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Physicochemical and Functional Properties of Native and Modified by Crosslinking, Dark-Cush-Cush Yam (*Dioscorea Trifida*) and Cassava (*Manihot Esculenta*) Starch

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Abstract Cush-cush yam (*Dioscorea trifida*), is a sub-utilized tropical crop, rich in starch, which grown in tropical areas. The exploitation of its starch has great potential. The objectives of this research was to modify starch isolated from dark-cush-cush yam, which grown in the Venezuelan Amazonian, and commercial cassava starch, by cross linking, in order to characterize and compare them with their native counterparts. The results showed that differences in the amylose content affected the composition, structure and functional properties of both starches. The degree of substitution (DS) was more significant in the cush-cush starch yam than that of the cassava starch, even if both were within the ranges allowed by the FDA. The granular size of cassava starch was slightly changed by modification. The rheological properties also changed, increasing the viscosity peak, breakdown, consistency and setback. Finally, that modification method conferred great stability to the starches gelatinization.

Keywords: *non-conventional starches, cross-linking, modified starches*

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1. Introduction

Starch is a popular ingredient used in food industry to impart and improve functional properties of several processed foods. Although, in nature there are many available botanical sources of this polymer, but only a few are used. Starch from corn (*Zea mays*), potato (*Solanum tuberosum*), sweet potato (*Ipomoea batatas*) and cassava (*Manihot esculenta*) are the most commonly used in the mentioned industry. Even if starch is the energy source of myriads of living organisms [1], their functional properties are the most appreciated by the food industry and consumers. This natural biopolymer is cheap, available, quite easy to handle and widely distributed in the nature.

There are a great number of plants with amylaceous storage organs containing almost 30% of starch (wet basis). One of them, cassava, is exploited commercially to produce starch in Brazil, Thailand and China. However, nearly the majority of starch sources have not been yet exploited despite their nutritional and functional properties. Among them, tubers from cush-cush yam (*Dioscorea trifida*), grown in artesian and local cultures, and wild in the Venezuelan tropics has been of great interest. Ref. [2] reported the waxy nature of starch from these tubers and, in turn, its potential interest for industrial processes.

Native starches have functional properties inherent to their botanical origin, that may be non specifics for some industrial applications. However, the modification of their properties can be brought about by using chemical, physical, or biological techniques, that may improve or introduce functionality in their structure. Once starch has been modified, must be characterized in order to validate its functional properties and compare them with those of the native counterpart, since this allows differentiating starch applications in industry [3]. In the particular case, modification by phosphating ensures that starches obtained are actually edible and, consequently, it is considered safe by the FDA [4].

The aim of this research was to study the influence of the modification by cross linking technique of isolated from dark-cush-cush yam (*Dioscorea trifida*) and cassava starches, in their physicochemical properties.

2. Experimental

2.1. Materials

Isolated and purified native starch, obtained from dark-cush-cush yams (*Dioscorea trifida*), grown in Venezuela, and cassava starch donated by Indelma industry (Cagua Venezuela) of Brazilian origin, were used in this research.

2.2. Starch Extraction

Starch was isolated and purified following the procedure described in [5]. Two batches of native starch were isolated from D. trifida tubers grown in Venezuelan farms. The cleaned tubers were peeled and their edible portions were sliced. Approximately 0.5 kg of the edible portion was mixed for 2 min in a waring blender with twice their volume of distilled water. This procedure was repeated six times to process approximately 3 Kg. The collected homogenate was passed through a 200-mesh sieve. The grinding and screening operations were repeated four more times. The resulting slurry was centrifuged at 1500 r/min for 15 min, at 28°C, to facilitate separation of the starch from the viscous mucilage. After carefully removing the remaining mucilaginous layer, the sediment was washed several times by suspending in distilled water and centrifuging until it appeared free of non-starch material. The sediment was then dried in a ventilated oven at 45°C. Starches were blended, passed through a 60-mesh sieve, and stored at room temperature in sealed plastic bags inside hermetic glass containers until subsequent analysis.

2.3. Starch Modification

Each starch was modified by the method described in [6] with modifications. Starch with dark-cush-cush (150.3 g) was mixed with distilled water (212 mL) at constant stirring and, then, sodium hydroxide (NaOH) to 2.5% and pH 10.5 was added. Afterwards, 7.5 g of sodium sulfate (Na_2SO_4) was added maintaining constant stirring. pH was adjusted again to 10.5 with NaOH to 2.5% and heated in a water bath at 45°C with constant agitation. Once this temperature was reached, 4.5 g of sodium trimetaphosphate (STMP) was added. The mixture was stirred for three hours. Then, it was neutralized to pH 7 with hydrochloric acid (HCl) at 2.5%. The starch obtained was centrifuged (Damon CRU-500 centrifuge, USA) at 1000 rpm and washed three times with distilled water. Subsequently, the obtained starch was placed in aluminum pans and dried in a tray dehydrator mark Mitchell, Model 645159 for 24 h. After that time, the modified starch was ground with a food processor form the brand Oster, and sieved through a 60 mesh screen. Finally, the starch was placed into clean containers and stored at room temperature.

For cassava starch, 300 g of starch was dissolved in 375 mL of distilled water and 15 g of Na_2SO_4; then, 9 g STMP was added and the procedure described above was followed.

2.4. Chemical Composition and Physicochemical Properties of the Starches

Chemical composition of native and modified starches were evaluated: moisture and ash by gravimetric method [7], crude protein (N x 6.25) by micro-Kjeldahl [7], fatty material by acid hydrolysis [8], apparent density, pH, titratable acidity (expressed as meq g-1), amylose content, using colorimetric micro-procedure that is based on the formation of the complex amylose and iodine [9], and purity. This last parameter was calculated as the sum of moisture content, crude protein, fatty materials and ash.

The phosphorous and the degree of substitution (DS) of the native and modified starches were determined by the colorimetric method [9] using the equations proposed in [10].

Color and white index (WI) of all the starches were obtained by a Colorimeter Macbeth model Color-eye 2445 with CIE LAB scale [11], and the equation expresed in [12].

The shape and the starch granule sizes have been determinated by an optical microscope equipped with Olympus BX60M and a video camera using an imaging system RS IMAGE Olympus. The magnification used was 50X. The granule size was calculated as the average granule diameter that was estimated by measuring at least 10 randomly selected granules from microphotographs of each.

The stability and clarity of starch pastes were determined at 25°C and 4°C following the methodology described in [13]. The starch pastes were prepared by suspending 0.2 g of the polymer in 5 mL of water in screw cap tubes and subjected to boiling water-bath at 98°C for 30 min. The tubes were shaken regularly every 5 min. During the heating time, the tubes were allowed to cool at room temperature for 15 minutes. The percentage (%) of transmittance at 650 nm was determined in spectrophotometer (Thermo Scientific Make, Model Genesis 10 S) using distilled water as a blank. Three samples were stored at both ambient temperature (25°C) and at 4°C, and the percent transmittance was measured at 24, 48 and 72 h at the same wavelength.

The rheological characteristics of dark-cush-cush yam and cassava, cross linked and native, pasta starches, were determined through the interpretation of data obtained from a Rapid-Visco-Analyser (RVA), Brabender R brand, model Micro Visco-Amylo-Graph, Duisbur's, Germany, executed under the program Viscograph Version 2.4.9. A suspension of 100 mL of 7% starch solids (dry basis to 14% moisture) was prepared, and was gradually heated in the team from 30°C to 90°C at a constant heating rate (6°C/min), from 90°C to 50°C. Finally, it was cooled using the same rat for cooling (6°C/min). Start of gelatinization: (A) maximum viscosity (B), stability "Breakdown" (BD), settling "Setback" (EB) and consistency (ED), were obtained.

The consistency of the gel was determined by the method described in [14].

2.5. Statistical Analysis

Analysis of variance at the significant level of 5% (0.05) was performed using the Statgraphics Program (Statically Graphics Educational, version 6.0 1992. Manugistics, Inc. and Statistical Graphics Corp., USA). When statistical differences were found, the Duncan's multiple range tests were applied at the significant level of 5% (0.05) to classify the samples.

3. Results and Discussion

The moisture content of both native and cross-linked cassava and dark-cush-cush yam starches was reported in Table 1. As can be seen, the humidity values of cassava were lower than those of dark-cush-cush yam. Similar results were reported for these types of starches in [5] and [15]. The found values of humidity, fall in the reported range for a stable shelf life [16].

Table 1. Chemical characterization on dry basis of native and modified starches from dark-cush-cush yam (*Dioscorea trifida*) and cassava (*Manihot esculenta*)

Parameter	Cush-cush yam native (%)	Cush-cush yam modified (%)	Cassava native (%)	Cassava modified (%)
Moisture	15.0 ± 0.2	7.9 ± 0.2	9.1 ± 0.5	7.1 ± 0.4^{d}
Crude protein	0.38 ± 0.03^{a}	0.35 ± 0.02^{a}	0.26 ± 0.01	0.12 ± 0.02
Crude fat	0.070 ± 0.006^{a}	0.09 ± 0.04^{a,b}	0.12 ± 0.04^{b}	0.3 ± 0.2^{b}
Ash	0.2 ± 0.1^{a}	4.2 ± 0.1	0.10 ± 0.02^{a}	0.92 ± 0.01
Purity	99.35 ± 0.05	95.35 ± 0.05	99.68 ± 0.02	98.66 ± 0.08
Apparent amylose	12 ± 3^{a}	11 ± 1^{a}	21 ± 3^{b}	22 ± 3^{b}
Phosphorous	0.006 ± 0.002	0.17 ± 0.08	0.014 ± 0.002	0.07 ± 0.01
DS	0.0006 ± 0.0002	0.017 ± 0.009	0.0015 ± 0.002	0.008 ± 0.001

Similar letters in the same row indicates no statistically significant difference ($p \leq 0.05$)

The crude protein content was low in all of the starches investigated, varying from 0.12 to 0.38% (Table 1). Taking into account those results, it can be assumed that the purification process was efficient. The cross-linked starches presented a reduction of the crude protein with respect to the native as a result of the modification. In the case of the dark-cush-cush yam, this effect was less noticeable. In cassava starch the reduction was around 50%.

The crude fat content of all the starches is also shown in Table 1. It is noteworthy that the native dark-cush-cush yam sample exhibited a value 50% lower than that of cassava. Despite that the fat content of the tapioca starch was higher, compared to the content of the dark-cush-cush yam starch, it is still lower as compared with other commercial starches. Although minor amounts of fat material could influence the gelatinization, the most remarkable effect is on the flavor profile of the starches.

As expected the ash, phosphorous content, and degree of substitution (DS) increased with modification, however as can be seen in Table 1, the increment was sharper in the dark-cush-cush yam starch compared with cassava.

The purity of the starches was very high for all of the materials, varying from 95.35 to 99.68%, corroborating the efficiency of the purification process.

The values of DS have been in the range permitted by FDA [4].

The apparent amylose content in dark-cush-cush yam starches were nearly 50% less than those of cassava. This fact may explain the differences in the other minor constituent of the starches. However, the structural properties of starch from dark-cush-cush yam were substantially modified, due to the higher DS presented. Finally, the modifications of the starches did not affect the amylose content in neither cases.

Table 2. Results of the physicochemical characterization of native and modified starches from dark-cush-cush yam (*Dioscorea trifida*) and cassava (*Manihot esculenta*)

Parameters	Cush-cush yam native	Cush-cush yam modified	Cassava native	Cassava modified
pH	6.63 ± 0.07^{a}	6.36 ± 0.01	6.8 ± 0.1^{a}	7.13 ± s0.02
Titratable acidity (meqNaOH/100g)	0.04 ± 0.01^{a}	0.09 ± 0.05^{a}	0.17 ± 0.04^{b}	0.13 ± 0.01^{b}
Luminosity (L)	96.8 ± 0.1^{a}	95.4 ± 0.1^{b}	99.3 ± 0.1^{c}	99.3 ± 0.1^{d}
Hue (a)	1.4 ± 0.01	1.5 ± 0.01	- 0.4 ± 0.1	- 0.1 ± 0.1
Chroma (b)	3.9 ± 0.1	4.8 ± 0.1	0.8 ± 0.1	0.1 ± 0.1
Total Color Difference (ΔE)	4.6 ± 0.1	4.4 ± 0.1	6.3 ± 0.1	6.0 ± 0.1
White Index (WI)	94.8 ± 0.01	93.2 ± 0.01	99.1 ± 0.01	99.3 ± 0.01
Apparent Density (g/ml)	0.98 ± 0.01	0.99 ± 0.01^{a,b}	1.01 ± 0.01^{b}	1.00 ± 0.01^{b}
Granular size (μm)	32.0 ± 6.0	31.0 ± 8.0	11.4 ± 0.5	12.1 ± 1.3

Similar letters in the same row indicates no statistically significant difference ($p \leq 0.05$)

Table 2 summarizes the physical and physicochemical properties of the starches. The values of pH and titrable acidity were in the range reported for commercial starches [2]. In regard to the color parameters, cassava (native and modified) have been whiter. They presented less pigment content than dark-cush-cush yam starches, due to the high WI and low *a**, and *b**. The high values of WI are indicative of white pure starches.

Table 2 shows the granular size of the starches, obtained by optical light microscopy. The granules of Cassava starch resulted three times smaller than those of the dark-cush-cush yam, with a mean diameter of 11.4 μm (Figure 1). The modification process slightly changed the size of cassava starch granules, but did not alter those of the cush-cush yam starch.

Finally, similars values of apparent density have been found varing from 0.98 to 1.01 g/ml.

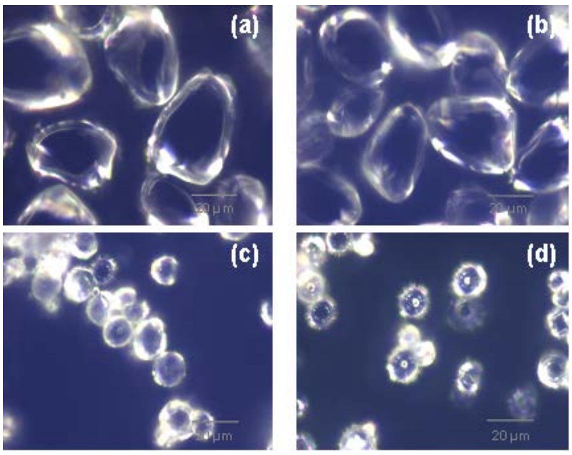

Figure 1. Optical light microscopy at 50 X of the granules of (a) dark-cush-cush yam native, (b) dark-cush-cush yam modified, (c) cassava native and (d) cassava modified starches

In Table 3 the pasta clarity (% Transmittance) of all the starches investigated, stored at 25 and 4°C is observed. As can be seen, there were significant differences in the pasta clarity, related to the botanical sources, modification treatment and storage temperatures. In the case of the dark-cush-cush yam, this parameter has been reduced by the effect of the temperature, but not affected by the modification process, in contrast to cassava starches. This behavior could also be attributed to the differences in the structure provided by the different concentration of amylose presented in each starch.

Table 3. Pasta clarity (% Transmittance) of native and modified starches, dark-cush-cush yam (*Dioscorea trifida*) and cassava (*Manihot esculenta*), stored at 25 and 4°C

Starch	Storage time (hs)			
	0	24	48	72
Cassava native at 25°C.	24.0 ± 9.0[a]	36.0 ± 12.0[a]	33.0 ± 9.0[a]	31.0 ± 7.0[a]
Cassava native at 4°C.	24.0 ± 9.0[a]	37.0 ± 3.0[a]	36.0 ± 3.0[a]	35.0 ± 3.0[a]
Cush-cush yam native at 25°C.	5.0 ± 2.0[b]	3.0 ± 2.0[b]	3.0 ± 1.0[b]	2.0 ± 1.0[b]
Cush-cush yam native at 4°C.	5.0 ± 2.0[b]	1.6 ± 0.7[b,c]	1.2 ± 0.5[c]	0.9 ± 0.3[b]
Cassava modified at 25°C.	3.4 ± 0.9[b]	2.6 ± 0,8[b,c]	3.1 ± 0,6[b]	5.0 ± 2.0[c]
Cassava modified at 4°C.	3.4 ± 0.9[b]	2.5 ± 0.7[b,c]	2.4 ± 0.8[b,c]	2.3 ± 0.8[b,c]
Cush-cush yam modified at 25°C.	7.8 ± 0.2[c]	4.4 ± 0.2[b]	3.5 ± 0.2[b]	2.9 ± 0.1[b,c]
Cush-cush yam modified at 4°C.	7.8 ± 0.2[c]	1.7 ± 0.1[b,c]	1.23 ± 0.06[c]	0.98 ± 0.04[b]

The values are the average of three determinations
Similar letters in the same column indicates no statistically significant difference ($p \leq 0.05$)

The modification of starches increased the noticeable peak of viscosity, the breakdown, consistency and setback of cush-cush yam. Similar effects on cassava starch were observed in the last two last parameters (Table 4). However, the structural properties of starch from dark-cush-cush yam were substantially modified due to the higher DS presented. As was note above, DS affects the starch properties. Crosslinking controls granular swelling and produces a starch that can tolerate high temperature, high shear, and acidic conditions. It can be observed in Table 4, that modification changes dramatically the gelatinization profile of the dark-cush-cush yam, and it starch has higher viscosity and low granular fragility (low breakdown) than cassava.

Table 4. Rheological (UB) and initial gelatinization temperature (°C) of 7 % solution of native and modified starches from dark-cush-cush yam (*Dioscorea trifida*) and cassava (*Manihot esculenta*)

Parameter	Dark-cush-cush yam native	Dark-cush-cush yam modified	Cassava native	Cassava modified
Initial gelatinization Temperature (°C)	74.7±0.1	78.8±0.9	68.9±0.1[a]	70.0±1.0[a]
Maximum viscosity (B) (UB)	74± 69	658±11	474±6[a]	467±4[a]
D	75±69	652±15	238±6	442±14
E	104±142	740±23	414±23	644±6
Breakdown (UB)	0±1[a]	4±3	235±1	28±11
Setback (UB)	30±73[a]	83±12[a]	- 60±16	178±2
Consistency (UB)	27±69[a]	86±8[a]	173±15	203±9

The values are the average of two determinations; similar letters in the same raw indicates no statistically significant difference ($n = 2$, $p \leq 0.05$).
where:
D = hot paste viscosity at the end of the plateau, at 90°C
E = cold paste viscosity at 50°C, the end of the cooling period
BD = maximum viscosity (B) - the hot paste viscosity at the end of the plateau at 90°C (D)
SB = cold paste viscosity at 50°C, the end of the cooling period (E) - maximum viscosity (B)
CS = the cold paste viscosity at 50°C (E) - hot paste viscosity at the end of the plateau at 90°C (D)

Finally, the consistency of all the gels was measured at a quite low concentration (100 mg/100mL = 0.1%). For cush-cush yam starchs, native and modified, were 50 ± 14 mm and 15.7 ± 0.6 mm, respectively; and for cassava, 112 ± 11 mm (native) and 51 ± 31 mm (fosfatized). An important decrease was evident in both, native and modified, cush-cush yam starches respected to cassava. Also, decrements of the concistency value due the modification were observed of both starches; contrary to the results of this parameter at 7% (Table 4).

4. Conclusions

The starches isolated and purified at the laboratory showed high purity and whiteness. The differences in the amylose content that was observed between both starches were reflected in the differences in their composition, structure and functional properties. The degree of substitution (DS) was more significant in the starch cush-cush yam than those shown by the cassava starch and both were between the ranges allowed by the FDA. There were significant differences in the brightness of the pulp, relative to the botanical sources, modification treatment, and storage temperature. Noteworthy differences in the paste clarity in regard to botanical source, modification treatment and storage temperature, were found. The granular size of the cassava starch was slightly changed by the cross linked, but this process did not alter that of the starch cush-cush yam. Moreover, modification treatment changed some of the starch functional properties. It increased the peak viscosity, breakdown, consistency and setback in the starches. Finally, the modification of starches conferred great stability at the gelatinization of the starches.

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