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Thermorheological characteristics and extrudability aptitude of a new amylose-free cassava starch

Características termoreologicas e aptidão de extrudabilidade de um novo amido de mandioca sem amilose

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ABSTRACT

Cassava crops have always been fundamental in human nutrition and industry. Nowadays, the development of new cultivars with specific properties has become a major research area. In this research, amylose-free cassava starch (WXCS) extracted from clone AM206-5 was evaluated with respect to its physicochemical, morphological, and thermorheological properties. The waxy nature of cassava starch was verified (0.54 ± 0.09% w/w amylose), showing a 16.92±0.20 µm average granule size and elliptical or spherical truncated shapes without granule aggregation. There were significant differences in the pasting profiles evaluated, with WXCS being thermally less stable (Breakdown = 698±2 cP) generating less viscous final pastes (731±16 cP) compared to a commercial amylose-free corn starch. The WXCS shear viscosity was determined in a capillary rheometer (Rheoplast®), showing an inverse linear temperature dependence, decreasing by a factor larger than 3 when the temperature changed from 100 to 120 °C, with a pseudoplastic flow described by the power law (n: 0.25–0.40), consistency index (32607 – 6695 Pa.s) and specific mechanical energy (124 - 75 J/g). The extensional viscosity was always higher than the shear viscosity, where increasing the strain rate and temperature enlarged the Trouton number (25–145). Complete WXCS transformation under real process conditions was achieved with a 30% w/w moisture content and 100 °C, which induced full granular integrity loss and crystalline structure destruction. The results confirmed a potential utilization for this new starch to obtain extruded-type food products or to serve as a biothickening agent.

Index terms: Amylopectin; extensional viscosity; extrusion; AM206-5; *Manihot esculenta*.

RESUMO

A cultura da mandioca sempre foi fundamental na alimentação humana e na indústria. Atualmente, o desenvolvimento de novas cultivares com propriedades específicas tornou-se uma grande área de pesquisa. Nesta investigação, o amido de mandioca sem amilose (WXCS) extraído do clone AM206-5 foi avaliado com respeito às suas propriedades físico-químicas, morfológicas e termorreológicas. Foi verificada a natureza cerosa do amido de mandioca (0.54 ± 0.09% p/p de amilose), mostrando um tamanho médio do grânulo de 16.92±0.20 µm e formas elípticas ou truncadas esféricas sem agregação de grânulos. Houve diferenças significativas nos perfis de colagem avaliados, sendo o WXCS termicamente menos estável (decomposição = 698±2 cP) gerando pastas finais menos viscosas (731±16 cP) em comparação com um amido de milho comercial sem amilose. A viscosidade de cisalhamento do WXCS foi determinada num reômetro capilar (Rheoplast®), mostrando uma dependência linear inversa da temperatura, diminuindo por um factor superior a 3 quando a temperatura mudou de 100 para 120 °C, com um fluxo pseudoplástico descrito pela lei de potência (n: 0.25-0.40), índice de consistência (32607 - 6695 K - Pa.s) e energia mecânica específica (124 - 75 J/g). A viscosidade de extensão foi sempre superior à viscosidade de cisalhamento, onde o aumento da taxa de deformação e da temperatura aumentou o número de Troutões (25-145). A transformação completa do WXCS sob condições reais de processo foi alcançada com um teor de humidade de 30% p/p e 100 °C, o que induziu a perda total da integridade granular e a destruição da estrutura cristalina. Os resultados confirmaram o uso potencial deste novo amido para a obtenção de produtos alimentares do tipo extrudido ou para servir como um agente bioespessante.

Termos para indexação: Amilopectina; viscosidade extensional; extrusão; AM206-5; *Manihot esculenta*.

INTRODUCTION

Cassava (Manihot esculenta Crantz) is considered a very important crop in tropical and subtropical countries as a starch source (Da Costa Nunes et al., 2021), as it is adapted to soils with low nutrient availability and drought tolerance (Tagliapietra; Zanon; Fernandes, 2021); in addition, it has become the third leading calorie source after rice and corn (excluding sugarcane) for vulnerable populations in Africa, Asia, and South America, and its use in the food and nonfood industries is growing (Versino; Urriza; García, 2019). It is increasingly suited to modern agriculture and competes with cereals such as rice (Jeong; Lee; Chung, 2021) and wheat (Tao et al., 2021), which have been further investigated seeking to improve its production and processing. Cassava, according to the Food and Agriculture Organization (FAO), is also the least expensive starch source and is actually being used in more than 300 industrial products. In 2018, cassava global production was approximately 300 million tons (fresh roots), and from these, it is estimated that 60.9% is produced in Africa (178 million tons), 29.4% in Asia (86 million tons), and only 9.8% in the Americas (28 million tons) (Food and Agriculture Organization - FAO, 2019).

Previous research findings on cassava root have reported an approximate composition as follows: moisture (70%), starch (24%), fiber (2%), protein (1%), minerals and other substances (3%), whereas starch (total dry weight) represents between 86 and 88% (Ceballos et al., 2007). The above data show that cassava starch extraction is a profitable option to increase the added value if field yields grow up and the starch extraction process improves. The International Center for Tropical Agriculture (CIAT) maintains an *in vitro* germplasm bank (Palmira - Colombia) with more than 6000 traditional, enhanced cassava varieties and *Manihot* genus wild relatives originating from Latin America, Asia, and Africa. This research institution seeks to develop competitive advantages on the cassava production chain. Inside this breeding program, some root genotypes have been identified with novel starch characteristics, given their high potential impact on the food industry (Sánchez et al., 2009).

Native cassava starch generally presents amylose contents between 13.6 and 25 (% w/w), reduced protein and lipid amounts, and low retrogradation rates, which make it quite different from cereal starches, increasing its potential applications (Chel-Guerrero et al., 2011; Oliveira et al., 2021). Currently, it competes with amylose-free corn starch, distinguished by its gel-forming ability and mild flavor (Breuninger; Piyachomkwan; Sriroth, 2009). Genetic modification applied to cassava varieties TMS60444 (Africa) and Adira 4 (Indonesia), through enzyme (GBSSI) inhibition, is responsible for amylose synthesis (Zhao et al., 2011). These cassava genotypes produce starches with a high amylopectin content, greater clarity and higher paste stability, characteristics desirable to produce sauces, soups, dairy products, textiles, and paper, avoiding environmentally unfriendly chemical starch modifications (Breuninger; Piyachomkwan; Sriroth, 2009).

The waxy cassava clwone named AM206-5 (CIAT), a natural product or spontaneous mutation developed by many self-pollinations, is an interesting scientific advance compared with other transgenic varieties. This waxy starch has almost 0% (w/w) amylose, with a more organized structure, higher crystallinity (40%), viscosity, instability, and swelling index values (Ceballos et al., 2007; Rolland-Sabaté et al., 2013), and features lower solubility than traditional cassava starches containing amylose. It has also been found to have thermomechanical properties (melting point, glass transition, mechanical relaxation temperature) similar to waxy corn starch (Pulido Díaz et al., 2017).

Starch processing through extrusion is one common technology in the food industry due to its scalability, high throughput, efficiency, and flexibility to produce many food types (Sun et al., 2021). Extruded food expansion and texture formation are complex processes even for products based on a single component (Starch); these starch phenomena are dependent on the viscoelastic characteristics, dough formulation, nucleation, bubble growth mechanism, and water plasticizing properties (Carvalho et al., 2010), all of which are relevant in later stages as a melt-to-viscoelastic transition and subsequently to a glassy state. The molten starch rheology results from transformations undergone by extruded material due to thermal and mechanical energy input. Therefore, it is important to describe the physicochemical transformations that occur in extrusion, with emphasis on the effects that these changes have on starch properties (Contreras-Gallegos et al., 2015). Equipment such as Rheoplast® has been designed to study the changes mentioned above, simulate processes and obtain essential (rheological) properties (Núñez; Della Valle; Sandoval, 2010), particularly for high-pressure and high-speed procedures such as food extrusion. These capillary rheometers guarantee real settings that are accurately representative of processing circumstances. As a result, they are essential for process optimization.

In the present research, a physicochemical, morphological, and thermorheological characterization of a new amylose-free cassava starch extracted from Clone AM206-5 (WXCS) was carried out. Corn starch is the most used industrially to manufacture extruded food products; for this reason, commercial amylose-free corn starch (WXMS)

was used as a physicochemical and morphological reference, because waxy cassava starch properties have been studied in detail for specific industrial purposes, being an alternative to waxy corn starch in periods with high prices or limited availability. It is expected that information generated in relation to this new raw material (WXCS) will be a starting point for future works about industrial transformation in food processes such as extrusion, packaging, and films, exploring its functional properties and potential applications.

MATERIAL AND METHODS

Starches

The study material was extracted from cassava roots *(Manihot esculenta* Crantz) AM206-5 (WXCS) Clone, harvested after a vegetative period of 17 months, and cultivated in an experimental plot in the International Center for Tropical Agriculture (CIAT) (Palmira - Colombia, annual rainfall 1021 mm, 1000 masl, average annual temperature 26 °C). Starch extraction followed these stages: washing, peeling, multistage centrifugation, sedimentation, drying, milling, sieving (mesh # 150), and sampling by quartering (Tran et al., 2015). Amylose-free corn starch (WXMS) was donated by Ingredion® Industries (Cali, Colombia).

Moisture content

Samples (2 ± 0.1 g) were weighed in aluminum crucibles and dried in a convection oven (UF30, Memmert, Germany) at 130 ± 0.5 °C for 3 hours. Subsequently, the crucibles containing the dried samples were covered and placed in a desiccator for 1 hour. Measurements were conducted in triplicate (wet basis).

Proximate composition

Ash content was determined following the gravimetric method (AOAC 942.05) by burning the samples in a furnace (FB1315 M, Thermo Scientific, USA) at 550 ± 1 °C for 3 hours. The residue was cooled in a desiccator for subsequent residual weight recording. Crude fiber was determined as the organic residue after starch digestion with H_2SO_4 (1.25%v/v) and NaOH (1.25% v/v) according to AOAC method 962.09. Protein was determined by the Kjeldahl method (AOAC 988.05), and digestion was performed with sulfuric acid (H_2SO_4), which converts nitrogen (N_2) into ammonia. This ammonia was determined by alkaline distillation and titration. The recorded value was multiplied by a factor of 6.25 (100/16), assuming that protein has a 16% nitrogen content. Ethereal extract was quantified in dried and homogenized starch subjected to extraction with petroleum ether (AOAC 960.39).

Amylose content

The amylose content was determined by differential scanning calorimetry (Creek et al., 2007). The starch (11± 0.01 mg) was weighed accurately in a pressure inox pan (70 µL), 50 µL of a 2% L-α-lysophosphatidylcholine solution was added, and the pan was hermetically sealed and stored for an hour. The sample pan was stabilized at 35 °C; in the reference cell, an empty inox pan was used. The thermal cycle was heating from 35 °C to 160 °C (15 °C/min), held at 160 °C for 2 min and then cooled to 60 °C (5 °C/min). Complex formation is an exothermic process that was measured between 65 and 91 °C. Finally, a calibration curve was performed with pure amylose (10120, Sigma Aldrich). All measurements were carried out in a Perkin Elmer Pyris 6 with nitrogen as the purge gas (20 ml.min-1).

Particle size

Starches particle size was determined by laser diffraction using a Mastersizer 2000 (Malvern Instruments, UK) operating at 1 minute ultrasound with 10 microns displacement. Each starch sample was suspended in 1.0 ml of water; the standard refractive indices used were 1.33 and 1.53 for water and starch, respectively. Starch granule volumes were calculated on the assumption that they were all spherical in shape.

Scanning electron microscopy (SEM)

Starches samples (10 ± 0.1 g) were subjected to drying (6 h at 40 ± 1 °C) in a vacuum oven (Isotem 282A, Fisherbrand, USA) and then cooled to room temperature in a desiccator; starch granules were coated with a gold layer using a metallizer (DESK IV, Denton Vacuum, USA), and microphotographs were obtained in a scanning electron microscope (JSM-6490, JEOL, Japan) with 20 KV as the accelerating voltage.

Pasting properties

Viscosity profiles were determined on a Rapid Viscosity Analyzer (RVA 4, Newport Scientific, USA). A 5% (w/v) starch-in-water suspension was prepared and subjected to the following cycles: 1) heating at 50 °C for 1 min, 2) increasing to 90 °C at a heating rate of 6 °C.min-1, 3) holding at 90 °C for 5 min, and 4) cooling to 50 °C (6 C.min-1). During the whole process, the suspension was stirred at 160 rpm (Ceballos et al., 2007). The following characteristics were obtained: 1) pasting temperature (PT), peak viscosity (PV), hot paste viscosity at 90 °C at the end of cycle 3 (HPV), and final viscosity (FV). Then, these parameters were calculated: Gel Instability Index or

Breakdown (BD = PV - HPV) and Gel Stability Index or Setback (SB = FV - PV). Extrudates obtained (capillary rheometer) were ground (6775 Freezer Mill, Spex, USA) for 5 min in liquid nitrogen. The powders were stored for 8 days at 20 ± 0.2 °C in a desiccator (NaBr) and analyzed for the gelatinization degree in excess water and the gelatinization profile.

Thermomechanical characterization

The starch (WXCS) was conditioned to 20% and 30% w/w (wet basis) moisture, for which it was placed in airtight glass bottles, and water was added dropwise with continuous stirring. The glass bottle was placed upside down and stored for at least 24 hours (2 ± 0.9 °C) to achieve uniform hydration. Hydrated samples were processed in a capillary rheometer with preshearing (Rheoplast®) that can simulate the extrusion treatment prior to viscosity measurement. The shear condition (100 rpm), temperature (100, 120 °C) and residence time (10 s) were constant for all moisture levels considered. The capillaries used were L/D 16, L/D 8, and L/D 0 (L/D: length/diameter ratio). The apparent shear velocity (γ_a) was calculated with Equation 1:

$$\dot{\gamma}_a = \frac{32Q_v}{\pi D^3} \quad (1)$$

where Q is the volumetric flow rate ($m^3 s^{-1}$) and D: capillary diameter (m). The shear stress (τ_w) was determined with Equation 2:

$$\tau_w = \frac{\Delta P}{4\frac{L}{D}} \quad (2)$$

The pressure gradient (ΔP/L) was calculated assuming a linear pressure profile. Bagley and Weissenberg-Rabinowitsch corrections (Equation 3) were performed to account for the entrance pressure effects on the shear velocity (γ_r):

$$\dot{\gamma}_r = \frac{3m+1}{4m} \quad (3)$$

Where, the slope (m) is Equation 4:

$$m = \frac{d\left(log\tau_w\right)}{d\left(log\dot{\gamma}_a\right)} \quad (4)$$

Finally, shear viscosity (η) was calculated from Equation 5:

$$\eta = \frac{\tau_w}{\dot{\gamma}_a} \quad (5)$$

Power-law parameters were determined using pressure gradient and flow velocity data. The extensional viscosity (η_e) and strain rate (ε) were calculated using the Equation 6 and Equation 7 presented below:

$$\eta_e = \frac{9\left(n+1\right)^2\left(\Delta P_{ln}\right)^2}{32\eta_a\dot{\gamma}_a{}^2} \quad (6)$$

$$\varepsilon = \frac{4\tau_w}{3\left(n+1\right)\Delta P_{ln}\dot{\gamma}_a} \quad (7)$$

where *n:* power law index, η_a: apparent viscosity, γ_a: shear rate, and ΔP_{ln} was determined from pressure plots with Bagley correction. The Specific Mechanical Energy (SME) is defined as the total mechanical energy input to the system per unit weight (extrudate), and the Equation 8 that allows its calculation is:

$$SME = \frac{2\pi V_\tau}{F_m} \quad (8)$$

where V : screw speed (rpm), τ: torque (N.m) and F_m: mass flow rate (g min^{-1}) (Berzin et al., 2010). The different material responses to stress or strain can be quantitatively characterized with the Trouton number (T_R), defined as the ratio of extensional viscosity (η_e) to shear viscosity (η), determined at equivalent strain ($\dot{\varepsilon}$) and shear ($\dot{\gamma}$) rates, was calculated from Equation 9.

$$T_R = \frac{\eta_e\left(\varepsilon\right)}{\eta\left(\dot{\gamma}_w = \dot{\varepsilon}\right)} \quad (9)$$

Gelatinization

WXCS powders were weighed (12 ± 1 mg) in stainless steel microcapsules, distilled water was added in a 1:3 ratio (w/v), and the capsule was hermetically sealed and incubated for 1 hour at room temperature to reach starch-water system equilibrium. Stabilized samples were analyzed in a differential scanning calorimeter (Q100, TA Instrument, USA) where a thermal sweep was made between 15 - 120 °C at 10 °C.min[1], assisted with N_2

flow (20 ml.min^{-1}). All measurements were performed in duplicate. Previously, the respective equipment verification was performed with Indium (In) (156.4 °C < To <156.8 °C, 28.2 J/g <ΔH <28.7 J/g).

Statistical analysis

All generated data were statistically analyzed using Statgraphics Centurion® 19 (StatPoint Technologies, USA) by analysis of variance and means comparison (LSD) test ($p < 0.05$) to verify if there were significant differences (proximate composition, pasting properties) between WXCS and WXMS. Regression analyses were also performed to identify the mathematical model that best described the WXCS molten flow.

RESULTS AND DISCUSSION

Physicochemical characteristics

The proximate analysis for amylose-free cassava (WXCS) and corn (WXMS) starches is presented in Table 1. There was a significant difference between the two starches. Further statistical tests revealed that WXCS presented higher dry matter, ash, protein, lipid, and amylose contents. The fact that WXCS presented higher ash, protein, and lipid contents was probably due to the semi-industrial extraction process used to obtain it, which was less standardized and did not achieve an optimal refinement level and could also be due to the specific cultivar used.

The amylose content was less than 0.6%, confirming the purity and waxy nature in both starches. This is an important quality control parameter, which allows the identification of possible adulterations (starch mixtures), remembering that the starch functional characteristics are determined by the whole granule properties and not only those of its individual components (amylose and amylopectin). Consistent with the literature, all parameters analyzed were in a normal range for this type of starch (Hoover, 2001); interestingly, from these experimental data, it is possible to observe that minor changes in proximate composition allow evidence of the origin differences between WXCS and WXMS.

Table 1: Proximate composition (% w/w) for cassava (WXCS) and corn (WXMS) amylose-free native starches.

Parameter	WXCS [a]	WXMS [a]	p value
Dry matter	89.20 (0.19)[b]	88.16 (0.03)[b]	0.0007
Ashes	0.26 (0.01)	0.09 (0.01)	0.0001
Protein	0.44 (0.01)	0.21 (0.04)	0.0006
Ethereal extract	0.96 (0.02)	0.79 (0.01)	0.0002
Crude fiber	0.14 (0.02)	0.09 (0.01)	0.0179
Amylose	0.54 (0.09)	0.35(0.07)	0.0034

[a]Wet Base [b]Standard deviation (SD) n = 3 replicates, means comparison (LSD) test at the 5% significance level ($p < 0.05$).

Turning now to the mean starch granule size (Figure 1), WXCS was larger (16.9 ± 0.2 µm) than WXMS (13.73 ± 0.02 µm) by approximately 23%; closer inspection shows than WXCS granule size full range from 5.01 to 39.81 µm, similar to other cassava starches obtained by genetic modification (Zhao et al., 2011), on traditional cassava starches, granule sizes between 12.9 - 17.2 µm are normal (Breuninger; Piyachomkwan; Sriroth, 2009), i.e., although WXCS is an amylose-free starch, granule size is preserved. WXMS presented an average granule diameter within the expected range (2–30 µm) for corn starches (Charles et al., 2005).

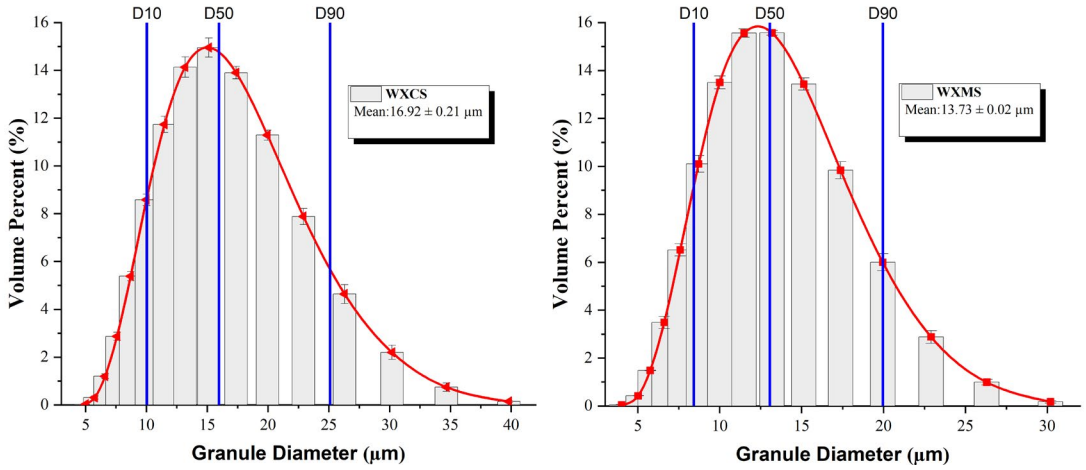

Figure 1: Granule size (µm) distribution profiles for amylose-free cassava (WXCS) and corn (WXMS) starches.

Table 2 provides the summary statistics for granule size distribution, what stands out in the presented data is that WXCS highest percentage (>66%) was between 10 and 20 µm, and the WXMS was close to 60% for the same interval. When the size distribution was analyzed by percentile, 90% of WXCS granules had a diameter smaller than 25.10 ± 0.49 µm. For all size distribution limits (percentile), WXMS granules were always smaller than WXCS granules, and these physical differences were confirmed by scanning electron microscopy.

Waxy cassava starch granules (Figure 2) presented elliptical, kettledrum or spherical truncated shapes, and some damaged granule particles were observed, probably as a result of damage by the mechanical extraction process. No aggregation was observed, and granules were mostly isolated from each other. Amylose-free corn starch granules (WXMS) showed spherical irregular shapes with smoothed edges with a tendency to form clusters, perhaps due to electromagnetic interactions or granule roughness; in addition, it was possible to observe some pores over granule walls. These pores can be found in cereal starches, and it has been proposed that starches with A-type crystallinity patterns (such as WXMS and WXCS) have porous internal structures, while B and C crystallinity types exhibit greater uniformity (Huber; BeMiller, 2000).

Pasting properties

Table 3 illustrates some of the main rheological characteristics studied, where all results were significant at the $p = 0.05$ level. There are several important differences between WXCS and WXMS related to pasting behavior, probably due to their biological origin, granule size, and/or amylopectin branched-chain distribution. Another possible explanation is that waxy cassava amylopectin has a slightly higher molar mass and branching degree than amylopectin from regular corn starch, which also contributes to the differences found (Morante et al., 2016). Additionally, the rheological behavior of the pastes can be attributed to the multiple polymerization degrees (DP) in amylopectin chains, with DP 6–9, DP 6–11, DP 12–24, and DP 25–36 common on corn and cassava waxy starches (Hsieh et al., 2019). These molecular chains in WXCS and WXMS expand when it becomes hot, allowing them to collide with others and form a network that thickens the fluid (gelatinization).

Table 2: Granule size distribution parameters for amylose-free cassava (WXCS) and corn (WXMS) starches.

Starch	Percentile (%)			Mean diameter
	10	50	90	
WXCS (µm)	10.04 ± 0.09	15.96 ± 0.17	25.10 ± 0.49	16.92 ± 0.21
WXMS (µm)	8.42 ± 0.01	13.06 ± 0.02	19.97 ± 0.03	13.73 ± 0.02
	Intervals (µm)			
	0 - 10	10 - 20	20 - 30	30 - 40
WXCS (%)	18.37	66.02	12.52	3.09
WXMS (%)	35.56	60.40	3.87	0.17

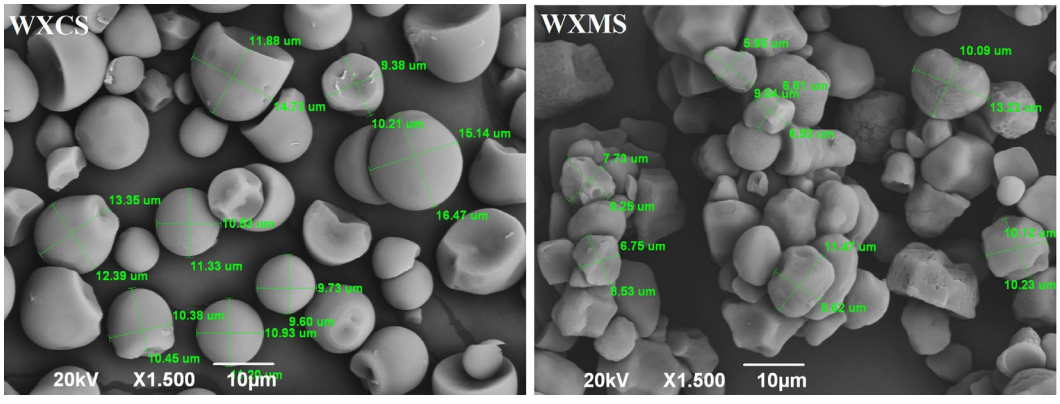

Figure 2: Granule morphology comparison for amylose-free cassava (WXCS) and corn (WXMS) starches.

Table 3: Pasting properties for native amylose-free cassava (WXCS) and corn (WXMS) starches.

Parameter	Unit	Starch*		p value
		WXCS	WXMS	
Pasting temperature (PT)	°C	66.6 (0.2)	71.1 (0.3)	0.0002
Peak viscosity (PV)	cP	1339 (17)	1186 (16)	0.0034
Hot Paste viscosity (HPV)	cP	641 (15)	788 (13)	0.0021
Final viscosity (FV)	cP	731 (16)	815 (9)	0.0013
Pasting start time (Pt)	min	4.10 (0.03)	4.87 (0.06)	0.0003
Peak viscosity time (PVt)	min	5.78 (0.07)	6.88 (0.01)	0.0001
Breakdown (BD)	cP	698 (2)	398 (3)	0.0001
Setback (SB)	cP	-608 (13)	-371(5)	0.0007

*Average results, standard deviations in parentheses n = 3, (-) symbol indicates a reduction means comparison (LSD) test at the 5% significance level ($p < 0.05$).

The two amylose-free starches analyzed exhibited a typical pasting curve (Figure 3) with a low pasting temperature (PT), setback (SB), peak viscosity (PV) and breakdown (BD). At the initial phase (heating up to 90 °C), the WXCS viscosity increased progressively at a higher rate than WXMS, reaching the maximum peak viscosity (PV) in a shorter time and greater magnitude. An increase in heating temperature triggered faster disintegration on swollen WXCS granules, increasing the gel instability index (BD).

It can be proposed for this case that larger granules (WXCS) gelatinize first and smaller granules (WXMS) later; although this is not a universal pattern, smaller granules have a higher solubility and water absorption capacity than larger granules, with which physicochemical properties, such as swelling power and water absorption capacity, are also correlated with the average granule size (Pulido Díaz et al., 2017). It has also been proposed that the gelatinization temperature and short chains in amylopectin have an inverse association, indicating that a lower amount of short amylopectin chains produces a higher initial pasting temperature (Santos et al., 2021).

The results suggest that WXCS presents a lower thermal stability (Breakdown) than WXMS (698 vs 398 cP), thus facilitating excessive granule swelling and developing a viscous paste that flows during cooking and does not gel upon cooling. Peak temperature (PT) can be a guide of the lowest temperature to cook a starch and their water-holding capacity, both important parameters at the industrial level, because it allows energy savings (66.6 vs 71.1 °C) and shorter times (5.78 vs 6.88 min) to reach the peak viscosity (1339 vs 1186 cP) when WXCS is used as the main ingredient (starch-based products) or additive in food manufacture.

After cooling, the WXCS and WXMS pastes viscosities increased slightly, a typical behavior when little amylose is present. Waxy cassava starch from Clone AM206-5 was less sensitive to retrogradation because its gel stability index or setback viscosity (608 cP) was higher than WXMS (371 cP), which makes it a no attractive ingredient for the frozen or refrigerated food products industry, because waxy cassava starch will be more prone to retrogradation due to higher setback viscosity than the corn starch, but its use as a texturizing and thickening agent has the potential to replace chemically stabilized starches. However, traditional chemical modification processes (acid hydrolysis and esterification) are also being applied to waxy cassava starches to improve the emulsifying properties (Fonseca-Florido et al., 2018).

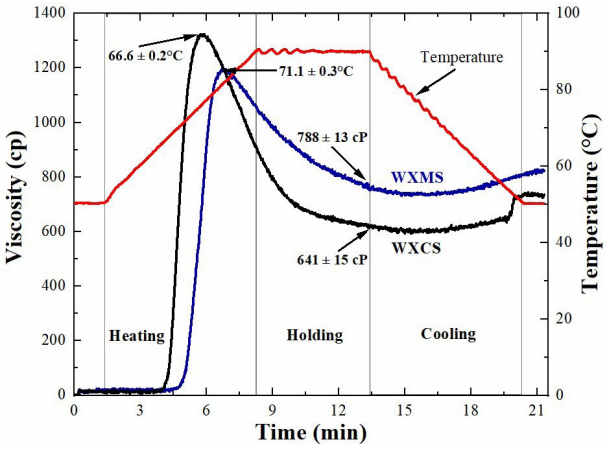

Figure 3: Pasting profile comparison for native amylose-free cassava (WXCS) and corn (WXMS) starches.

Thermomechanical characterization

Knowledge about melted waxy starches flow properties is essential as they influence the industrial process analysis and optimization, per example, food extrusion, in which there is a continuous starch transformation. Flow curves determined by capillary rheometry (Rheoplast®) show that at 30% (w/w) moisture, WXCS molten shear viscosity (η) present a linear decrease (log/log scale), 1 – 300 s^{-1} interval, as a shear rate (γ_r) function (Figure 4), hence exhibiting a pseudoplastic behavior which can be described by the power law $\eta = K(\gamma_r)^{n-1}$, where K (Pa.s^n) and n are the consistency and flow indices, respectively. The most interesting aspect of this graph is that shear viscosity decreases by a factor larger than 3 when temperature is increased from 100 to 120 °C, which reflects a WXCS strong thermal dependency. Further analysis showed that apparent viscosity declines as temperature rises and consequently the specific mechanical energy (SME) decreases possibly due to starch depolymerization or dextrinization.

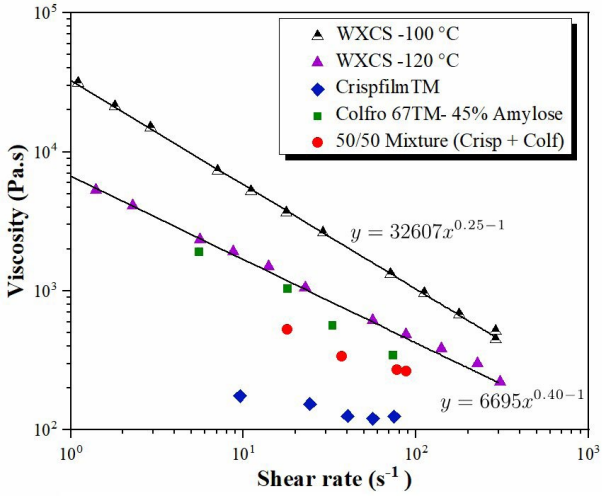

Figure 4: Viscosity as shear rate function for amylose free cassava starch (WXCS, Moisture: 30% w/w) and corn starches with different amylose-amylopectin ratio (Campanella et al., 2002).

Regarding WXCS (30% w/w moisture), as the temperature increased (100 to 120 °C), the power law parameters and flow behavior index (n) increased (0.25 - 0.40), whereas the consistency index (32607 – 6695 K - Pa.s^n) and specific mechanical energy (124 - 75 J/g) decreased. Despite this low SME (75 J/g) carried out in Rheoplast®, WXCS viscosity was higher compared to other waxy starches, suggesting that WXCS is more sensitive to mechanical degradation (Pulido Díaz et al., 2017). In contrast, as SME increases (≤124 J/g), a viscosity reduction is induced, which is related to starch destructuring, specifically macromolecular chain splitting, i.e., depolymerization. As SME controls the treatment intensity undergone by WXCS, it is very important to scale and compare processes, since it has been found that at the same SME, similar changes in starch properties take place (Della Valle et al., 1996). Another significant aspect of SME is that it allows understanding the relationship between thermomechanical treatment factors such as screw speed, barrel temperature, flow rate and final extrudate properties.

Moving on now to consider WXCS rheological behavior against other commercially sourced corn starches, such as Colfro 67TM (45% amylose), CrispfilmTM (2% amylose), and a 50/50 mixture (Campanella et al., 2002), it was clearly observed that corn amylopectin (CrispfilmTM) had the lowest viscosity. Taking into account that the thermal history of these corn starches is unknown, it can be stated that WXCS does not generate viscosities as low as waxy corn starch (molecular weight: 520.7×10^6 g mol^{-1}) despite being lighter (408×10^6 g.mol^{-1}) (Rolland-Sabaté et al., 2013).

When comparing data from (Della Valle et al., 1996) for "starch D" (actually waxy corn starch), which gives K= 1600-825 and n=0.65-0.76 for 100-120 °C (SME=900-500 kJ/kg), the corresponding flow curves match those shown here (Figure 4). These results are likely related to the amylopectin chain structure being more branched on corn starch than on cassava starch; both have long chains (DP ≥37) at a low percentage. This parameter value is higher for cassava starch (9.2%) than for corn starch (7.4%), triggering a mobility diminution over the amorphous phase, enabling chain agglomeration with viscosity increase (Della Valle et al., 1998).

If WXCS consistency (K= 6695) and flow indices (n= 0.40) at T:120 °C are compared to traditional cassava starch (K = 7785; n = 0.44) with amylose (20% w/w) at similar conditions (water content: 30%, T: 110 °C) and capillary rheometer (Sandoval; Farhat; Fernandez, 2007), these values present a certain similarity suggesting a potential starches exchange in already developed food formulations. Unfortunately, it was not possible to perform Rheoplast® tests when WXCS starch was hydrated at 20% (w/w), as too much pressure variability was registered; closest hypothesis in response to this behavior could be excessive power requirements inside the Rheoplast®, so that an extreme hydraulic energy is required to move the stucked central piston when it is surrounded with melted starch. Therefore, no data were obtained for n and K under these conditions, but respective product transformation analyses were performed on the obtained samples.

The section below describes extensional flow curves from the same experiments, with data processed according to Equation 6. From Figure 5, it can be seen that the WXCS extensional viscosity (η_e) follows a log/log linear power law model, but one unanticipated finding occurred: η_e was one order of magnitude higher than the shear viscosity (η) for identical extensional strain and shear rates. This study confirms that the WXCS extensional deformation resistance is greater than the frictional resistance at equivalent shear or tensile stress. Similar behavior has been observed in wheat starch (Martin; Averous; Della Valle, 2003), corn extrudates with fiber (Pai et al., 2009), waxy corn and casein blends (Chan et al., 2007), and cassava flours (Sandoval; Farhat; Fernandez, 2007). It is important to highlight the difficulty in comparing extensional and shear rheological data, considering that the equipment used (capillary or in-line rheometer) and applied conditions affect the thermomechanical history of the samples. Consequently, it is normal to find a large variability in the rheological data of starchy products.

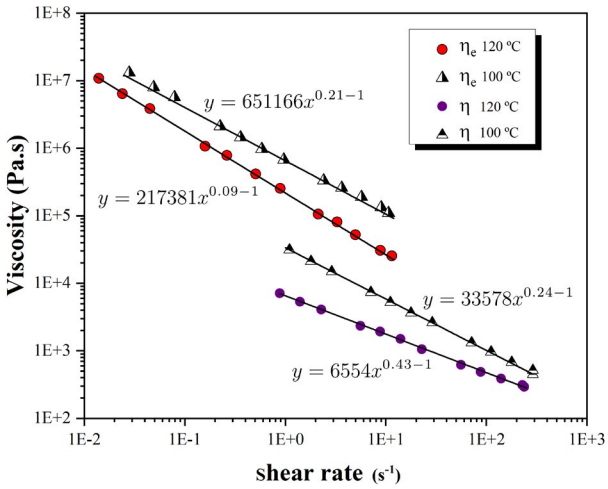

Figure 5: Viscosity as strain rate function for amylose free cassava starch (WXCS, Moisture: 30% w/w). Note: Extensional (ηe) and shear viscosity (η).

A Trouton number (T_R) equal to 3 is expected for a Newtonian fluid. For the conditions evaluated, the Trouton number calculated at 100 °C, and a 0.3–11 s^{-1} strain rate, the T_R records are in the 20–26 interval and increase from 25 to 145 at T=120 °C (0.02–8.8 s^{-1} strain rate). T_R plays a critical role in thermoplastic polymers, and the large values found for WXCS indicate a strain hardening trend, an important property to process films and foams (McCann et al., 2018). At present, studies related to the extensional viscosity of commercial food starchy products are limited due to technical difficulties and measurement limitations, despite the importance of molten starch properties in later expansion process stages, i.e., while elastic thin walls exert resistance to bubbles or cell growth generated by water vapor (Kapoor; Bhattacharya, 2000).

The most obvious finding to emerge from WXCS rheological analysis is that the pseudoplastic flow and Trouton number (TR) are temperature dependent, suggesting a higher elastic nature when thermomechanical treatment rises. It may be concluded that WXCS is suitable for manufacturing expanded food by the extrusion process, but it should be noted that WXCS molten flow is greatly affected by small temperature and strain rate increases, i.e., high sensitivity to degradation. Finally, it is important to remember that native waxy starches have low thermal processing potential. To reduce these limitations, waxy starches need to be subjected to a conversion, which can be done by extrusion, either pure or mixtures.

Starch transformation

The last research stage was focused on checking the residual waxy starch present in extrudates obtained using Rheoplast®, therefore DSC thermograms shown in Figure 6 were performed. Before continuing, it is important to remember that in gelatinization measurements carried out under excess water, the crystalline structure generally disappears around to 60 –70 °C, in native starches or without any transformation (Della Valle et al., 1996).

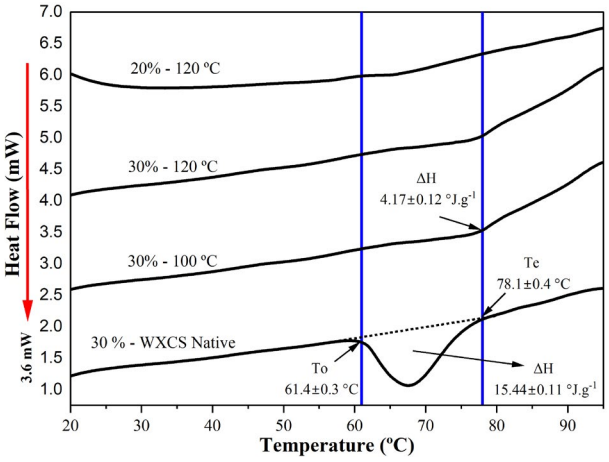

Figure 6: Gelatinization profile comparison for WXCS starch extruded by Rheoplast® at different moisture contents (% w/w) and temperatures compared to the native state.

In a previous work carried out by our group with raw WXCS (Pulido Díaz et al., 2017), thermograms performed at 20% moisture content report T_o: 144.80 °C (start) and T_e: 157.58 °C (end) melting temperatures, being higher than those applied by Rheoplast® in this research. Nevertheless, no peak was detected when WXCS was processed at 120 °C (SME = 200 kJ/kg), regardless of moisture content, unmodified starch was no detected, confirming a full crystalline structure loss. Actually, the last DSC thermogram (20% w/w - 120 °C) indicates a flat peak close to 70-78 °C which may indicate an annealed crystals disruption (starch remnants formed at high temperature in Rheoplast® treatment), moreover, DSC does not truly give indication about granular structure (fragments and phantoms).

The above phenomenon can be explained in two different ways: 1) Differential scanning calorimetry is a tool that by itself generates an incomplete modifications description that starch undergoes across the extrusion process, since material is only exposed to heating by thermal energy and 2) The shear impact (assessed by SME) at granular level is due to the WXCS fragmentation as a solid friction consequence and may not be detected by DSC. For larger water content (30% w/w), at 100 °C, there was still a remnant, as indicated by the gelatinization enthalpy which decreased by 73% (4.17 ± 0.12 J/g) with respect to the native state enthalpy (15.44 ± 0.10 J/g). This result is explained by considering that at larger mositure content, there is less solid friction in WXCS, since water fills the intergranular spaces and acts more as a lubricant.

These results were confirmed by a paste viscosity analysis (Figure 7), the flat viscosity profile for treatment at 20% (w/w) moisture and 120 °C (200 kJ/kg SME), show that peak viscocity remained only 6.56% compared to WXCS native state (1326 cP), validating that WXCS transformation was complete, and that it had lost its granular and crystalline structures, as well as initial low paste viscosity also suggests that a significant depolymerization, due to shear, may have occurred. These properties are similar to extruded starchy products, which suggests that WXCS can be processed by extrusion.

Conversely, at 30% moisture content (100 or 120 °C), extruded WXCS profiles still displayed a viscosity peak at 428 or 334 cP, respectively, which is in agreement with granule fragments presence. Indeed, the maximum viscosity peak decreased by 68% with respect to native starch, for the same moisture content but at 120 °C, the reduction was 75%; since granules were not totally destroyed, their swelling increased the final viscosity. At 30% moisture content a 20 °C degrees increase (100 to 120 °C), produces a 94 cP or 22% reduction in the peak viscosity, whereas lowering the water content from 30% to 20% (120 °C fixed), causes a 74% decrease in maximun viscosity.

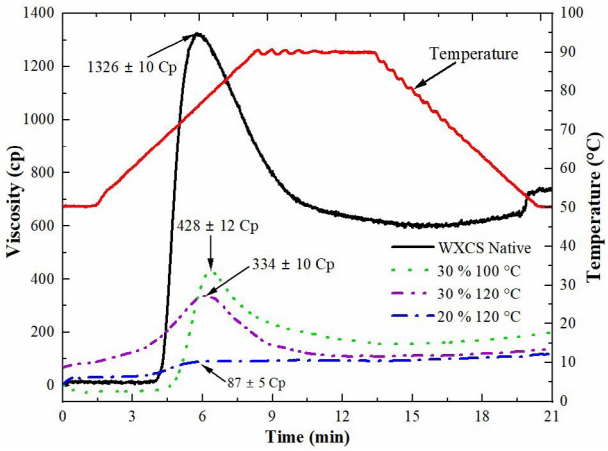

Figure 7: Pasting profile comparison for WXCS extruded by Rheoplast® at different moisture contents (% w/w) and temperatures compared to the native state.

CONCLUSIONS

The waxy cassava starch (AM206-5 cultivar) shows a morphology characterized by elliptical or spherical granules. Rheological and thermal studies indicate that WXCS has lower temperature stability than waxy maize starch. At a low hydrated molten state, WXCS exhibits a pseudoplastic flow behavior and follows the power law, with a consistency index that decreases with increasing temperature. The Trouton number indicated a strain hardening, which suggests the WXCS capacity to create stable foam structures, similar to other industrial extruded starches.

AUTHOR CONTRIBUTION

Conceptual Idea: Pulido-Diaz, A.; Methodology design: Pulido-Diaz A.; Della-Valle, Guy.; Data collection: Pulido-Diaz A.; Della-Valle, Guy.; Data analysis and interpretation: Pulido-Diaz A.; Della-Valle, Guy.; Forero-Longas, F and Writing and editing: Pulido-Diaz A.; Della-Valle, Guy.; Forero-Longas, F.

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