Research Article

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Starch determination, amylose content and susceptibility to *in vitro* amylolysis in flours from the roots of 25 cassava varieties

Luisa Elena Mejía-Agüero,[a,b] Florangel Galeno,[b] Oswaldo Hernández-Hernández,[b] Juan Matehus[a] and Juscelino Tovar[b*†]

Abstract

BACKGROUND: Cassava cultivars are classified following different criteria, such as cyanogenic glucoside content or starch content. Here, flours from the roots of 25 cassava varieties cultivated simultaneously in a single plantation, were characterized in terms of starch content (SC), amylose content (AC), α-amylolysis index (AI) and gel formation ability. Resistant starch content (RS) was measured in 10 of the samples.

RESULTS: Cassava flours exhibited high SC, low AC and low AI values, with differences among varieties. Cluster analysis based on these parameters divided the cultivars in four groups differing mainly in SC and AC. AI and AC were inversely correlated ($r = -0.59$, $P < 0.05$) in 18 of the cultivars, suggesting AC as an important factor governing the susceptibility to enzymatic hydrolysis of starch in raw cassava. Differences in susceptibility to amylolysis, assessed by RS, were also recorded in the sample subset analyzed. Most flours yielded pastes or gels upon heating and cooling. Gels differed in their subjective grade of firmness, but none exhibited syneresis, confirming the low retrogradation proclivity of cassava starch.

CONCLUSION: Some differences were found among cassava samples, which may be ascribed to inter-cultivar variation. This information may have application in further agronomic studies or for developing industrial uses for this crop.

Keywords: cassava starch; flours; amylose content; resistant starch; amylolysis rate

INTRODUCTION

Cassava (*Manihot esculenta Crantz*) is a traditional and important crop in different regions of the world. It has been cultivated for years by rural inhabitants in countries with economies in transition, representing one of the main components of the diet in tropical and subtropical areas.[1,2] It has many production advantages, such as adaptability to poor soils, drought resistance and high return per unit of energy used in its cultivation.[1]

Cassava is an important source of calories[3,4] a feature deriving from the high starch content of its roots.[5] However, cassava is not only used for direct human consumption, since a substantial part of its global production is also processed and manufactured as starch, thus becoming available for the food and feed industries.[6–9] Cassava starch is also important for economic activities not associated with food. This is the case of fuel alcohol production,[10] as well as the textile and paper industries.[1,7] Nonetheless, a number of factors such as stress conditions during growing and harvesting together with the crop age may affect the synthesis and quality of starch, making it difficult to optimize the commercial viability of cassava as a raw material for industrial purposes.[11–13] Furthermore, recent studies have pointed out the existence of some inter-cultivar variability in physico-chemical characteristics of cassava starch.[2,14]

Given the growing industrial use of cassava, it is important to investigate, in depth, the characterization of different agronomic varieties, which may help in the selection of raw materials for particular purposes and in the development of agronomic improvement programs. Besides the content of potentially toxic cyanogenic glycosides, cassava cultivars may show other compositional variations.[4] Therefore, the objective of this study was to characterize 25 cassava varieties grown in Venezuela, through the quantification of the starch content and susceptibility to α-amylolysis of flours prepared from freshly harvested roots. The amylose content and the tendency for gel formation were also evaluated. Simultaneous cultivation and harvesting at a single location guaranteed minimal influence of environmental factors on the investigated root properties.

* *Correspondence to: Juscelino Tovar, Functional Food Science Centre, Lund University. P.O. Box 124, SE-22100 Lund, Sweden. E-mail: juscelino.tovar@ffsc.lu.se*

† *Present address: Functional Food Science Centre, Lund University. P.O. Box 124, SE-22100 Lund, Sweden.*

a *Fundación Instituto IDEA, Carretera Nacional Hoyo de la Puerta-Baruta, Valle de Sartenejas, Caracas 1015A, Venezuela*

b *Institute of Experimental Biology, Faculty of Sciences, Universidad Central de Venezuela, P.O. Box 47114, Caracas 1041A, Venezuela*

MATERIALS AND METHODS

Source of material

The 25 cassava varieties were supplied by the Instituto de Estudios Avanzados (IDEA, Caracas, Venezuela). Twenty-one of the samples have been coded according to the Centro Internacional de Agricultura Tropical (CIAT, Cali, Colombia). IAC14 and IAC15 were coded according to Instituto Agronomico do Campinas (Brazil) and were supplied by Agropecuaria Mandioca C.A. Talibana and Roja were also supplied by Agropecuaria Mandioca C.A.

Planting and harvesting

Cassava plants were planted in December 2004 at Finca Agropecuaria Mandioca, located in Temblador, Monagas State, Venezuela (8° 28′ N; 62° 12′ W). Plots (11 × 5 m) were laid out in a random block arrangement in three replications; 55 plants were planted per block, at a density of 1 plant m^{-2}. During the harvest (December 2005) tree cassava plants from each cultivar were selected at random from one of the blocks. One root per plant was used for preparing the flours to be analyzed.

Sample preparation

The three roots chosen from each cultivar were peeled manually and chipped into slices of 0.5 cm thickness. Approximately 500 g of root slices were prepared per cultivar. These were first sun-dried for 6 h and then further dried at 45 ± 2 °C in an forced-air oven (BLUE M, Gravity Oven, Stabil-therm; Thermal Product Solutions, New Columbia, PA, USA) for 24 h. Dried samples were milled using an industrial mill (Retsch Verder SR 300; Retsch GmbH, Haan, Germany) and sieved (60 mesh). Finally, sub-samples were milled again (40 mesh) with a mill for analytical preparations and kept in polyethylene bags at room temperature, until further analysis.

Moisture

The moisture content was determined gravimetrically after heating at 130 °C until constant weight, using 1 g of sample. Measurements were done in triplicate.

Starch content

Starch content was assessed following the enzymatic protocol of Holm *et al.*,[15] based on the sequential digestion of flour suspensions with thermostable α-amylase (Termamyl, Novozymes A/S, Bagsvaerd, Denmark; at 98 °C for 20 min) and amyloglucosidase (A-7255; Sigma Chemical Co., St Louis, MO, USA; at 60 °C for 1 h), yielding free glucose. For calculation of the starch content, the released glucose was determined using a glucose oxidase–peroxidase kit (Qualitest, Caracas, Venezuela).

α-Amylolysis index

The rate of hydrolysis by hog pancreatic α-amylase (Sigma Chemicals) was measured by the method proposed by Holm *et al.*,[16] in flour samples containing equivalent amounts of starch (500 mg).

Resistant starch

The total resistant starch content of the raw flours was assessed with the method of Goñi *et al.*,[17] which applied to raw samples provides type II resistant starch values.[18]

Amylose content

The apparent amylose content was determined colorimetrically after iodine binding, following the method proposed by Juliano[19] using a standard curve prepared with potato amylose–amylopectin (Sigma Chemicals) blends.

Gel formation ability and syneresis tendency

The method proposed by Yeh and Yeh[20] was followed. Flours were dispersed in distilled water (8% w/v) using 30 mL centrifuge tubes. The samples were incubated in a boiling water bath for 30 min with stirring every 10 min, cooled to room temperature, centrifuged (1500 × *g* for 30 min at 4 °C) and the supernatant was discarded. The pelleted gel was stored at 4 °C for 24 h and centrifuged (1500 × *g* for 30 min at 4 °C) in order to visualize the presence of excluded water.

Statistical analysis

One-way analysis of variance (non-parametric) and correlation analysis were applied to the results; means were compared by Tukey's HDS and Kruskal–Wallis tests, using StatSoft (Version 8, 2001; StatSoft, Tulsa, AZ, USA). Hierarchical cluster analysis considering starch content, amylose content and amylolysis index was performed according to Ward,[21] using Paleontological Statistics Software (http://www.nhm.uio.no/norlex/past/download.html); the clusterization result is presented graphically as a dendrogram.

RESULTS AND DISCUSSION

Starch content

The starch content (SC) of the root flours from the 25 cassava varieties ranged between 742 and 814 g kg^{-1} (dry basis) (Table 1). Compared to other tubers, these SC levels are similar to those typically reported in potato (530–800 g kg^{-1})[22] and yam (750–840 g kg^{-1})[23] and greater than in sweet potato (470–740 g kg^{-1}).[24] Roja, Talibana, IAC15 and BRA383 exhibited the lowest SC, being significantly different from at least five of the cassava samples studied. In particular, SC in BRA383 was significantly lower ($P < 0.05$) than those exhibited by 14 other cultivars. Since all the samples included in this study came from plants grown simultaneously and at the same location, the observed differences in starch content among varieties must respond to inter-cultivar variability, with minimal influence of environmental factors. In spite of these differences, all values fell within the range reported in the literature for a large number of cassava cultivars.[2,7,25] The high starch content in all cassava varieties stresses the potential of this crop as a valuable energy source for humans and animals, as well as a suitable raw material for thickening agents[3,7,25,26] bio-fuels[10] or new product development by the food industry.[3,7,8] Nevertheless, the relative large variability existing among cassava cultivars may constitute a restrictive factor for industrial food manufacturing.[27] Hence, the evaluation of the starch quality beyond simple quantification provides valuable information concerning the versatility of use of a particular cassava cultivar. In this study, two additional quantitative indicators of starch properties were evaluated in order to broaden the functional characterization of the different varieties. These were the apparent amylose content in the different cultivars and the initial rate of hydrolysis by α-amylase *in vitro*.

Table 1. Characterisation of cassava flours

Cultivar	Starch content (g kg^{-1}, dry basis)	Amylose content (g kg^{-1}, starch basis)	α-Amylolysis index (g kg^{-1} maltose, starch basis)
BRA 383*	742 (7)^{e}	68 (19)^{abcde}	65 (5)^{bcdefghi}
PER 183	797 (9)^{abcd}	33 (4)^{d}	50 (7)^{jk}
TAI 8*	814 (15)^{a}	75 (5)^{abcde}	64 (3)^{cdefghij}
CM-507-37*	765 (17)^{bcde}	55 (4)^{cde}	65 (3)^{abcdefgh}
CM 523-7*	805 (16)^{abc}	102 (8)^{abcd}	54 (9)^{fghijk}
CM 3306-4*	811 (17)^{ab}	120 (8)^{a}	51 (9)^{ijk}
CM 4574-7	778 (11)^{abcde}	77 (7)^{abcde}	66 (2)^{abcdef}
CM 4843-1*	798 (24)^{abcd}	83 (8)^{abcde}	54 (9)^{efghijk}
CM 5306-8	812 (19)^{ab}	74 (7)^{abcde}	70 (4)^{abcd}
CM 6119-5*	780 (15)^{abcde}	66 (7)^{abcde}	78 (6)^{ab}
CM 6438-14*	807 (34)^{abc}	75 (10)^{abcde}	51 (3)^{hijk}
CM 6740-7	800 (14)^{abc}	102 (4)^{abcd}	65 (3)^{abcdefg}
CM 6921-3*	812 (17)^{ab}	89 (1)^{abcde}	52 (5)^{ghijk}
CM 7073-7*	785 (102)^{abcde}	113 (7)^{ab}	41 (2)^{k}
CM 7514-7*	782 (18)^{abcde}	73 (8)^{abcde}	71 (3)^{abcd}
CM 7514-8*	797 (11)^{abcd}	99 (4)^{abcde}	58 (2)^{efghij}
CM8027-3*	803 (6)^{abc}	67 (3)^{abcde}	68 (2)^{abcde}
SM 805-15*	806 (5)^{abc}	90 (8)^{abcde}	57 (7)^{defghij}
SM909-25*	813 (30)^{ab}	27 (3)^{e}	76 (6)^{abc}
SM1565-15*	796 (17)^{abcd}	67 (1)^{abcde}	79 (4)^{a}
Roja*	762 (32)^{cde}	93 (4)^{abcde}	51 (5)^{hijk}
IAC14	783 (18)^{abcde}	59 (4)^{abcde}	51 (4)^{ijk}
IAC15*	751 (23)^{de}	25 (15)^{e}	70 (6)^{abcd}
Talibana	761 (15)^{cde}	65 (4)^{abcde}	54 (7)^{fghijk}
CM 430-37	790 (8)^{abcde}	106 (4)^{abc}	71 (2)^{abcd}

Values are expressed as means (standard deviation in parentheses).
$^{a-k}$ Means in columns sharing at least one letter in common do not differ significantly ($P < 0.05$).
The α-amylolysis index is defined as the amount (g) of starch hydrolysed by α-amylase in 30 min kg^{-1} initial starch.
* Cultivars showing inverse correlation ($r = -0.59$) between amylose content and α-amylolysis index.

Amylose content

Amylose content (AC) ranged between 25 and 120 g kg^{-1} (starch basis), with cultivar IAC15 having the lowest value and CM3306-4 the highest (Table 1). Statistical analysis showed significant differences ($P < 0.05$) among the AC values of CM-507-37, PER183, SM909-25 and IAC15 and those varieties with the highest AC (CM33064, CM70737). Furthermore, SM909-25 and IAC15 exhibited the lowest values, showing significant differences with five (CM33064, CM70737, CM43037, CM67407, CM5237) of the cassava flours analyzed (Table 1). Again, since all cultivars were grown and harvested under identical conditions, the differences observed must be due the agronomic variety. The markedly low proportion of linear glucans (amylose) exhibited by IAC14, CM507-37, PER183, SM909-25 and IAC15, would permit the expectation of interesting physico-chemical characteristics for them, which could be associated, for instance, with low temperature gelatinization. Such a potential must be explored with the actual physico-chemical assessment.

Forty percent of the evaluated varieties fell within the amylose level range (80–160 g kg^{-1}) reported by Gallant *et al.*[28] for starch obtained from different *Manihot utilissima* samples. Furthermore, four of the cultivars (CM523-7, CM 3306-4, CM 7073-7 and CM 430-37) showed amylose contents resembling those of the low-amylose varieties reported by Aryee *et al.*[2] However, in general terms the ACs found in this work were lower than those reported previously in different cassava varieties,[2,7,9,29–31] which showed values in the 170–280 g kg^{-1} range. Although these differences may be explained by agronomical variability, the influence of the analytical method used in the different studies cannot be disregarded. In this work, amylose was determined in non-defatted samples of root flours, following the simplification established by Juliano[19] for materials with very low crude fat contents (0.3–0.55%), as those recorded for different cassava cultivars.[31–33] Since a substantial part of amylose in cassava starch forms complexes with cognate starch lipids,[31] and considering that more than half of our varieties exhibited markedly low amylose content (Table 1), it is plausible that the AC values found here for whole flours are influenced by the putative presence of significant levels of endogenous amylose–lipid complexes. Nonetheless, AC levels reported in this work allow differences to be established among the native flours from the different cassava varieties. In future investigations attention should be paid to the analytical aspects mentioned.

Rate of α-amylolysis

The initial *in vitro* enzymatic hydrolysis (α-amylolysis) was also evaluated in the native flours from the different cassava varieties. The percentage of conversion ($t = 30$ min) from starch to maltose, or amylolysis index (AI), ranged from 41 to 79 g kg^{-1}. Such values can be considered low, as the native corn starch reference showed a 280 g kg^{-1} index. Significant differences ($P < 0.05$) were shown among the AIs of the various cultivars (Table 1). Differences in the *in vitro* α-amylolysis among native starches has been attributed to the interplay of a number of factors, such as granular shape and size, crystalline organization, amylose-to-amylopectin ratio and extent of distribution of $\alpha(1-6)$ branch points, among others.[28,31,32] Cassava starch granules exhibit an X-ray diffraction pattern corresponding to the C-type allomorph,[34] a crystalline arrangement characterized by its limited digestibility.[28,35] As a matter of fact, Valetudie *et al.*[32] compared the α-amylolytic susceptibility of starches from various tubers and roots, reporting a very slow digestion for native cassava starch, whose hydrolysis rate (0.18 min^{-1}) was in the same range as those recorded here for most of the cultivars.

Amylose is characterized by an essentially linear, packed and also more compact structure than amylopectin.[28] Hence, the α-amylolysis rate of native starches generally decreases with the amylose content.[36] According to the statistical analysis of present data, an inverse correlation ($r = -0.59$, $P < 0.05$) between AC and AI could be established for the flours from 18 of the cultivars (Table 1). Consequently, the AC in these varieties appears to be an important factor governing the enzymatic susceptibility. Nevertheless, other extrinsic factors, such as starch–lipid and starch–protein interactions described for cassava starch[31,37] may also play a role in these differences in propensity to amylolysis. Interestingly, for the cultivars CM430-37, CM6740-7, CM4574-7, CM5306-8, Talibana, IAC14 and PER183 no correlation was found between the two parameters under consideration. Further studies looking at starch properties and fine composition of these varieties may be interesting.

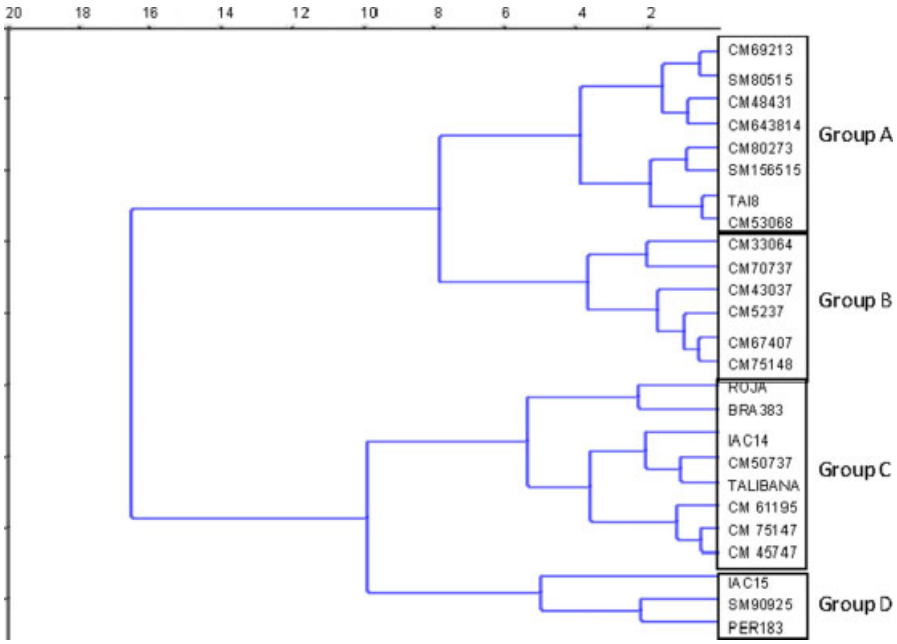

Figure 1. Cluster analysis of cassava varieties based on SC, AC and AI, following Ward's method. Group A: cultivars with intermediate AC (67–90 g kg^{-1}) and high SC values (≥796 g kg^{-1}). Group B: cultivars with high AC (≥99 g kg^{-1}) and high SC values (≥796 g kg^{-1}). Group C: cultivars with intermediate/low AC (55–93 g kg^{-1}) and intermediate/low SC (761–783 g kg^{-1}). Group D: cultivars exhibiting the lowest AC values (≤33 g kg^{-1}).

Cluster analysis based on starch content, amylose content and rate of α-amylolysis

The cluster analysis represented in Fig. 1 pointed out SC and AC as the most significant descriptors permitting classification of the cassava cultivars into four groups, stressing the relatively ample inter-cultivar variation of cassava starch properties. The groups established corresponded to cultivars with high SC and high AC, high SC and intermediate AC, intermediate SC and AC, and a group with extremely low AC values, respectively. Inter-cultivar distances within each group (A, B, C and D) were defined by IA. The information provided by this analysis may be of use for further agronomic development of cassava cultivars with focus on potential industrial applications based on these physico-chemical characteristics. Since an interesting feature of cassava starch is its low amylose content and consequent limited retrogradation proclivity, cultivars included in group D appear as promising candidates for industrial use.

Gel formation ability

The amylose and amylopectin contents of a particular starch dictate the gelatinization behavior and rheological characteristics of the resultant paste or gel. The capacity of the flours to form gels was also evaluated in this work. After heat gelatinization and cooling at room temperature, the flours formed a soft gel, except for IAC14, Roja, IAC15, Talibana, BRA 383 (which exhibited intermediate AC), and CM430-37 (among those of highest AC samples). IAC14 and Roja developed only a very viscous paste but not a real gel, while IAC15 and Talibana formed a more fluid paste. BRA383 and CM430-37 did not develop noticeably viscous pastes. These observations confirm the generally acknowledged limited gel-forming capacity of cassava starch, but also reveal interesting inter-varietal differences.

Once kept under refrigeration (4 °C for 48 h) all gelatinized samples formed true gels, differing in their subjective grade of firmness. Bra 383, CM4303-7, CM4574-7 and CM80273 formed soft clots whilst the rest of the samples formed firm gels. Interestingly, among this last group, CM3306-4 and CM4843-1 showed the firmest gels, which is in agreement with the relatively high amylose content showed by these cultivars. Also, CM3306-4 was the only variety yielding a gel of whiteness appearance, notably different to the rest of the samples which resulted in translucent gels.

The gel forming tendency of cultivars Bra 383, Roja, CM4574-7, CM430-37 and CM80273, was not in accordance with that observed for other samples with similar AC. CM430-37 was classified in the group with higher AC, while Bra 383, Roja, CM4574-7 and CM8027-3 belong to the group with intermediate AC. However, none of them produced real gels after boiling and initial cooling at ambient temperature, and exhibited a very soft gel after 2 days under refrigeration. Hence, factors other than amylose content appear to influence the gel formation capacity in cassava flours.

The separation of aqueous phase (syneresis) from gels obtained by thermic gelatinization of the flours is an indirect indicator of the tendency of the starch components to recrystallize (retrograde).[38] In the present study, none of the good gel-yielding cultivars exhibited syneresis, confirming the low retrogradation proclivity of cassava starch.[7]

Resistant starch

Starches from different botanical sources may differ in their susceptibility to enzymatic digestion. This is valid both for native (raw) and heat-processed materials.[26,33] In raw samples, as those studied here, a variable fraction of the starch is resistant to hydrolysis due to intrinsic structural features of the starch granules. This type of resistant starch (RS) is known as type-2 resistant starch (RSII).[35] Since no information is available for the RS content of raw cassava flours, 10 of the samples evaluated in this study were randomly selected for assessing this parameter (Table 2). Relatively high levels of RS values were registered in all samples, ranging from 50 to 196 g kg^{-1}. As mentioned before, cassava starch granules

Table 2. Resistant starch content in the flours from 10 cassava varieties

Cultivar	Resistant starch (g kg^{-1}, dry basis)
TAI 8	104 (16)^{b}
CM-507-37	50 (5)^{cde}
CM 523-7	124 (14)^{b}
CM 3306-4	196 (21)^{a}
CM 4574-7	100 (11)^{b}
CM 4843-1	120 (10)^{b}
Roja	133 (27)^{b}
IAC14	105 (8)^{b}
IAC15	76 (4)^{cd}
Talibana	85 (8)^{c}

Values are expressed as means (standard deviation in parentheses). $^{a-e}$ Means in columns sharing at least one letter in common do not differ significantly ($P < 0.05$).

show a C-type X-ray diffraction pattern,[31] which is associated with slow and incomplete digestion features *in vitro* and *in vivo*.[35]

Significant differences ($P < 0.05$) were found among RS contents of the cassava flours, with CM507-37 showing the lowest and CM3306-4 showing the highest levels. These cultivar-associated differences suggest RS content as a potentially useful parameter for the classification of cassava varieties. Further studies are envisaged to evaluate this possibility.

An inverse correlation ($r = -0.65, P < 0.05$) was found between RS content and the AIs of the flours, an observation that may be associated with the suggested limited accessibility of the amylolytic enzymes to the RS-rich zones of the granule.[28]

CONCLUSION

Significant differences in starch content, amylose levels and α-amylolysis rates were found among 25 cassava varieties. Using cluster analysis, these parameters divided the samples in four major groups. This preliminary classification may be of use, for instance, in further agronomic studies or for developing new industrial uses for cassava.

Significant variability was also noted in the *in vitro* resistant starch content of a subset of 10 samples, suggesting a potential use of this parameter as a complementary criterion for the characterization of cassava varieties.

The study also confirmed the ability of starch in heated samples of most cassava flours to form soft gels upon cooling/storage, with noticeable varietal differences.

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