International Journal of Biological Macromolecules 139 (2019) 63–74

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Combining ozone and ultrasound technologies to modify maize starch

Nanci Castanha [a], Dâmaris Carvalho Lima [a], Manoel Divino Matta Junior [a],
Osvaldo H. Campanella [b], Pedro Esteves Duarte Augusto [a,c,*]

[a] *Department of Agri-food Industry, Food and Nutrition (LAN), Luiz de Queiroz College of Agriculture (ESALQ), University of São Paulo (USP), Pieracicaba, SP, Brazil*
[b] *Department of Food Science and Technology (FST), College of Food, Agricultural, and Environmental Sciences (CFAES), The Ohio State University (OSU), Columbus, OH 43210-1007, USA*
[c] *Food and Nutrition Research Center (NAPAN), University of São Paulo (USP), São Paulo, SP, Brazil*

ARTICLE INFO

Article history:
Received 7 June 2019
Received in revised form 3 July 2019
Accepted 25 July 2019
Available online 29 July 2019

Keywords:
Physical modification
Chemical modification
Combined technologies

ABSTRACT

In this work, maize starch was modified using ultrasound (US) and ozone (O_3) treatments, each one alone and also in combination. The starch molecular structure, granule characteristics and properties were evaluated. The US treatment alone did not show influence on the starch physical characteristics. On the other hand, the O_3 treatment, alone or in combination with US, led to significant changes on starch molecules by increasing carbonyl and carboxyl groups and the apparent amylose content, while decreasing pH and the starch molecular size distribution. The granules' particle size distribution (PSD), their morphology and crystallinity were not affected by any of the treatments. Regarding the starch properties, water absorption index (WAI), water solubility index (WSI), pasting properties and gel strength were clearly more affected by the ozone treatment as compared with the ultrasound treatment. However, the paste clarity was significant higher when the combined treatments were applied, especially when US was used before O_3. These results are prompting the hypothesis that the US treatment improved the subsequent action of O_3.

© 2019 Elsevier B.V. All rights reserved.

1. Introduction

Starches are the main energy reserve of plants and are abundantly found in nature. Obtained from renewable sources, being inexpensive and very versatile material in terms of functional properties, starches are used in several industries, such as food, paper, textile and pharmaceutical [1,2]. The modification of starches is a highly industrially employed technique, since it is an applicable form to improve the functional properties of native starches [3,4] — which are limited by nature. However, nowadays as important as to obtain starches with better properties, is using efficient, safe and environmentally friendly methods to achieve it.

Among the starch modification methods, the most industrially employed are the oxidative ones [3]. However, these processes are usually performed using chemical agents, and are being questioned regarding their impacts on consumers and the environment, which has led to a search by new technologies [3,5]. Among the possible alternatives to chemical methods, ozone (O_3) has been shown to be one of the most promising, as it is highly oxidative [6] and has the advantage of being an environmentally friendly technology, since it quickly decomposes to oxygen [7]. Ozone has already been studied as an oxidizing agent for the modification of a wide range of starch sources, such as potato [8,9], rice [10] and cassava [11]. Starch modification caused by this treatment has been attributed to the generation of electronegative groups and the hydrolysis of the glycosidic bonds of the starch molecule, thus reducing the starch molecular size [8,10,12,13]. However, it is important to emphasize that the botanical source and, consequently, the structure of the starch, has a great influence on the ozone action, and further studies in this field are still required.

On the other hand, physical modification methods, among which we highlight US, can be used to produce modified starches with specific functional properties, having as a positive aspect the fact that no chemical agent is used in the process [3]. High intensity ultrasound treatment is able to change the material properties (i.e. physical disruption, acceleration of some chemical reactions), due to a formation of intense cavitation [14,15]. The modification of starch technological properties using ultrasound has already been studied and proposed for different starch sources, such as rice [16], oat [17], cassava [18], and maize [19,20]. US is claimed to cause starch modifications such as disintegration of granules [21–23], and molecular size reduction [21,24]. However, structural modifications of biopolymers by US treatment are reported to be highly dependent on the US conditions and the material source [25,26]. In fact, some of the results presented in the literature are contradictory. These differences in the observed results emphasize that the lack of standardization during these treatments does not enable to establish definite conclusions about the treatment efficacy and its effects on the starch molecular structure. Moreover, some works in the literature do not follow good practices in ultrasound processing, which can compromise the obtained results [27].

---

[*] Corresponding author at: Avenida Pádua Dias, 11, Piracicaba, SP 13418-900, Brazil.
E-mail address: pedro.ed.augusto@usp.br (P.E.D. Augusto).

https://doi.org/10.1016/j.ijbiomac.2019.07.161
0141-8130/© 2019 Elsevier B.V. All rights reserved.

Finally, the combination of ozone and ultrasound treatments have been little explored. Only two published works explore this subject [28,29]. However, both papers report only rheological and thermal properties of the modified starches, but do not provide information on structural changes neither potential correlations between the modified starch structure and its properties. Consequently, the mechanisms of the combined action of ultrasound and ozone on starch, remains unexplored.

In order to meet the industrial demand for starches with specific characteristics, considering safe and environmental needs, the objective of this work aims to evaluate the structure and the properties of modified starches using combined ultrasound and ozone treatments. Due to its high industrial importance, maize (corn) starch was used as the targeted starch.

2. Material and methods

2.1. Starch sample and processing conditions

Native maize (corn) starch (~28% of amylose, Argo CS 3400) was provided by "Ingredion Brasil Ingredientes Ltda".

The ozone (O_3) treatment used was similar to the one reported in previous work [8], which is schematically illustrated in Fig. 1. In short, 700 mL of starch suspension (10% m/m, wet basis) in distilled water was placed in a glass reactor (capacity: 2 L, height: 56 cm, internal diameter: 6 cm) and processed for 15 min at 25 °C. The ozone was generated from industrial oxygen (95% purity; constant flow at 1 L/min) using an ozone generator unit (Ozone & Life, O&G Model RM 3.0, São Jose dos Campos, Brazil). The gas ($O_2 + O_3$) stream, with an ozone concentration

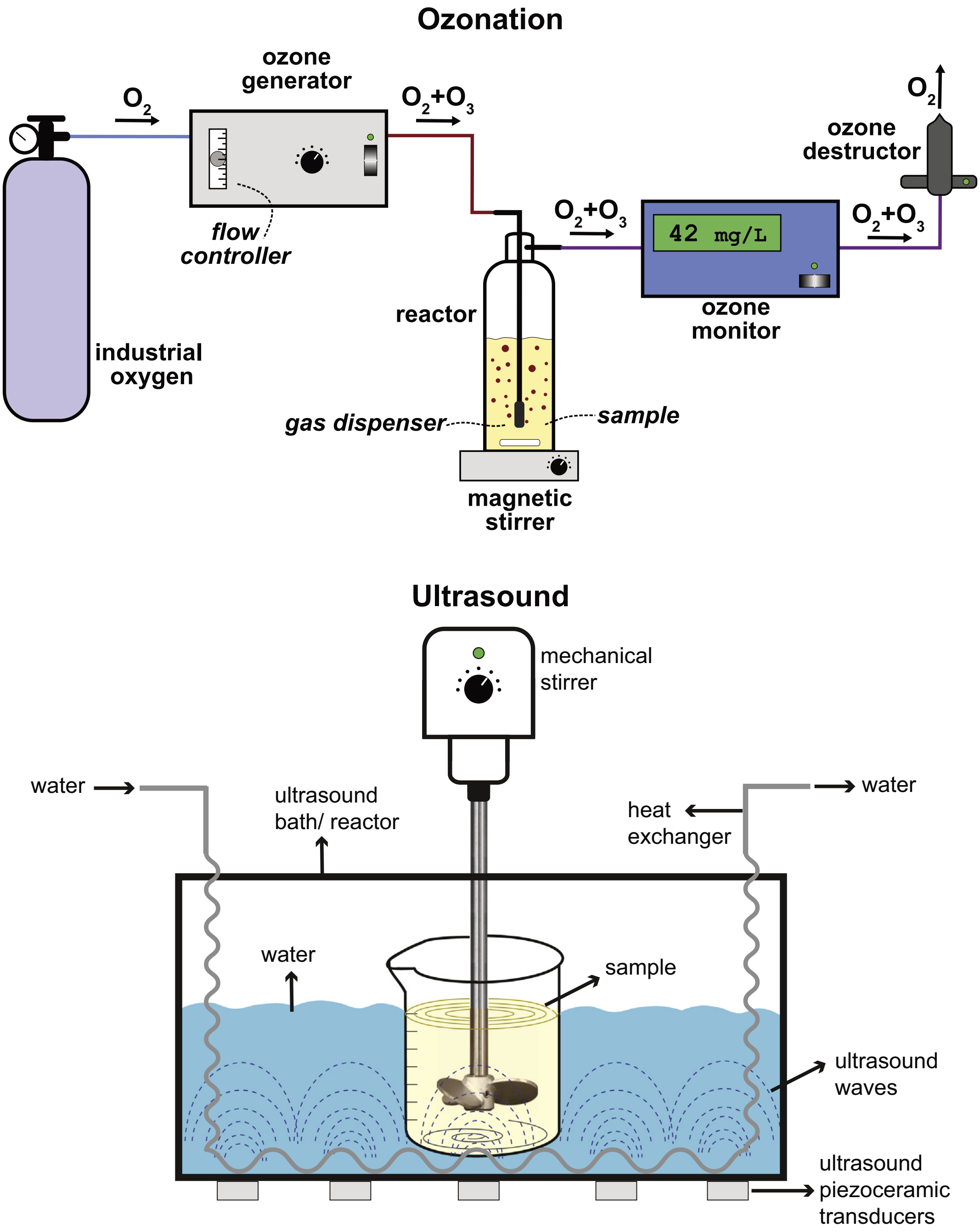

Fig. 1. General aspect of the ozonation (up) and ultrasound (down) systems.

of 42 mg O_3/L of mixture, was introduced in the reactor, being bubbled in the starch suspension. After processing, the water was separated from the sample, and an air circulation oven (at 35 °C) was used to dry the starch (until ~12% of moisture content). The samples were sieved (250 μm) before the analyses.

To perform the physical modification using ultrasound (US), a starch suspension in distilled water (700 mL, 10% w/w) was prepared inside a glass beaker and processed in an ultrasonic bath (Q13/25, Ultronique, Brazil). The US conditions were: processing time of 8 h, temperature of 24–26 °C, frequency of 25 kHz and volumetric power of 72 W/L — which was calculated by the calorimetric method, according to the described by O'Donnell et al. [30]. The temperature was controlled using a heat exchanger inside the US bath (the recirculating water was provided by an external water bath). The ideal location of the samples inside the US bath was evaluated according to the good practices described in the literature [27], ensuring homogeneity and the exposure of the sample to the highest US intensity. The bath was filled with 6 L of distilled water mixed with 15 drops (~0.5 g) of dishwashing detergent (lemon dishwasher, Ypê, Brazil), to decrease the surface tension of the solvent. To prevent decantation of the starch granules during the treatment, a mechanical stirrer was used and located as illustrated in Fig. 1. After processing, the water was separated from the sample, and an air circulation oven (at 35 °C) was used to dry the starch (until ~12% of moisture content). The samples were sieved (250 μm) before the analyses.

Maize starch was treated by US or O_3 individually. Furthermore, the combination of these two treatments was performed as follows: immediately after ozone treatment, the starch sample was transferred to a beaker and treated by ultrasound, using the same conditions previously described. Moreover, the order of the combined treatment changes. That is, another sample, after being processed by ultrasound, was transferred to the reactor and ozonated. The ozone processing (indicated as O_3) was combined with the ultrasound processing (indicated as US), is distinguished as US-O_3 or O_3-US according to the order of the treatments. In summary four different treatments (O_3, US, US-O_3 and O_3-US) were used to assess the physical modifications achieved by these treatments.

2.2. Starch molecular evaluation

2.2.1. Carbonyl and carboxyl groups

The methodology described by Chattopadhyay, Singhai and Kulkami [31], with modifications, was used to evaluate the carboxyl content. In details, ~3 g of starch was suspended in 25 mL of 0.1 M HCl and stirred for 30 min. The samples were then filtered (205 μm) and washed with distilled water. The retained samples were mixed with 300 mL of distilled water and gelatinized in a boiling water bath for 15 min. The sample, still hot, was titrated with NaOH (0.1 M) until pH 8.3, using an automatic titrator (Plus Titrino 848, Metrohm, Herisau, Switzerland). A non-modified sample (control) was regarded as the blank. Using Eq. (1), the "carboxyl groups per 100 glucose units (COOH/100GU)" was calculated. In the equation: Vs = volume of NaOH used for the sample, Vb = volume of NaOH used for the blank, M = molarity of NaOH, and S = the mass of sample (dry basis).

$$\frac{\mathrm{COOH}}{100\mathrm{GU}} = \frac{(\mathrm{Vs}-\mathrm{Vb})\cdot \mathrm{M}\cdot 0.045\cdot 100}{\mathrm{S}} \tag{1}$$

The methodology described by Smith [32], with modifications, was used to evaluate the carbonyl content. In details, ~4 g of starch was suspended in 100 mL of distilled water and completely gelatinized in a boiling water bath for 30 min. The gels were then cooled (to ~40 °C) and their pH was adjusted to 3.2 using HCl 0.1 M. The samples were then mixed with 15 mL of hydroxylamine solution (10 g of hydroxylamine hydrochloride +40 mL of 0.5 M NaOH solution + distilled water until reach 200 mL) and kept in a water bath at 40 °C for 4 h. The sample was then titrated with HCl (0.1 M) until pH 3.2, using an automatic titrator (Plus Titrino 848, Metrohm, Herisau, Switzerland). A mix of all the reagents, without any starch sample, was regarded as the blank. Using Eq. (2), the "carbonyl groups per 100 glucose units (CO/100GU)" was calculated. In the equation: Vb = the volume of HCl used to test the blank, Vs = the volume of HCl required in the titration, M = molarity of HCl, and S = the mass of the sample (dry basis).

$$\frac{\mathrm{CO}}{100\mathrm{GU}} = \frac{(\mathrm{Vb}-\mathrm{Vs})\cdot \mathrm{M}\cdot 0.028\cdot 100}{\mathrm{S}} \tag{2}$$

2.2.2. pH

The pH was determined as specified in Adolfo Lutz Institute [33], using a starch dispersion (10%) and a calibrated potentiometer (Tecnal, TEC-5 mode).

2.2.3. Molecular size distribution

A gel permeation chromatography was used to evaluate the molecular size distribution of the samples. A CL-2B gel (Sigma, Sweden) was packed in a GE XK 26/70 column (diameter: 2.6 cm, height: 70 cm). The samples' preparation was performed as follows: 0.1 g of starch was mixed with 10 mL dimethylsulfoxide (DMSO, 90%) and heated in boiling water bath for 1 h. The obtained gels were slowly stirred overnight at room temperature (~25 °C). After this process, an aliquot of 3 mL of sample (containing ~30 mg of starch) was mixed with 10 mL of ethanol (99.5%), which promoted the starch precipitation. To isolate the precipitated starch, the samples were centrifuged at 1210 RPM for 30 min. Before injecting the samples in the column, the precipitated starch was mixed with 9 mL of hot distilled water kept in a boiling water bath until complete dissolution, according to Song and Jane [34]. 4 mL of the obtained solution (starch concentration of ~3 mg/mL) was then upwardly eluted in the column, using a solution of 25 mmol/L of NaCl + 1 mmol/L of NaOH as mobile phase, at a rate of 60 mL/h. A fraction collector (Gilson model FC203B, Middleton, England) was used to collect 4 mL of samples/ tube, and the content of each tube was analysed using the blue value method [35], at 630 nm wavelength, in a spectrophotometer (Femto, Model 600S, São Paulo, Brazil).

2.2.4. Apparent amylose content

The ISO methodology [36] was used to analyse the apparent amylose content of the samples. In short, the starch sample was mixed with ethanol and sodium hydroxide and gelatinized in a boiling water bath for 10 min. After the gelatinization, the obtained sample was mixed with of NaOH 0.09 M, and an aliquot of this new solution was then mixed with acetic acid (1 M), iodine solution (10% KI and 5% I_2 in distilled water) and distilled water (until reach 100 mL). The intensity of the obtained blue colour (starch iodine complex) was estimated by measuring the samples' absorbance at 620 nm using a spectrophotometer (Femto, Model 600S, São Paulo, Brazil). A standard curve of amylose/amylopectin previously prepared was used as reference.

2.3. Starch granules evaluation

2.3.1. Granule morphology

Images of the starch granules, showing their morphology and the possible changes in the starch surface after processing, were obtained by scanning electronic microscopy using a FEI Inspect F50, (Japan) operating at an acceleration voltage of 2.0 kV and available at LNNano, Campinas, Brazil.

To prepare the samples, a double sticky carbon tape was fixed on a circular aluminium stub, and dry starch was sprinkled on the tape. A brush was used to ensure a uniform layer of powder particles. The stubs were then coated with gold (~4 mm) and then evaluated in the microscope using an Everhart-Thornley Detector (ETD) for secondary electrons.

2.3.2. Granule relative crystallinity

The crystallinity patterns of the starch granules were evaluated using an X-ray diffractometer (Rigaku Miniflex 600 RXD, Tokyo, Japan), using copper radiation and the following test conditions: scan rate of 2°/min at an angle 2θ ranging from 3 to 50°, with 40 kV and 15 mA. To ensure a constant moisture of the samples before the assays, the starch samples were kept for 10 days in a desiccator containing saturated $BaCl_2$ solution (25 °C, $a_w = 0.9$) at the bottom. The relative crystallinity (RC) of the starch granules was calculated, as described by Nara and Komiya [37], using the Origin software version 9.1 (Microcal Inc., Northampton, MA, USA). The 2θ angle selected for the calculation ranged from 3 to 36°

2.3.3. Granules size distribution

The particle size distribution (PSD) of the starch granules was measured using a Laser Analyser equipment (Partica LA-950V2 Laser Particle Size Analyser, HORIBA, Japan). To avoid increase in the granules' size due to water uptake, the samples were dispersed in ethanol (99.5%). The obtained data were evaluated using the software LA-950 for Windows (HORIBA, Japan). The area-based mean diameters (D [2,3]) calculated by Eq. (3)) were evaluated, where n_i was the number of particles with diameter d_i.

$$D[3,2] = \frac{\sum_i n_i d_i^3}{\sum_i n_i d_i^2} \tag{3}$$

2.4. Starch properties evaluation

2.4.1. Pasting properties

The pasting properties were determined using the RVA-4 equipment (Newport Scientific Pvt. Ltd., Australia, with the Thermocline for Windows software, version 3.0). The Standard 2 program was selected (held at 50 °C for 1 min, heating to 95 °C at 6 °C/min, held at 95 °C for 5 min, cooled to 50 °C at 6 °C/min, and held at 50 °C for 2 min). In each cycle, 3 g of starch sample (14% moisture basis) and 25 g of distilled water were used.

2.4.2. Gel texture

The gel strength was determined using a Texture Analyser (TA.XT Plus, Stable Micro Systems Ltd., Surrey, UK) with a load cell of 50 kgf (~490 N). The samples obtained after the RVA assays were stored in 30 × 20 mm (diameter × height) plastic cups for 24 h at 5 ± 2 °C. To ensure uniform moisture of the samples, they were held in a desiccator with water at the bottom. After storage, the samples were carefully removed from the cups, smeared with mineral oil, and uniaxially compressed until 75% of its height using a plate probe (P/100) with 100 mm of diameter. The stress was calculated as the ratio of the force measured by the equipment and the samples' transversal area. The relative strain was calculated as the ratio of the maximum strain measured by the equipment and the samples' height.

2.4.3. Paste clarity

The method described by Craig et al. [38], with the modifications proposed by Aplevicz and Demiate [39], was used to measure the paste clarity of the samples. This method is based on the transmittance measurement (T%) of the starch pastes, which were obtained after the gelatinization of 0.2 g of starch in 20 mL of distilled water, using a boiling water bath for 30 min. To ensure homogeneity of the samples, each tube was individually stirred 5 times every 5 min. Before the assays, the tubes were cooled to ambient temperature (~25 °C). The pastes' absorbance was measured at 650 nm using a spectrophotometer (Femto, Model 600S, São Paulo, Brazil).

2.4.4. Water absorption index (WAI) and water solubility index (WSI)

The method described by Anderson et al. [40], with modifications, was used to evaluate the WAI and the WSI of the starch samples. In details, 0.5 g of starch (dry basis) was mixed with 6 mL of distilled water in pre-weighed centrifuge tubes. The samples were then heated using different temperatures (60, 70, 80 and 90 °C) for 30 min in a water bath with slow stirring. After heating, the tubes were centrifuged (1210 RPM for 10 min), and both supernatant and precipitated were collected. The supernatant phase (containing the soluble starch) was dried at 105 °C in pre-weighted glass plates and the soluble fraction (SF) was determined, being used to calculate the WSI (Eq. (4)). The precipitated fraction (PF) retained in the tube (containing the starch insoluble fraction and the water retained by it) was also weighed to calculate WAI (Eq. (5)). For both equations: M is the mass of the starch sample (dry basis).

$$\mathrm{WSI}\ (\%) = \frac{SF}{M} \cdot 100 \tag{4}$$

$$\mathrm{WAI}\left(\frac{\text{g water}}{\text{g IS}}\right) = \frac{PF-(M-SF)}{(M-SF)} \tag{5}$$

2.5. Experimental design and statistics

Each process (O_3, US, O_3-US and US-O_3) was performed in triplicate and the analyses were performed at least in duplicate for each sample. The averages and the standard deviations were calculated and, when appropriate, the Tukey's multiple comparisons were used. A significance level of 5% was considered. A non-modified maize starch sample (indicated as "control") was used for comparisons.

3. Results

3.1. Starch molecular structure

Results of the starch molecular structure evaluated using carbonyl and carboxyl groups content, pH, apparent amylose content and molecular size distribution are shown in Fig. 2.

3.1.1. Molecular oxidation: Carbonyl, carboxyl and pH

Considering the carbonyl content (Fig. 2A), the US sample did not present a significant difference ($p < 0.05$) compared to the control sample, while the O_3, O_3-US and US-O_3 samples had an increased content of carbonyl groups compared to the control and the US treated sample. Concerning carboxyl groups (Fig. 2A), the O_3 and the US-O_3 samples had a significant increase ($p > 0.05$) when compared to the control, while the US and the O_3-US samples did not. The pH (Fig. 2B) of the samples decreased after the modification, except for the US sample, which did not show a significant variation when compared to the control.

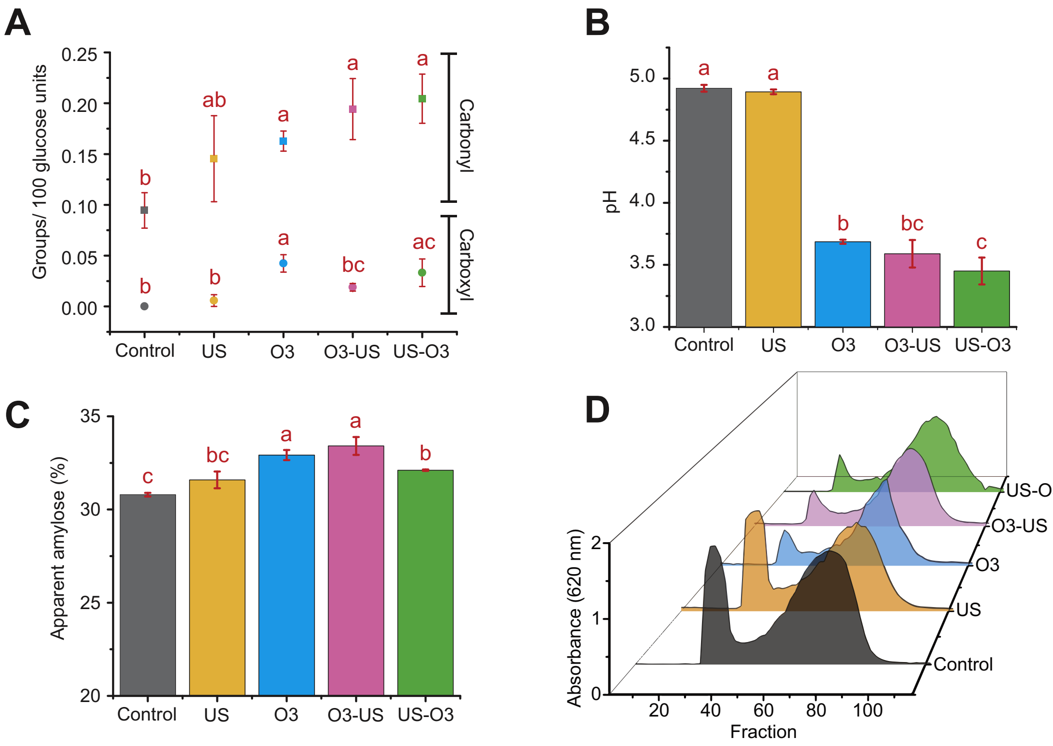

Fig. 2. Carbonyl and carboxyl groups (A); pH (B); apparent amylose content (C); and molecular size distribution profile (blue value method) (D) of the control and modified (US, O_3, O_3-US and US-O_3) maize starch samples. Vertical red bars represent the standard deviations. Results labelled with the same small letter do not differ significantly ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In fact, the carbonyl and carboxyl groups are expected to increase after oxidative processes, as a consequence of the oxidation of the hydroxyl groups [41,42]. Also, as those groups increase, the pH of the samples tends to decrease, due to the formation of carboxylic acids, which is in accordance with results obtained in this work. Therefore, the modification involving the use of ozone (alone or combined with US), in general, led to an increase of the carbonyl and carboxyl groups (Fig. 2A) and a decrease of pH (Fig. 2B). The only exception was the O_3-US sample, whose carboxyl content was not statistically different from the control sample, despite presenting a higher value than the control. One possible explanation for this behavior can be that the combination of US and O_3 led to a higher formation of carbonyl groups, rather than carboxyl groups, as can be observed in the Fig. 2A. Even so we highlight the differences are small, and they can also be related with sample/process variability. On the other hand, US processing alone, as expected, did not affect the hydroxyl groups of the starch molecules.

3.1.2. Molecular depolymerization: apparent amylose content and size exclusion chromatography

Proceeding with the molecular evaluation, another change that usually occurs with modified starches is the hydrolysis of their glycosidic bonds, leading to a depolymerization of their molecules (both amylose and amylopectin), resulting in shorter molecular chains [8,41,43,44]. The blue value method, based on the starch iodine complex, is one of the most useful methods to evaluate the changes in this degree of polymerization [45,46]. Therefore, the modified samples were evaluated using two different approaches, both based on the blue value method: the apparent amylose content and size exclusion chromatography.

The apparent amylose content (Fig. 2C) increased after the modification processes. The exception, once again, was the US treatment, which did not show a significant change ($p < 0.05$) when compared to the control. As previously mentioned, the apparent amylose analysis is, in fact, an indirect evaluation of the iodine affinity of the starch molecules, which can increase or decrease depending on its molecular size. Therefore, the ozone process partially depolymerized the starch molecules, whose residues showed higher iodine affinity, when compared to the US and control samples. In fact, the most notable differences on the molecular size distribution profile (Fig. 2D) were observed on samples that were treated with ozone (O_3, O_3-US and US-O_3).

The apparent amylose content variation after an ozonation process is contradictory in the literature. Some works reported increased values after the modification processes [12], while other report decreases [8]. Since this analysis is based on the iodine affinity of the amylose and amylopectin molecules, it is possible to assume that the contradictory results may be related to the molecular chain-length and electronegative groups obtained after the modification process. Castanha et al. [8], for example, studied the modification of potato starch, which is well known to have amylopectin molecules with long chains and high iodine affinity [47], in addition of having a high level of phosphate groups. All those characteristics may have influenced the measured apparent amylose decrease, since the molecules may have become less prone to bind iodine after the modification. An opposite behavior was observed by Oladebeye et al. [12], indicating that ozone, as in our case, improved the iodine affinity of the molecules — more than simple affirm “ozone increased the amylose content”. Summarizing, this analysis seems to be dependent of the starch source, and consequently of the molecular size distribution and electronegative groups, and processing conditions. Consequently, it appears the apparent amylose content assay may not be a conclusive response of the effects of the ozone modifications. To overcome that weakness in the analysis, gel permeation chromatography was used to help the understanding of starch molecular changes upon the modification processes.

Chromatography results (Fig. 2D) showed a notable difference between the ozonated samples (O_3, O_3-US and US-O_3) when compared to the US and control samples, with a clear decreasing of the first peak. Gel permeation chromatography analysis divides the starch molecules based on their sizes and ramifications: long and branched molecules elute first, forming the first peak (usually associated with amylopectin molecules), while shorter and less branched molecules elute later, forming the second peak (usually associated with amylose molecules) [48]. Therefore, the decrease of the first peak after the ozone treatment indicates that the molecules with longer chain-lengths (probably mostly amylopectin) are depolymerized by this process.

3.1.3. Molecular structure evaluation: overview

Considering all the molecular results (Fig. 2), it is possible to observe some patterns. In general, the effect of combining the technologies (US + O_3) did not present a significant difference if compared to the O_3 results or among themselves. It indicates that ozone processing, at the conditions applied in this work, had a higher influence on the starch molecular modification when compared to the ultrasound processing. Furthermore, the order at which the processes were combined (US-O_3 or O_3-US) apparently had no influence on starch molecular changes.

It is important to notice, however, that despite of no statistical definitive, the US-O_3 treated sample showed a tendency to have a higher carbonyl content (Fig. 2A) and a lower pH (Fig. 2B), when compared to the other samples. This sample showed a significant decrease in the apparent amylose content (Fig. 2C) when compared to the O_3 and O_3-US samples, and its molecular-size distribution showed a second peak eluting later, when compared to the other samples (Fig. 2D), indicating that the molecular size of this sample was smaller when compared to the other ones. All these results, despite apparently not significant, played an important role on the properties of the US-O_3 sample, as discussed in next sections. Furthermore, this information can indicate that the

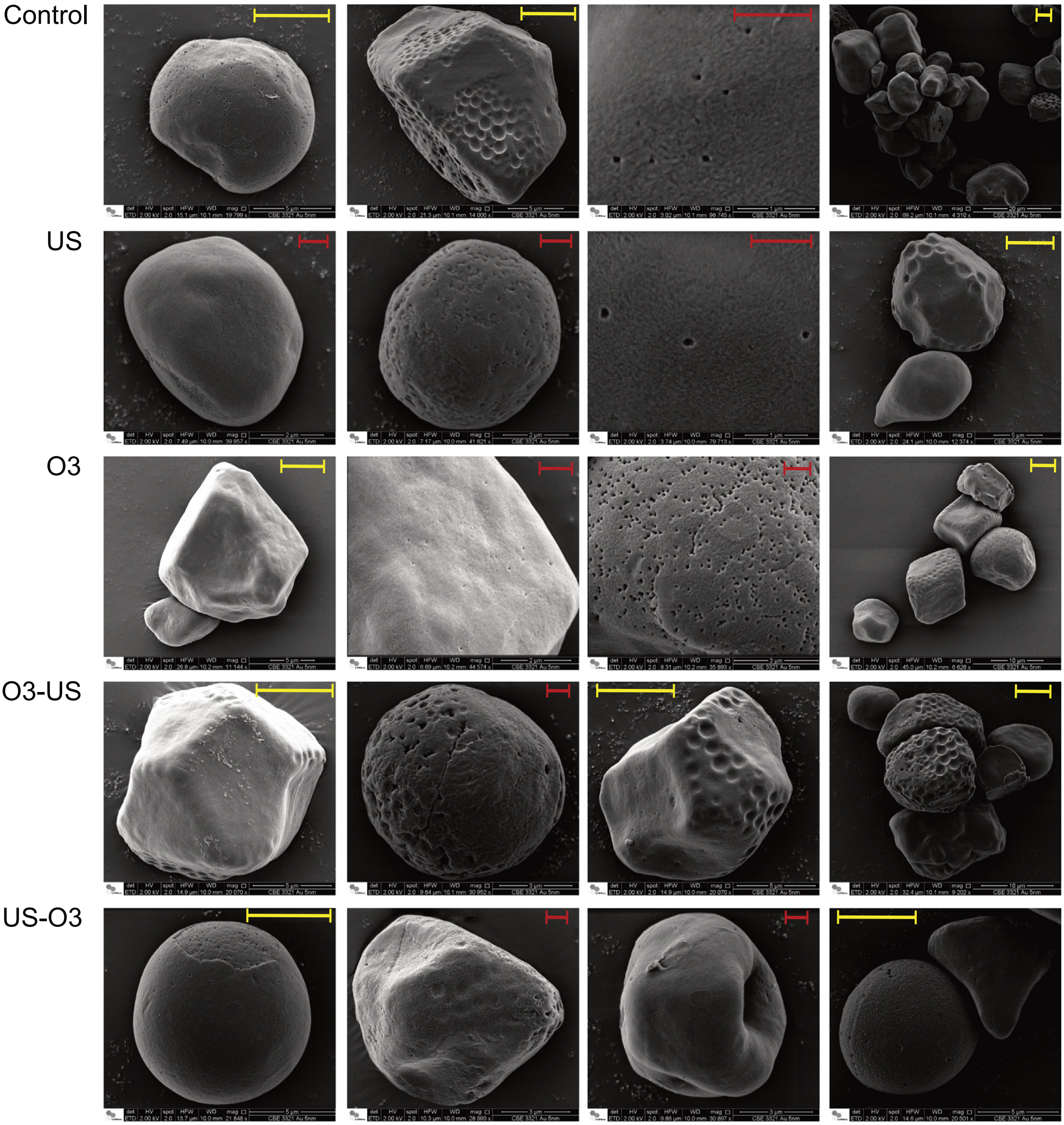

Fig. 3. Scanning electronic microscopy (SEM) images of the control and modified (US, O_3, O_3-US and US-O_3) maize starch samples. Yellow lines measure 5 μm, and red lines measure 1 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

US alone processing does not change the starch molecules, but it can promote changes on the starch granules, that affect the way how ozone processing interacts with it (i.e. US-O_3 treatment).

Summarizing, US process by itself did not led to any significant changes in the starch molecules structure. The carbonyl and carboxyl groups, and consequently the pH, remained unchanged, as well as its molecular size distribution and apparent amylose content. Conversely, ozonated samples (O_3, O_3-US and US-O_3), with few exceptions, showed significant changes ($p > 0.05$) in all the evaluated parameters, indicating that the ozone processing, alone or in combination with the US, led to significant molecular changes in maize starch.

Therefore, it is important to evaluate the effect of these processes on the starch granule, in order to better understand each effect and test our hypothesis concerning the US treatment, as follows.

3.2. Characteristics of the starch granules

The characteristics of the starch granules were also investigated, in order to elucidate if the modification processes had any influence on their morphology. As discussed in the Material and methods section, methods used were scanning electronic microscopy (SEM, Fig. 3), particle size distribution (PSD, Fig. 4) and X-ray analysis to measure granule crystallinity (Fig. 5).

3.2.1. Granule morphology: scanning electronic microscopy (SEM)

The starch granule morphology is illustrated in Fig. 3. The control sample presented small and irregular granules, as expected for native maize starch, and some of the granules also presented pores, roughness, craters and depressions on their surfaces. Similarly, the modified samples exhibited irregular granules whose surface had pores, roughness, craters and depressions, meaning that no notable changes were observed between the control and the modified samples.

Other works on the literature reports the appearance of pores on the surface of different starch samples after ultrasound processing [44,49,50]. Some hypothesis can be raised to explain why no differences were observed in the present work. The first one is related with the naturally heterogeneous and irregular granules shown by the native maize starch, which makes it difficult to notice any changes on their characteristics (in special using a qualitative tool such as SEM). Another explanation is that the US and O_3 treatments, with the conditions applied in this work, were not enough to change the maize starch granules' morphology. In fact, Sujka and Jamroz [44], by comparing the US effect on the surface characteristics of different starch samples, observed that potato and wheat starches where more affected by the US treatment than rice and maize starches, which have smaller granules.

It is important to mention, however, that although US and O_3 processing was not sufficient to change the granule morphology, results presented next and related to the starch properties reinforces the hypothesis that US processing promotes some changes on the granules, which further affects how ozone processing interact with them (US-O_3 treatment). Our hypothesis is that those changes are originated at the centre of the granules. Sujka and Jamroz [44], using transmission electronic microscopy (TEM), observed not only pores, but also a cavity of irregular shape in the centre of the granules after ultrasound processing, and those cavities where independent of the starch botanical origin. Further studies are needed to clarify this topic.

3.2.2. Granule size: particle size distribution (PSD)

PSD results of the control and modified starch samples, as well as its mean particle diameters, are illustrated in Fig. 4. As illustrated, PSD of the modified samples did not present any remarkable difference when compared to the control (Fig. 4), regardless of having been processed with ozone and/or ultrasound. Luo et al. [50] did not observe changes on the maize starch shape and particle size after processing with ultrasound. Castanha et al. [9], on the other hand, reported a decrease on the volume-based mean particle diameter of potato starch processed with ozone, indicating that the process decreased the size of the bigger granules, but not of the smaller ones. The authors concluded that the bigger granules could be more accessible to ozone treatment. It is interesting to note that Carmona-Garcia et al. [51] indicates that larges granules are also more affected by the US waves than smaller ones, which can partially explain the results obtained in this work (potato starch granules are much higher than maize).

Results showed that the US and O_3 treatments, did not promote apparent changes on the granules' morphology, neither changed their sizes and shapes.

3.2.3. Granule crystallinity: X-ray diffraction

The crystallinity characteristics of the native and modified starch granules are illustrated in Fig. 5. Firstly, control and modified starches can be classified as samples of an A-type, which is typical of cereals [52,53]. In fact, after the modification processes, no changes were observed on the X-ray diffraction patterns of the samples, which maintained the same pattern and relative crystallinity ($p < 0.05$).

Furthermore, some authors [54,55] have suggested a new approach to evaluate the X-ray diffraction patterns of starches. In this approach, the X-ray patterns are compared to an α-amylose standard (PDF 43-1858), available in a database of Powder Diffraction Files (PDF-4) [56]. It worth to be mentioned that the standard amylopectin pattern is still

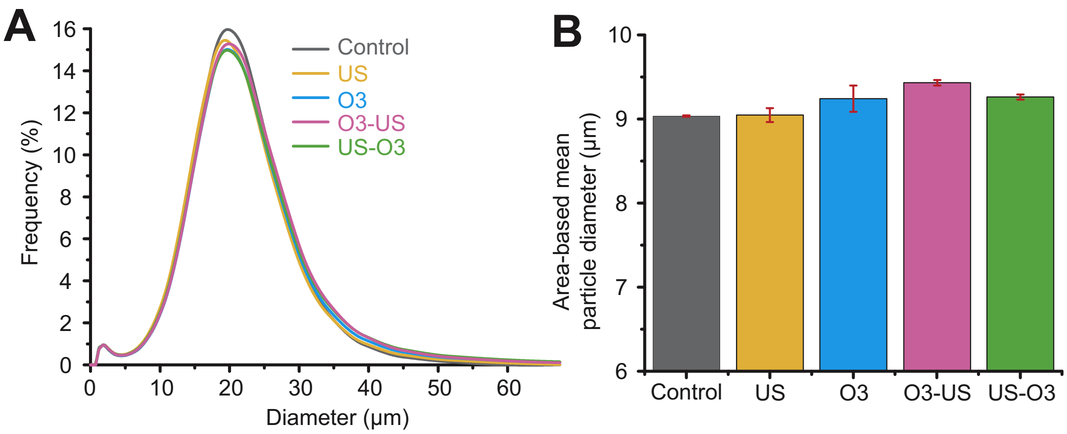

Fig. 4. Particle size distribution (PSD) based on the volume (A); and area-based mean particle diameter (D [2,3]) (B) of the control and modified (US, O_3, O_3-US and US-O_3) maize starch samples. Vertical red bars represent the standard deviations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

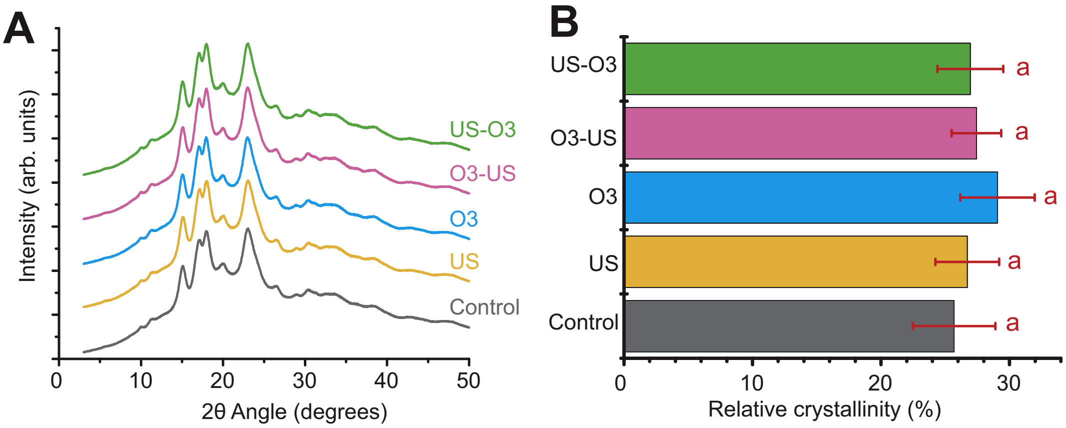

Fig. 5. X-ray diffraction patterns (A); and relative crystallinity (B, calculating from 2θ angle from 3° to 36°) of the control and modified (US, O_3, O_3-US and US-O_3) maize starches. Horizontal red bars represent the standard deviations. Results followed by the same small letter do not differ significantly ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

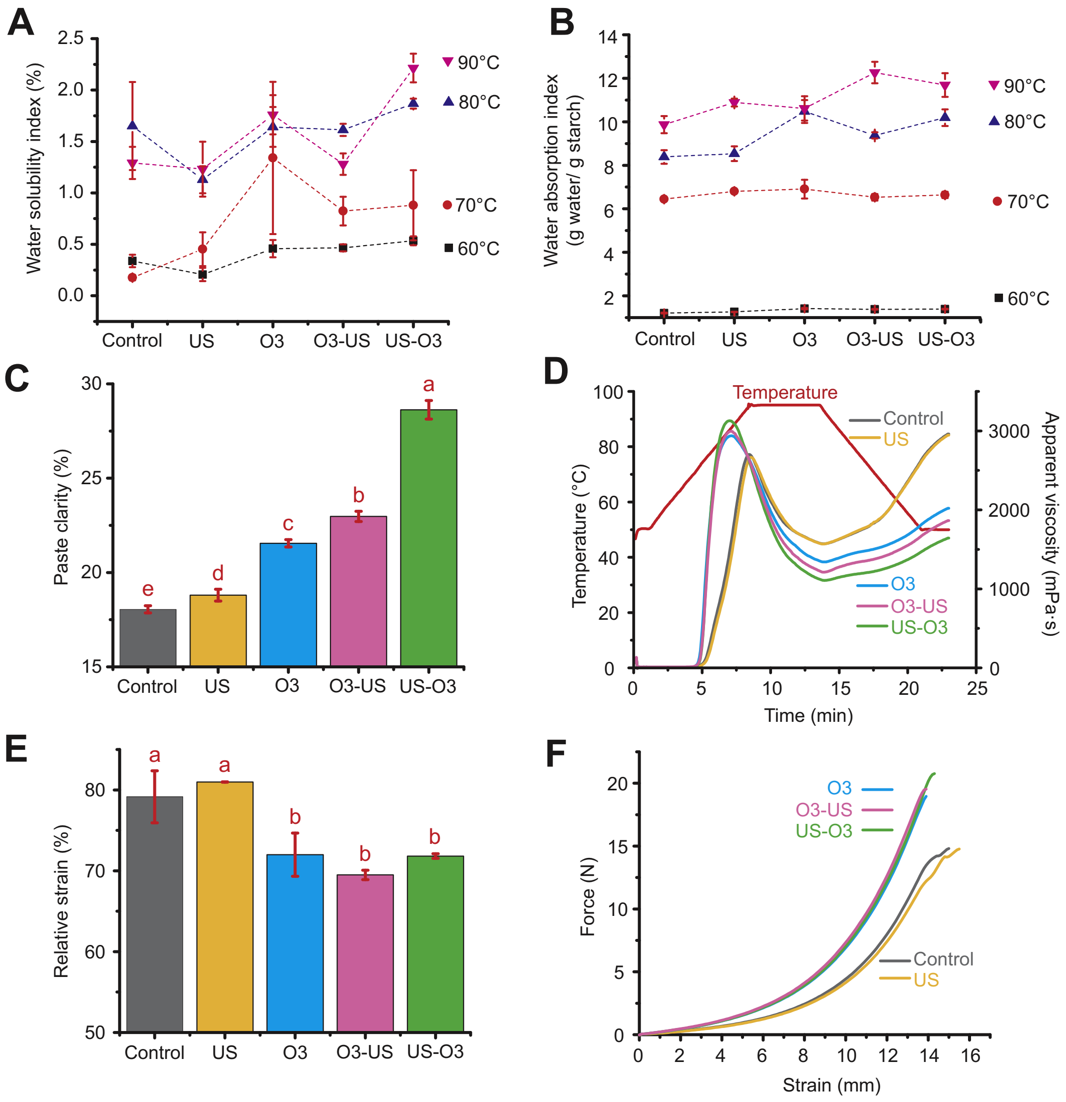

Fig. 6. Water solubility index (WSI) (A); water absorption index (WAI) (B); paste clarity (C); pasting properties (D); relative strain of rupture during compression of the starch gels (E); and stress until rupture of the starch gels (F) of the control and modified (US, O_3, O_3-US and US-O_3) maize starch samples. Vertical red bars represent the standard deviations. Results followed by the same small letter do not differ significantly ($p < 0.05$). Dashed lines on (A) and (B) are traced only to facilitate the interpretation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Water absorption index (WAI) and water solubility index (WSI) of the control and modified (US, O_3, O_3-US and US-O_3) maize starch samples. Average ± standard deviation. Results followed by the same small letter do not differ significantly ($p < 0.05$).

Water absorption index (g water/g starch)

Samples	60 °C				70 °C				80 °C				90 °C			
Control	1.2	±	0.0	c	6.4	±	0.1	a	8.4	±	0.3	b	9.9	±	0.4	c
US	1.3	±	0.0	b	6.8	±	0.1	a	8.5	±	0.3	bc	10.9	±	0.2	bc
O_3	1.4	±	0.0	a	6.9	±	0.4	a	10.5	±	0.5	a	10.6	±	0.6	bc
O_3-US	1.4	±	0.0	a	6.5	±	0.2	a	9.4	±	0.1	cd	12.3	±	0.5	a
US-O_3	1.4	±	0.0	a	6.6	±	0.1	a	10.2	±	0.4	ad	11.7	±	0.5	ab

Water solubility index (%)

Samples	60 °C				70 °C				80 °C				90 °C			
Control	0.3	±	0.1	cb	0.2	±	0.0	b	1.7	±	0.4	ab	1.3	±	0.2	bc
US	0.2	±	0.1	c	0.5	±	0.2	ab	1.1	±	0.1	b	1.2	±	0.3	b
O_3	0.5	±	0.1	ab	1.3	±	0.7	a	1.6	±	0.2	ab	1.8	±	0.2	ac
O_3-US	0.5	±	0.0	ab	0.8	±	0.1	ab	1.6	±	0.1	ab	1.3	±	0.1	bc
US-O_3	0.5	±	0.0	a	0.9	±	0.3	ab	1.9	±	0.0	a	2.2	±	0.1	a

under study, and that the amylose sample used as reference for the PDF database was not from maize starch. Nevertheless, this approach allows to compare each peak with its corresponding source. For example, the peaks located at ~20° and ~22.5° are related to the amylose crystalline structure. Therefore, by analysing results using this approach, it is possible to say that neither the amylose nor the amylopectin crystals were affected by the modification process, since the peaks locations were maintained. These results are also supported by the relative crystallinity results (Fig. 5B). Similar results (no changes on the crystalline domains) were found by Luo et al. [50], while studying maize starch modification using ultrasound, and by Castanha et al. [8], while studying potato starch modification using ozone.

3.2.4. Granule structure evaluation: overview

To sum up, regarding the starch granules, it was possible to conclude that their characteristics were apparently maintained, since no changes on their morphology, PSD and crystallinity were observed after the modification processes. However, internal changes were not measured, but as stated by other researchers (e.g. Sujka and Jamroz [44]) using TEM, observed internal modifications could be responsible for observed changes on the starch molecules and their properties after treatments.

3.3. Starch properties

Practical applications of modified starches are one of the main goals of the modification processes. Therefore, some of the functional properties of the modified starches were evaluated and correlated with the structure characteristics described earlier. Water absorption (WAI) and solubility (WSI) indexes, paste clarity, pasting properties (RVA) and gel strength are illustrated in Fig. 6. Tables containing the main results and statistical analysis concerning the WAI and WSI (Table 1) and RVA (Table 2) analyses are also presented.

3.3.1. Water absorption (WAI) and water solubility (WSI) indexes

As expected, WSI (Fig. 6A) increased with increasing temperature. At 60 °C, the ozonated samples (O_3, O_3-US and US-O_3) showed a tendency to present a higher solubility, although the changes did not result statistically different. At 70 °C, the same behaviour was observed, being the O_3 sample the only that presented a significant difference ($p > 0.05$) when compared to the control sample. At 90 °C, the US-O_3 sample presented the higher solubility of all samples, being statistically equal only to the O_3 sample. Overall, results did not follow a linear increase and it is possible to conclude that O_3 processing apparently increased the starch solubility. On the other hand, US processing alone had no influence on the starch solubility, which is in accordance with results previously discussed (no molecular or granular changes were observed for this sample).

WAI results (Fig. 6B) were also temperature dependent and increased with temperature. The highlights, once again, are observed in the ozonated samples (O_3, O_3-US and US-O_3). At 80 °C, the O_3 and the US-O_3 treatments showed the highest WAI, followed by the O_3-US treatment, whereas at 90 °C, the O_3-US and US-O_3 treatments showed the highest WAI values, being the only treatments different from the control ($p > 0.05$). The US treatment showed no significant differences with the control at temperatures >70 °C. However, at 60 °C, this sample presented a higher absorption capacity when compared to the control sample, indicating that the US treatment may have affected the starch structure (as previously discussed) in a way that promote the increase of the granules water uptake capacity. In fact, as this analysis is temperature-dependent, those results can be related with the integrity

Table 2
Main parameters of the pasting properties of the control and modified (US, O_3, O_3-US and US-O_3) maize starch samples. Average ± standard deviation. Results followed by the same small letter do not differ significantly ($p < 0.05$). AV = apparent viscosity. Relative breakdown was calculated as: (breakdown / peak AV) · 100. Relative setback was calculated as: (setback / trough AV) · 100.

Samples	Peak AV (mPa·s)				Trough AV (mPa·s)				Relative breakdown (%)				Final AV (mPa·s)				Relative setback (%)				Pasting T (°C)			
Control	2703	±	16	c	1571	±	48	a	42	±	2	c	2961	±	28	a	89	±	5	a	76	±	1	a
US	2694	±	2	c	1615	±	18	a	40	±	1	c	2975	±	17	a	84	±	3	a	75	±	3	ab
O_3	2960	±	1	b	1350	±	47	b	54	±	2	b	2041	±	5	b	51	±	5	b	72	±	1	b
O_3-US	3162	±	13	a	1170	±	35	c	63	±	1	a	1721	±	4	c	47	±	5	b	72	±	1	b
US-O_3	3206	±	23	a	1106	±	17	c	66	±	0	a	1629	±	18	d	47	±	4	b	71	±	3	b

of the granules, since a granule with a damaged structure can present better water absorption at lower temperatures than at higher ones where they may breakdown faster [57].

Sujka et al. [44], processed maize starch in water using an ultrasonic homogenizer, observing an increase of the swelling power and solubility of the samples. Similarly, Luo et al. [50] observed an increase of the swelling power and solubility of normal maize starch processed with ultrasound. Both authors hypothesized (but did not demonstrate) that the results may be related to depolymerization of the starch molecules after ultrasound processing, which was not observed in our work (Fig. 2C and D). It is important to remark that in the mentioned studies the concentration of the processed starch slurries was higher (30%). This and a different system used compared to our work may have influenced the results.

On the other hand, increasing solubility after ozonation were observed by different authors [9,12,13], while the WAI presented different results depending on the starch source and process conditions. Despite different results, the authors related the observed behaviour with the depolymerization of the starch molecules and with the oxidation of the hydroxyl groups to carbonyl and carboxyl groups after the ozone processing, which is in accordance with our results (Fig. 2).

3.3.2. Paste clarity

Paste clarity (Fig. 6C) was significant higher ($p > 0.05$) for all modified starches when compared with the control. The US-O_3 sample stood out, presenting the highest value, which was ~61% higher than the control sample. This is a remarkable result by considering the importance of the paste clarity for several starchy final food products [44].

Our theory to explain this property (US-O_3 sample presenting a higher paste clarity) is once more related to the possible modification caused by the previous US treatment on the internal structure of the maize starch. Although no apparent increase in pores on the surface of the samples was observed, results are showing that the US treatment apparently promoted ozone action, by facilitating its action on the interior of the starch granule. In fact, the weakening of the granules' structure induced by the US treatment has been reported in several works, and it is already being used to facilitate other subsequent processes, such as to facilitate both acid and enzymatic starch hydrolysis and to obtain porous starch granules using amylase, or starch nanoparticles [57,58].

The evidence for this hypothesis is the US-O_3 sample presenting some particularities when compared to the O_3-US sample, like for example the tendency of this sample to have a higher carbonyl content (Fig. 2A) and a lower pH (Fig. 2B), a significant decrease on the apparent amylose content (Fig. 2C), an apparent lower molecular size (Fig. 2D), and higher paste clarity.

3.3.3. Pasting properties and gel texture

The RVA (Fig. 6D) and the gel texture (Fig. 6E and F) presented the same pattern observed throughout this work, in which the ozonated samples (O_3, O_3-US and US-O_3) stood out from the others, agreeing with the previous results. In fact, the US sample did not differ significantly from the control sample in any pasting or gel texture parameters. In general, the ozonated starch samples (O_3, O_3-US and US-O_3) presented higher paste viscosities, lower relative setbacks and harder and less elastic gels (evidenced by the lower relative strain, Fig. 6E). Those characteristics can be useful in some food applications, such as gravies, dips, sauces and puddings [59].

However, the effect of combining the technologies was evidenced on the RVA analysis, indicating that, despite no molecular or granular significant differences between them or when compared to the O_3 sample, their pasting properties had some particularities. Among the results, we highlight the peak apparent viscosity (AV), which was higher when applying the combination of the technologies (US-O_3 and O_3-US), and their relative setback, which was lower. Those results indicate that the starches were modified by the combined treatments and showed a more consistent paste and a lower tendency to retrogradation. Our results are in discordance with the observed by İbanoğlu et al. [28] while studying the effects of the combination of ozone and ultrasound on the rheological properties of rice starch. The authors observed a higher viscosity after the ultrasound processing, while after the ozone processing and its combination with ultrasound, the starch viscosity decreased. Also, in their work, the order at which the processes were applied had no influence on the results.

3.3.4. Starch properties evaluation: overview

Summarizing, in general the starch properties were affected by the treatments. The ozone process, alone or in combination, once more showed different results when compared to the control and starches treated only with US, which was similar to the control sample. However, results show that the US treatment is apparently able to change the starch structure, improving ozone action. As previously discussed, the main hypothesis for this behavior is possible internal modifications within the granules caused by the ultrasound action. Therefore, although the US technology seemed to be ineffective when using alone, it could be very useful to potentialize the ozone effects, proving to be a potential effective alternative for starch modification.

4. Conclusions

The results illustrated that, at the conditions applied in this work, the US technology by itself had no measurable influence on the starch structure and properties. On the other hand, the ozone proved to be an effective technology to modify maize starch, alone or in combination with US. The ozonated samples (O_3, O_3-US and US-O_3) exhibited significant changes on the starch's molecular structure (carbonyl and carboxyl groups, pH, molecular size distribution and apparent amylose content) and properties (WAI, WSI, pasting properties, gel strength and paste clarity). On the other hand, the surface morphology, PSD and crystallinity of the maize starch samples were not affected by any treatment, alone or combined.

It is worth highlighting that the paste clarity was significant higher when a combination of the treatments was applied, especially when the US was used prior the O_3 treatment, illustrating the hypothesis that US changed the starch structure, improving the ozone action. However, further studies are needed to better understand the phenomena and optimize processing, comprising conditions of processing and starch sources.

Declaration of Competing Interest

The authors have declared no conflict of interest.

Acknowledgements

The authors are grateful to:

- the São Paulo Research Foundation (FAPESP, Brazil) for funding the project n° 2016/18052-5;
- the National Council for Scientific and Technological Development (CNPq, Brazil) for funding the project n° 401004/2014-7 and the productivity grant of P.E.D. Augusto (306557/2017-7);
- this study was financed in part by the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil)" - Finance Code 001, through the N. Castanha and D.C. Lima Ph.D. scholarships;
- the Technology Center of Cereal and Chocolate of Food Technology Institute (ITAL), and Dra. Izabela Dutra Alvim, for assistance and facilities for the PSD analysis;

- the "Laboratório Multiusuário de Caracterização de Materiais" (MultiMat/ZEB-FZEA/USP), in the name of Mr. Leonardo Martins Pozzobon and Prof. Dr. Elíria M. J. A. Pallone, for assistance and facilities of X-ray diffraction analysis;
- The LNNano (CNPEM, Brazil) for technical support and facilities during electron microscopy work (Project SEM-24192);
- Dr. Caio C. L. Silva and M.Sc. Meliza L. Rojas, for general support.

References

[1] H. Kochkar, M. Morawietz, W.F. Hölderich, Oxidation of potato starch with NO_2: characterization of the carboxylic acid salts, Appl. Catal. A Gen. 210 (2001) 325–328, https://doi.org/10.1016/S0926-860X(00)00815-2.

[2] S.D. Zhang, Y.R. Zhang, X.L. Wang, Y.Z. Wang, High carbonyl content oxidized starch prepared by hydrogen peroxide and its thermoplastic application, Starch/Staerke 61 (2009) 646–655, https://doi.org/10.1002/star.200900130.

[3] B. Kaur, F. Ariffin, R. Bhat, A.A. Karim, Progress in starch modification in the last decade, Food Hydrocoll. 26 (2012) 398–404, https://doi.org/10.1016/j.foodhyd.2011.02.016.

[4] O.B. Wurzburg, Modified Starches: Properties and Uses, CRC Press, Boca Raton, FL, 1986.

[5] Q. Liu, E. Weber, V. Currie, R. Yada, Physicochemical properties of starches during potato growth, 51 (2003) 213–221.

[6] A.K. Mahapatra, K. Muthukumarappan, J.L. Julson, Applications of ozone, bacteriocins and irradiation in food processing: a review, Crit. Rev. Food Sci. Nutr. 45 (2005) 447–461, https://doi.org/10.1080/10408390591034454.

[7] H. Çatal, Ş. İbanoğlu, Effect of aqueous ozonation on the pasting, flow and gelatinization properties of wheat starch, LWT - Food Sci. Technol. 59 (2014) 577–582, https://doi.org/10.1016/j.lwt.2014.04.025.

[8] N. Castanha, M.D. Matta Junior, P.E.D. Augusto, Potato starch modification using the ozone technology, Food Hydrocoll. 66 (2017) 343–356, https://doi.org/10.1016/j.foodhyd.2016.12.001.

[9] N. Castanha, D.N. Santos, R.L. Cunha, P.E.D. Augusto, Properties and possible applications of ozone-modified potato starch, Food Res. Int. (2018)https://doi.org/10.1016/j.foodres.2018.09.064 #pagerange#.

[10] H. Çatal, Ş. İbanoğlu, Effects of ozonation on thermal, structure and rheological properties of rice starch in aqueous solution, 38 (2013) 63–70.

[11] B. Klein, N.L. Vanier, K. Moomand, V.Z. Pinto, R. Colussi, E. Da Rosa Zavareze, A.R.G. Dias, Ozone oxidation of cassava starch in aqueous solution at different pH, Food Chem. 155 (2014) 167–173, https://doi.org/10.1016/j.foodchem.2014.01.058.

[12] A.O. Oladebeye, A.A. Oshodi, I.A. Amoo, A.A. Karim, Functional, thermal and molecular behaviours of ozone-oxidised cocoyam and yam starches, Food Chem. 141 (2013) 1416–1423, https://doi.org/10.1016/j.foodchem.2013.04.080.

[13] H.T. Chan, R. Bhat, A.A. Karim, Physicochemical and functional properties of ozone-oxidized starch, J. Agric. Food Chem. 57 (2009) 5965–5970, https://doi.org/10.1021/jf9008789.

[14] D.J. McClements, Advances in the application of ultrasound in food analysis and processing, Trends Food Sci. Technol. 6 (1995) 293–299, https://doi.org/10.1016/S0924-2244(00)89139-6.

[15] Z.J. Dolatowski, J. Stadnik, D. Stasiak, Applications of ultrasound in food technology, Acta Sci. Pol. 63 (2007) 89–99.

[16] S. Yu, Y. Zhang, Y. Ge, Y. Zhang, T. Sun, Y. Jiao, X.Q. Zheng, Effects of ultrasound processing on the thermal and retrogradation properties of nonwaxy rice starch, J. Food Process Eng. 36 (2013) 793–802, https://doi.org/10.1111/jfpe.12048.

[17] S.R. Falsafi, Y. Maghsoudlou, H. Rostamabadi, M.M. Rostamabadi, H. Hamedi, S.M.H. Hosseini, Preparation of physically modified oat starch with different sonication treatments, Food Hydrocoll. 89 (2018) 311–320, https://doi.org/10.1016/j.foodhyd.2018.10.046.

[18] Y. Monroy, S. Rivero, M.A. García, Microstructural and techno-functional properties of cassava starch modified by ultrasound, Ultrason. Sonochem. 42 (2018) 795–804, https://doi.org/10.1016/j.ultsonch.2017.12.048.

[19] A. Hu, S. Jiao, J. Zheng, L. Li, Y. Fan, L. Chen, Z. Zhang, Ultrasonic frequency effect on corn starch and its cavitation, LWT - Food Sci. Technol. 60 (2015) 941–947, https://doi.org/10.1016/j.lwt.2014.10.048.

[20] A.R. Jambrak, Z. Herceg, D. Šubarić, J. Babić, M. Brnčić, S.R. Brnčić, T. Bosiljkov, D. Čvek, B. Tripalo, J. Gelo, Ultrasound effect on physical properties of corn starch, Carbohydr. Polym. 79 (2010) 91–100, https://doi.org/10.1016/j.carbpol.2009.07.051.

[21] Y. Iida, T. Tuziuti, K. Yasui, A. Towata, T. Kozuka, Control of viscosity in starch and polysaccharide solutions with ultrasound after gelatinization, Innov. Food Sci. Emerg. Technol. 9 (2008) 140–146, https://doi.org/10.1016/j.ifset.2007.03.029.

[22] J. Zhu, L. Li, L. Chen, X. Li, Study on supramolecular structural changes of ultrasonic treated potato starch granules, Food Hydrocoll. 29 (2012) 116–122, https://doi.org/10.1016/j.foodhyd.2012.02.004.

[23] Y.Y.J. Zuo, P. Hébraud, Y. Hemar, M. Ashokkumar, Quantification of high-power ultrasound induced damage on potato starch granules using light microscopy, Ultrason. Sonochem. 19 (2012) 421–426, https://doi.org/10.1016/j.ultsonch.2011.08.006.

[24] R. Czechowska-Biskup, B. Rokita, S. Lotfy, P. Ulanski, J.M. Rosiak, Degradation of chitosan and starch by 360-kHz ultrasound, Carbohydr. Polym. 60 (2005) 175–184, https://doi.org/10.1016/j.carbpol.2004.12.001.

[25] B.K. Tiwari, K. Muthukumarappan, C.P. O'Donnell, P.J. Cullen, Rheological properties of sonicated guar, xanthan and pectin dispersions, Int. J. Food Prop. 13 (2010) 223–233, https://doi.org/10.1080/10942910802317610.

[26] A. Vercet, C. Sánchez, J. Burgos, L. Montañés, P. Lopez Buesa, The effects of manothermosonication on tomato pectic enzymes and tomato paste rheological properties, J. Food Eng. 53 (2002) 273–278, https://doi.org/10.1016/S0260-8774(01)00165-0.

[27] M. Vinatoru, Ultrasonically assisted extraction (UAE) of natural products some guidelines for good practice and reporting, Ultrason. - Sonochemistry. 25 (2015) 94–95, https://doi.org/10.1016/j.ultsonch.2014.10.003.

[28] Ş. İbanoğlu, Z.T. Özaslan, E. İbanoğlu, Combined effect ozonation and ultrasonication on rheological and thermal properties of rice starch in aqueous phase, Qual. Assur. Saf. Crop. Foods. (2018) 1–6, https://doi.org/10.3920/QAS2017.1096.

[29] Ş. Ibanoğlu, Z.T. Özaslan, E. Ibanoğlu, Effects of ultrasonication and aqueous ozonation on gelatinization and flow properties of potato starch effects of ultrasonication and aqueous ozonation on gelatinization and flow, Ozone Sci. Eng. 00 (2017) 1–8, https://doi.org/10.1080/01919512.2017.1398634.

[30] C.P. O'Donnell, B.K. Tiwari, P. Bourke, P.J. Cullen, Effect of ultrasonic processing on food enzymes of industrial importance, Trends Food Sci. Technol. 21 (2010) 358–367, https://doi.org/10.1016/j.tifs.2010.04.007.

[31] S. Chattopadhyay, R.S. Singhal, P.R. Kulkarni, Optimisation of conditions of synthesis of oxidised starch from corn and amaranth for use in film-forming applications, Carbohydr. Polym. 34 (1997) 203–212, https://doi.org/10.1016/s0144-8617(97)87306-7.

[32] R.J. Smith, Characterization and analysis of starches, in: R.L. Whistler, E.F. Paschall (Eds.), Starch Chem. Technol, 1st ed.Academic Press, New York 1967, pp. 569–635.

[33] I. Adolfo Lutz, Normas analíticas do Instituto Adolfo Lutz: Métodos físicos e químicos para análises de alimentos, 2008 1020.

[34] Y. Song, J. Jane, Characterization of barley starches of waxy, normal, and high amylose varieties, Carbohydr. Polym. 41 (2000) 365–377, https://doi.org/10.1016/S0144-8617(99)00098-3.

[35] B.O. Juliano, A simplified assay for milled-rice amylose, Cereal Sci. Today. 16 (1971) 334–340 (doi:0009-0360).

[36] I. Organization for Standarization-ISO, Determination de La teneur em amylose. Norme ISO 6647, 1987.

[37] S. Nara, T. Komiya, Studies on the relationship between water-satured state and crystallinity by the diffraction method for moistened potato starch, Starch - Stärke 35 (1983) 407–410, https://doi.org/10.1002/star.19830351202.

[38] S.S.A. Craig, C.C. Maningat, P.A. Seib, R.C. Hoseney, Starch paste clarity, Cereal Chem. 66 (1989) 173–182.

[39] K.S. Aplevicz, I.M. Demiate, Caracterização de amidos de mandioca nativos e modificados e utilização em produtos panificados, Ciên. Tecnol. Aliment. 27 (2007) 478–484, https://doi.org/10.1590/S0101-20612007000300009.

[40] R.A. Anderson, H.F. Conway, A.J. Peplinski, Gelatinization of corn grits by roll cooking, extrusion cooking and steaming, Starch - Stärke 22 (1970) 130–135, https://doi.org/10.1002/star.19700220408.

[41] M. Boruch, Transformations of potato starch during oxidation with hypochlorite, Starch - Stärke 37 (1985) 91–98, https://doi.org/10.1002/star.19850370306.

[42] O.B. Wurzburg, Converted starches, in: O.B. Wurzburg (Ed.), Modif. Starches Prop. Uses, 1st ed.CRC Press, Boca Raton, FL 1986, pp. 17–40.

[43] P. Forssell, A. Hamunen, K. Autio, P. Suortti, K. Poutanen, Hypochlorite oxidation of barley and potato starch, Starch - Stärke 47 (1995) 371–377, https://doi.org/10.1002/star.19950471002.

[44] M. Sujka, J. Jamroz, Ultrasound-treated starch: SEM and TEM imaging, and functional behaviour, Food Hydrocoll. 31 (2013) 413–419, https://doi.org/10.1016/j.foodhyd.2012.11.027.

[45] Q. Liu, Understanding starches and their role in foods, in: S.W. Cui (Ed.), Food Carbohydrates Chem. Phys. Prop. Appl, 1st ed.CRC Press, Boca Raton, FL 2005, pp. 309–355.

[46] J.C. Shannon, D.L. Garwood, C.D. Boyer, Genetics and physiology of starch development, in: J.N. BeMiller, R.L. Whistler (Eds.), Starch Chem. Technol, 3rd ed.Academic Press, London, UK 2009, pp. 23–82.

[47] J. Jane, Y.Y. Chen, L.F. Lee, A.E. McPherson, K.S. Wong, M. Radosavljevic, T. Kasemsuwan, Effects of amylopectin branch chain-length and amylose content on the gelatinization and pasting properties of starch, Cereal Chem. 52 (1999) 555, https://doi.org/10.1094/CCHEM.1999.76.5.629.

[48] S. Hizukuri, J. Abe, I. Hanashiro, Starch: analytical aspects, in: A. Eliasson (Ed.), Carbohydrates Food, 2nd ed.CRC Press, Boca Raton, FL 2006, pp. 305–390.

[49] M. Sujka, Ultrasonic modification of starch — impact on granules porosity, Ultrason. Sonochem. 37 (2017) 424–429, https://doi.org/10.1016/j.ultsonch.2017.02.001.

[50] Z. Luo, X. Fu, X. He, F. Luo, Q. Gao, S. Yu, Effect of ultrasonic treatment on the physicochemical properties of maize starches differing in amylose content, Starch/Staerke 60 (2008) 646–653, https://doi.org/10.1002/star.200800014.

[51] R. Carmona-Garcia, L.A. Bello-Perez, A. Aguirre-Cruz, A. Aparicio-Saguilan, J. Hernandez-Torres, J. Alvarez-Ramirez, Effect of ultrasonic treatment on the morphological, physicochemical, functional, and rheological properties of starches with different granule size, Starch - Stärke 68 (2016) 972–979, https://doi.org/10.1002/star.201600019.

[52] H.F. Zobel, Starch crystal transformations and their industrial importance, Starch - Stärke 40 (1988) 1–7, https://doi.org/10.1002/star.19880400102.

[53] N.W.H. Cheetham, L. Tao, Variation in crystalline type with amylose content in maize starch granules: an X-ray powder diffraction study, Carbohydr. Polym. 36 (1998) 277–284, https://doi.org/10.1016/S0144-8617(98)00007-1.

[54] L. Alonso-Gomez, A.M. Niño-López, A.M. Romero-Garzón, P. Pineda-Gomez, A. del Real-Lopez, M.E. Rodriguez-Garcia, Physicochemical transformation of cassava starch during fermentation for production of sour starch in Colombia, Starch/Staerke 68 (2016) 1139–1147, https://doi.org/10.1002/star.201600059.

[55] S.M. Londoño-Restrepo, N. Rincón-Londoño, M. Contreras-Padilla, A.A. Acosta-Osorio, L.A. Bello-Pérez, J.C. Lucas-Aguirre, V.D. Quintero, P. Pineda-Gómez, A. del Real-López, M.E. Rodríguez-García, Physicochemical, morphological, and rheological characterization of Xanthosoma robustum Lego-like starch, Int. J. Biol. Macromol. 65 (2014) 222–228, https://doi.org/10.1016/j.ijbiomac.2014.01.035.

[56] J. Faber, T. Fawcett, The powder diffraction file: present and future, Acta Crystallogr. Sect. B Struct. Sci. 58 (2002) 325–332, https://doi.org/10.1107/S0108768102003312.

[57] J.N. Bemiller, Physical modification of starch, in: M. Sjoo, L. Nilsson (Eds.), Starch Food, 2nd ed.Elsevier Ltd, United Kingdom 2018, pp. 223–253, https://doi.org/10.1016/B978-0-08-100868-3.00005-6.

[58] J.N. Bemiller, K.C. Huber, Physical modification of food starch functionalities, Annu. Rev. Food Sci. Technol. 6 (2015) 19–69, https://doi.org/10.1146/annurev-food-022814-015552.

[59] Y. Chen, L. Kaur, J. Singh, Chemical modification of starch, in: M. Sjoo, L. Nilsson (Eds.), Starch Food, 2nd ed.Elsevier Ltd, United Kingdom 2018, pp. 283–321, https://doi.org/10.1016/B978-0-08-100868-3.00007-X.