sweet taste and ease of applicability in dietary uses. Reducing sugars have been found to affect a number of starch retrogradation kinetics (Farhat *et al.*, 2000) and increase the viscosity of starch depending on the type of sugar in question (Abu-Jdayil *et al.*,2004). The reducing sugar content was positively correlated with the percentage cold water soluble materials (r = 0.454) (Table 3). This was so because reducing sugars are soluble at room temperature and contribute significantly to the total amount of soluble matter in starch. In the selection of cassava for food and industrial use, the percentage of reducing sugars in a particular variety is not considered in many cases. However, it is important especially in the boil and cook varieties, where it determines the sweetness of a particular variety. In such cases, high reducing sugar content is necessary and varieties with such a characteristic are sought for. In cases where starch is required for industrial use and in large enough quantities, varieties with a low or no reducing sugar content reducing are required; in this case selections from the parental lines such as NASE 10 and NASE 12 are important.

Protein content ranged from 0.22 to 0.43% in the parental lines, and varied considerably among them. However, the protein content among the progenies ranged from 0.24 – 0.30% and no significant differences were observed among them (Table 5). There were no significant variations in the protein content among the parents and the F_1 families, thus making selections for protein contents among both the parents and the F_1 families difficult.

The results for protein content were much lower than results reported by Ceballos *et al*. (2006) but were in agreement with those reported by Peroni *et al*. (2006) and Moorthy (2001). Various studies (Christine *et al*., 2007; Lim *et al*., 2008; Perez *et al*., 2003) conducted to ascertain the effect of protein content on starch properties in cereal starches revealed that protein affects a number of pasting properties but does not effect starch content and the starch yield. This implies that protein content is important in the use of starch. However, for cassava, there is a need for increased protein content in starch to cater for the nutritional needs of the people who depend greatly on cassava for food. In the use of starch for industrial uses and pharmaceuticals, cassava starch from both the parents and the F_1 families would be ideal since it contains lower amounts of protein compared to cereal starches (Moorthy, 2001) and would thus require less processing procedures. The negative correlations observed between the protein content and cold water soluble materials (r = -0.476) signify the relative insolubility of proteins at low temperatures. However, at increased temperature, the protein content is positively correlated to solubility (r = 0.689) (Table 3) implying that at a high temperature, protein contribute to the total amount of soluble matter.

The amylose content observed in both the parents and the progenies ranged from 18.8 -25% (Table 5). Significant ($P<0.05$) variations were observed in the amylose content of both the parents and the F_1 families, although wider variations were observed among the F_1 families. This is important in the selection of cassava varieties among the F_1 families where both low and high amylose containing starches can be selected for different uses.

Among the parents, only Bamunanika showed significant differences compared to the F_1 families; amylose content being lower than the rest of the parents. However, among the F_1 families, low amylose contents below 10.0% and high amylose contents, above 25% were observed in some progeny accessions, giving more room for selections. Increased amylose content is important in production of amylose extender mutants (Vandeputte and Delcour, 2004) for production of starch with a low glycemic index (Baguma *et al*., 2003). Low amylose content cassava can be used to produce waxy starch (Ceballos *et al*., 2006), which has a number of applications in industry such as the production of adhesives and in the use of starch as a binder.

The amylose content of starch affects starch solution properties such as starch solubility and swelling power, which depend on the leaching of amylose out of the crystalline network of amylopectin into solution (Moore and Amante 2005). Since the amylose content affects the total amylopectin content in starch, the selection of cassava starch with particular amylose contents depends on its use in industry or for food as shown above. Thus, selections for varieties depending on amylose puts into consideration the fact that the cassava starch produced has completely no amylose (waxy starch) or it has high amounts of amylose (amylose extender mutants). In particular, waxy starch has numerous industrial uses and ease in applicability making it more important today (Ceballos *et al.*, 2006).

ACKNOWLEDGEMENT

This work was supported by the Swedish International Cooperation Agency/Department for Research Cooperation (Sida/SAREC) under the BIO-EARN Programme.

REFERENCES

Abu-Jdayil, B., Mohameed, A. and Eassa, A. 2004. Rheology of wheat starch–milk–sugar systems: effect of starch concentration, sugar type and concentration, and milk fat content *Journal of Food Engineering* 64:207-212.

Baguma,Y., Sun, C., Ahlandsberg, S., Mutisya, Palmqvist, S., Rubaihayo, P., Magambo, M., Egwang, T., Larson, H. and Christer, J. 2003. Expression patterns of the gene encoding starch branching enzyme II in the storage roots of cassava *(Manihot esculenta)*. *Plant Science* 164:833-839.

Benesi, M. 2005. Characterization of Malawian Cassava Germplasm for diversity, Starch extraction and its Native and modified properties. PhD thesis, Free State University, South Africa.

Cardoso, R., Carvalho, B. and Agostini, M. 2006. Sugary cassava (*Manihot esculenta* Crantz): preliminary morphological characterization and agronomic evaluation. Embrapa Biotechnological Centre.

Ceballos H, Morante N, Perez J, Calle F, Jaramillo G, Arias B, and Belloti A (2005). Diallele analysis in cassava adapted for the mild altitude valleys environment. *Crop Science* 45:1058-1063.

Ceballos, H., Sanchez, T., Morante, N., Fregene, M., Dufour, D., Smith, A., Denyer, K., Perez, J., Calle, F. and Mestres, C. 2006. Discovery of an Amylose-free Starch Mutant in Cassava (*Manihot esculenta* Crantz) *Journal of Agriculture and Food Chemistry* 55:7469 - 7476.

Christine, R., Jenny, T., Helene, C., Ihwa, T., Peter, H., Michael, G., Behjat, K., Wang, H., Oscar, L., Joseph.I., Steve, M., Ahmed, R., Sadequr, R., Matthew, M. and Li, Z. 2007. Effects of starch synthase IIa gene dosage on grain, protein and starch in endosperm of wheat.

Theoretical and Applied Genetics 115:1053-1065.

Dubois, M., Gilles, K., Hamilton, K., Rebers, A., and Smith, F. 1956. Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry* 28:350-356.

FAO production year book (2000) FAOSTAT database,http://apps.fao.org.

Farhat, I., Blanshard, J., Descamps, M. and Mitchell, J. 2000. Effect of sugars on retrogradation of waxy maize starch-sugar extrudates. *Cereal* 77:202-208.

Helrich, K. 1990. Official Methods of Analysis 15th Edition. Association of Official Analytical Chemist Inc., Virginia. pp. 70.

ISI, 1999-2001. Tapioca (Cassava) starch. Available at URL:http://home3.inet.tele.dk/starch/isi/

Karim, A., Toon, L., Lee, V., Ong, W., Fazilah, A. and Noda, T. 2007. Effects of Phosphorus Contents on the Gelatinization and Retrogradation of Potato Starch. *Journal of Food Science* 72:132 -138.

Karkalas, J. 1985. An improved enzymatic Method for the Determination of Native and Modified Starch. *J. Sci. Food Agric.* 36:1019.

Leach, H., McCowan,L. and Schoch, T. 1959. Swelling and solubility patterns of various starches. *Cereal Chemistry* 36:534-544.

Mariscal, A. M., Bergantin, R. V. and Troyo, D. A. 2002. Cassava Breeding and varietal release in the Philippines. Asia- cassava workshop. PDF Paper 42.

Moore, G. and Amante, L. 2005. Cassava and corn starch in maltodextrin production. *Quimica Nova* 28:596-600.

Moorthy, S. 2001. Tuber crop starches, Tech Bulletin No. 18 CTCRI, Trivandrum.

Mukerjea, R., Slocum, G. and Robyt, J. 2007. Determination of the maximum water solubility of eight native starches and the solubility of their acidic-methanol and -ethanol modified analogues. *Carbohydrate Research* 342:103–110.

Nanda. K, Balagopalan. C, Padmaja. G, Moorthy. S. and Sajeev, S. 2001. PostHarvest Management of Cassava for Industrial Utilization in India, Central TuberCrops Research Institute (CTCRI), India.

Niba, L., Bokanga, M., Li, B. and Schlimme, D. 2007. Physicochemical properties and starch granular characteristics of flour from various cassava *(Manihot esculenta)* genotype, Donald Danforth Plant Science Center.

Perez, O., Haros, M., Suarez,C. and Rosell,C. 2003. Effect of steeping time on the starch properties from ground whole corn. *Journal of Food Engineering* 60:281-287.

Rampersad, R., Badrie, N. and Comissiong, E. 2003. Characteristics of Flavored Snacks from Extruded Cassava/Pigeonpea Flour. *Journal of Food Science* 68:363-367.

Riley, C., Wheatley, A. and Asemota, H. 2006 Isolation and Characterization of starch from eight *Dioscorea alata* cultivars grown in Jamaica. *Afr J. Biotech* 5:1528-1536.

Tester, R., Morrison,W. and Schulman, A. 1993. Swelling and gelatinization of cereal starches V. Riso mutant of Bomi and Carlsberg II barley cultivars. *Journal of Cereal Science* 17:1-9.

Van der Veen. M, Veelaert. S, Van der Goot. A. and Boom. R. 2005. Starch hydrolysis under low water conditions: A conceptual process design, Wageningen University, Bomenweg 2, 6703 HD Wageningen, The Netherlands.

Vandeputte, G.. and Delcour, J. 2004. From sucrose to starch granule to starch physical behaviour: a focus on rice starch. *Carbohydrate Polymers* 58:245–266.

Vigneshwaran, N., Nachane, P., Balasubramanya, H. and Varadarajan, V. 2006. A novel one-pot 'green' synthesis of stable silver nanoparticles using soluble starch. *Carbohydrate Research* 341:012–2018.

Wholey, D. and Booth, R. 2006. A comparison of simple methods for estimating starch content of cassava roots. *Journal of the Science of Food and Agriculture* 30:158 - 164.

Willett, J. and Doane, M. 2002. Effect of moisture content on tensile properties of starch/poly(hydroxyester ether) composite materials. *Polymer* 43:4413-4420.