Full Length Research Paper

Characterization of the roots and starches of three cassava cultivars

Taiana Ladeira[1], Hugo Souza[2], Rosinelson Pena[1]*

[1]Faculdade de Engenharia de Alimentos, Instituto de Tecnologia, Universidade Federal do Pará, Belém, Pará, Brazil.
[2]Departamento de Ciência de Alimentos, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

Cassava (*Manihot esculenta* Crantz) is a plant of high genetic variability. The cultivars collected in the Amazon are highly valued for their quality and adaptability to local ecological conditions. Among the products of cassava, the most versatile and valuable is starch. The aim of this study was to obtain and characterize the starches of three cultivars of cassava (Jurará, Santarém SI and Maranhense II cultivars) produced in the state of Pará, aiming at technologically harnessing this crop in that region. The roots and starches were analyzed for physicochemical properties, and the starches were morphologically characterized. In general, the roots of the three cultivars had different compositions ($p \leq 0.05$). All of the physicochemical parameters of the starches reached the standards set by Brazilian law. According to optical and electron microscopy, the morphology of the granules from the three starches are characteristic of native cassava starches.

Key words: *Manihot esculenta*, microscopy, physicochemical, property.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a shrubby perennial heliophyte of the family Euphorbiaceae. Brazil has more than 4,000 cataloged cassava varieties, and different species are suitable for cultivation, depending on the region and its respective ecological parameters (Amaral et al., 2007). Globally, cassava is the sixth most important crop after wheat, rice, potato, corn and barley, and it is a staple food for more than 800 million people around the world, mainly in tropical countries (Lebot, 2009).

In addition to its recognized social and economic importance as a producer of food and industrial crops, Brazil is considered the center of cassava diversity (Rogers and Appan, 1973) while the Amazon is probably the origin of the species (Allem, 1994). Moreover, Nigeria is the largest producer of cassava in the world (Osundahunsi at al., 2011).

The characterization and assessment of the germplasm and its potential uses involves a strategic combination of research and development to add ever increasing value and permit vertical integration in production (Morales 1996, Unpublished manuscript). The biodiversity is an important socio-economical asset (Costa et al., 2003). Under this framework, the Amazon serves as a large repository of cassava genetic resources and as such, the cultivars collected in the Amazon are highly valuable in terms of their quality and adaptability to local ecological conditions.

Among cassava's products and by-products, the *fécula*, which is legally defined in Brazil (by Brazil 2005) as the starch fraction that originated from roots and tubers, is the most versatile and valuable. The value comes from its multiple applications, which range from culinary to industrial uses (Souza et al., 2005). *Fécula* is obtained from cassava roots that have been processed by peeling, grinding, purification, sieving, centrifuging, and drying (Camargo et al., 1984).

The aim of the present study was to obtain and characterize starches from three cassava cultivars (namely, Jurará, Santarém SI and Maranhense II) highly produced in the state of Pará, Brazil, with the aim of increase the technological harnessing of this crop in that

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*Corresponding author. E-mail: rspena@ufpa.br; rspena70@hotmail.com. Tel: +55 9132018055; Fax: +55 9132018055.

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        ┌─────────────────────┐
        │   Cassava's roots   │
        └─────────────────────┘
                  │
                  ▼
   ┌────────────────────────────┐
   │ Desintregration in a industrial │
   │  blender (1kg raw material/L │
   │     water) for 5 minutes     │
   └────────────────────────────┘
                  │
                  ▼
          ┌──────────────┐
          │  Separation  │
          │  (60 mesh)   │
          └──────────────┘
            │           │
            ▼           ▼
┌──────────────┐   ┌──────────────┐
│ Solid fibrous │   │  Starch milk │
│   residue    │   └──────────────┘
└──────────────┘          │
        │                 ▼
        ▼          ┌──────────────┐
┌──────────────┐   │  Purification │
│   Storage    │   │  (200 mesh)  │
└──────────────┘   └──────────────┘
                          │
                          ▼
┌──────────────┐   ┌──────────────┐
│ Residual water│◄─│   Washing    │
└──────────────┘   │  (4 times)   │
                   └──────────────┘
                          │
                          ▼
                ┌──────────────────┐
                │  Drying process  │
                │ (Spouted bed cone-│
                │ cylindder at 70°C)│
                └──────────────────┘
                          │
                          ▼
                 ┌──────────────┐
                 │ Native starch│
                 └──────────────┘
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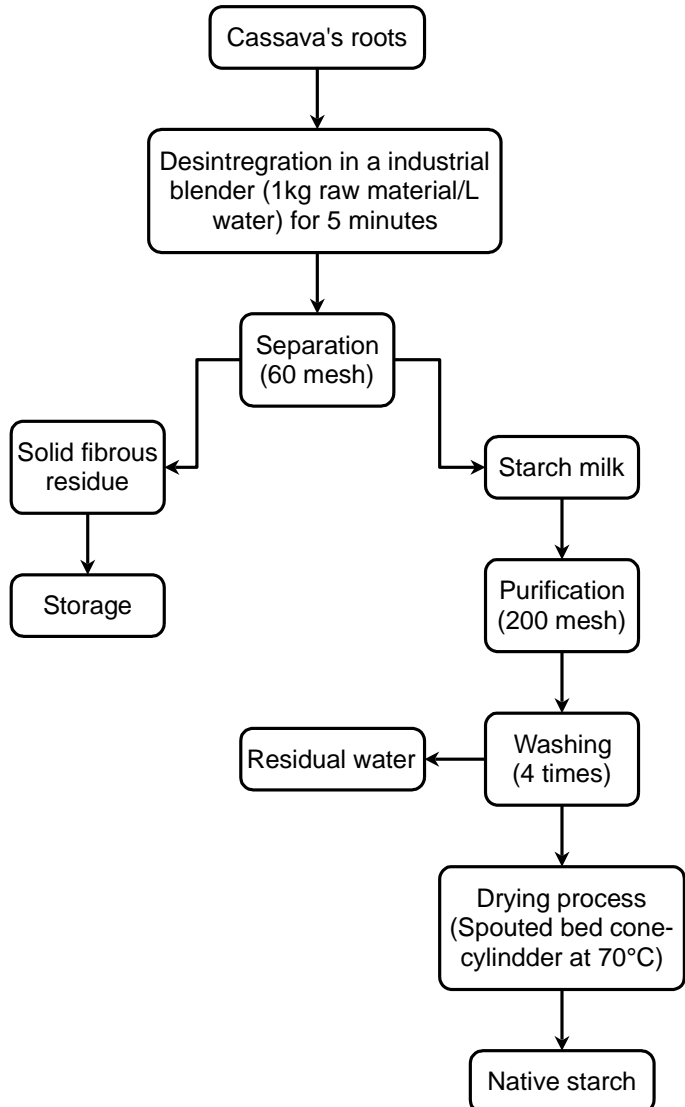
Figure 1. Flowchart for the production of cassava starch.

region. These cultivars are used in the production of cassava flour from dry group, cassava flour from water group and *tapioca* flour, typical widely-consumed starchy products from Amazonian region of Brazil.

MATERIALS AND METHODS

Raw material

Cassava roots from three cultivars used in this study were obtained at Active Germplasm Bank of Brazilian Agricultural Research Corporation (EMBRAPA/CPATU). The cultivars were Maranhense II, Santarém SI and Jurará; each one was derived from three or four clones.

The roots were transported in 50 kg plastic bags at room temperature (≈30°C) to the Food Engineering

Laboratory, where they were washed with running water to remove loose dirt, sanitized for 15 minutes in a solution of 160 mg/L active chlorine (Oliveira et al., 2003) and rinsed. After cleaning, the roots were packed, frozen at -5ºC and kept at that temperature until just before use.

Characterization of roots and starches

The following analyses were conducted to characterize the cassava roots and starches. Water activity (a_w) was measured with a hygrometer (Aqualab 3TE, Decagon, Pullman, WA) at 25°C. Moisture content was evaluated using a gravimetric method in an air-circulation oven of FABBE Trademark, model 110 at 130°C (protocols n. 44-15A). Ash was also evaluated using a gravimetric method by incineration of the sample in Muffle furnace at 525ºC, for 12 h (protocol n. 08-17). Total protein was measured using the Kjeldahl method, with a 6.25 nitrogen-protein correlation factor (protocol n. 46-10). Total titratable acidity was evaluated with a titration method (protocol n. 02-31), following the AACC (1995). Total lipids were analyzed according to Bligh and Dyer (1959). pH was measured by a direct reading on a MA PA 200 Marconi pH meter, following the AOAC (1997) protocol n. 943.02. Starch was analyzed using acid hydrolysis, according to Rickard and Behn (1987). Color was evaluated using tristimulus colorimetry in a colorimeter (Konica Minolta CR 400, Singapore), with L*, a* and b* values based on the parameters D65 (day light) and 10º (observer angle) according to CIE Lab standards Chroma Meter (1989). Total and reducing sugars were evaluated with the Lane-Eynon titration method, according to AOAC (1997) protocol n. 920.183b. All analyses were performed in triplicate.

Obtaining the cassava starch

The steps performed to obtain the cassava starch are described in the flowchart in Figure 1 and are based on the methodology used by Leonel (2007). The obtained products were dried in a cylindrical conical spouted bed drying, with a forced 70°C airflow and 1.37 kg of inert polypropylene particles.

Morphological characterization of starches

Optical microscopy

Sample surface was observed by optical microscopy using a DM/LP LEICA microscope with transmitted polarized in an industrial blender (Veiga Santos et al., 2005).

Scanning electron microscopy (SEM)

The starch sample was fixed on a sample holder using a double-sided sticky tape. The holders were loaded and

Table 1. Physicochemical characterization of roots from Maranhense II, Jurará and Santarém SI cultivars.

Properties	Cultivars		
	Maranhense II	**Jurará**	**Santarém SI**
Moisture content (%)	73.25±0.08[b]	63.13±1.14[c]	75.62±1.08[a]
Water activity (a_w)	0.98±0.02[a]	0.98±0.02[a]	0.98±0.02[a]
pH	6.66±0.03[b]	6.96±0.01[a]	6.60±0.03[b]
Soluble solids content[d]	7.42±0.10[a]	4.43±0.51[b]	4.28±0.24[b]
Total titratable acidity[e]	1.21±0.06[b]	1.43±0.09[b]	2.88±0.35[a]
Ashes (%)	0.28±0.02[a]	0.12±0.007[b]	0.28±0.01[a]
Lipid (%)[f]	0.65±0.07[b]	0.93±0.13[a]	0.91±0.12[a]
Protein (%)[f]	1.26±0.03[b]	0.94±0.02[b]	2.92±0.13[a]
Starch (%)[f]	76.94±0.20[a]	74.22±0.24[b]	68.30±0.11[c]
Total sugars (%)	4.02±0.04[b]	4.30±0.05[a]	3.71±0.01[c]
Reducing sugar (%)	1.18±0.02[b]	2.10±0.02[a]	0.89±0.01[c]
Non reducing sugars (%)	2.70±0.02[a]	2.08±0.03[b]	2.68±0.02[a]
L	72.43±4.98[a]	79.85±4.29[a]	81.19±2.03[a]
a*	-1.63±0.69[a]	-1.53±0.12[a]	-0.9±0.03[a]
b*	16.29±0.60[a]	14.08±0.27[b]	15.52±0.78[a]

Mean values in the same line followed by different subscript letters are significantly different ($p \leq 0.05$).
[d] Brix at 20°C
[e] meq NaOH/100g
[f] Dry basis.

sputtered with platinum powder. Sample surface was observed using a scanning electron microscope (1450 VP LEO) and images were taken at 5-10 kV.

Statistical analysis

For the physicochemical results, analysis of variance (ANOVA) and Tukey's post-hoc test were used to compare means. Analyses were performed using the software STATISTICA Kernel Release 7.1 (StatSoft Inc., 2006, Tulsa, OK) for Windows XP.

RESULTS AND DISCUSSION

Characterization of roots

Table 1 shows the mean values and the respective standard deviation for the physicochemical parameters of the roots from the Jurará, Santarém SI and Maranhense II cassava cultivars.

There is a statistically significant difference in moisture content among the three cassava cultivars ($p \leq 0.05$). The high level of moisture found in the three starches promotes the growth of microorganisms, especially mold and yeast, as confirmed by water activity ($a_w > 0.6$). The three cassava cultivars are classified as perishable based on the observed a_w values (Damodaran et al., 2007). These results suggest that cassava should be processed or kept under controlled temperatures after harvest to

ensure conservation until further processing or use. The moisture values were similar to those found by Padonou et al. (2005), who observed mean values of 69.4% when studying 12 cultivated varieties of Benin cassava, and by Sánchez et al. (2009), who reported mean moisture values of 66.4% for more than 3,000 varieties of cassava roots.

There were statistically significant differences in pH between the three cassava cultivars ($p \leq 0.05$); however, all are classified as low-acidity foods (pH > 4.5). These foods provide a suitable environment for the development of microorganisms, including pathogenic bacteria, mold and yeast. For comparison, Camacho and Cabello (2009) reported a pH of 6.0 for the root of the *Fécula Branca* variety of cassava.

The soluble solids content of the Maranhense II cultivar was higher than that of the Jurará and Santarém SI cultivars, which were statistically indistinguishable (p>0.05). Oliveira et al. (2003) reported an average value of soluble solids of 4.33 °Brix for minimally processed cassava at the beginning of storage.

The total titratable acidity of the Santarém SI cultivar was higher than that of the Jurará and Maranhense II cultivars, which were not statistically differents (p>0.05). The values agree with those reported by Oliveira and Morais (2009), who obtained values between 1.55 and 2.70 mL NaOH/100 g for the roots of the IAC 576-70 variety of cultivated cassava at different harvest times.

The average ash content reported by Oliveira and

Morais (2009) ranged from 0.88% to 1% for cassava roots, which was at least three times higher than the values found for the three roots tested in this study. Other authors reported an ash content of 0.5% for roots of the *Fécula Branca* variety of cassava (Camacho and Cabello, 2009). Such result is closer to those observed in this study.

Ceni et al. (2009) observed lipid contents ranging from 0.33% to 2.4% in five varieties of cassava. The values found for the three cultivars in this study were within this range. A lower value was reported by Padonou et al. (2005), who found an average of 0.56% in cassava roots. Higher values were observed for the Jurará and Santarém SI cultivars (0.93 and 0.95%, respectively), which were not statistically significant (p>0.05).

The Jurará and Maranhense II cultivars had protein contents that were not statistically significant (p>0.05), which were similar to the values reported for different cassava varieties (from 1.2% to 1.8%) (Ceni et al., 2009). The Santarém SI cultivar had a higher value; however, the protein content was similar to that observed for different cassava roots (3% dry basis) (Cereda, 2001).

The analyses of variance showed that there was a significant difference between the starch contents of the three cassava cultivars (p≤0.05). According to Holloway et al. (1985), cassava root has a high percentage of starch (84.9% dry basis). Sánchez et al. (2009) reported an average of 85.5% starch for more than 3,000 cultivars of cassava roots. The results obtained for the three cultivars in this study were lower, and the Santarém SI cultivar exhibited the lowest starch content.

The contents of ash, lipid, protein, starch and sugar, on a dry basis, were 94.92%, 88.08% and 88.50% for Maranhense II, Jurará and Santarém SI roots, respectively. The remaining amount may be attributed to the fibrous fraction of the roots, not measured. It was observed an average of 11.78% (dry basis) for the fibrous fraction in a study of the composition of fibrous residue from the cassava starches (Leonel and Cereda, 2000).

There was a significant difference in total and reducing sugar contents between the cultivars studied (p≤0.05). The values found in this study agree with those reported by Sánchez et al. (2009), who found values ranging from 0.2 to 18.8% for total sugars and from 0.0 to 15.7% for reducing sugars in more than 4,000 genotypes of cassava roots. In this study, the highest total and reducing sugar contents was observed in the Jurará cultivar.

In terms of instrumental color, L values above 70 were found for all the roots *in natura,* and the highest value (81.19) was observed for the Santarém SI cultivar. The chromaticity coordinate a*, which ranges from -100 (green) to +100 (red), was similar for all cultivars, and tended to a very light green. The chromaticity coordinate b*, which ranges from -100 (blue) to +100 (yellow), indicated a higher intensity of yellow for the Maranhense II and Santarém SI cultivars.

Extraction of cassava starch

The native starch extracted from the roots of the cassava cultivars sedimented quickly. The starch appeared to be free from impurities and dyes. These are some of the important characteristics of cassava starch compared to the starch from other botanical sources, which are slow to sediment, give low yields or may be colored by contamination from proteins and lipids (Moorthy, 1994).

The yield obtained in the starch extraction is presented in Table 2. According to Leonel et al. (1998), the starch extraction yield from cassava roots was 22.5% at the industrial level; in our study, the Jurará cultivar was closer to the industrial level.

Physicochemical characterization of starches from Maranhense II, Jurará and Santarém SI cassava cultivars

Table 2 presents the results of the physicochemical characterization of starches from the three cassava cultivars. Despite the significant difference between the moisture content of the three starches, all of them met the maximum limit of 14% (w/w) stipulated by Brazilian law for cassava starch (Brazil, 2005). The moisture values are less than those reported by Nwokocha et al. (2009) for cassava starch (14.6%). However, the Maranhense II and Jurará cultivars had contents similar to those found by Leonel et al. (1998) for cassava starch (12.37%, dry basis). The pattern of moisture content resulted in a significant difference (p≤0.05) in a_w. All of the values were below 0.6, which ensures the microbiological stability of the products (Rockland and Nishi, 1980; Scott, 1957).

The starches obtained from the three cassava cultivars met the pH limits established by Brazilian law (Brazil, 2005), which allows a range between 4.0 and 7.0 for the product. The pH of starches from the Maranhense II and Jurará cultivars were statistically similar and higher than the value for Santarém SI (p≤0.05), but all of them agree with their respective *in natura* root pH (Table 1). Nwokocha et al. (2009) reported a pH of 5.56 for cassava starch.

The starch obtained from the Santarém SI cultivar showed a statistically higher acidity, whereas the starch from the Jurará cultivar had lower ash content. Nevertheless, the ash content of the starches from the three cassava cultivars met the maximum value of 0.7% established by Brazilian law (Brazil, 2005).

There was a statistically significant difference (p≤0.05) between the protein contents of the three starches. The protein content of the starch from the Jurará cultivar agreed with the average value of 0.27% reported by Abera and Rakshit (2003) for cassava starch. The protein contents of the Maranhense II and Santarém SI cultivars were higher. The lipid content, which did not show significant differences (p>0.05) across the three cultivars, were also higher than found in Abera and Rakshit (2003),

Table 2. Physicochemical and yield characterization of starches from Maranhense II, Jurará and Santarém SI roots.

Properties	Cultivars		
	Maranhense II	Jurará	Santarém SI
Yield (%)	12,31%	21.76%	17,94%
Moisture content (%)	11.25±0.08[a]	10.48±0.23[b]	8.9±0.19[c]
Water activity (a_w)	0.56±0.001[a]	0.41±0.005[b]	0.30±0.002[c]
pH	6.72±0.01[a]	6.79±0.06[a]	6.45±0.04[b]
Total titratable acidity[d]	1.0±0.06[b]	1.0±0.12[b]	2.0±0.25[a]
Ashes (%)[e]	0.35±0.05[a]	0.14±0.02[b]	0.38±0.05[a]
Protein (%)[e]	0.43±0.02[b]	0.26±0.05[c]	0.77±0.86[a]
Lipid (%)[e]	0.19±0.07[a]	0.26±0.05[a]	0.36±0.02[a]
Starch (%)[e]	97.36 ± 0.12[a]	98.44 ± 0.25[a]	97.62±0.10[a]
L	87.22±0.007[a]	83.65±0.19[b]	86.69±1.40[a]
a*	-0.24±0.01[a]	+0.33±0.01[b]	+0.01±0.01[c]
b*	+4.71±0.16[a]	+3.57±0.18[b]	+5.02±0.35[a]

Mean values in the same line followed by different subscript letters are significantly different ($p \leq 0.05$)
[d] mL NaOH/100g
[e] Dry basis.

who reported a range of 0.08 to 0.1% for starch from three cassava varieties.

Starch contents of the three starches were the same statistically and above the 80% minimum required by Brazilian law (Brazil, 2005) for cassava starch. The high starch content indicates a high efficiency in the extraction and purification stages of starch obtained from roots of Maranhense II, Jurará and Santarém SI cultivars. The lower the content of other substances (i.e., lipid, protein and minerals, among others) show the better quality of the starches.

The starches of the Maranhense II and Santarém SI cultivars showed the highest values for the luminosity parameter L (87.22 and 86.69, respectively), indicating an increased whiteness of the products. The same starches also showed higher values for the chromaticity coordinate b* (+4.71 and +5.02, respectively), which indicates an increased tendency toward yellow, possibly due to the presence of a natural dye such as β-carotene drawn through during the extraction process (Oliveira et al., 2010). That same tendency was shown for the roots of these cultivars (Table 1).

According to the results obtained for moisture, starch content, ash content and pH, the starche obtained from the Santarém SI cultivar may be classified as "Type 1", demonstrating its high quality. By contrast, starches from the Maranhense II and Jurará cultivars can only fit into the "Type 3" classification, mainly due to their pH value and ash content (Brazil, 2005).
and non polarized light of starches obtained from the three cassava cultivars. All of them exhibited the presence of birefringence under polarized light (Figures

2A, 2C and 2E), which indicates semi-crystalline starch

Morphological characterization of the starches

Optical microscopy

Figure 2 shows the optical micrographs under polarized granules (Cereda, 2001; Damodaran et al, 2007; Souza and Andrade, 2000).

Optical microscopy under non-polarized light (Figures 2B, 2D and 2F) showed the homogeneity of starch granule shape across the three cultivars and revealed the presence of a central hilum in most of them. Beninca et al. (2008) observed the same pattern for corn starch, as did Lacerda et al. (2008) for partially hydrolyzed cassava starch. Fissures in the hilum and some radial cracks were observed. These were attributed to the compression to which the starch granules were subjected while preparing the sections.

Scanning electron microscopy

Figure 3 shows the electron micrographs of the starches from the three cassava cultivars. The starch granules from all the cultivars were circular in shape and had some concave-convex characteristics, as observed by other authors (Freitas and Leonel, 2008; Leonel, 2007; Pimentel et al., 2007).

The diameter of the granules varied considerably between starches from the three cassava cultivars. For the starch from the Jurará cultivar, the smallest observed diameter was 9.4 µm and the largest was 25.2 µm (average size of 17.3 µm). For the Maranhense II cultivar,
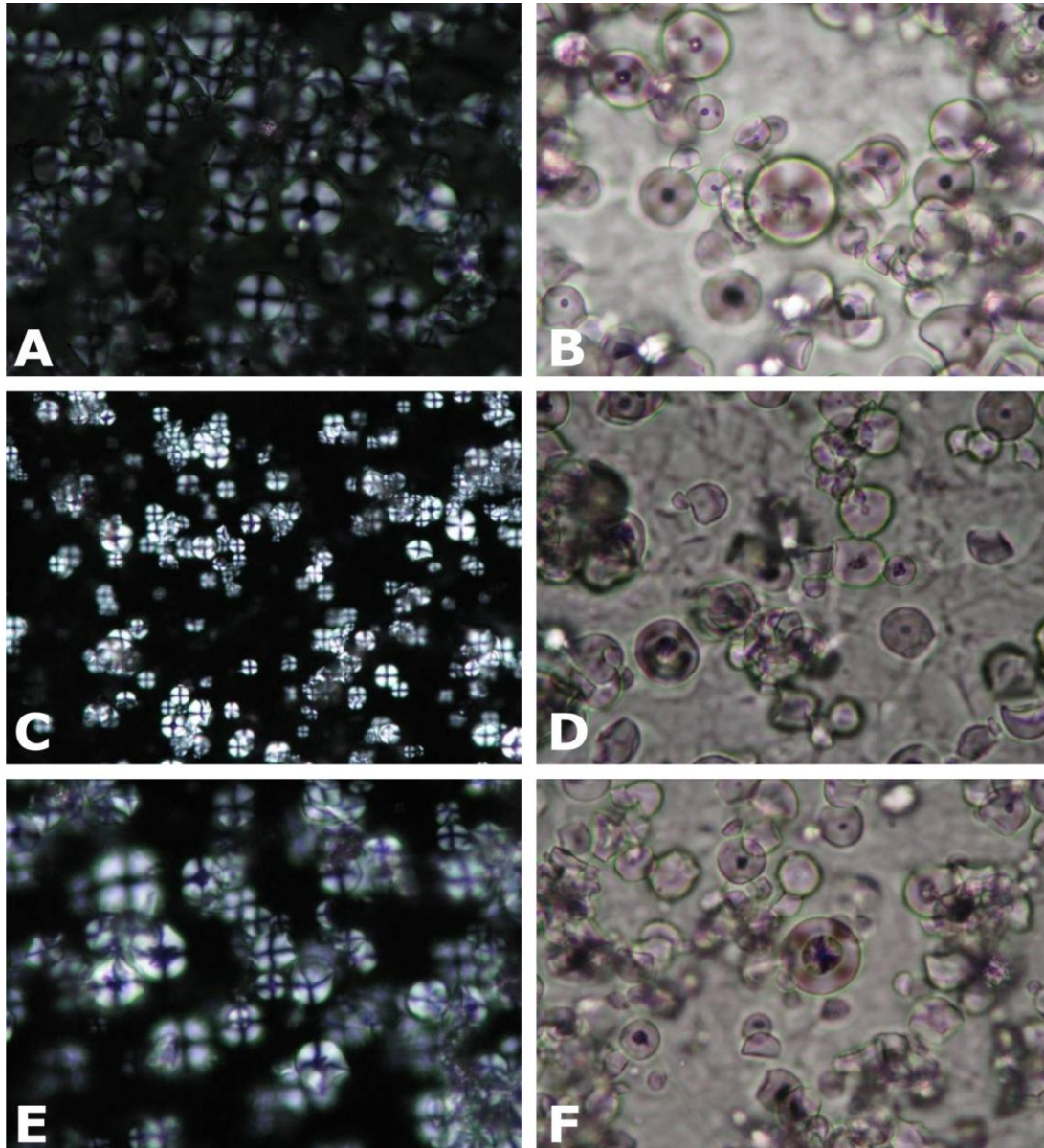
Figure 2. Optical micrographs of the three cassava starches studied: Jurará: (A) polarized light 50x and (B) non polarized light 50x; Maranhense II: (C) polarized light 20x and (D) non polarized light 50x; Santarém SI: (E) polarized light 50x and (F) non polarized light 50x.

the diameter of the granules ranged from 9.9 to 21.3 μm (average size of 15.6 μm). For the starch from the Santarém SI cultivar, the range was from 9.9 to 17.2 μm (average size of 13.6 μm). Similar results were obtained by Leonel (2007) and Nwokocha et al. (2009).

Conclusions

The cassava roots from the Maranhense II, Jurará and Santarém SI cultivars showed distinct physicochemical characteristics relative to one another, but they were in agreement with the characteristics exhibited by other cassava varieties.

The starches obtained from the three cassava cultivars met the identity and quality standards for cassava products established by law in Brazil. Additionally, the high starch content and the low content of non-starch constituents confirm the quality of the products.

The Jurará presented similar yield to industrial sample, with the lowest yellow color intensity among the studied cultivars, which makes it an excellent alternative for starch production.

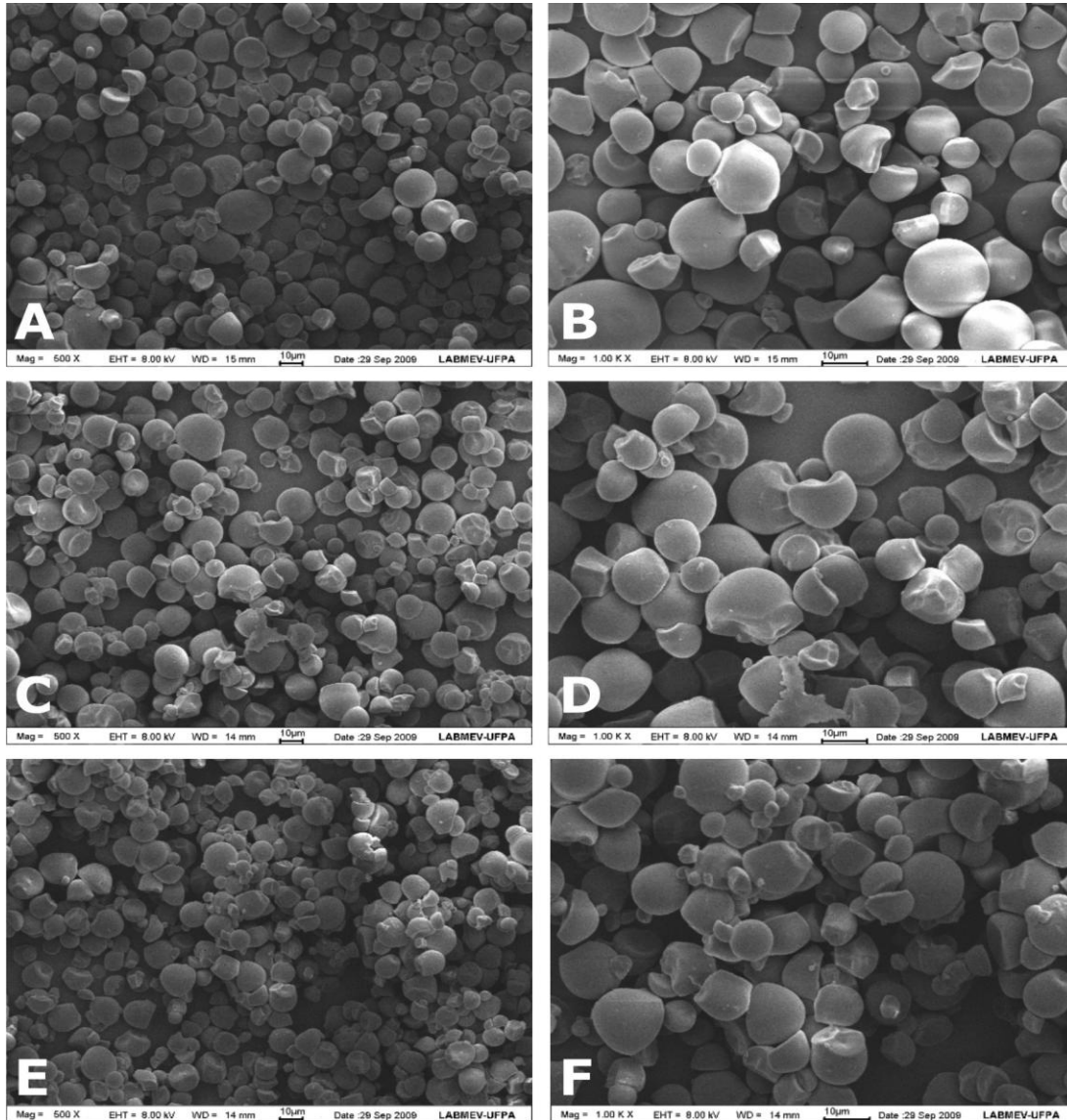According to the identity and quality standards for cassava product, the starches obtained from the Jurará

Figure 3. Electron micrographs of the starches from the cultivar Jurará: (A) 500x and (B) 1000x; Maranhense II: (C) 500x and (D) 1000x; Santarém SI: (E) 500x and (F) 1000x.

and Santarém SI cultivars were classified as "Type 1," whereas the starche obtained from the Santarém SI cultivar was classified as "Type 3", according to Brazilian law.

The starch granules from the three starches showed the characteristic morphology of native starches from other cassava varieties under both optical and electron microscopy.

ACKNOWLEDGEMENTS

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