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Microstructural and techno-functional properties of cassava starch modified by ultrasound

Yuliana Monroy[a], Sandra Rivero[a,b], María A. García[a,b,*]

[a] CIDCA (Centro de Investigación y Desarrollo en Criotecnología de Alimentos), 47 y 116 S/N, La Plata (B1900AJJ), Buenos Aires, Argentina – Centro Científico Tecnológico La Plata (CCT-La Plata) CONICET, Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), Argentina
[b] Facultad de Ciencias Exactas, Universidad Nacional de La Plata (UNLP), Argentina

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ABSTRACT

This work was focused on the correlation between the structural and techno-functional properties of ultrasound treated cassava starch for the preparation of tailor-made starch-based ingredients and derivatives. Furthermore, the effect of treatment time, sample conditioning and ultrasound amplitude was studied. Ultrasonic treatment of cassava starch induced structural disorganization and microstructural changes evidenced mainly in the morphological characteristics of the granules and in their degrees of crystallinity. These structural modifications were supported by ATR-FTIR and SEM and CSLM studies as well as DRX and thermal analysis. The selection of the processing conditions is critical due to the complete gelatinization of the starch was produced with the maximum amplitude tested and without temperature control. Rheological dynamical analysis indicated changes at the molecular level in starch granules due to the ultrasound treated, revealing the paste stability under refrigeration condition. PCA allow to establish the interrelationships between microstructural and techno-functional properties. In summary, different starch derivatives could be obtained by adjusting the ultrasound treatment conditions depending on their potential applications.

1. Introduction

Starch-based derivatives are nowadays used for many applications in food processing in order to achieve particular technological properties. In this way, the native starch granules can be modified by applying physical, chemical or enzymatic modifications. Starch modifications promote molecular disorganization, polymer degradation, molecular rearrangement, polymer crosslinking, and oxidation or addition of chemical groups [1,2]. Through these modifications, starch derivatives are obtained and characterized in order to explore their functionality and versatility [3].

According to Wurzburg [4] and Singh et al. [1], the chemical modification of starches involves etherification, esterification, crosslinking and grafting reactions that allow the introduction of functional groups in the starch chains, or decomposition reactions (acid or enzymatic hydrolysis and oxidation). Pedrosa Silva Clerici [5] has proposed different conventional methods of modification, its use for the production of thermoplastic starch by extrusion and other potential uses. In this sense, Pérez-Sira and González-Parada [6] have modified starches by a combination of pregelatinization and extrusion. Currently, there is a wide range of modified starches on the market, mainly destined to the food, pharmaceutical, paper and textile industries, most of them from maize, potatoes and wheat. The physical modification of cassava starch and the study on their functional properties have hardly been reported and requires a deep analysis.

According to official data, a growth of the modified starch market is expected to reach 4.1% during 2017 and it is projected to achieve USD 12.14 billion up to 2022 [7], indicating the high demand for this type of ingredients. However, the highest proportion of the products marketed correspond to chemically modified starches involving the use of toxic substances and non-environmentally friendly procedures regarding the waste that they generate and the energy consumption involved. In this sense, one of the most widely used modified starch requires the use of crosslinking agents such as phosphorus oxychloride and epichlorohydrin, which are recognized by their toxicity [8,9].

The ultrasound is the sound waves at a frequency which exceeding the audible threshold of the human hearing range. Ultrasound treatment (UT), is a physical method of starch modification that has shown many advantages in terms of higher selectivity and quality, reduced use of chemicals and processing time, and finally serving as an environment-friendly processing [10]. The effects of UT results from acoustic cavitation, which is the fast generation, growth, and finally implosive

* Corresponding author at: CIDCA (Centro de Investigación y Desarrollo en Criotecnología de Alimentos), 47 y 116 S/N, La Plata (B1900AJJ), Buenos Aires, Argentina – Centro Científico Tecnológico La Plata (CCT-La Plata) CONICET, Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), Argentina
E-mail address: magarcia@quimica.unlp.edu.ar (M.A. García).

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collapse of bubbles in liquid that generates heat (up to 5000 K) and pressure (up to 20 MPa) just in a short time [11]. During UT, ultrasound energy can be transferred to starch granules through a process called cavitation, which refers to the formation, growth, and rapid collapse of microbubbles [12]. As a result of this treatment, the polymer chains near the collapsing microbubbles are caught in a high gradient shear field, which leads to the breakage of macromolecular C—C bonds, and formation of long-chain radicals [13].

To the best of our knowledge, scarce studies that make a comprehensive analysis of the microstructural changes and its relationship with the functional properties of cassava starch modified with ultrasound are reported. Moreover, published data are centered mainly on the effect of UT on the physical properties and the changes experienced in corn, potato, and rice starch properties.

This work was focused on the correlation between the structural and techno-functional properties of physically modified cassava starch (*Manihot esculenta*), as a contribution to the application of ultrasound treatment, a simple and environment-friendly approach for the preparation of tailor-made cassava starch-based derivatives.

2. Materials and methods

2.1. Cassava starch ultrasound treatment

Cassava starch was provided by the Cooperativa Agrícola Mixta de Montecarlo (Misiones, Argentina), containing 17% amylose. Aqueous cassava starch suspensions at a concentration of 5% (w/v) were treated with ultrasound at a power of 750 W and 40% amplitude during different times (5, 10 and 20 min) in a Sonics VCX-750 ultrasonic equipment (Vibra Cell, USA). Additionally, the condition of 60% for 20 min was also analyzed in order to study the ultrasound amplitude influence. Two conditions were assayed: with or without cooling down during sonication by immersion in an iced-water bath of the sample container, considering that, as it is well known, the ultrasound application raises the temperature of treated samples. Table 1 shows the processing conditions and the nomenclature used in each case. Samples were centrifuged at 2500 rpm for 10 min and dried in an oven at 37 °C up to a constant weight in order to obtain the modified starches, which were then ground in a mortar until a fine powder was obtained, which was sieved through a sieve of 53 µm (ALEIN International, Argentina).

2.2. Structural studies of modified cassava starches

2.2.1. Microscopic analysis: SEM and CLSM

Starch granule morphology was analyzed by SEM using a low vacuum FEI model Quanta 200 scanning electron microscope (Eindhoven, The Netherlands). Starch samples were mounted on bronze stubs with a double-sided adhesive tape and then coated with a thin gold layer. Samples were examined at an acceleration voltage of 20 kV.

Likewise, confocal Laser Scanning Microscopy (CLSM) was used to complement the starch granule morphology studies. All samples were examined after binding with fluorescein isothiocyanate (FITC), a fluorescent label freshly prepared in 0.3 mg/mL pH 9 buffer ($NaHCO_3$ 50 mM and NaCl 100 mM). Native and modified starches (5 mg/mL) were suspended in milli-Q water and 1000 µL of the suspensions were stained by the addition of 40 µL of FITC. Starch granules were visualized using a LEICA TCS SP5 (Mannheim, Germany) inverted microscope equipped with an Ar laser, at excitation and emission wavelengths of 488 and 518 nm, respectively. Images were acquired using a HCX PL APO CS63.0 × 1.40/UV/oil immersion objective with 1024 × 1024 pixel resolution, in a constant z-position. Software Leica Application Suite Advanced Fluorescence (LAS AF), version 2.2.1. build 4842 was employed in the image analysis.

2.2.2. Granule size distribution

The size of the starch granules was determined (expressed in % volume) was determined by Dynamic Light Scattering (DLS) with a particle size analyzer (Malvern Mastersizer 2000E, Malvern Instruments Ltd., Worcestershire, U.K.). Measurements were performed in quadruplicate at room temperature. The refractive indices of 1.33 for water and 1.52 for starch were used as standards according to Torres et.al [14].

2.2.3. ATR-FTIR

Molecular interactions study was carried out by means of ATR-FTIR technique. Spectra were recorded using a Nicolet, iS10 Thermo Scientific (Madison, USA) by the accumulation of 32 scans at 4 cm^{-1} resolution in the 4000–400 cm^{-1} wavenumber range. Starch powders were placed onto a diamond ATR crystal using a top-plate and pressure-arm accessories (Smart iTX accessory) for the Nicolet™ iS™10 (Thermo Scientific™, Madison, USA). Data were analyzed by using the software Omnic 8 (Thermo Scientific, Madison, USA).

The spectral deconvolution of the peaks within the region 1065–950 cm^{-1} was performed by using curve fitting algorithms. Inverted second derivative spectra were used to estimate the number, position and relative contribution of individual components.

The software iterated the curve-fitting process by adjusting the high and width of the peaks to achieve the best Gaussian-shaped curves that fit the original spectrum.

2.2.4. X Ray diffraction

The characteristic patterns of native and modified cassava starch were evaluated by X-ray diffraction in a X'Pert Pro Analytical Model PW3040/60 (Almelo, The Netherlands). The CuKα radiation (1.542 Å) was generated at 30 mA and 40 kV, recording the relative intensity in the scattering range of (2θ) 3–60° with a step size of 2θ = 0.02°. The relative crystallinity was determined as the ratio of the crystalline area to the total area by using the Origin software (Version 7.0, Microcal Inc., Northampton, MA, USA) and expressed as (%) [3].

2.3. UT modified cassava starch characterization

2.3.1. Differential scanning calorimetry (DSC)

Thermal properties of native and modified starches were conducted by using a DSC model Q100 controlled by a TA 5000 module (TA Instruments, New Castle, Delaware, USA), with a quench cooling accessory, under a N_2 atmosphere (20 mL min^{-1}). Samples were analyzed between 10 °C and 120 °C, at a heating rate of 10 °C/min.

About 10 mg of 20% (w/w) aqueous suspensions of native or modified starch were weighed in preweighed aluminum pans which were hermetically sealed. An empty pan was used as reference. From

Table 1
Nomenclature used and temperatures reached for cassava starch granules modified under different ultrasound conditions.

	Conditions		Nomenclature	Process temperature (°C)	
	Amplitude	Time (min)		Initial	Final
Native			N		
Without ice bath condition	40%	5	U5	22	33
		10	U10	22	40
		20	U20	22	50
With ice bath condition	40%	5	UB5	22	21
		10	UB10	22	28
		20	UB20	22	37
Without ice bath condition	60%	20	U 60%	22	65
With ice bath condition			UB 60%	22	24

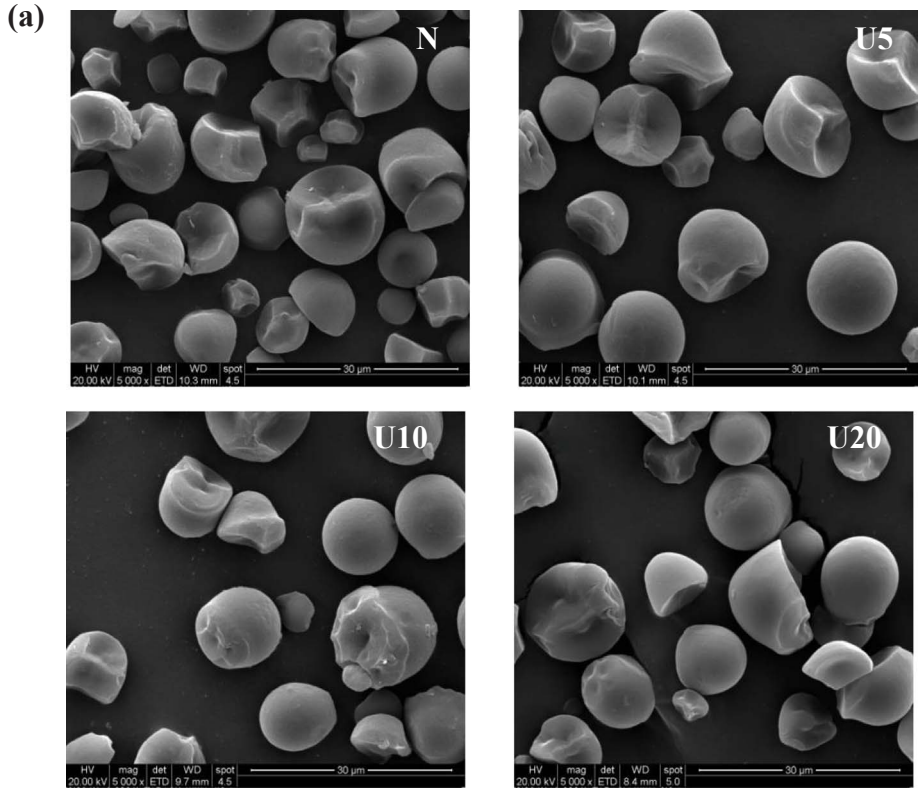

Fig. 1. Effect of ultrasound treatment time on: **a)** microscopic structure, and **b)** size distribution profiles of native cassava starch granules (N), and samples treated with UT for 5, 10, and 20 min (U5, U10, U20). Magnifications are indicated in the micrographs.

the thermograms, the enthalpy (ΔH, J g^{-1} of dry starch) and the thermal transition parameters such as onset temperature (T_o), peak temperature (T_p), and final temperature (T_f) for gelatinization were obtained using the Universal Analysis V1.7F software (TA Instruments). Assays were performed at least by triplicate.

2.3.2. Swelling power

For swelling power determinations, native or modified starches (0.1 g) were suspended in 8 mL of distilled water at 25 °C for 24 h with occasional stirring. Each suspension was centrifuged at 2500 rpm for 10 min; the decanted was weighed and the supernatant was drying in an oven at 105 °C until achieving the constant weight. Measurements were conducted at least by triplicate and samples swelling power was determined by gravimetric weight gain and it was expressed in (%).

2.3.3. Rheological characterization

The rheological assays were performed in a Rheo Stress 600 ThermoHaake (Haake, Germany) rheometer using a plate–plate sensor PP35 at controlled temperature (20 °C). Starch suspensions (5% w/v) were gelatinized at 90 °C for 20 min. The measurement gap distance was fixed at 1 mm. Rotational mode was used to investigate time-dependent behavior of the native and modified starch pastes. Rheological behavior was mathematically fit by using the Ostwald de Waele model and the corresponding thixotropic or antithixotropic indexes were determined [15]. For non-Newtonian systems apparent viscosity was calculated at 500 s^{-1}.

Viscoelastic behavior of starch pastes was studied through dynamic assays. In order to determine the linear viscoelasticity range, a stress sweep (0–100 Pa) at constant frequency (1 Hz) was performed.

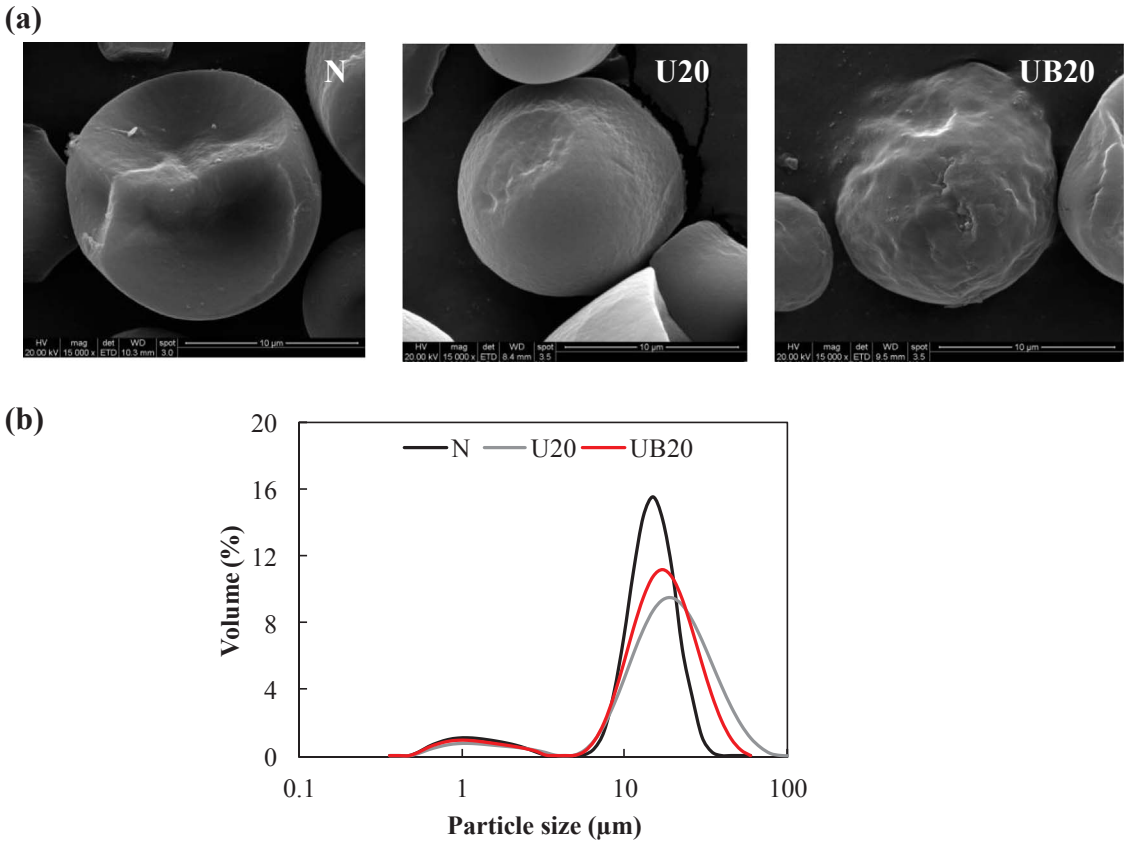

Fig. 2. Influence of sample conditioning with ice bath during the ultrasound treatment on: **a)** microscopic structure, and **b)** size distribution profiles of native cassava starch granules (N), and samples treated with UT for 20 min with (UB20) and without cooling bath (U20). Magnifications are indicated in the micrographs.

Furthermore, frequency sweeps (0.01–100 Hz) were made at constant stress (1Pa). The dynamic rheological parameters recorded were the storage modulus (G'), the loss modulus (G"), and the tan δ (tan δ = G"/G'). Mechanical spectra were obtained by plotting G' and G" as a function of the frequency (ω). The rheological tests were carried out at least in duplicate. In addition, storage under refrigerated conditions (96 h at 4 °C) was studied in order to evaluate the starch pastes retrogradation.

2.4. Statistical analysis

All experiments were performed at least in duplicates, with individually prepared cassava starch treated samples as replicated experimental units, as described previously in each determination. The InfoStat Software (Version 2009) (InfoStat Group, Agricultural Sciences College, National University of Cordoba, Argentina) was used. Analysis of variance (ANOVA) and comparison of means with the Fisher's least significant difference (LSD) test were conducted, at a significance level $p = 0.05$. The results were subjected to a Principal Components Analysis (PCA) in order to observe the differences and similarities of the analyzed samples. The PCA was conducted on mean values using Infostat v2009 software (Córdoba, Argentina).

3. Results and discussion

Taking into account that ultrasound promotes the generation of caloric and mechanical energy and cavitation [16], an increase of the suspension temperature was observed (Table 1). After 20 min of treatment at 60% amplitude without the ice bath, it was observed that the final temperature was 65 °C causing the complete starch gelatinization meanwhile the treatment at 40% amplitude induced the partial gelatinization of samples (Table 1). For this reason the ice bath condition was assayed in order to control the temperature. This result could be attributed to the physical damage that ultrasound produces in the granule structure, which would facilitate the penetration and diffusion of water and, therefore its greater hydration. The increase in the swelling capacity could be ascribed to the modifications caused at the crystalline structure level such as the intermolecular bonds rupture, morphological and permeability changes in the starch granules. Several authors proposed that these changes would induce a greater accessibility of the water molecules to interact with free hydroxyl groups of amylose and amylopectin polymers [10,13,17,18].

3.1. Microstructural analysis

Microscopic analysis has been widely used to detect structural changes caused by physical, chemical or enzymatic modification processes [19]. Fig. 1 shows the scanning electron micrographs of native and modified cassava starch granules and the size distribution profiles associated in each case. The morphology of the studied native granules was oval, oval-truncated, or rounded, in accordance to Sívoli et al. [19,20]. Additionally, SEM showed a smooth granule surface without cracks for the native cassava starch. Micrographs of treated samples suggested that modifications induced by UT energy are of superficial and microstructural nature, without affect the integrity of the starch granules (Fig. 1). In general, the physical modification did not induce changes in the characteristic shape of the granule, but an increase on the surface roughness was detected, which was intensified with the treatment time (Fig. 1 a); similar results were reported by Sujka [18]. It has been reported that ultrasound treatment generates frictional forces that cause structural modifications of starch granules by collapsing and cavitation of bubbles that induce high pressure gradients and high local velocities of the liquid layers in their surrounding area. This fact causes the formation of shear forces sufficient for polymer degradation

exhibiting the granules small fissures and depressions on the surface [17,18,21].

DLS analysis showed that native cassava starch granules presented a bimodal size distribution with two distinct groups centered at 1.1 and 15.5 µm. Siroth et al. [22] informed that the type of size distribution of the granules of some tubers, as cassava, depends on the phase that the crop is undergoing at the time of its harvest and its variety. According to Carmona-García et al. [23], the size of starch granules plays an important role in the modification of the microstructure due to a larger capacity for trapping kinetic energy.

Although the bimodal distribution was maintained after the ultrasound treatment, size profiles were broadened, being the largest granules the most susceptible (Fig. 1b). The higher the treatment time the wider the distribution. Thus, for the most drastic condition (U20) the distribution obtained was centered at 0.74 and 9.5 µm. This tendency was more marked in the case of samples treated without cooling down (Fig. 2b). Ultrasonic treated samples conditioned with ice bath exhibited similar patterns in spite of the treatment time; these results correlate with SEM morphological observations (Fig. 2a). Amplitude increment did not affect the size distribution profile of treated starch granules (data not shown).

For all studied samples granule size diameters obtained by SEM differed from those determined by DLS. For example, by SEM cassava native starches exhibited a mean granule diameters centered at 6 and 12 µm. This fact has been previously reported [24] on standard and waxy maize starches treated by ultrasound. The authors explained that particles with size > 10 µm could not be properly detected by DLS as they settle down rapidly.

The analysis by CLMS allowed the detection of granular internal structures and the presence of cavities and cracks. Thus both microscopic techniques (SEM and CLMS) provide complementary information. It was observed that the native starch showed no damaged granule surface (Fig. 3). Ultrasound caused physical alterations; increasing the treatment intensity formed cracks, channels that were deepened from the center of the granule as cracking increased (Fig. 3). UT could affect the structure of cassava starch granules and induce cavities on their surface which provided channels, facilitating water diffusion into granules. Similar results were informed by Hu et al. [25] and Huang et al. [26]. The sonic energy is trapped by the dispersed granules, producing high-frequency vibrations that eventually break the starch granule. More severe granule cracks were observed in regions near the hilium (Fig. 3), which has been indicated as the more fragile surface structure of the native granule [25].

As it can be seen in Table 2, the superficial changes support the higher swelling values obtained for modified UT starches. Although the starch granules are not necessarily broken, the increase in the extent of surface fractures, the disruption and mechanical damages can lead to increases the swelling power [13,23]. At the longest ultrasound time, starch granules presented an increase in swelling power, indicating higher granule disorganization which promotes the interaction of water molecules with free hydroxyl groups of amylose and amylopectin through hydrogen bonds [13].

3.2. Structural and thermal properties of UT modified starch

ATR-FTIR spectra presented bands associated to stretching, flexion and deformation corresponding to the main functional groups characteristic of the polymer starch (Fig. 4a). For treated samples, from ATR-FTIR spectra it was possible to establish that the positions of the characteristic absorption peaks have not experienced changes after the sonication treatment, although a slight diminution in their intensities was detected. Similar results were reported by Bai et al. [27].

The broad band appearing at 3400 cm^{-1} corresponds to the stretching of the –OH groups of amylose and amylopectin; similar results were reported by Enriquez [28]. On the other hand, bands located at 2956 and 2930 cm^{-1} corresponding to C—H bonds vibration [29] were observed. Additionally, the band located at 1153 cm^{-1} signed to C—O bonds flexion of the hydroxyl groups.

ATR-FTIR spectra of starch has been shown to be sensitive to changes in structure on a molecular level, such as starch chain

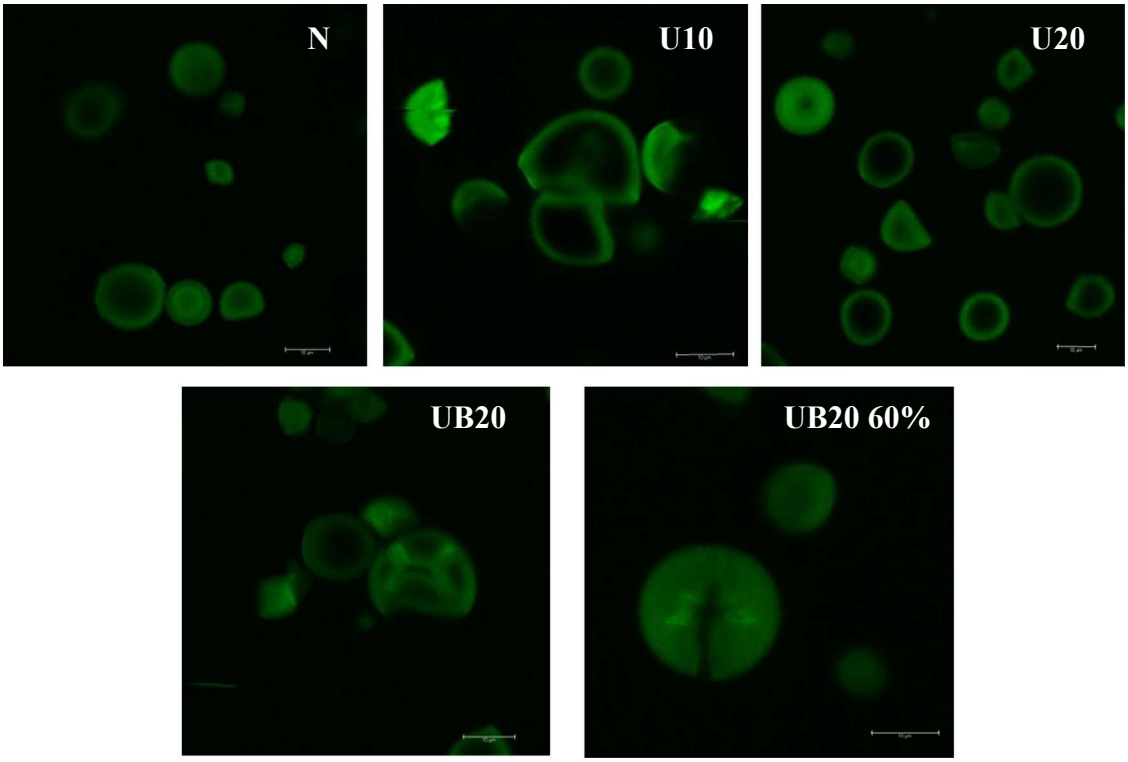

Fig. 3. CLMS analysis of native cassava starch granules (N), and samples treated analyzing the influence of the ultrasound time (U10, U20), the ice bath conditioning (UB20), and the UT amplitude (UB20 60%). Magnifications are indicated in the images.

Table 2
Thermal parameters and swelling power of native and physically modified cassava starch granules.

Starch	T_o (°C)	T_p (°C)	T_f (°C)	ΔH (J/g, dried basis)	% Swelling power
N	57.5 ± 0.4^{a}	65.2 ± 0.5^{a}	78.8 ± 0.2^{ab}	14.44 ± 0.04^{c}	192.11 ± 1.42^{a}
U5	56.9 ± 0.4^{a}	65.0 ± 0.3^{a}	76.9 ± 1.2^{a}	13.58 ± 0.16^{ab}	195.69 ± 6.09^{ab}
U10	56.9 ± 1.1^{a}	65.1 ± 0.9^{a}	79.5 ± 1.8^{ab}	13.47 ± 0.04^{a}	202.67 ± 6.21^{abc}
U20	58.3 ± 0.1^{a}	64.5 ± 0.7^{a}	77.3 ± 0.4^{ab}	13.30 ± 0.29^{a}	221.00 ± 10.68^{d}
UB5	56.7 ± 0.2^{a}	65.1 ± 0.1^{a}	79.1 ± 0.7^{b}	13.33 ± 0.75^{b}	198.03 ± 4.05^{ab}
UB10	56.9 ± 0.5^{a}	64.7 ± 0.4^{a}	75.8 ± 0.1^{a}	13.50 ± 0.09^{a}	206.31 ± 1.05^{bc}
UB20	56.9 ± 0.9^{a}	64.4 ± 1.4^{a}	80.3 ± 1.6^{ab}	13.59 ± 0.25^{ab}	201.90 ± 4.08^{abc}
U20 60%	nd	nd	nd	nd	nd
UB20 60%	56.9 ± 0.1^{a}	64.8 ± 0^{a}	75.4 ± 0.4^{a}	13.36 ± 0.01^{a}	223.63 ± 4.67^{d}

T_o: onset temperature, T_p: peak temperature and T_f: final temperature. ΔH: enthalpy. nd: not determined. Reported results correspond to mean ± standard deviation. Different letters within the same column indicate significant differences ($p < 0.05$).

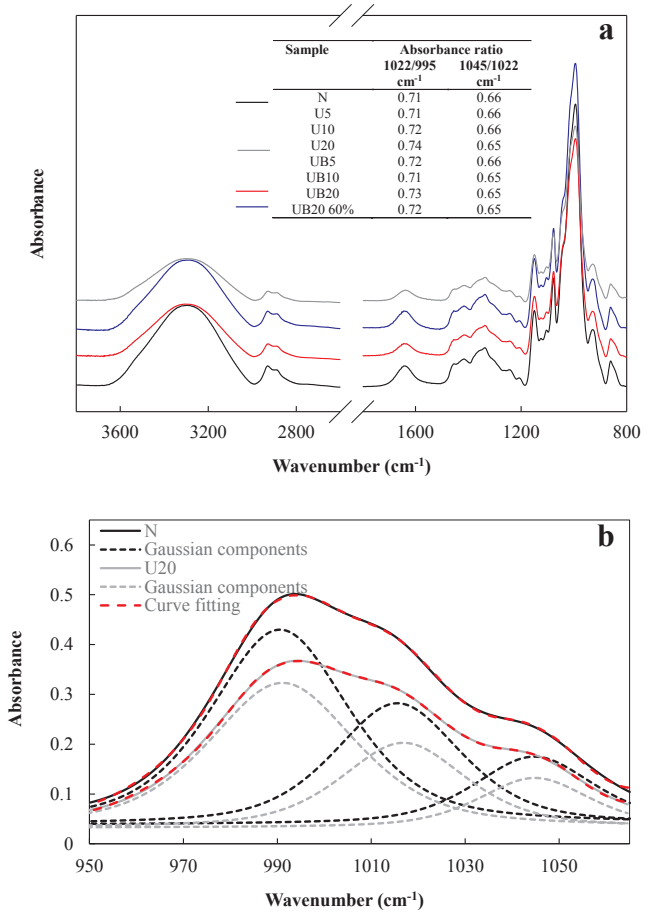

Fig. 4. ATR-FTIR spectra of a) Unresolved FTIR spectra and b) curve fitting analysis in the 950–1065 cm^{-1} region. Experimental data (black and grey full lines) and individual Gaussian components (dotted lines) are shown for N and U20 samples.

conformation, helicity, crystallinity, retrogradation processes, and water content. According to Wang et al. [30,31], the wavenumber range from 900 to 1300 cm^{-1} corresponds mainly to C—O and C—C stretching vibrations, being this region sensitive to changes in polymer conformation (Fig. 4). This region of the FTIR spectra samples was described by three main modes with maximum absorbance at 1045, 1022, and 995 cm^{-1}. The band at 1045 cm^{-1} is ascribed to the ordered regions in starch [30,31]. On the other hand, the 1022 cm^{-1} absorbance band arises as a result of absorption by stretching modes in amorphous starch, and is therefore sensitive to amorphous structure. The band at 995 cm^{-1} results from bonding in hydrated carbohydrate helices.

The bands at 1045 and 1022 cm^{-1} have been linked with order/crystallinity and amorphous regions in starch, respectively. Intensity ratio for 1045/1022 cm^{-1} is associated with crystalline, while ratio for 1022/995 cm^{-1} is associated with amorphous region. The absorbance ratios of 1045/1022 and 1022/995 cm^{-1} are assumed to represent, the order in more crystalline regions and the state of organization of the double helices localized inside crystallites, respectively [32]. The absorbance at the three wavenumbers was obtained from the IR spectra and the ratio of absorbance 1045/1022 and 1022/995 cm^{-1} were calculated and listed in Table inset in Fig. 4a.

The increase of values for the ratio of absorbance 1022/995 cm^{-1} for UT samples indicated a higher proportion of amorphous to ordered structure zones in the starch granules. Furthermore, a slight decrease in the ratio of 1045/1022 cm^{-1} in treated samples was observed (Table inset in Fig. 4a). Additionally, the spectral deconvolution of the peaks revealed that the individual peak areas experienced a diminution with the ultrasound application for samples treated during 20 min (Table inset in Fig. 4a).

On the other hand, microstructural characterization of ultrasound treated starch was completed with DSC and XRD analysis.

Cassava starch suspension evidenced an endothermic transition associated to gelatinization in the range temperature from 57.5 to 78.8 °C coinciding with that reported in the literature (Table 2), [15,33]. As expected, the starches treated with ultrasound in the most drastic condition (U 60%) showed no thermal transition, confirming that the complete gelatinization of the starch was achieved during processing. For the other conditions studied, the thermograms presented the typical gelatinization endotherms, without variation in the peak temperature, observing a decrease of the enthalpy associated with the native one. In the assays performed by DSC, there was a decrease in enthalpy of gelatinization in the treated samples compared to the control, being this difference statistically significant ($p > 0.05$). It has been proposed that ultrasound treatment causes a structural disturbance in the crystalline regions of the granules preceded by the reversible hydration of the amorphous phases [17]. Similar elucidation has been proposed by Luo et al. [34]. The reduction in the enthalpy values, indicates that the organization of the starch components plays an important role in the gelatinization process of starches modified by UT. These results are in agreement with the FTIR-ATR findings, particularly with the reduction of the ratio of 1045/1022 cm^{-1}.

Carmona-García et al. [23] reported that ultrasound power decreased the onset temperature and enthalpy of the gelatinization of non-waxy rice starch. Jambrak et al. [17], informed that ultrasound treatment barely affected the gelatinization temperatures of corn starch granules. However in this study, the transition temperatures did not show significant ($p > 0.05$) changes; even though UT modified the size distribution profiles of starch granules (Figs. 1 and 2).

Amylose and amylopectin chains can be arranged in a semicrystalline structure forming a matrix of starch granules with the ability to diffract the X-rays, thus allowing to obtain information from its structure [35]. Generally, tuber starches present a polymorphism Type B or

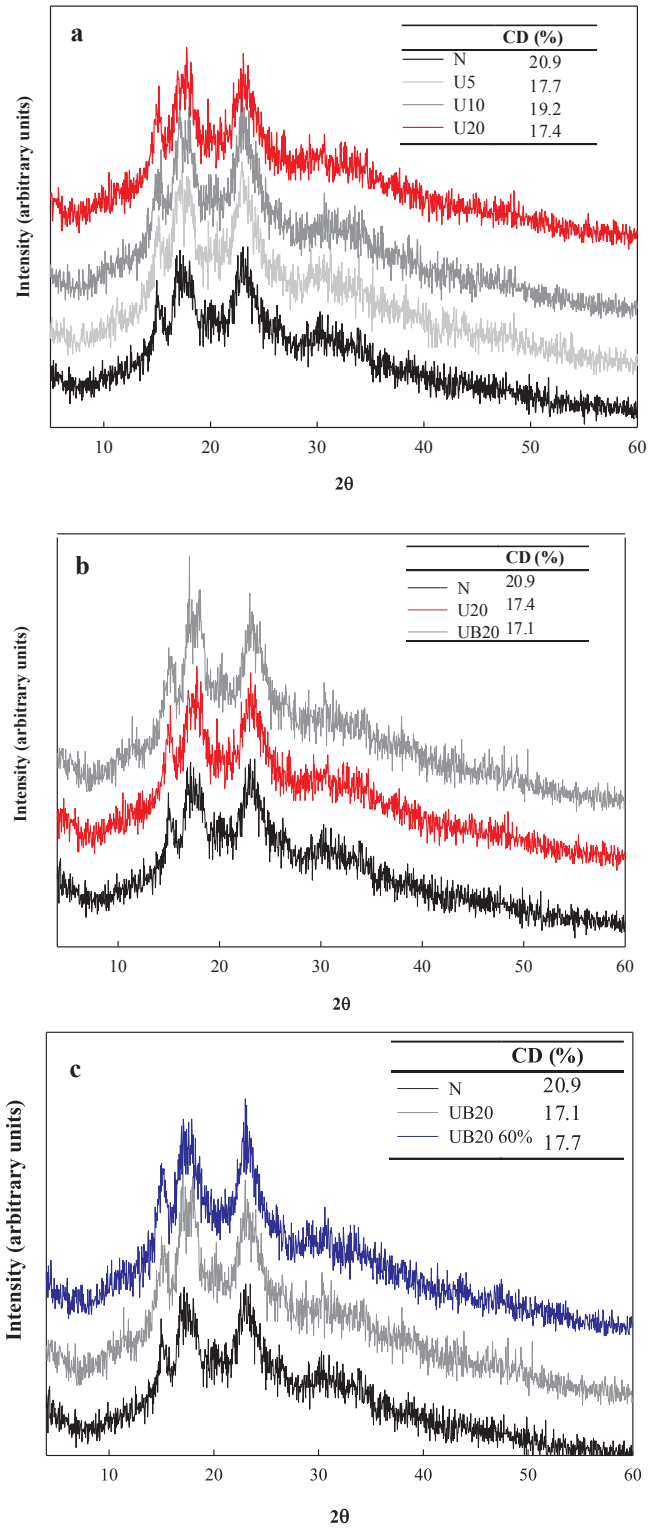

Fig. 5. X-ray diffraction patterns of native (N), and sonicated cassava starch granules. Tables inserted show the crystallinity degree (%). Effects of **a)** ultrasound treatment time (U5, U10, and U20); **b)** ice bath conditioning (U20, UB20) and **c)** the amplitude of treatment (UB20, UB20 60%).

C [36,37].

Figure 5 shows the X-ray diffractograms of the native starch and the samples sonicated at different times. The evolution pattern and the peak intensities in approximately 2θ = 15, 17, 18, 20, 23, 30° indicated that the native cassava starch had a C-type crystallinity pattern and that it was not modified with the treatment. A similar trend was observed by

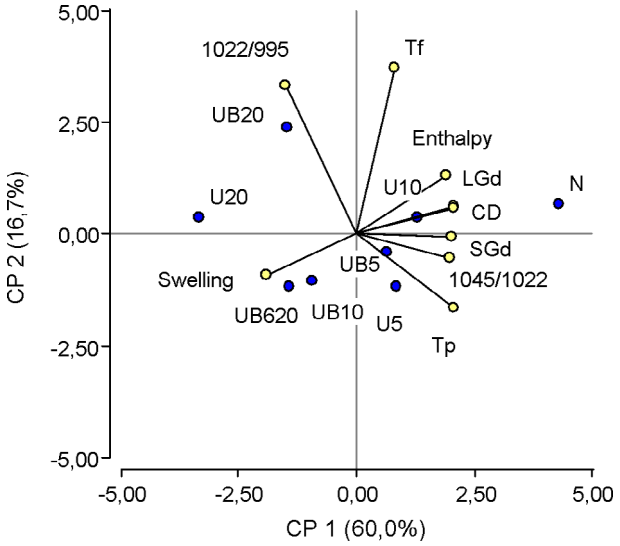

Fig. 6. PCA biplots summarizing the relationships between the samples (native and modified starch granules) and their microstructural and physicochemical properties. Samples nomenclature used is described in Table 1.

Boufi et al. [38]. In the Table inset in Fig. 5 the crystallinity degree (CD) of the samples are presented. In general, it was observed that increasing the treatment time, the samples CD decreased (Fig. 5a). A similar trend was obtained for the ice bath condition (Fig. 5b) meanwhile a slight effect was observed with the UT amplitude increase (Fig. 5c). This behavior could be attributed to the destabilization of the lamellar array of starch granules induced by the treatment. The results of X-rays are consistent with those obtained by DSC and FTIR spectra (Table 2 and Fig. 4).

According to Luo et al. [34] the X-ray diffraction spectra could be attributed to preferential degradation of the amorphous regions by ultrasonic treatment.

A PCA was carried out, taking into account the obtained microstructural results as well as those of thermal analysis in order to compare the main characteristics of UT cassava starches. PC1 and PC2 explained the 76.7% of the total variance. In general, PC1 which explained the 60% of the total variance could be related to more drastic UT conditions clustering U20, UB20 and UB20 60% treatments (Fig. 6). PC2, which explain a much lower percentage of variance (16.7%), and could be related to samples treated or not under ice-bath. The value of the cophenetic correlation (0.990) indicates that the variable reduction carried out was adequate. Linear correlations were observed between thermal parameters (T_f and enthalpy), the mean size of the large granules and crystallinity degree. Besides, the 1045/1022 cm^{-1} ratio of the ATR-FTIR bands and the gelatinization peak temperature also show a linear correlation. On the contrary, inverse correlations were detected between the ATR-FTIR bands ratios (1022/995 cm^{-1} and 1045/1022 cm^{-1}) and swelling power, gelatinization enthalpy and the diameter of the large granules. Thus, PCA allowed to establish a strong relationship between microstructural analysis and the main physicochemical characteristics of UT cassava starches.

3.3. Rheological behavior

Rotational tests allowed to analyze the rheological behavior of the pastes under large shearing deformations. Samples with and without treatment showed a rheological behavior of a thixotropic pseudoplastic type, which could adjust satisfactorily to the Ostwald de Waele model or power law (Table 3). The treatment allowed to obtain higher viscosity pastes, this behavior was more intensified in the samples treated with ice bath. Both, thixotropy and consistency indexes followed a similar trend than apparent viscosity, being the flow behavior index the

Table 3
Rheological properties and Ostwald de Waele model fitting parameters of pastes prepared with native and treated cassava starch granules.

Sample	Apparent viscosity at 500 s^{-1} (mPa s)	Thixotropy (Pa s^{-1})	Ostwald de Waele model		
			K* (Pa s^{-n})	n**	r^2
N	412.61 ± 12.18^{a}	24,265 ± 841^{a}	5.71 ± 0.05^{a}	0.566 ± 0.003^{c}	0.9986
U5	513.37 ± 31.64^{bc}	26,250 ± 169^{ab}	7.06 ± 0.94^{bc}	0.569 ± 0.009^{cd}	0.9989
U10	496.77 ± 16.92^{b}	37,155 ± 5805^{c}	6.59 ± 0.32^{bc}	0.572 ± 0.001^{cd}	0.9986
U20	439.10 ± 5.19^{a}	25,115 ± 1209^{ab}	6.61 ± 0.02^{b}	0.557 ± 0.008^{b}	0.9989
UB5	557.34 ± 16.18^{d}	26,470 ± 1979^{ab}	7.25 ± 0.02^{c}	0.577 ± 0.003^{d}	0.9989
UB10	526.71 ± 6.85^{bcd}	24,655 ± 756^{ab}	7.25 ± 0.01^{c}	0.570 ± 0.001^{cd}	0.9991
UB20	539.12 ± 17.57^{cd}	30,330 ± 3253^{b}	7.00 ± 0.01^{bc}	0.578 ± 0.005^{d}	0.9990
UB20 60%	406.48 ± 15.59^{a}	22,040 ± 283^{a}	6.39 ± 0.15^{ab}	0.546 ± 0.002^{a}	0.9986

Reported results correspond to mean ± standard deviation. Different letters within the same column indicate significant differences ($p < 0.05$).
* K: consistency index and
** n: flow behavior index.

less affected parameter. The sharp decrease in the apparent viscosity values of gelatinized starch suspensions treated during 20 min (U20 and UB20 60%) suggested that ramified amylopectin chains were largely affected by the sonic energy, which generated linear starch chains. The reduction of the apparent viscosity shown in Table 3 can be attributed to fragmentation of amylose chains and debranching of amylopectin molecules caused by the effect of ultrasound waves in C–O–C linkages [39].

Moreover, after the treatment in the most drastic condition (U20 60%) the pastes presented a Newtonian behavior indicating the granular structure destruction and depolymerization of the main components.

On the other hand, the oscillatory tests allowed to evaluate the viscoelastic behavior of the samples submitted to small deformations (within the linear viscoelastic range). Fig. 7 shows the mechanical spectra corresponding to the native and modified starches with ultrasound. For all assayed conditions, the elastic modulus (G') was higher than the viscous one (G") in the whole analyzed frequency range. Both exhibited a weak dependence on the frequency throughout the test, indicating the development of a gel-like structure.

Analyzing the influence of the treatment time, it was observed higher G' values for the most drastic condition associated to the greater strength of gel (Fig. 7a). This behavior could be attributed to the structural disorganization caused by the treatment and the consequent association of the polymeric chains to form the viscoelastic network. Similar hypothesis was argued by Carmona-García et al. [23] who stressed that viscoelastic moduli of UT starch pastes was more affected for plantain than taro starch, which exhibited large and small granule size respectively.

Mechanical spectra of the pastes prepared from treated starch with ultrasound in an ice-bath or 10 and 20 min did not shown differences in relation to native starch ones (Fig. 7b and d). However, the spectra of the samples treated during 5 min with and without the ice bath showed lower values of G' in comparison to native ones (data not shown).

The ultrasound amplitude effect was analyzed in Fig. 7c, showing that more elastic networks were developed in pastes derived from UB20 60%, since under this drastic condition starch polymer chains fragmentation and amylopectin debranching occurred, which would facilitate the gelation process and subsequent retrogradation phenomenon [25]. Thus, comparing the G' value at a fixed frequency, it is observed that with increasing treatment time a stronger gel was developed (Fig. 7d).

Concerning to the increase in the G' values observed (Fig. 7), under the UT condition studied the starch macromolecules fragmentation could be induced leading to short polymeric chains capable to re-associate through hydrogen-bonds and thus to form a more elastic three-dimensional network. On the other hand, when UT is performed without the ice-bath the fragmentation would be greater, due to the higher starch granules swelling as well as the amylose lixiviation, which increase the UT efficiency to modify the polymeric chains. Likewise, the changes revealed by structural analysis (ATR-FTIR and DRX results) as well as DSC studies support this hypothesis.

With regard to the storage effect of the pastes under refrigeration conditions, there were no significant differences ($p > 0.05$) in the treated samples compared to the native one except for those treated for 5 min (Fig. 7d). In contrast to these results, the samples that were subjected to ultrasound with ice bath exhibited lower values of G' after storage, indicating a less tendency to retrograde. The pastes of the modified starches were more stable because under cooling conditions at 96 h the G' values increased 27% and 106% for the samples treated with ultrasound during 20 min to 40% of amplitude and the native respectively. As was previously mentioned, the fragmentation induced by UT under the ice-bath condition occurs in a minor extension, leading to polymeric chains with a restricted reassociation capacity. This fact explains the formation of a less compact network due to the decreasing of chain interactions through hydrogen bonds. Fig. 8 presents the PCA of the first two principal components (PC1 and PC2) of the rheological parameters studies as well as the swelling power of ultrasound treated starch suspensions. The value of the cophenetic correlation (0.974) indicated that the variable reduction carried out was adequate; PC1 and PC2 explained 90.5% of the total variance. In general, PC1 which explained the 76.5% of the variance could be again associated to the UT severity grouping the treatments with higher time and amplitude performed (U20 and UB20 60%). PC2, which explain a much lower percentage of variance (14%), only separate samples treated under ice-bath. A linear correlation was observed between the G' values of retrograded samples and those corresponded to the granules' swelling power, while an inverse correlation was observed between the first parameter and apparent viscosity of gelatinized starch suspensions.

4. Conclusions

Ultrasonic treatment of cassava starch induced structural disorganization and changes of their techno-functional properties. The physical treatment of the starch produced microstructural changes evidenced mainly in the morphological characteristics of the granules and in their degree of crystallinity. Exhibiting cassava starch a bimodal distribution, it was observed that UT treatment caused a more appreciable effect in the large size granules.

These structural modifications were supported by ATR-FTIR and SEM and CSLM studies. The selection of the processing conditions is critical due to the complete gelatinization of the starch was produced with the maximum amplitude tested and without temperature control (U 60%). Rheological dynamical analysis indicated changes at the molecular level in starch granules due to the ultrasound treated, revealing the paste stability under refrigeration condition which is of particular interest for food uses. PCA allow to establish the interrelationships between microstructural and techno-functional

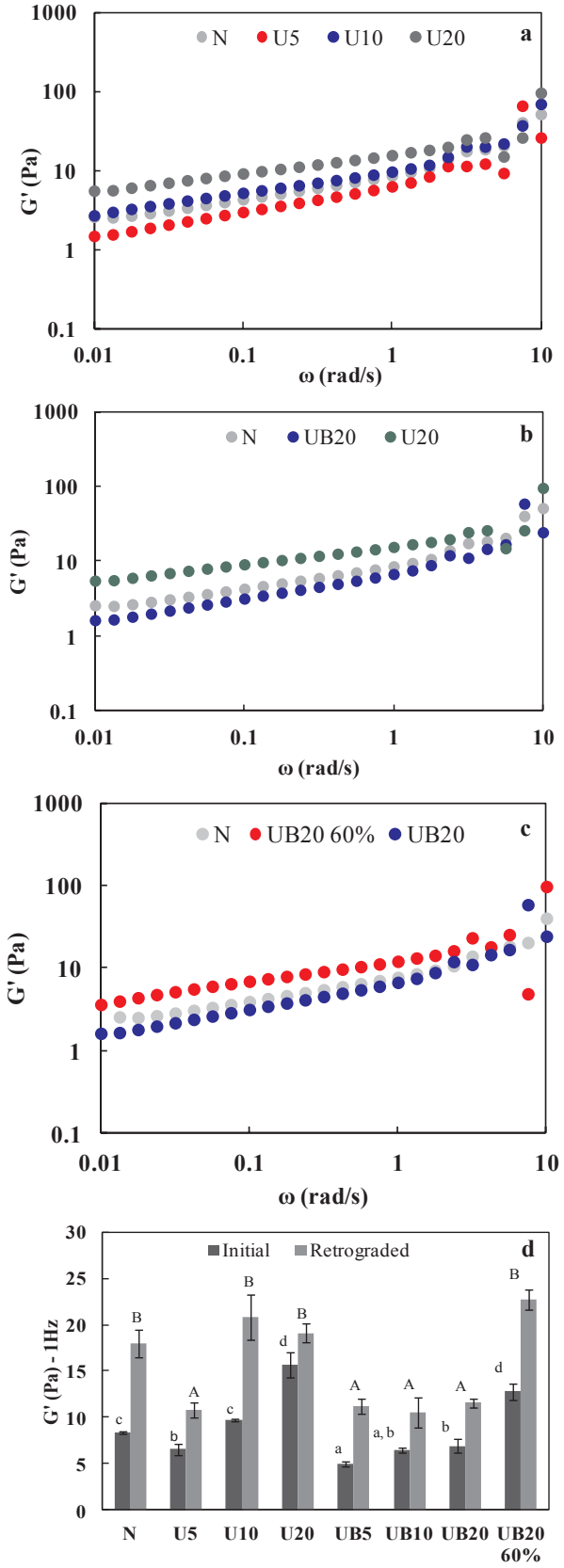

Fig. 7. Frequency sweep of native (N) and sonicated starch granules studying the effect of: **a)** ultrasound treatment time (U5, U10, and U20); **b)** ice bath conditioning (U20, UB20) and **c)** the amplitude of treatment (UB20, UB20 60%). **d)** Elastic modulus (G') at a fixed frequency of 1 Hz for pastes freshly prepared and stored during 96 h at 4 °C.

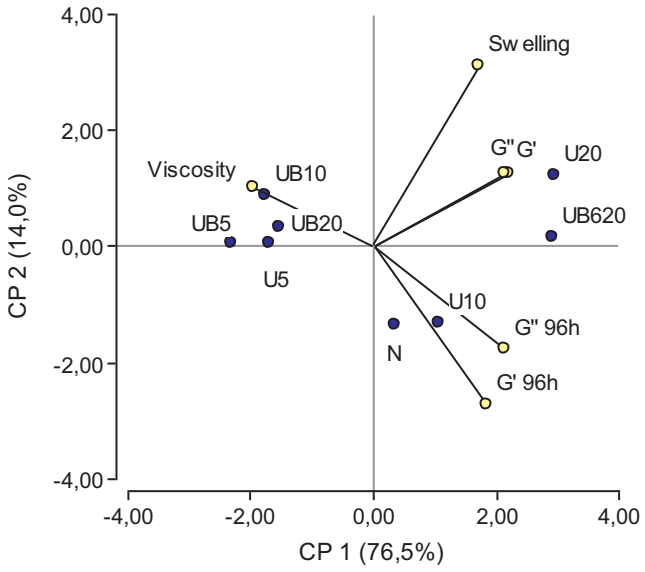

Fig. 8. PCA biplots showing the relationships between the samples (native and modified starch granules) and their main rheological parameters and swelling power. Samples nomenclature used is described in Table 1.

properties.

The results obtained in the present work indicate that UT, a simple and eco-compatible technique, allowed to physically modify cassava starch. In this way, different starch derivatives could be obtained by adjusting the ultrasound treatment conditions depending on their potential applications.

Conflict of interest

The authors have declared no conflict of interest.

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