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Screening of Starch Quality Traits in Cassava (*Manihot esculenta* Crantz)

Cassava (*Manihot esculenta* Crantz) is one of the most important sources of starch in the tropics. There is limited and contradictory information regarding cassava starch characteristics. The International Center for Tropical Agriculture (CIAT) holds in trust FAO's cassava germplasm collection. Starches from 3272 landraces (including 12 wild relatives) and 772 improved clones were extracted and analyzed over a period of several years. In most cases only one starch sample per genotype was analyzed. Average cyanogenic potential was 327 ppm but considerably higher in the landraces (340 ppm) than in improved clones (267 ppm). Average total and reducing sugars were slightly higher in improved clones (4.06 and 1.56%, respectively) than in landraces (3.68 and 1.25%, respectively). Amylose content was similar in both types of germplasm with an average of 20.7%. Average pasting temperature was 65.3°C. Maximum viscosity was 777.5 mPa s, breakdown was 298.1 mPa s, consistency was 155.8 mPa s and setback was -144.5 mPa s. The large sample of starches analyzed provides very robust information regarding the actual characteristics of cassava starch.

Keywords: Cassava starch; Amylogram; Pasting properties; Genetic resources

1 Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most important sources of commercial production of starch in tropical and subtropical countries [1]. About 73.7 to 84.9% of the dry root weight of cassava is starch [2]. Compared with other tropical root and tuber crops, cassava starch and its biosynthesis have been well studied [1, 3-9]. The starch granules are generally round (oval), with a flat surface on one side (truncated). The size of individual granules ranges from 5 to about 40 μm, with reported averages varied from 5.4 to 17.2 μm [1, 3, 8, 9]. However, recently a mutation with considerably smaller starch granule size has been reported [10].

There is a widespread variation of starch biochemical and functional properties reported in the literature. Amylose content, for example, has been reported to range from 18.6 to 23.6% [3, 11]; 17 to 25% [12]; 18 to 25% [1]; 15.9 to 22.4% [9] or 13.6 to 23.8% [2]. An amylose-free natural mutation has also been reported recently [13] as well as a high amylose/small granule induced mutation [10]. Earlier, *N. Zakhia* and co-workers [14] reported root and starch quality traits from the cassava core germplasm collection of CIAT (from 502 to 565 genotypes depending on the trait). Average amylose content was around 22%. Most reports in the literature are based on starch samples from just a single or few genotypes. Perhaps the only exception is the 22.7-32.4% range of variation reported from CIAT germplasm [14], or the 160 Indonesian genotypes analyzed by *Sudarmonovati* et al. [15]. When characterizing cassava starch, therefore, it is difficult to define what a "typical" or "normal" cassava starch is.

The cassava-breeding project at the International Center for Tropical Agriculture (CIAT) has implemented several strategies to develop high-value cassava clones to take advantage of the new opportunities opened to cassava by the globalization of the economies in many tropical countries [16-18]. The main objective is to develop not only clones with high and stable productivity, but also with root characteristics that better fit the needs of the different industries. For the feed industry high-protein clones have been identified [19]. For the starch industry, different approaches to develop and identify clones with novel starch properties have been gradually introduced in the cassava-breeding project [17, 18]. In addition, the identification of those genotypes, where interesting starch quality variations are expressed, requires the availability of special tests. As a result of these activities two commercially relevant mutations have been recently reported [10, 13] and valuable information on starches from a large number of cassava genotypes has been collected.

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CIAT holds in trust the worldwide cassava germplasm collection with more than 6000 accessions. Three main types of accessions can be mentioned: a) wild relatives of cassava within the *Manihot* genus; b) traditional landraces grown by farmers in Africa, Asia or Latin America and the Caribbean (LAC); and c) improved cassava germplasm produced by breeding projects in the continents mentioned above. The collection is maintained *in vitro* at the CIAT experimental station located in Palmira, Valle del Cauca, Colombia [20].

The objective of this study is to report the starch characteristics of more than four thousand cassava clones (landraces and improved varieties) and few wild species, which had been evaluated in the root quality laboratory at CIAT.

2 Materials and Methods

As part of the project to identify high-value cassava germplasm, with special emphasis in starch quality and other root-traits, CIAT initiated the screening of starch quality traits in accessions from the germplasm collection as well as improved cassava varieties developed by CIAT, the International Institute of Tropical Agriculture (IITA) or two breeding projects in Thailand at Department of Agriculture and Kasetsart University [17, 18].

The first step was to retrieve the germplasm from the *in vitro* condition and grow the plants in the field for starch extraction and root evaluation. Plantlets from the *in vitro* collection were hardened in greenhouse conditions following standard protocols [21]. After a week of gradual hardening, plantlets were transferred to green house conditions for about two months and then transplanted to the field. Initially five plantlets per genotypes entered the hardening process. But, unavoidably, some of these plants were lost in the process. Therefore, analyses reported herein are based on one to five plants per genotype. Plants were harvested at 10-12 months of age from 1999 to 2003 near Palmira, Colombia (1000 m above sea level). Tab. 1 provides information about the origins of the cassava genotypes evaluated in this study. The variation in the number of clones from different countries is representative of the diversity of germplasm in the collection. As expected, most of the material comes from LAC, given the American origin of the genus *Manihot* [22, 23]. The most common origins were Colombia and Brazil with 1230 and 919 clones, respectively. Peru and Venezuela were represented by more than 100 genotypes. On the other hand, only two clones represented Chile and China. Among the materials evaluated there were 12 genotypes representing four different wild *Manihot* species *(M. chlorosticta, M. carthaginensis*, *M. esc. flabellifolia* and *M. filamentosa*). Root and starch analyses follow the general protocols described in [24].

2.1 Root and starch moisture content

Up to five roots from one to five plants per genotype were peeled and immediately cut into small pieces and mixed. Moisture content was determined after drying 50 g of sample (freshly cut pieces or starch) at 60°C for 48 h [25].

Tab. 1. Summary of the origins from the materials evaluated in this study and the number of clones representing them.

Country	# clones	Country	# clones	Country	# clones
Argentina	42	Fiji	5	Thailand	12
Bolivia	4	Philippines	5	USA	7
Brazil	919	Guatemala	61	Venezuela	158
Chile	2	Indonesia	33	Vietnam	4
China	2	Malaysia	46	CG [1]	46
Colombia	1230	Mexico	66	CM [1]	364
Costa Rica	86	Nigeria	13	GM [1]	188
Cuba	64	Panama	30	SG [1]	28
Dominican Rep.	4	Paraguay	94	SM [1]	138
Ecuador	83	Peru	281	Other. [2]	12
El Salvador	5	Puerto Rico	9	*Manihot* sp	12

[1] Improved clones developed at CIAT. CG, CM and GM identify clones whose mother and father are known. For SG and SM only the mother is known.
[2] Mostly a group of improved clones not developed at CIAT.

2.2 Flour extraction

Freshly cut pieces from the harvested peeled root(s) were lyophilized during 24 h at – 30°C and then ground. A FreeZone Stoppering Tray Drier - Model 79480 and a 6-L Freeze Dry System – Model 77530 (Labconco Corporation, Kansas City, USA) were used. The flour thus obtained was stored in plastic bags for total and reducing sugar and starch analyses.

2.3 Starch isolation

Freshly cut pieces of peeled roots were suspended in tap water and crushed in an Osterizer blender. The slurry was filtered through a 100 μm sieve. The starch was allowed to settle and the supernatant decanted off and dried in an oven with fan-forced ventilation at 40°C during 48 h.

2.4 Determination of total and reducing sugars

Contents of total and reducing sugars were determined according to *Cronin* and *Smith* [26]. Sugars were extracted from 2 g of root flour using an 80% ethanol solution, a Fehling reagent and a glucose standard curve. A Cecil spectrophotometer model CE 2021-Series 2000 (Cambridge, UK) was used in the determination.

2.5 Determination of starch content

Starch was measured after incubation with thermostable α-amylase and then with amyloglucosidase. The released glucose was measured with a spectrophotometer (Cecil CE 2021, 2000 Series, Cambridge, UK) after reaction with 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) reagent containing glucose oxidase and peroxidase [27]. Starch content was calculated as 90% of glucose content.

2.6 Paste clarity

The methodology suggested by *Craig* et al. [28] was used. A 1% (db) aqueous dispersion of starch was boiled at 97°C (1000 m above sea level) with shaking thoroughly every 5 min for 30 min. Transmittance was measured after cooling to room temperature at 650 nm.

2.7 Colorimetric amylose determination

Amylose content in the starch was measured following standard procedures [29]. Starch granules were first dispersed with ethanol and then gelatinized with sodium hydroxide. An aliquot was then acidified and treated with an iodine solution, which produces blue-black stain coloration. The color intensity, which is related to amylose content, was then measured with a spectrophotometer (λ: 620 nm) and compared with standard curves obtained using purified amylose (ICN) and amylopectin (SIGMA A0512) extracted from potato tubers. Three different quantifications per starch sample were made and mean values were then calculated.

2.8 Pasting properties

Hot starch dispersion viscosity profiles were obtained with a Rapid Visco Analyser model RVA-4 Series (Newport Scientific, Warriewood, Australia). Starch (1.25 g, db) was dispersed in distilled water (about 23 cm^3) to yield a 5% suspension. Viscosity was recorded using the temperature profile: holding at 50°C for 1 min, heating from 50°C to 90°C at 6°C/min, holding at 90°C for 5 min, and then cooling down to 50°C at 6°C/min with continuous stirring at 160 rpm. Four parameters were measured: pasting temperature (PT), peak viscosity (PV), hot paste viscosity at the end of the plateau at 90°C (HPV) and the cool paste viscosity raising 50°C (CPV). With them, three additional parameters were calculated: breakdown (BD), estimated as PV-HPV; setback (SB), estimated as CPV-PV; and consistency (CS), estimated as CPV-HPV; ease of cooking estimated as time to PV – time to PT.

2.9 Swelling power, solubility and dispersed volume fraction measurements

Swelling power and solubility patterns [30] were determined using 1.5% (w/w, db) starch dispersions (0.42 g dry matter dispersed in 27.58 g distilled water). The paste was prepared in a Rapid Visco Analyser (RVA) holding at 35°C for 1 min, heating to 75°C (complete gelatinization of starch) at 6°C/min, holding at 75°C for 2.5 min. The paste was immediately transferred to a 50 cm^3 centrifuge tube. The supernatant and sediment after centrifugation for 5 min at 6000 × *g* at 25°C were collected and weighed (W_{su} and W_{se}, respectively) then dried at 100°C for 24 h and 48 h respectively and weighed (D_{su} and D_{se}, respectively). Three parameters were calculated: concentration of soluble material in the supernatant (solubility), the swelling power and the volume fraction of the dispersed phase (Φ).

$$\text{Solubility (\%db)} = 100 \cdot D_{su}/0.42$$

$$\text{Swelling power} = (W_{se} - D_{se})/D_{se}$$

$$(\Phi) = (27.86 - (W_{su} - D_{su})/27.86$$

Factor 27.86 is calculated as total volume ($cm^{3)}$) of the paste.

Starch specific density is 1.5 g/cm^3

27.86 = 27.58 + (0.42/1.5) cm^3

2.10 Total cyanide content

Approx. 60 g, freshly cut cassava pieces, 1 cm^3 cubes, were homogenized in 150 cm^3, 0.1 M H_3PO_4 (25%, v/v, ethanol), followed by centrifugation. The supernatant was analyzed for cyanogenic compounds after appropriate dilution, by hydrolysis with exogenous linamarase prepared in the lab from cassava peel extraction. HCN was evaluated by colorimetric measurement after coloration with 1,3-dimethyl barbiturate/isonicotinate reagent according to *Essers* et al. at λ = 605 nm [31].

3 Results and Discussion

The wild Manihot species reported variation which fell within the range of values for *Manihot esculenta* accessions and therefore no distinction has been made between them and cultivated cassava.

Tab. 2 summarizes the results of the most relevant root quality traits. Average dry matter content was 33.6%, with a range of variation from 14.28 to 48.12%. There was a slight asymmetry in the frequency distribution with a tendency of longer tails to the left (skewness -0.40). Cyanogenic potential ranged widely from 14 to 3274 ppm, with an average of around 327 ppm. Distribution was highly asymmetrical (skewness 2.96) with a long tail to the right (Fig. 1). Total and reducing sugars also showed an asymmetrical distribution with longer right tails, particularly in the case of reducing sugars (skewness 1.76 and 3.08, respectively). Average values were 3.75 and 1.31% for total and reducing sugars, respectively. Average starch content was 84.5%, with a tendency of values to concentrate towards the higher values (skewness -0.65), which accentuated a similar tendency observed for dry matter content.

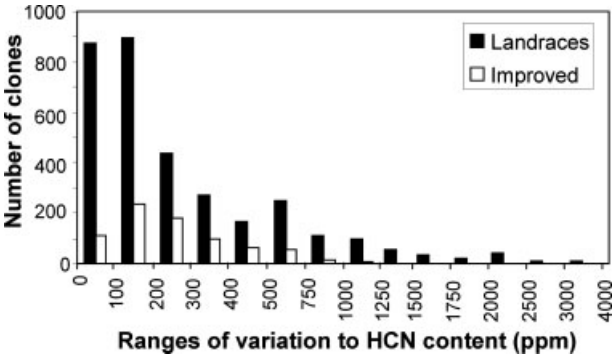

Fig. 1. Histogram illustrating distribution of cyanogenic potential (HCN in ppm) of landraces and improved cassava. There is a clear asymmetry with a long tail to the right and the distribution of improved clones tends to be more concentrated around lower HCN values.

Amylose content ranged from 15.2 to 26.5% with an average of 20.7% (Tab. 3). The average amylose content of 20.7% is a very robust estimate since it is based on such a large sample of genotypes. Distribution was practically normal with a very slight asymmetry towards a longer right tail (Fig. 2). No more than 1.5% of the samples had amylose values below 17.5 or above 24.5%. Water solubility and swelling power showed a more asymmetrical distribution with skewness values ranging from 1.53 to 1.77 (longer right tails). Average paste clarity was 45.2% with a large variation ranging from 12.5 to 96.6%.

Pasting properties of the samples analyzed are listed in Tab. 4. Average pasting temperature was 65.3°C and ranged from 58.8 to 71.2. Distribution frequency was

Tab. 2. Root quality traits from more than 4000 cassava genotypes expressed on Dry Basis.

Parameter	Dry matter content [%]	Cyanogenic potential [ppm]	Sugars content		Starch content [%]
			Total [%]	Reducing [%]	
Maximum	48.1	3274	18.8	15.7	91.0
Minimum	14.3	14	0.2	0.0	65.0
Average	33.6	327.4	3.8	1.3	84.5
Standard deviation	6.47	397.7	2.32	1.43	3.34
Skewness	-0.40	2.96	1.76	3.08	-0.65
Count	4051	4050	4049	4049	4049

Tab. 3. Starch quality traits from more than 4000 cassava genotypes.

Parameter	Amylose content [%]	Water solubility [%, db]	Swelling power [%, g/g]	Paste clarity [%]
Maximum	26.5	16.6	15.5	96.6
Minimum	15.2	0.2	0.8	12.5
Average	20.7	2.2	4.6	45.2
St. Dev.	1.61	1.59	2.31	10.54
Skewness	0.22	1.77	1.53	-0.30
Count	4042	4050	4050	4044

relatively symmetrical as for the other parameters described in Tab. 4. Maximum viscosity averaged at 777.5 mPa s, with a wide range of variation form 146 up to 1505 mPa s. Breakdown ranged from 28.1 to 859.0 mPa s with an average around 298.1 mPa s. Consistency ranged from 626 down to 0 mPa s with an average of 155.8 mPa s. Finally average setback was -144.5 mPa s with a minimum of -702.0 and a maximum observed value for 273.0 mPa s.

When clones were grouped by country or region of origin some weak trends could be observed in some cases. For example, clones from Brazil and Indonesia had the highest averages for HCN (508 and 408 ppm, respectively), whereas Ecuador, Peru as well as improved clones, showed the lowest averages (158, 162 and 219 ppm, respectively). Wild relatives of cassava tended to have higher levels of total sugars (average 4.80%), while that for improved clones was 4.05%. The Peruvian clones showed the lowest average for total sugars (3.22%), along with non-Indonesian clones from Asia (3.24%), which also showed the lowest averages for reducing sugars (0.98%). Wild cassava relatives also had high levels of reducing sugars (2.09%). Water absorption tended to be higher in South American clones (4.79; 4.81; 4.84 and 5.05% for Brazil, Peru, Colombia and Ecuador, respectively) than in Central (3.59%) and North American (3.62%) as well as clones from Indonesia (3.52%). A similar trend was observed for swelling power. Starches from clones originating in the southern region of South America tended to have low averages for maximum viscosity (727 mPa s) and breakdown (257 mPa s), whereas clones from Peru and Ecuador tended to have the highest averages (>830 mPa s for maximum viscosity) and 362 mPa s for breakdown of clones from Ecuador. Finally, wild relatives tended to have low averages for consistency values (131 mPa s), and ease of cooking (2.53 min).

Tab. 5 presents relevant information for the contrast between landraces and improved clones. For most variables there is not much difference between these two groups of cassava genotypes. Perhaps the only cases where some interesting differences could be observed were for dry matter content (32.8 versus 36.7% for landraces and improved clones); and cyanogenic potential (340 versus 267 ppm respectively for landraces and improved clones). In an earlier study, *Bokanga* [32] analyzed 881 genotypes from IITA's collection, 559 accessions from CIAT core collection, 184 landraces from Cameroon and 144 Nigerian local cultivars and in no case found acyanogenic cassava. Amylose content averages were very similar (20.89 and 20.02%) as were most of the other starch characteristics including pasting temperature and maximum viscosity. Clarity was slightly lower for landraces (44.5%) compared with improved clones (48.1%).

The results presented in this article are exploratory. In most cases samples were un-replicated. However, when unusual data was observed, because of the particular interest in identifying unusual starch types, it prompted a second analysis to confirm the data. This approach, although it is limited from the experimental point of view, allowed the analysis of such a large number of genotypes,

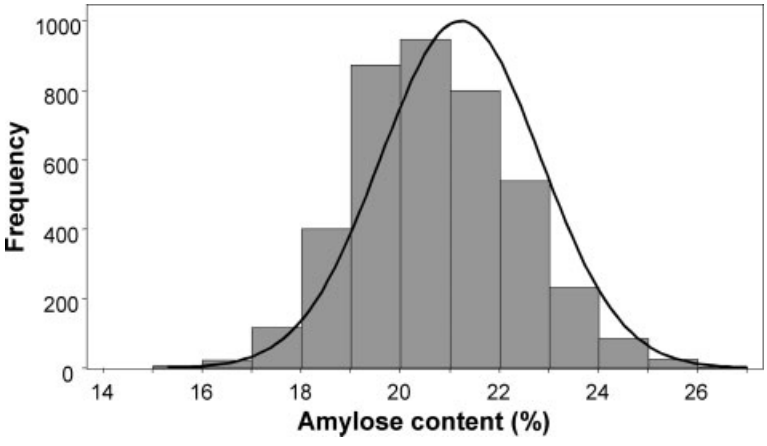

Fig. 2. Histogram illustrating distribution of amylose (% of total starch) in starch samples from 4046 different cassava accessions. Frequency distribution is close to that expected from the normal distribution curve (provided in the figure). The right tail tends to be slightly longer.

Tab. 4. Pasting properties from starches of more than 4000 cassava genotypes.

Parameter	Pasting temperature [°C]	Maximum viscosity [mPa s]	Breakdown [mPa s]	Consistency [mPa s]	Setback [mPa s]	Ease of cooking [min]
Maximum	71.2	1505.0	859.0	626.0	273.0	5.6
Minimum	58.8	146.0	28.1	0.0	-702.0	1.1
Average	65.3	777.5	298.1	155.8	-144.5	2.8
St.Dev.	1.75	165.03	107.1	57.8	96.2	0.72
Skewness	-0.13	0.22	0.81	0.94	-0.38	0.33
Count	4051	4051	4051	4051	4051	4051

Tab. 5. Comparison between landraces and improved clones root quality traits.

Parameters	3272 landraces				772 improved clones			
	Mean	St. dev.	Min.	Max.	Mean	St. dev.	Min.	Max.
Root traits								
Dry matter [%]	32.8	± 6.4	14.3	47.9	36.7	± 5.9	14.7	48.1
HCN content [ppm]	340	± 428	14	3274	267	± 212	28	2147
Flour traits								
Total sugar [%]	3.68	± 2.33	0.20	18.8	4.06	± 2.26	0.63	13.45
Reducing sugars [%]	1.25	± 1.43	0	15.7	1.56	± 1.41	0.01	8.24
Starch [%]	84.5	± 3.4	65.0	91.0	84.4	± 3.0	73.0	91.0
Starch traits								
Amylose [%]	20.89	± 1.6	15.15	26.46	20.02	± 1.44	15.75	25
Water solub. [%, db]	2.15	± 1.51	0.2	12.3	2.25	± 1.85	0.24	16.6
Swell. power [%, g/g]	4.57	± 2.35	0.79	15.4	4.65	± 2.14	2.08	14.7
Clarity [%]	44.5	± 10.7	12.5	86.3	48.1	± 9.25	21.7	96.6
Pasting temp. [°C]	65.2	± 1.75	58,7	71,1	65.4	± 1.69	60.0	69.7
Maximum viscosity	776	± 169	152	1401	783	± 148	146	1505
Breakdown [mPa s]	300	± 109	35	859	290	± 99	28	795
Consistency [mPa s]	158	± 59	0	626	147	± 52	1	394
Setback [mPa s]	-142	± 95	-702	273	-153	± 102	-584	103
Ease of cook. [min]	2.76	± 0.68	1.14	5.61	2.98	± 0.84	1.14	4.89

and provides very reliable information since at least outlaying data points were confirmed. One problem that remains unsolved is the possibility of a plant, for unknown reasons, producing starch samples with characteristics that may not be representative of that genotype. By and large, however, average values presented in this study should be very robust and properly represents starch characteristics of cassava. Range of variation is also useful to provide an idea of what traits may offer alternatives for further genetic improvement.

Although this study was not set up for comparing landraces and improved clones, the fact that the latter show a relatively higher dry matter content (36.7%) compared with landraces (32.8%) is highly suggestive, as it is the reduced cyanogenic potential (267 versus 340 ppm). These two characteristics have been key breeding objectives over the years and the differences observed would reflect the efficiency of breeding approaches, and the responsiveness of cassava to them. In other words, heritability of these traits must have been appropriate to allow for these genetic improvements.

On the other hand, there have been no efforts to change starch characteristics (until now) in cassava breeding projects. The only successful approach in that area has been the genetic transformation to produce amylose-free cassava [33] and along with the discovery of amylose-free and high-amylose mutations [10, 13] opens up a new world of opportunities for the cassava and starch sectors. But the germplasm analyzed in this study was not affected by these recent discoveries. The fact that there is no

much difference between averages for landraces and improved clones is not irrelevant. After four decades of genetic improvement of cassava some changes could have occurred if in fact these parameters were negatively (or positively) and strongly correlated with agronomic performance. The absence of significant shifts in the averages for these traits, even if they were not selected criteria, would indicate that they are relatively neutral for agronomic performance. In other words, there is no indication that these traits have been selected for indirectly through any association between them and agronomic performance.

For some traits (water solubility, swelling power, paste clarity, paste breakdown, consistency, and setback) there is large range of variation. It is very interesting to examine the relationships of these attributes from data of all evaluated clones (e.g. breakdown versus swelling power). More investigation and comparison on starch structure of clones with a great difference in functionalities are of great interest.

4 Conclusion

Analysis of a large number of cassava samples (including few wild relatives) has been conducted. The size of this analysis provides reliable information regarding average and ranges of distributions for the most relevant starch and root parameters. Average dry matter content was found to be 33.6% of which an average of 84.5% was starch. However, average dry matter content of landraces and wild relatives was lower (32.8%) than that of improved clones (36.7%). Average amylose content across the entire evaluation was 20.7% with no major difference between landraces and improved clones. Maximum viscosity was 777.5 mPa s, breakdown was 298.1 mPa s, consistency was 155.8 mPa s and setback was -144.5 mPa s. The large number of starch samples analyzed provides very robust information regarding the actual characteristics of cassava starch. The ranges of variation for different variables suggest that there may be possibility for further improvements such as the one observed for dry matter content.

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References

[1] S. N. Moorthy: Tropical sources of starch, in *Starch in Food* (Ed. A. C. Eliasson). CRC Press, Boca Raton, Fl, **2004**.

[2] J. E. Rickard, M. Asaoke, J. M. V. Blanshard: The physicochemical properties of cassava starch. *Trop. Sci.* **1991**, *31*,189–207.

[3] R. Hoover: Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydr. Polym.* **2001**, *45*, 253–267.

[4] Y. Baguma, S. Chuanxin, S. Ahlandsberg, J. Mutisya, S. Palmqvist, P. R. Rubaihayo, M. J. Magambo, T. G. Egwang, H. Larsson, C. Jansson: Expression patterns on the gene encoding starch branching enzyme II in the storage roots of cassava (*Manihot esculenta* Crantz). *Plant Sci*. **2003**, *164*, 833–839.

[5] T. R. I. Munyikwa, S. Lageveld, S. N. I. M. Salehuzzaman, E. Jacobsen, R. G. F. Visser: Cassava starch biosynthesis: new avenues for modifying starch quantity and quality. *Euphytica* **1997**, *96*, 65–75.

[6] S. N. I. M. Salehuzzaman, E. Jacobsen, R. G. F. Visser: Isolation and characterization of a cDNA-encoding granule-bound starch synthase in cassava (*Manihot esculenta* Crantz) and its antisense expression in potato. *Plant Mol Biol* **1993** *23*, 947–962.

[7] K. Sriroth, V. Santisopasri, C. Petchalanuwat, K. Kurotjanawong, K. Piyachomkwan, C. G. Oates: Cassava starch granule structure-function properties: influence of time and conditions at harvest on four cultivars of cassava starch. *Carbohydr. Polym.* **1999**, *38*, 161–170.

[8] F. H. G. Peroni, T. S. Rocha, C. M. L. Franco: Some structural and physicochemical characteristics of tuber and root starches. *Food Sci. Tech. Int.* **2006**, *12(6)*, 505–513.

[9] A. L. Charles, Y. H. Chang, W. C. Ko, K. Sriroth, T. C. Huang: Influence of amylopectin structure and amylose content on the gelling properties of five cultivars of cassava starches. *J. Agric. Food Chem.* **2005**, *53*, 2717–2725.

[10] H. Ceballos, T. Sánchez, A. P. Tofiño, E. A. Rosero, K. Denyer, A. M. Smith, D. Dufour, N. Morante, J. C. Pérez, B. Fahy: Induction and identification of a small-granule, high-amylose mutant in cassava (*Manihot esculenta* Crantz). *J. Agric. Food Chem.* **2008**, *56(16)*, 7215–7222.

[11] I. Defloor, I. Dehing, J. A. Delcour: Physico-chemical properties of cassava starch. *Starch/Stärke* **1998**, *50*, 58–64.

[12] A. Fernandez, J. Wenham, D. Dufour, C. C. Wheatley: The influence of variety and processing on the physicochemical and functional properties of cassava starch and flour in *Cassava flour and starch: progress in research and development* (Ed. D. Dufour, G. M. O'Brien, R. Best) CIRAD/CIAT Montpellier, France/ Cali, Colombia, **1996**.

[13] H. Ceballos, T. Sánchez, N. Morante, M. Fregene, D. Dufour, A. M. Smith, K. Denyer, J. C. Pérez, F. Calle, C. Mestres: Discovery of an amylose-free starch mutant in cassava (*Manihot esculenta* Crantz). *J. Agric. Food Chem.* **2007**, *55(18)*, 7469–7476.

[14] N. Zakhia, C. Wheatley, G. O'Brien, D. Dufour: Screening of CIAT cassava germplasm diversity: the relationship between cyanogenic potential and cassava starch functional properties, in *The cassava biotechnology network. Proceedings of the second international scientific meeting, 1994/08/22-26, Bogor, Indonesia*, CIAT Document n°150, 823–830, **1995**.

[15] E. Sudarmonowati, N. S. Hartati, L. Sukmarini: Amylose content variation of Indonesian cassava genotypes and its correlation with RAPD and AFLP markers, in *Proceedings of the First International Meeting on Cassava Breeding, Biotechnology and Ecology,* Brasilia, **2006**.

[16] H. Ceballos, C.A. Iglesias, J. C. Pérez, A. G. O. Dixon: Cassava breeding: opportunities and challenges. *Plant Mol. Biol.* **2004**, *56*, 503–515.

[17] H. Ceballos, M. Fregene, Z. Lentini, T. Sánchez, Y. I. Puentes, J. C. Pérez, A. Rosero, A. P. Tofiño: Development and Identification of High-Value Cassava Clones. *Acta Horticulturae* **2006**, *703*, 63–70.

[18] H. Ceballos, M. Fregene, J. C. Pérez, N. Morante, F. Calle: Cassava genetic improvement, in *Breeding Major Food Staples* (Ed. M. S. Kang, P. M. Priyadarshan). Blackwell Publishing. Ames, IA, **2007**.

[19] H. Ceballos, T. Sánchez, A. L. Chávez, C. Iglesias, D. Debouck, G. Mafla, J. Tohme: Variation in crude protein content in cassava (*Manihot esculenta* Crantz) roots. *J. Food Compos. Anal.* **2006**, *19*, 589–593.

[20] http://isa.ciat.cgiar.org/urg/main.do?language=es.

[21] R. J. Segovia, A. Bedoya, W. Triviño, H. Ceballos, G. Gálvez, B. Ospina: Metodología para el Endurecimiento de 'vitroplantas' de yuca, in *La Yuca en el Tercer Milenio* (Eds. B. Ospina, H. Ceballos) CIAT, Cali, Colombia, **2002**.

[22] H. Brücher: *Useful plants of neotropical origin and their wild relatives*, Springer-Verlag, Berlin -New York, **1989**.

[23] A. C. Allem: The origin of *Manihot esculenta* Crantz (Euphorbiaceae). *Genetic Resources and Crop Evolution* **1994**, *41*, 133–150.

[24] J. Aristizábal, T. Sánchez: Guia técnica para producción y análisis de almidón de yuca. *Boletin de Servicios Agrícolas de la FAO*. Roma, Food and Agriculture Organization of the United Nations, **2007**.

[25] M. L. Prieto, C. E. Valencia: Obtención de un método patrón y desarrollo de un método rápido y práctico para la determinación del contenido de humedad en yuca (*Manihot esculenta* Crantz). Universidad del Valle and Universidad Nacional de Colombia. Degree Thesis. **1988**, 101 pp (Available at CIAT, Apartado Aéreo 6713, Cali. Colombia).

[26] D. A. Cronin, S. Smith: A simple and rapid procedure for the analysis of reducing, total and individual sugars in potatoes. *Potato Res.***1979**, *22(2)*, 99–105.

[27] I. L. Batey: Starch analysis using thermostable α-amylases. *Starch/Stärke* **1982**, *34*, 125–128, modified by Mestres as reported in: C. Mestres Gélification d'amidons de maïs modifiés thermiquement. Application à la fabrication de pâtes alimentaires sans gluten. Ph. D. Thesis, France, 1982.

[28] S. A. S. Craig, C. C. Maningat, P. A. Seib, R. C. Hoseney: Starch paste clarity, *Cereal Chem*, **1989**, *66(3)*, 173–182.

[29] ISO 6647 Riz: Détermination de la teneur en amylose, **1987**.

[30] C. Mestres, M. Nago, N. Akissië, F. Matencio: End use quality of some African corn kernels. Cooking behavior of whole dry-milled maize flours; incidence of storage. *J. Agric. Food Chem.* **1997**, *45*, 565–571

[31] S. A. J. A. Essers, M. Bosveld, R. M. Van Der Grift, A. G. J. Voragen: Studies on the quantification of specific cyanogens in cassava products and introduction of a new chromogen. *J. Sci. Food Agric.* **1993**, *63 (3)*, 287–296.

[32] M. Bokanga: Distribution of cyanogenic potential in cassava germplasm, in *International Workshop on Cassava Safety.* IITA, Ibadan, Nigeria. *Acta Horticulturae* **1994**, *375*, 117–123

[33] C. J. J. M. Raemakers, E. Sofiari, N. J. Taylor, G. G. Henshaw, E. Jacobsen, R. G. F. Visser: Production of transgenic cassava plants by particle bombardment using luciferase activity as the selection marker. *Mol. Breed.* **1996**, *2*, 339–349.

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