International Journal of Biological Macromolecules 138 (2019) 1087–1097

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Hydrogels based on ozonated cassava starch: Effect of ozone processing and gelatinization conditions on enhancing 3D-printing applications

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ARTICLE INFO

Article history:
Received 13 May 2019
Received in revised form 20 July 2019
Accepted 20 July 2019
Available online 21 July 2019

Keywords:
Cassava starch
Starch modification
Ozone
Oxidation
3D printing

ABSTRACT

Ozone is an interesting alternative for modifying starch, as it is considered an emerging and environmentally friendly technology. New applications for food ingredients are receiving attention, such as 3D printing. Consequently, the impact of emerging technologies on new applications must be understood. In this work, cassava starch was modified by ozone to evaluate its printability. Increasing ozonation time produced a starch with higher carbonyl and carboxyl contents, lower pH and molecular size, and gels with different behaviors (stronger and weaker than the native ones, as a function of processing time). The hydrogels obtained were evaluated in relation to pasting and gel properties, including their printability. The effects of starch concentration, gelatinization temperature and storage period were also evaluated. Starch ozonated for 30 min showed the lowest peak apparent viscosity at all the temperatures and starch concentrations evaluated, and provided the strongest gel. Gels produced by native starches and starches ozonated for 30 min showed good printability when the gelatinization temperature used was 65 °C, but up to this temperature, only starch ozonated for 30 min produced gels with good printability. This work highlights that, by using the ozone process to modify starch and varying the process conditions, it is possible to obtain hydrogels with enhanced pasting properties, gel texture, and printability, thereby expanding the potential of starch applications.

1. Introduction

Starches are biodegradable biopolymers obtained naturally from different sources, and widely used in different industries, such as foodstuffs, animal feed, chemicals, petrochemicals, textiles, paper, and in other materials. In the search to increase the application potential of starches for different sectors and discover new functionalities, starch modification is conducted using various methods, including chemical, physical, enzymatic and genetic modifications. Ozone technology is considered an innovative chemical treatment for modifying starch, as it is considered a green technology because ozone is rapidly decomposed into oxygen, without leaving residues in food or in the environment [1]. Ozone is a very strong oxidizing agent and is an interesting alternative to the traditional oxidation processes used in the starch industry.

Ozone processing can promote starch modification through the cleavage of the glycosidic bonds of both amylose and amylopectin molecules (by decreasing their sizes and distribution), and it also leads to the replacement of hydroxyl groups by carbonyl and carboxyl groups (by changing their charges and chemical affinity), mainly in the amorphous regions of the granules [2]. This process can be used safely in both gaseous and aqueous forms [3].

Ozone treatment has shown good results for starch modification and contributes interesting properties for different applications. Chan et al. [4] stated that no residues were observed when ozonized starches were introduced into food products. Oladebeye et al. [5] treated cocoyam and yam starches with ozone, and Klein et al. [6] worked with cassava starch, although in systems different from that used in the present work. Oladebeye et al. [5] conducted direct reactions of starch with ozone gas, while Klein et al. (2014) [6] showed that the efficiency of ozone oxidation of starch under aqueous solution is dependent on pH. Amorim et al. [3] carried out ozone treatment on cassava starch, without observing changes of color; however, they observed changes of odor. Çatal & İbanoğlu [7] observed that ozone treatment produced a modified wheat starch with higher cooking stability and lower retrogradation tendencies. These authors also reported different pasting properties, as well as structural changes in starch granules. Matta Junior

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https://doi.org/10.1016/j.ijbiomac.2019.07.124

et al. [8] conducted a mild ozone treatment on cassava starch, resulting in higher clarity, better oven expansion and a softer texture in the biscuits obtained. Castanha et al. and Castanha et al. [2,9] studied potato starch processed by ozone. They obtained materials with a low peak apparent viscosity and gels stronger than native starch, thus with characteristics interesting for certain applications, such as the 3D printing highlighted in the present proposal.

Among possible applications for starch, 3D printing is gaining attention in the food sector as it can provide new products, such as food structure customization, and customized and digitalized nutrition, thereby broadening the source of food raw materials and shortening the food supply chain [10–12]. According to Azam & Zhang [13], starch can improve the rheological properties of food matrixes to achieve higher printability for 3D applications and improve their sensory attributes. The literature comprises many works involving the application of native starches for 3D food printing [13–17]. However, the use of modified starches for this application still needs investigation. Besides its application in the food sector, the 3D printing of starch gels has been evaluated and considered for tissue engineering, as a scaffold [18] and for cartilage [19] production. The use of modified starches has not been investigated for 3D printing, though it can expand the range of starch gel applications.

Cassava (*Manihot esculenta* Crantz), also called tapioca, yucca or manioc, is a perennial shrub which is currently the sixth most important world food crop, providing sustenance for >800 million people in tropical and sub-tropical Africa, Asia and Latin America [20,21]. Cassava presents high tolerance to drought and harsh climatic conditions and around-the-year availability, making it a reliable crop for ensuring food security [22]. Cassava starch, the most essential component of cassava, is commonly used as a thickener and stabilizer in food processing to improve the convenience of the process and quality of final products. It is also used as an additive in the textile and papermaking industries [23]. Another advantage of exploiting cassava as a resource is to greatly increase the choice and diversity of staple foods for a large population, especially as a gluten-free food for people with celiac disease [24].

In this context, the aim of this work was to explore the modification of cassava starch by using ozone technology and evaluate the potential of the hydrogels obtained for 3D food printing. To this end, the effect of ozonation was first evaluated in starch structure and properties. Then, the hydrogels obtained from the modified starches were evaluated for their paste and gel properties, including the printability of the gels. The effects of starch concentration, gelatinization temperature and storage period were also evaluated.

2. Material and methods

2.1. Cassava starch and ozone processing

Native cassava starch (Amilogill 1500) was supplied by Cargill Agrícola – Brazil (moisture: 13.2 g/100 g, amylose content: 28.9 g/100 g and amylopectin content: 71.1 g/100 g). All the chemicals were of analytical grade.

The ozone processing of cassava starch was conducted using a method similar to that described by Castanha et al. [2], shown in Fig. 1. Ozone processing was carried out in a reactor with cassava starch suspended in distilled water (10 g dry starch/100 g suspension; 1500 mL) under constant stirring using a magnetic stirrer. Ozone was produced in an ozone generator unit (Ozone & Life, O&G Model RM 3.0, São José dos Campos, Brazil) using the coronal-discharge method from industrial oxygen (95% purity). The ozone-rich gas current was directed to a cylindrical glass reactor (2 L capacity, 56 cm high, and 6 cm internal diameter) via a glass tube ending in a porous gas disperser. The gas flow in the reactor was maintained constant at 1.0 L min^{-1}, and the ozone concentration in the gas current was kept at 43 mg O_3 L^{-1}. The gas flow leaving the reactor was directed to an ozone destructor, which thermally destroys the residual ozone in oxygen. After the ozone treatment, the reactor was supplied with oxygen only for a further 10 min, in order to eliminate the residual ozone. The cassava starch was processed for 15 and 30 min. Native cassava starch, referred to here as “0 min” processing, was also considered in the evaluation.

After processing, the starch suspension was maintained at rest for decanting, and the supernatant liquid was discarded later. The starch was dried in an air circulation oven (MA 035, Marconi, São Paulo, Brazil) at 35 °C until reaching a moisture content of approximately 12%. The dried starch was then macerated, sifted (250 μm) and stored for further analysis in glass containers.

2.2. Molecular characteristics of starch: determination of pH, and carbonyl and carboxyl contents

The pH of the starch suspension (10.7 g starch/100 g suspension) was obtained using a potentiometer (Tecnal, model TEC-5 mode, Piracicaba – Brazil) under constant stirring.

Carbonyl and carboxyl contents were obtained according to the method described by Smith [25] and Chattopadhyay et al. [26], respectively, with small modifications.

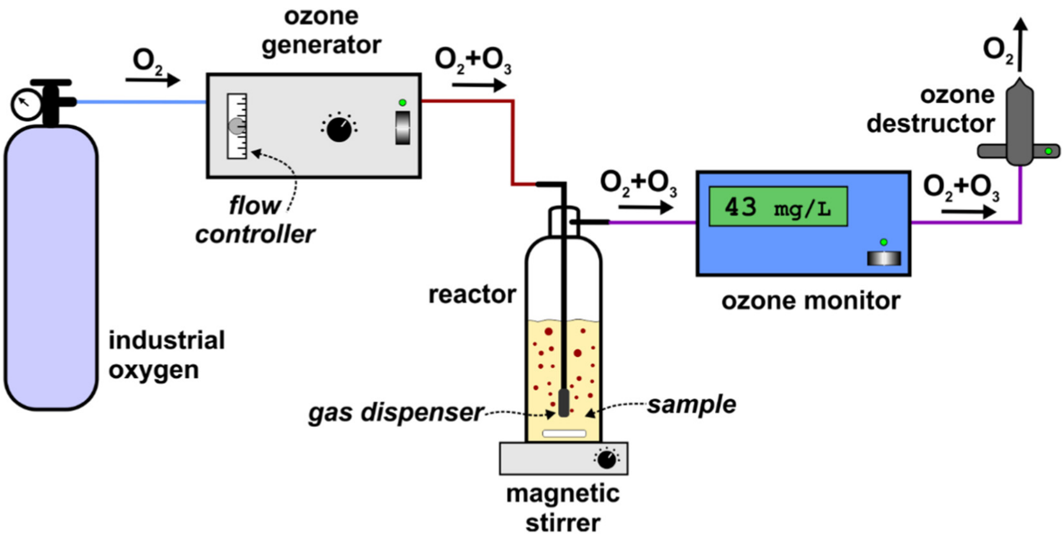


Fig. 1. Schematic representation of the ozone processing system.

For carbonyl content determination, 2 g of sample was dispersed in 100 mL of distilled water and heated in a bath with boiling water for 30 min under constant stirring. The sample was cooled to 40 °C, the pH was adjusted to 3.2 using HCl solution (0.1 M), and 15 mL of hydroxylamine chloride solution (25 g of hydroxylamine hydrochloride diluted in 100 mL of NaOH 0.5 M) was added. The samples were incubated at 40 °C for 4 h and then the excess hydroxylamine was titrated with 0.1 M HCl until pH 3.2 using an automatic titrator (Plus Titrino 848, Metrohm, Herisau, Switzerland). A blank was made without the sample but following the same procedures used for the samples. The carbonyl content was calculated as the amount of carbonyl groups in relation to 100 units of glucose (CO/100 GU) according to Eq. (1):

$$\frac{CO}{100\ GU} = \frac{(V_B - V_S).M, 0.028.100}{W} \tag{1}$$

where:

Vs = the volume of NaOH required for the ozonated samples;

Vb = the volume of NaOH used in the blank;

M = the molarity of NaOH;

W = the sample mass in dry basis.

To determine the carboxyl content, 2 g of sample was dispersed in 25 mL of HCl solution (0.1 M) in water under stirring (25 °C) for 30 min and centrifuged. The residue was filtrated using a vacuum system with a qualitative filter paper (205 μm), and then washed with 400 mL of distilled water. Next, 300 mL of distilled water was added and the dispersion was heated in a bath with boiling water under continuous stirring for 15 min. While it was still hot and under stirring, the sample was titrated with 0.01 M NaOH until reaching pH 8.3 using an automatic titrator (Plus Titrino 848, Metrohm, Herisau, Switzerland). A blank was made with the non-modified starch but following the same procedures used for the other samples. Carboxyl content was expressed as the amount of carboxyl groups in relation to 100 units of glucose (COOH/100 GU), according to Eq. (2):

$$\frac{COOH}{100GU} = \frac{(V_S - V_b).M.0.045.100}{W} \tag{2}$$

where:

Vb = the volume of HCl used in the blank;

Vs = the volume of HCl required for the sample;

M = the molarity of HCl;

W = the sample mass in dry basis.

The analyses were done in triplicate.

2.3. Starch molecular characteristics: molecular size distribution

The molecular size distribution profile of the native starch (control) and ozonated starches (15 and 30 min) were determined using a gel permeation chromatography (GPC) system, according to Song & Jane [27], with some modifications.

A glass column (diameter: 2.6 cm and high: 70 cm) packed with Sepharose CL-2B gel (Sigma, Sweden) was used. A solution with 0.1 g of starch with 10 mL of Dimethylsulfoxide (DMSO; 90%, Labsynth, Brazil) was prepared. The solution was heated in a bath with boiling water for 1 h and then kept at 25 °C for 24 h under constant stirring. An aliquot of 3 mL of this starch solution was mixed with 10 mL of absolute ethanol and then centrifuged (30 min, 3000 g). The precipitated starch was dissolved in boiling distilled water (9 mL) and heated in a bath with boiling water for 30 min. This starch solution (4 mL) was added in the chromatographic column with an eluent solution (25 mmol·L^{-1} of NaCl and 1 mmol·L^{-1} of NaOH), at a rate of 60 mL·h^{-1}. Aliquots of 4 mL were collected using a fraction collector (Gilson, model FC203B, Middleton, England) and then analyzed by the blue value method [28], using a spectrophotometer at a wavelength of 620 nm (Femto, model 600S, São Paulo - Brazil). Glucose was used as marker to indicate the end of the analysis.

2.4. Differential scanning calorimetry (DSC)

The thermal characteristics during starch gelatinization were determined by using a Multi-Cell Differential Scanning Calorimeter (MC-DSC) – (TA Instruments, Lindon, UTAH, USA). Before analysis, the samples were weighed in the ampoules (total mass (starch + water) so that each contained 700 mg, keeping the relation of 10.7 g starch/100 g). An ampoule with alumina powder was used as reference and three runs for each sample were analyzed. The MC-DSC heating program consisted in going from 30 to 130 °C at a rate of 2 °C/min. The onset temperature (To), the peak temperature (Tp), the conclusion temperature (Tc), and the enthalpy (ΔH) associated with the starch gelatinization interval were calculated with the aid of the Universal Analyzer software (TA Instruments).

2.5. Pasting properties

Starch pasting properties were determined using a Rapid Visco Analyzer - RVA-4 (Newport Scientific Pvt. Ltd., Australia, with Thermocline for Windows software, version 3.0).

In the second part of this work (characterization of the modified starches obtained), the standard conditions for starch characterization were employed, using a suspension of 10.7 g starch/100 g (correct to 14% moisture basis) and following the procedure: temperature held at 50 °C for 1 min, then increased to 95 °C (6 °C·min^{-1} and maintained for 5 min), followed by cooling to 50 °C (6 °C·min^{-1}), and finally holding at 50 °C for 2 min.

In the third part of this work (evaluation of the hydrogels obtained), the RVA behavior of starch suspensions was characterized using different concentrations (3.6 and 10.7 g/100 g, correct to 14% moisture basis) and at different gelatinization temperatures (65, 75, 85, and 95 °C). The procedure consisted in keeping the starch suspension at 50 °C for 1 min, then heating it to the specific gelatinization temperature (at 6 °C·min^{-1}), maintaining it at these temperatures for 5 min, followed by cooling to 50 °C (6 °C·min^{-1}), and finally maintaining it at 50 °C for 2 min.

The starch concentration of 10.7 g starch/100 g suspension is a standard concentration conventionally used to determine the pasting properties of the starch under standard conditions of analysis. However, the standard concentration used in the RVA assays is higher than the regular concentration of industrial applications, especially for food products. Therefore, the starch concentrations analyzed (10.7 and 3.6 g starch/100 g suspension) were used as a tool to compare the behavior of the samples under different conditions, as the standard conditions may not be representative for all industrial applications.

2.6. Gel firmness

The hydrogel firmness (strength) was determined in the different starches and conditions of gelatinization, using the gels obtained after the RVA analysis.

Immediately after finishing the RVA analysis, the gels were placed in plastic cups (40 mm diameter × 20 mm height) and stored for different periods (1 and 7 days) in the refrigerator (5 ± 2 °C) before texture evaluation. The cups were kept in a desiccator with water at the bottom to ensure uniform moisture.

The samples were then removed from the refrigerator and analyzed immediately to ensure a constant temperature. Gel firmness was determined by a puncture assay using a texture analyzer TA TX Plus (Stable Micro Systems Ltd., Surrey, UK) with a load cell of 50 kgf (490.3 N). The samples were penetrated until a distance of 4 mm using a cylindrical probe (P/0.5R, 0.5 cm of diameter) at 1 mm·s^{-1}. The equipment measured the force as a function of penetration depth. Gel firmness was evaluated by the energy required to penetrate the material (calculated by the area below the curve force versus distance of penetration).

2.7. Gel structure: optical microscopy

The gels obtained by the RVA analysis were observed using a light microscope (model L1000, Bioval, Brazil; with a 20 W halogen lamp, the magnification of 40×) and a microscope digital camera of 5.1 megapixels (ECMOS C-Mount, Sony, Japão). The material analyzed consisted of the gels obtained immediately after RVA analysis, diluted with distilled water (1:1, v/v) and colored with Lugol's solution. These samples were placed on a glass slide, covered by a glass cover slip and directly evaluated. Images were obtained after verifying their representativeness.

2.8. Hydrogel application: evaluating printability

The gels stored for 1 and 7 days with different starch concentrations (3.6 and 10.7 g/100 g suspension) and gelatinization conditions were placed in a plastic syringe with a die diameter of 1.5 mm and extruded over a glass slide for visual evaluation of the gel's consistency and moldability at room temperature. To ensure the reproducibility and veracity of the procedure, manual extrusion using the syringe was carried out ten times (lines with ~6 cm), and photographs were taken to identify the most predominant visual aspect of the extruded material. Although simple, this approach allowed evaluating the printability of the hydrogels obtained (Fig. 9), which is the first step toward 3D printing. Based on this approach, several conditions were selected for 3D printing.

The selected gels were processed in a 3D printer Stampante 3D (3DRAG V1.2, Futura Elettronica, Italy). The gels were produced following the same proportion of 10.7 g starch/100 g. The suspension was stirred and heated until reaching the desired gelatinization temperature, and maintained in this condition for 5 min. The gels obtained were transferred to the printer syringes (60 mL) that were closed using parafilm plastic, and then stored in a refrigerator (5 ± 2 °C) for 7 days. The syringes were then removed from the refrigerator and immediately processed in the 3D printer: a 0.8 mm diameter nozzle was used; the nozzle height was set at 18 mm with a speed of 20 mm/s and an extrusion rate of 30 mm/s at 20 °C. The physical model (star) was created using the Repetier Host V2.0.1 and Slic3r software (Hot-World GmbH & Co. KG, Willich, Germany) and the dimensions of the star shape were 5 cm × 5 cm × 2 mm (Length × Width × Height).

2.9. Experimental design and statistical analysis

A completely randomized design was applied with four replicates for each processing condition. To evaluate the differences between the materials analyzed, an analysis of variance (ANOVA) and Tukey's test at a 5% significance level were performed with the software *Statistic* 13 (StatSoft, USA).

3. Results and discussion

3.1. Ozonated cassava starch characterization

3.1.1. Starch molecular characteristics

According to Oladebeye et al. [5], carbonyl and carboxyl are crucial for starch oxidation, as they are responsible for the depolymerization of starch chains and the conversion of hydroxyl to carbonyl and carboxyl. Therefore, considering that ozone is a recognized oxidizing agent, these functional groups were determined to identify the extension of oxidation in cassava starch by ozone processing.

Fig. 2 shows the pH, carbonyl, and carboxyl contents of the cassava native starch ozonated for 15 and 30 min. The pH decreased, and the carbonyl and carboxyl group contents increased as a function of ozonation processing time, confirming the high level of starch oxidation. The reduction of starch pH can be associated with the higher carboxyl content, a functional group acidic nature.

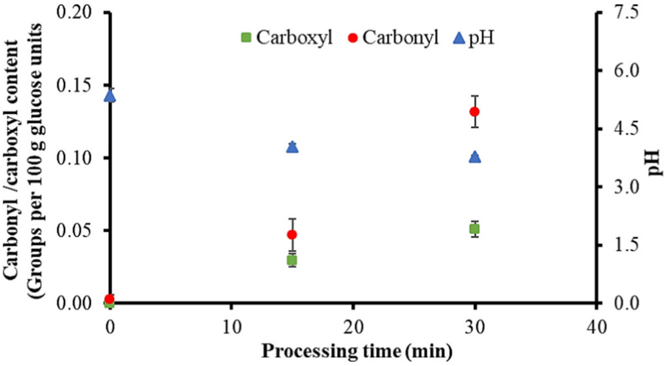


Fig. 2. pH and contents of carbonyl, and carboxyl groups of control (native) and cassava starches ozonated for 15 and 30 min. Carboxyl groups in native starches are considered as negligible and thus zero. The dots are the mean values and the vertical bars are the standard deviations.

In this work, we produced modified starches with two different oxidation levels. This strategy was aimed at extending the cassava starch application as the proportion of carbonyl and/or carboxyl groups present in oxidized starches directly influences their physicochemical properties, potentially fitting the products for different industrial uses [29].

According to Wurzburg [30], two important reactions occur during starch oxidation: (1) the oxidation of hydroxyl groups to carbonyl and carboxyl groups (already mentioned in Section 3.1.1), and (2) the de-polymerization of starch molecules by the cleavage of (1 → 4)-α-D and (1 → 6)-α-D glycosidic linkages. In the case of ozone processing, starch de-polymerization is even more substantial and the oxidation of hydroxyl groups to carbonyl and carboxyl occurs simultaneously [2].

The ozonated cassava starches obtained in this work showed slightly higher contents of carbonyl and carboxyl groups than the potato starch ozonized for 30 min (0.11 and 0.04 per 100 g glucose unit for carbonyl and carboxyl contents, respectively) in the work of Castanha et al. [2].

The ozone action mechanism on the starch molecule proposed was schematized by Castanha et al. [2]. Briefly, the starch molecule undergoes two main transformations: (1) the depolymerization of both amylose and amylopectin, resulting in smaller molecules with a different distribution; and (2) oxidation in the hydroxyl groups resulting in carbonyl and carboxyl groups, promoting different interactions between the molecules.

In order to evaluate this potential de-polymerization, the starch molecular size distribution was studied by gel permeation chromatography (Fig. 3). Using this technique, the starch molecules are separated and eluted through a column filled with a gel of known porosity to allow separation based on molecular size: the higher molecular weight fractions are eluted first, since they rarely penetrate the pores, while the lowest molecular weight fractions are retained longer in the column [31]. Therefore, as can be seen in Fig. 3, we can observe two peaks: the first consists of molecules of larger size and more ramifications, which can be associated with amylopectins, while the second peak represent molecules of smaller size and a linear structure, which can be associated with amyloses.

The first peak of the ozonated cassava starches decreased, while the second peak increased, according to the increase in ozonation process time. Another observation is that the second peak moved to the right (i.e. eluting later) as a function of the increase in ozonation process time. These results indicate the ozone treatment reduced the size of the molecules, attributable to the cleavage of the glycosidic bonds of the ozonated starch molecules [2]. Sandhu et al. [32] also obtained similar results when treating wheat starch with ozone gas.

Once the ozonation process has changed the molecular structure of the cassava starch, the effects on its properties must be evaluated.

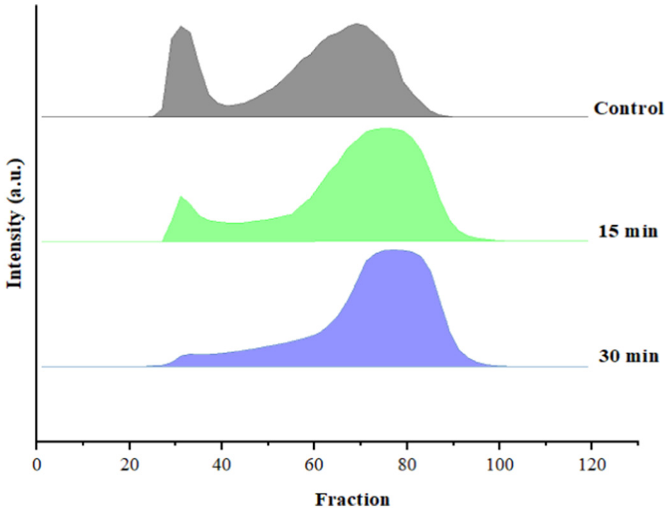


Fig. 3. Molecular size distribution determined by the blue value (BV) during gel permeation chromatography of control (native) and cassava starches ozonated for 15 and 30 min. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.1.2. Pasting properties and gel firmness

The RVA curves, firmness, and images of the gel obtained for the native and ozonated starch for 15 and 30 min are shown in Fig. 4. Table 1 shows the pasting properties of native and ozonated cassava starches. We can observe that the ozonation process affected the pasting properties of the cassava starch differently when different processing times were applied. The peak apparent viscosity of the ozonated starch for 30 min decreased significantly when compared with native and ozonated starch for 15 min (Fig. 4C). Castanha et al. [2] attributed this result to the partial cleavage of the glycosidic bonds promoted by the ozone treatment, as observed in Fig. 3. Therefore, the decrease in the apparent viscosity might reflect the weakening of starch granules, implying a reduction of shear resistance to the point where granule integrity cannot be maintained [33]. Chan et al. [34] also found a reduction of the peak apparent viscosity for tapioca starch processed with ozone in the gaseous phase.

The trough, final, and setback apparent viscosities showed similar tendencies to the peak viscosity, decreasing significantly ($p < 0.05$) as the exposure time to ozone increased. On the other hand, the pasting temperature increased significantly as a function of ozonation process time. Castanha et al. [2] associated this behavior with the presence of smaller size molecules in the ozonated starches (molecular depolymerization), which need more energy to gelatinize than large size molecules.

The Relative Breakdown (RBD) (calculated by the ratio between the values of Breakdown (BD) and the Peak Apparent Viscosity (PAV)) is a parameter which can be associated with the facility of starch granule disruption [2]. The ozonated cassava starches showed higher values for the RBD than the native starch. This can be associated with the fact that the ozone treatment confers a new ability of intermolecular association with the polymers due to the replacement of the hydroxyl group by carbonyl and carboxyl groups (Fig. 2) and a change in molecular size distribution (Fig. 3) [2,34,35].

Gel firmness was higher for the cassava starch ozonated for 30 min and lower for the cassava starch ozonated for 15 min, when compared with the native cassava starch (Fig. 4B). This result is very interesting since both stronger and weaker gels can be obtained by changing the process conditions, which can cover different industrial applications. This behavior is once again linked to the changes in both molecular size distribution and chemical affinity, including charges (by oxidation of hydroxyl groups to carbonyl and carboxyl), in molecular interaction and gel formation. Specific molecular size distribution results in different rearrangements, with stronger or weaker interactions, as can be see for the difference between the ozonated samples for 15 and 30 min. Besides having a smaller molecular size than the other treatments, the starches ozonated for 30 min are composed of a higher fraction of molecules with similar sizes (Fig. 3), which may have promoted better pairing to form the network in gel. This behavior can be observed in Fig. 4(C and D) which shows the visual consistency of these gels and the energy required to deform them, respectively, in line with the results obtained (Fig. 5).

From these results, we can see that the paste obtained from ozonated starch is less consistent, and the gel obtained from the starch ozonated for 30 min is the strongest, displaying good behavior for a material intended for 3D printing. However, different aspects such as gelatinization temperature and storage time can change the hydrogel's behavior, making it necessary to carry out a further evaluation.

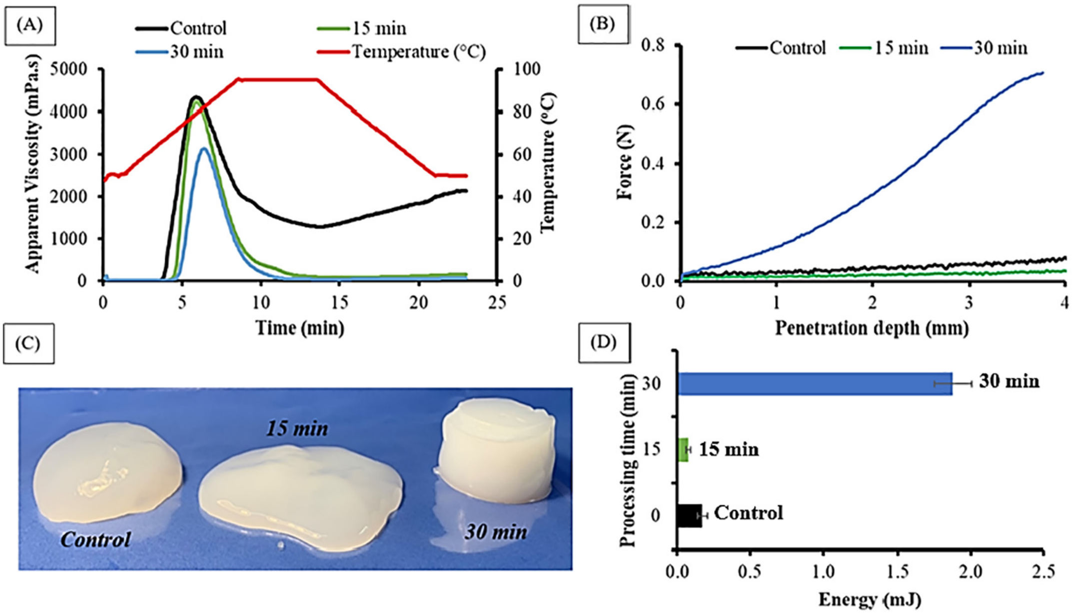


Fig. 4. (A) RVA curves, (B) Gel firmness, (C) images of the gels obtained, and (D) the energy required in a puncture assay of the control (native) and cassava starches ozonated for 15 and 30 min gels (10.7 g starch/100 g suspension).

Table 1
Pasting properties of native and ozonated cassava starches (average ± standard deviation).

Processing time (min)	PAV (mPa·s)	TAV (mPa·s)	RBD (mPa·s)	FAV (mPa·s)	SB (mPa·s)	PT (°C)
0	4308.50 ± 61.39^{a}	1264.25 ± 19.07^{a}	70.54 ± 0.96^{c}	2111.50 ± 71.71^{a}	847.25 ± 56.96^{a}	65.99 ± 0.79^{c}
15	4314.60 ± 106.45^{a}	90.40 ± 14.12^{b}	97.89 ± 0.90^{a}	164.80 ± 21.86^{b}	74.60 ± 10.85^{b}	70.14 ± 0.26^{b}
30	3414.60 ± 167.05^{b}	40.40 ± 1.52^{c}	99.03 ± 1.07^{b}	64.60 ± 2.88^{c}	24.20 ± 1.92^{c}	71.47 ± 0.37^{a}

Peak Apparent Viscosity (PAV), Through Apparent Viscosity (TAV), Relative Breakdown (RBD), Final Apparent Viscosity (FAV), Setback (SB) and Pasting Temperature (PT).
a– c: different small caps in the same column indicate significant difference among treatments, as revealed by Tukey's test, $p < 0.05$.

3.1.3. Thermal properties

The DSC curves are shown in Fig. 6 and the gelatinization properties of the samples (native and cassava starches modified by ozone) are summarized in Table 2.

Fig. 6 shows endothermic peaks, indicating the transition from ordered to disordered structures for all the samples. Regarding the gelatinization parameters in Table 2 (To, Tp, Tf and gelatinization enthalpy (ΔH)), significant differences ($p < 0.05$) between the control and the ozonated starches were found. Matta Junior et al. (2019) [8] observed similar values for certain gelatinization parameters of native cassava starch (Tp = 68.1 °C and ΔH = 13.3 J/g).

The To, Tp, and Tf increased as the ozonation processing time increased, which indicates the ozone processing resulted in starch with higher thermal stability. The results obtained in this study suggest that the process of gelatinization, which involves the destruction of starch crystallite and the loss of helical conformation [5], was affected by the ozonation. Regarding the peak temperature (Tp), the values and behavior were similar to those found for the pasting temperature obtained in the RVA analysis (Table 1). The enthalpy of gelatinization represents the amount of thermal energy involved in the gelatinization process and can provide an indirect measure of the number of crystalline regions [36,37] and the structure of double helices of the starch broken during heating [38]. The smallest gelatinization enthalpy value (ΔH) found for the starch ozonated for 30 min clearly corroborated the hypothesis that in addition to the depolymerization of starch chains, some double helices disassociated within the crystalline and non-crystalline regions of the starch, leading to a decrease in crystallinity [36,37].

3.2. Effect of gelatinization temperature, starch concentration and storage time on hydrogel properties

3.2.1. Pasting properties

Fig. 6 shows the RVA curves for the different starch concentrations at gelatinization temperatures of 65, 75, 85, and 95 °C.

We can observe in Fig. 6 that the starch concentration at 3.6% suspension resulted in much lower apparent viscosity (~20×) than when using a 10.7% suspension, as expected. This behavior is related to the smallest interaction between the swollen granules and the continuous phase, as usually occurs at low concentrated suspensions. As the concentration increases, starch–starch interactions become dominant and a larger number of water molecules are immobilized, resulting in an additional increase of apparent viscosity [39].

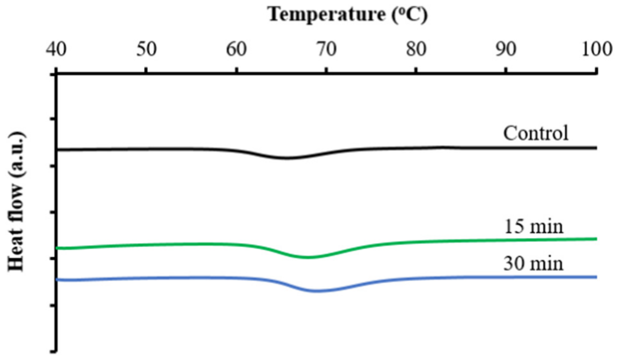


Fig. 5. DSC representative curves of the gelatinization properties of the native and ozonated starch samples, in arbitrary units (a.u.) of heat flow.

The difference of the apparent viscosity between the modified starches in the lowest starch concentration was not as evident as in the highest concentration. For the starch concentration of 3.6%, the ozonated starches always presented a lower apparent viscosity than the native one, followed by the cassava starch ozonated for 15 min and for 30 min. For the starch concentration of 10.7%, the cassava starch ozonated for 15 min showed results similar to those of the native starch when gelatinized at 85 and 95 °C.

By analyzing the sample's behavior at different gelatinization temperatures, it can be observed that there is an increase in the apparent viscosity when the gelatinization temperature changes from 65 to 75 °C. However, it did not vary significantly between 75 and 95 °C, except for the starch ozonated for 15 min at the higher starch concentration, which showed an increase in apparent viscosity when the temperature changed from 75 to 85 °C. When the starch granules under suspension are heated, the apparent viscosity increases due to the gelatinization process, which occurs through the absorption of a large amount of water, resulting in swelling and increased granule size [40]. The notable change of viscosity between 65 and 75 °C can be associated with the temperature zone for which the starches present the highest increase in swelling power, though for temperatures above 75 °C there were no significant changes to this property.

Fig. 7 shows the optical microscopy images used to understand how the starch granules are changed to a gel during the RVA analysis.

According to Atkin et al. [41], starch gelatinization is accompanied by water uptake, swelling, the irreversible disruption of internal molecular order through the dissociation of double helices and the melting of crystallites, the leaching of polysaccharides, the disintegration of swollen granules, and the formation of a paste or gel.

At 65 °C, the microscopies of all the starch gels showed swollen granules for both starch concentrations. At 75 °C, native and cassava starch ozonated for 15 min showed apparently swollen starch granules but cassava starch ozonated for 30 min showed less defined granule shapes (less marked surfaces), indicating a more significant breakdown of these structures.

At the lowest starch concentration at 85 and 95 °C, the cassava starches presented only “ghosts”; “Ghosts” is name given to “empty shell” fragments of starch granules found in the medium [42]. At the highest starch concentration, at 85 and 95 °C, native starch still presented apparent swollen starch granules but the ozonated cassava starches presented only “ghosts”. The same behavior was observed by Castanha et al. [9], when comparing native potato starch with ozonated potato starches. These authors explained that unmodified granules require more energy to disrupt the bonds of the crystalline region, a finding confirmed by the lower RBD value calculated for the native starch when compared with the ozonated starches. This means that native starches show greater resistance to starch granule disruption.

For all the samples, the increasing temperature was positive for the granular rupture and greater amylose leaching, mainly between 65 and 75 °C. As can be seen for the pasting properties, the transition from 65 to 75 °C was more decisive for granule swelling and rupture.

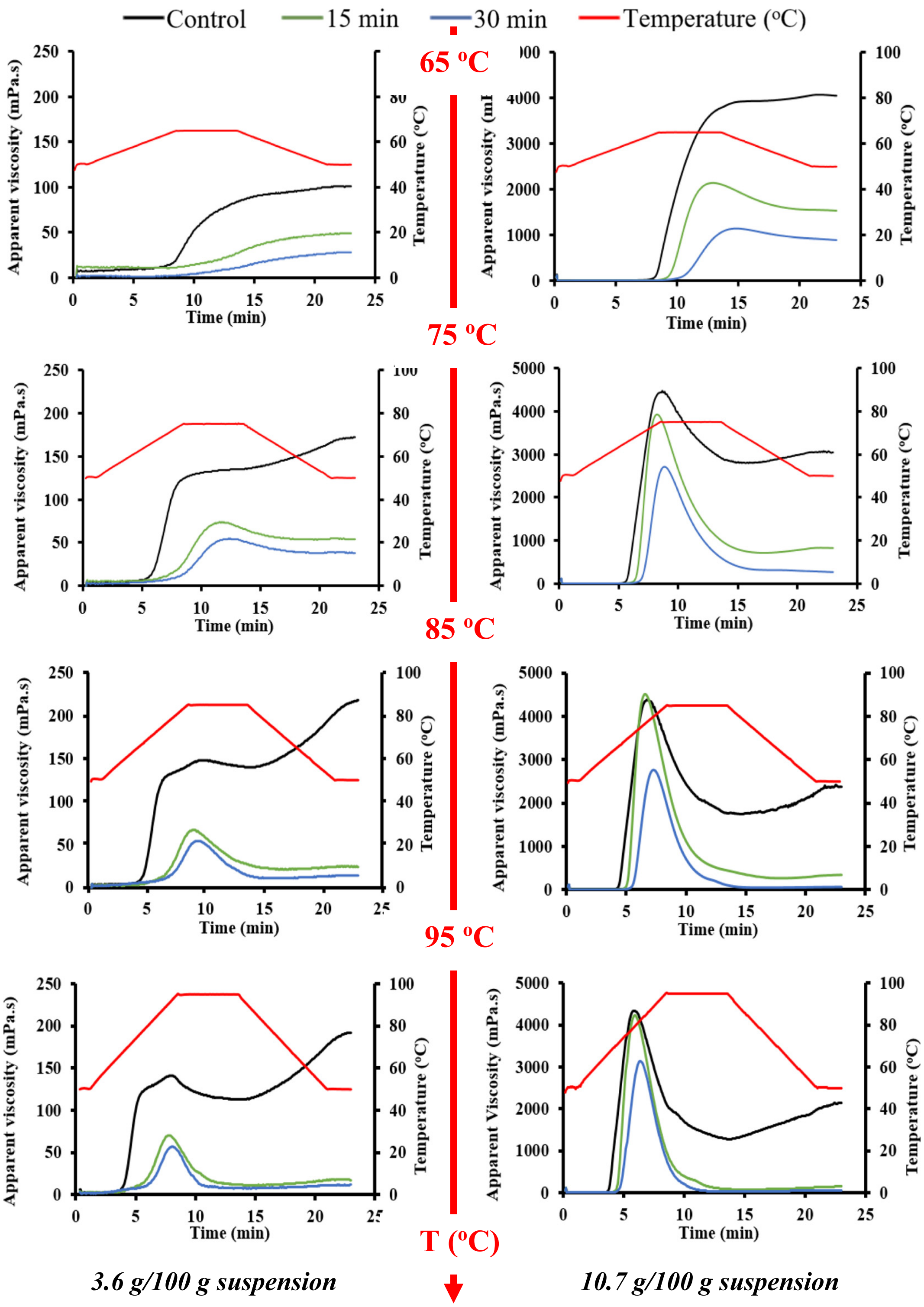
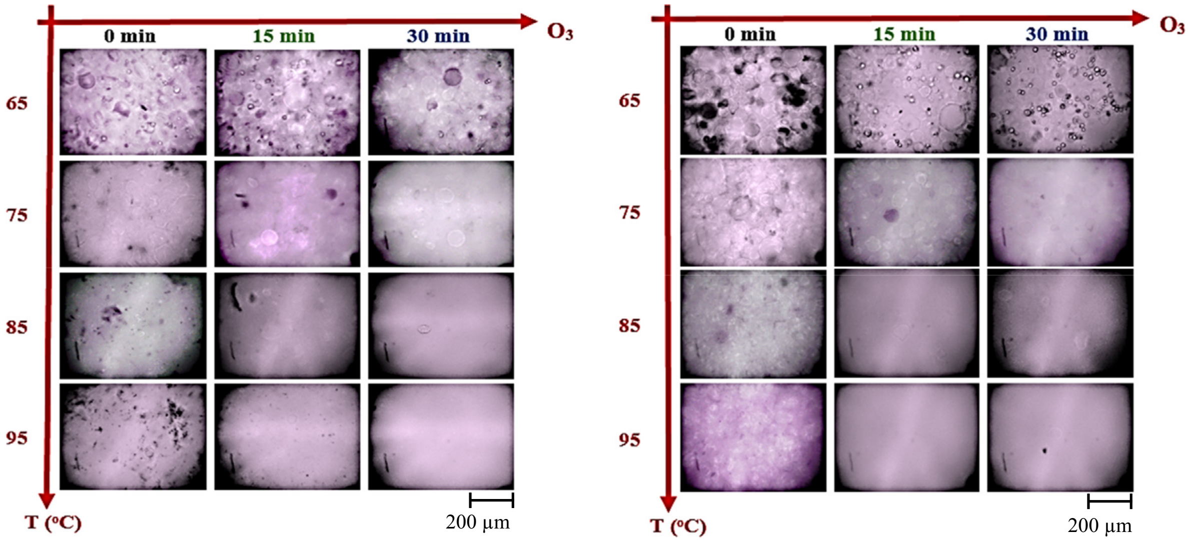


Fig. 6. RVA curves for the different starch concentrations at the gelatinization temperatures: 65, 75, 85, and 95 °C.

Table 2
Gelatinization properties of native and ozonated cassava starches (average ± standard deviation).

Processing time (min)	To (°C)	Tp (°C)	Tf (°C)	ΔH (J/g)
0	55.30 ± 0.40^{c}	65.77 ± 0.08^{c}	81.14 ± 0.56^{b}	12.61 ± 0.22^{a}
15	56.41 ± 0.46^{b}	67.98 ± 0.26^{b}	81.57 ± 0.30^{b}	12.22 ± 0.32^{a}
30	59.36 ± 0.62^{a}	69.06 ± 0.26^{a}	84.26 ± 0.79^{a}	11.03 ± 0.29^{b}

a–c: different small caps in the same column indicate significant difference among treatments, as revealed by Tukey's test, $p < 0.05$.
To = onset temperature, Tp = peak temperature, Tf = final temperature and ΔH = enthalpy.



3.6 g/100 g suspension *10.7 g/100 g suspension*

Fig. 7. Optical microscopy for the gels produced after the RVA analysis.

Therefore, we obtained starches with different rheological behaviors that may lead to diverse applications. In the case of 3D food printing, the ideal viscosity of the material should be low enough to permit easy extrusion through a small nozzle and high enough to be cohesive without deformation of the layers deposited previously [43]. Thus, the starch ozonated for 30 min can be a good alternative to form a gel with lower viscosity at the temperatures evaluated.

3.2.2. Gel firmness

This criterion is used to evaluate the strength of the hydrogels produced after RVA. After the starch undergoes gelatinization in the presence of water, the subsequent cooling causes retrogradation, i.e. a process in which the disordered macromolecular components of starch are reorganized (amylose or amylopectin) [44]. Thus, gel firmness depends on the reorganization and network of these structures.

Fig. 8 shows the strength of the different hydrogels obtained. Firstly, it is important to compare the order of magnitude of axis y for the starch suspension of 3.6 g/100 g and 10.7 g /100 g. By increasing the starch content 3 times, a significant increase (~2 until 40×) in gel strength was reached for all the starches (native and ozonated). The higher starch concentration led to an increase in the number of starch molecules per unit volume, and increased the probability of intermolecular hydrogen bonding, leading to a more compact network structure with increased gel strength [15].

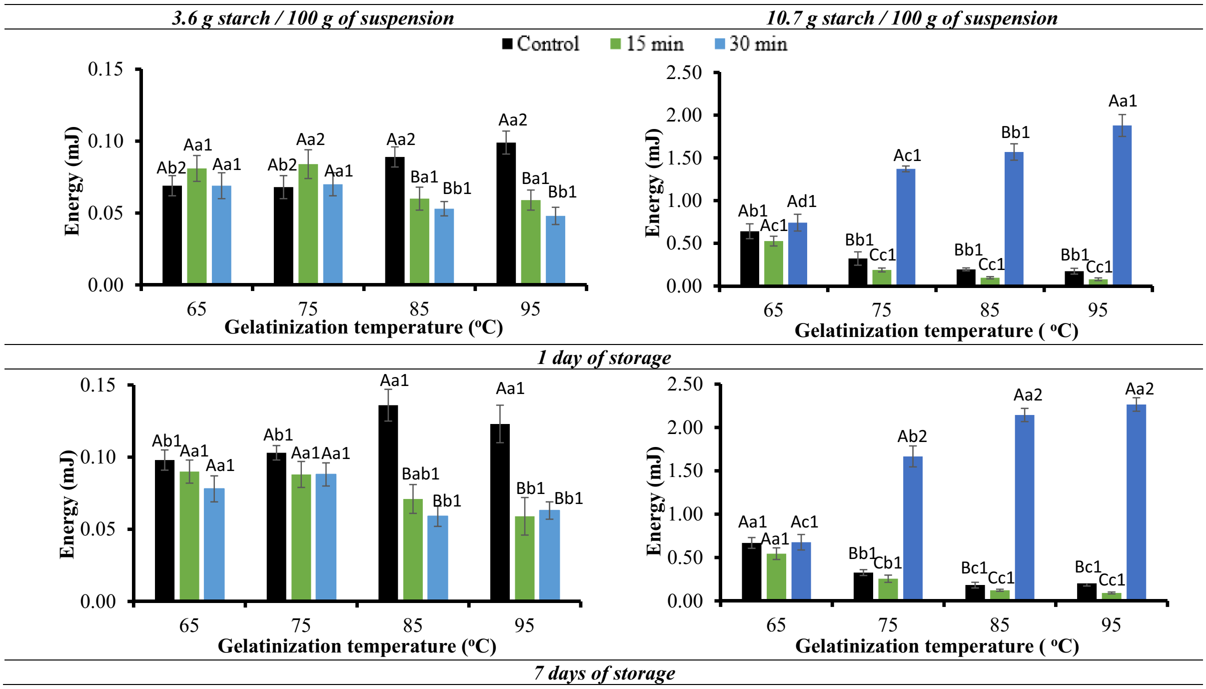


Fig. 8. Strength of the hydrogels obtained: effect of ozone processing, gelatinization temperature and storage time. (A–C: different large caps indicate significant differences between the different starches at the same gelatinization temperature, as revealed by Tukey's test, $p < 0.05$; a - c: different small caps indicate significant differences between the same starches at different gelatinization temperatures, as revealed by Tukey's test, $p < 0.05$; 1–2: different numbers indicate significant differences between the same starches at the same gelatinization temperature but with different storage periods, as revealed by Tukey's test, $p < 0.05$).

The storage period had a small effect on the gel strength, which can be interesting when considering industrial product storage. On the other hand, the effect of gelatinization temperature was considerable (although to different extents as a function of the different treatments).

For the gels produced with the lowest starch concentration (3.6%), the increase in gelatinization temperature had a small effect on gel strength, with the exception of the native starch from 75 °C to 85 °C, which increased ~30%. On the other hand, by increasing the gelatinization temperature, stronger gels were obtained at 10.7%, in particular in the case of the cassava starch ozonated for 30 min. The strongest gel was obtained with 10.7%, with ozonation for 30 min and gelatinization at 95 °C. Its strength was ~4 times higher than the stronger gel obtained from the native starch (gelatinization at 65 °C) and ~10 times the gel obtained from the native starch at the same temperature. This indicates that a specific molecular size distribution and charges were reached in this treatment to ensure better rearrangement, promoting better pairing to form the network in the gel. It is worth mentioning that this result is remarkable from the industrial standpoint.

Finally, using three variables: starch type, starch concentration and gelatinization temperature, we observed different behaviors of the gel strength obtained, thus extending the possible applications of gels. Their printability was then evaluated.

3.2.3. Evaluating the printability of the gels

Printability, or the potential of a material to be used for printing, requires that the dimensional stability of the material in question prevents it spreading on surfaces, and that it is capable of supporting its own weight during layer-by-layer deposition [11].

The gels produced with the lowest starch concentration (3.6%) were not able to form a straight line when extruded and spread on a surface by a syringe. Therefore, the evaluation was conducted with the gels produced with the highest concentration of starch (10.7%). Fig. 9 shows images of the simulated printing with the gels stored for 1 and 7 days at this concentration, considering different ozonation times and gelatinization temperatures.

In Fig. 9, we can see that it was possible to extrude all the starch gels produced in a straight line and with a defined shape. However, it can be noticed that the line produced by the cassava starch ozonated for 15 min resulted in less defined and regular lines, for all the gelatinization temperatures employed. On the other hand, the gels produced with the native starch and starch ozonated for 30 min show a regular structure, especially the ozonated starch. Furthermore, the gels stored for 7 days show a more regular structure than gels stored for 1 day. Indeed, these results agree with the gel strengths obtained (and also demonstrate the important of evaluating gel strength with respect to printability).

At a gelatinization temperature of 65 °C, the gels produced by native cassava starch and cassava starch ozonated for 30 min resulted in good candidates for use in 3D printing. For process temperatures up to 75 °C, the cassava ozonated starch for 30 min was the best candidate for 3D printing.

To evaluate if the ozone treatment really improves the printability of these hydrogels, 3D stars were printed with selected gels. Fig. 10 shows images of the stars produced based on gels obtained from the control (native) starch and those processed for 30 min with ozone, at gelatinization temperatures of 65 and 95 °C, and a storage period of 7 days in a refrigerator (5 ± 2 °C).

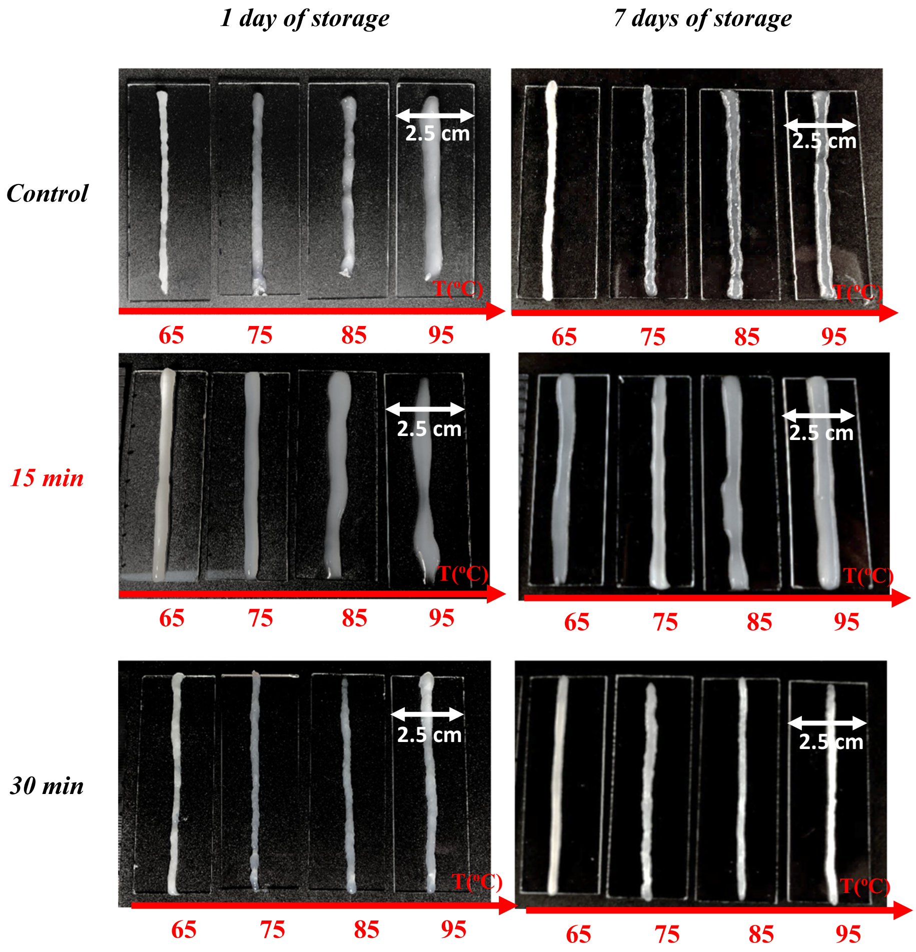


Fig. 9. Images of the simulation of gel printability. The images were taken for storage periods of 1 and 7 days with starch concentration of 10.7 g/100 g suspension.

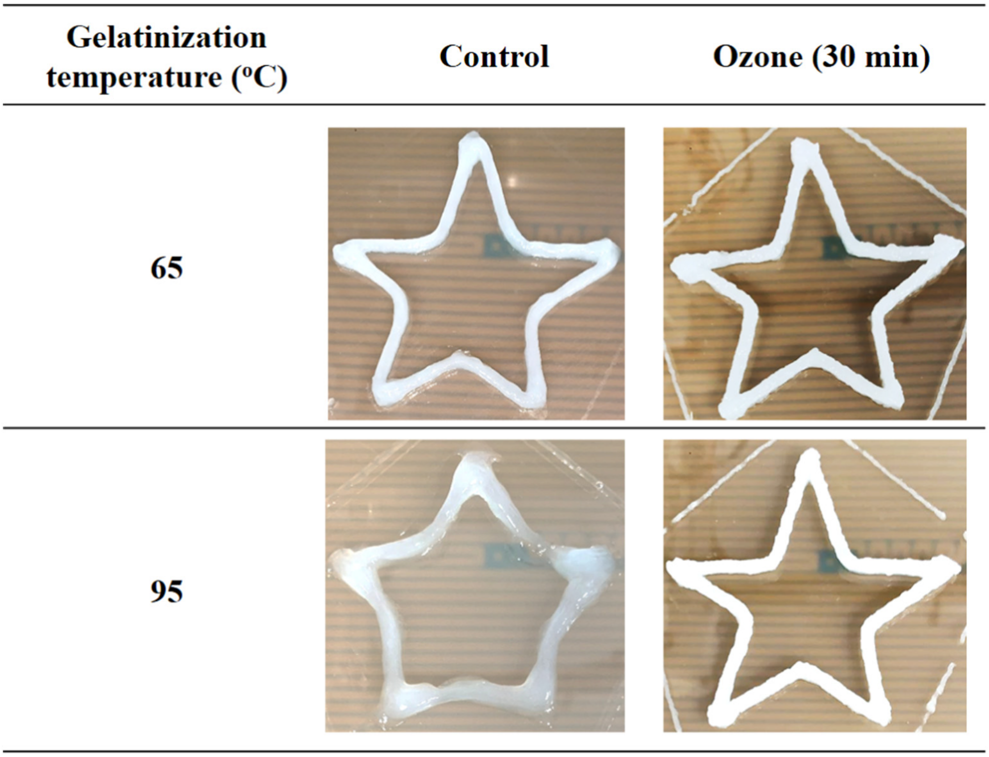


Fig. 10. Images of the stars obtained by a 3D printer of cassava starch gels: control (native starch) and starch ozonated for 30 min, produced at gelatinization temperatures of 65 and 95 °C, and stored for 7 days at 5 °C before printing.

Fig. 10 shows that when the gels were produced using the lowest gelatinization temperature (65 °C), it was possible to print stars with both gels. However, it is notable that the star produced with the ozonated starch showed better resolution (well-defined angles). For the gels produced using the highest gelatinization temperature (95 °C), the native starch did not present good printability and spread over the surface. On the other hand, the hydrogels produced with starch ozonated for 30 min continued to exhibit good printability, resulting in a star with better resolution when compared with the others printed in this work.

Consequently, this work successfully developed ozonated starches for use as ingredients with enhanced properties for 3D printing.

4. Conclusion

This work demonstrated that ozone treatment is a relevant strategy for modifying cassava starch intended for 3D printing. The effect of ozonation processing was evaluated on the starch biopolymer, paste and gel properties. Then, the obtained hydrogels were evaluated by also considering different starch concentrations, gelatinization temperatures and storage times. The results obtained were correlated, in order to better understand the changes made to the paste, gel strength, structure and printability properties of the ozonated starches, when compared to the native one. This work also revealed that starch concentration was an important parameter for the gelling stage and that the gelatinization temperature was changed for the native and ozonated starches. Ozone processing promoted starch oxidation, reduced starch molecular size (depolymerization), and the starch ozonated for 30 min exhibited a lower peak apparent viscosity. The lowest starch concentrations studied were not capable of forming consistent gels with the requisite characteristics of printability. On the other hand, the highest starch concentration formed stronger and more printable gels. The stabilization of the gels for 7 days was found to be preferable, resulting in a lower propensity for spreading and better consistency thanks to the retrogradation of starch biopolymers. The gels produced by native and starches ozonated for 30 min presented good printability when the gelatinization temperature used was 65 °C, but up to this temperature, starch ozonated for 30 min produced more printable gels. The gels produced by starch ozonated for 15 min did not present good printability. Finally, ozone processing can be an alternative for obtaining starches with new and potentially interesting properties, thereby expanding the potential of using cassava starch in 3D printing.

Acknowledgments

The authors are grateful to the Région Pays de la Loire/RFI "FOOD 4 TOMORROW" for funding the Post-doctoral fellowship "STARCH-3D" of B. Maniglia, to the São Paulo Research Foundation (FAPESP, Brazil) for funding project n° 2016/18052-5, and to the National Council for Scientific and Technological Development (CNPq, Brazil) for funding the productivity grant of P.E.D. Augusto (306557/2017-7). This study was funded in part by the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES)" - Finance Code 001, through the D.C. Lima Ph.D. scholarship.

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