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Properties and possible applications of ozone-modified potato starch

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ABSTRACT

In this work, different properties of potato starch modified by ozone oxidation were evaluated and described aiming to represent different possibilities of industrial application. The most promising results were observed regarding the pasting properties and the gel texture of the starch samples ozonated for 15 and 30 min. These samples presented a higher apparent viscosity and a higher gel strength when gelatinized at 65 and 70 °C, if compared to the native sample. Furthermore, the 15 and 30-min samples retained more water at mild temperatures (~60 °C) than the other samples. These results could be related to the less compact structure of the oxidized starches after the ozone processing due to the cleavage of their glycosidic bonds and the presence of electronegative groups. Not only do these characteristics facilitate the water absorption and gelatinization of the samples at mild temperatures, they also favour the granular disruption at higher temperatures (above 85 °C). The data reported in this work broadens the understanding of the ozone-modification process, as well as suggesting possibilities of industrial applications using ozonated potato starch.

1. Introduction

Native starches have specific and limited properties. Consequently, they do not meet all industrial demands. As a result, starch modification became essential.

Starch modification by chemical methods is widely used in many food and non-food applications (Masina et al., 2016). However, the consequent waste generation and high costs demand new alternatives for most of these processes (Liu, Weber, Currie, & Yada, 2003). The ozone is an alternative to current oxidative methods as it is considered a simple technique with environmental claim (Mahapatra, Muthukumarappan, & Julson, 2005).

Starch modification through ozonation is highly relevant from both academic and industrial points of view. Applications of ozone for starch modification have been reported by different authors using different sources, such as rice, maize, potatoes, yams and cassavas (An & King, 2009; Çatal & İbanoğlu, 2012, 2013, 2014; Hui T. Chan, Bhat, & Karim, 2009; Hui Tin Chan et al., 2011; Hui Tin Chan, Fazilah, Bhat, Leh, & Karim, 2012; Klein et al., 2014; Oladebeye, Oshodi, Amoo, & Karim, 2013).

All the results reported in the literature reinforce the effectiveness of the ozone as a potential oxidizing agent in the modification of starches. However, little progress has been made considering the technological properties of ozonated starches regarding their characteristics under different conditions (different temperature or different starch/water concentrations, in relation to standard of analyses).

In a previous study (Castanha, Matta Junior, & Augusto, 2017), the effect of the ozone on the structure and properties of potato starch was evaluated. The main results are the replacement of the hydroxyl groups by carbonyl and carboxyl groups, as well as to the cleavage of the glycosidic bonds of both amylose and amylopectin molecules (decreasing their sizes), mainly in the amorphous regions of the granules. Considering the properties, significant differences were observed in the starch pasting properties, gel texture and paste clarity, proving the efficacy of the ozone processing. However, to the best of our knowledge, further properties of the ozonated potato starch, such as its pasting properties at different temperatures and starch concentration, as well as the gel texture and the water holding capacity in these conditions, have not been evaluated in the literature. Consequently, this is the objective of this work.

There are several reasons that explain the importance of evaluating modified starch properties under different conditions, instead of using standard protocols of analysis: I. mostly starchy systems undergo mechanical and thermal processes before being used, because in native conditions they are insoluble in water at room temperature and less digested by the human body (Malumba, Doran, Danthine, Blecker, &

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Béra, 2018); II. to achieve the required characteristics for different applications, starch thermal processing is performed under different conditions, which vary widely among industries and among products; III. the behaviour of the starch samples is influenced by factors such as the rate of increase in temperature, starch concentration and shear forces (Anuntagool, Alvarez, & Flick, 2017), making it difficult to predict and control; IV. the starch modification processes can change the granule size, morphology and composition, significantly affecting its gelatinization behaviour (Jane et al., 1999).

Therefore, the standard conditions may not be representative for all industrial applications and evaluation under different conditions may be important to understand possible applications of the modified starches. For example, in a Rapid Visco Analyser (RVA) analysis, the starch concentration is usually fixed at ~10%, although it widely varies in the industry. This variation in the analysis conditions is rarely conducted in the literature.

Considering this scenario, the aim of this paper was to evaluate and describe the properties of potato starch modified by ozone oxidation under different analyses conditions (at different temperatures and starch concentration). In addition, this study aimed to broaden the understanding concerning the impact of this type of modification on the potato starch properties and applications by correlating these properties with the previously reported structural changes undergone by them.

2. Material and methods

2.1. Material

Potato starch was extracted from native potatoes (*Solanum tuberosum* L., Monalisa cultivar) and the proximal composition of both potato tubers and potato starch was described in our previous study (Castanha et al., 2017) as the same batch was used. The main characteristics of the potato starch were: ~28% of apparent amylose content, ~800 $mg \cdot kg^{-1}$ of phosphorus content and ~99.5% of purity.

All the chemicals were of analytical grade.

2.2. Starch modification

First of all, 700 mL of the starch suspension in distilled water (10% m/m in dry basis) was ozonated for 15, 30, 45 and 60 min, under constant stirring, with a gas flow of 0.5 $L \cdot min^{-1}$ and an ozone concentration in the gas current of 47 mg $O_3 \cdot L^{-1}$. The ozone was produced in an ozone generator unit (Ozone & Life, O&G Model RM 3.0, São José dos Campos, Brazil) by the corona discharge method from industrial oxygen (95% purity). The glass reactor was cylindrical, with a capacity of 2 L, 56 cm high and 6 cm in internal diameter. The gas was dispersed in the sample through a glass tube, ending in a porous gas disperser to create small bubbles aiming to increase the ozone diffusion in the suspension. After processing, the starch samples were allowed to settle, the upper water was poured, and the samples were dried in an air circulation oven at 35 °C until ~12% of moisture content.

Further details of the ozonation process can be found in our previous paper (Castanha et al., 2017).

2.3. Starch evaluation

2.3.1. Granule properties: particle size distribution (PSD) and zeta potential (ζ)

The PSD of the starch samples was determined using a Laser Analyser (Partica LA-950V2 Laser Particle Size Analyser, HORIBA, Japan) and the data were evaluated using the software LA-950 for Windows (HORIBA, Japan). The samples were dispersed in ethanol (99.5%). The volume-based mean diameter (D[4,3], Eq. 1) and the area-based mean diameter (D[3,2], Eq. 2) were evaluated, where n_i was the number of particles with diameter d_i.

$$D[4,3] = \frac{\sum_i n_i d_i^4}{\sum_i n_i d_i^{3.}} \tag{1}$$

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

The ζ of the starch granules was measured using Zetasizer equipment (Zetasizer Nano ZS, Malvern Instruments, United Kingdom). The starch suspensions were prepared in Milli-Q water (0.01% m/m) and the pH was adjusted 24 h before the analysis to 3.0, 4.0, 5.0, 5.5, 6.5 and 7.0 using 0.1 M HCl or 0.1 M NaOH.

2.3.2. Water absorption index (WAI) and Water solubility index (WSI)

The WAI and the WSI were evaluated as described by Anderson, Conway, and Peplinski (1970), with modifications. 0.5 g of starch (dry basis) was mixed with 6 mL of distilled water in pre-weighed centrifuge tubes. The tubes were then put in the water bath and stirred for 30 min at different temperatures (30, 55, 60, 65, 70, 85 and 95 °C). After heating, the tubes were centrifuged at 3000*g* for 10 min. After centrifugation, 2 phases were obtained: the supernatant phase, which contains the starch soluble fraction in water; and the precipitated phase, containing the starch insoluble fraction and the water retained by it.

The soluble starch fraction (SS) of the supernatant was determined after drying at 105 °C, to calculate the WSI (Eq. 3). Therefore, the WSI represents the soluble portion (%) of the starch at the given temperature. The precipitated (PT) sample retained in the tube was also weighed. The WAI is an indicator of the starch swelling power and represents the amount of water that 1 g of insoluble starch (IS) was able to absorb and retain (Eq. 4). For both equations: MS is the mass of the starch sample, in dry basis.

$$\mathrm{WSI}\,(\%) = \frac{SS}{MS} \bullet 100 \tag{3}$$

$$\mathrm{WAI}\left(\frac{\text{g water}}{\text{g IS}}\right) = \frac{\mathrm{PT} - (\mathrm{MS} - \mathrm{SS})}{(\mathrm{MS} - \mathrm{SS})} \tag{4}$$

2.3.3. Pasting properties

The pasting properties were determined using a Rapid Visco Analyser (RVA-4, Newport Scientific Pvt. Ltd., Australia) with the Thermocline for Windows software (version 3.0). 1 g of starch (correct to 14% moisture basis) was suspended in 27 g of distilled water and analysed (starch concentration ~3.6%). It is important to highlight that the water/sample proportion was different from that conventionally used as the pasting properties of the starch under standard conditions of analysis have already been explored in previous studies (Castanha et al., 2017; Çatal & İbanoğlu, 2012). Furthermore, the standard concentration used in the RVA assays is higher than the regular concentration of industrial applications, especially for food products. Therefore, the RVA analysis was used as a tool to compare the behaviour of the samples under different conditions, and not as a standard analysis to characterize the modified starches.

The suspensions were first held at 40 °C for 1 min, then heated to different maximum temperatures (55, 60, 65, 70, 85 and 95 °C) for 7 min and 30 s. They were then kept at the maximum temperature for 5 min, followed by cooling to 40 °C for 7 min and 30 s, and finally held at 40 °C for 2 min. The maximum temperatures were based on the gelatinization temperature measured using Differential Scanning Calorimeter equipment (DSC-60, Shimadzu, Japan). For the DSC analysis, 3 mg of dry starch was placed in an aluminium pan with 7 μL of deionized water. The samples in the hermetically sealed pans were equilibrated at room temperature for 1 h before the measurements were taken. The scanning temperature was from 30 to 100 °C and the heating rate was 10 $°C \cdot min^{-1}$. An empty pan was used as a reference. The DSC results can be found in the Supplementary material (Fig. S1, Table S1).

It is important to highlight that the DSC indicates the temperature at which the starch granules begin to irreversibly change, under quiescent conditions. Therefore, the DSC analysis was performed under standard conditions in order to characterize the gelatinization temperatures of the samples. Moreover, the results were used as a reference to determine the temperature profiles of the RVA analysis.

2.3.4. Optical microscopy

The starch granule surface and swelling characteristics were observed using a light microscope (model L1000, Bioval, Brazil) with a 20 W halogen lamp. 1 mL of the starch dispersion/gel obtained after each Rapid Visco Analyser (RVA) assay was dispersed in 1 mL of distilled water with 50 μL of Lugol. A drop of this material was placed on a glass slide and covered by a glass cover slip. The magnification used was 100× and a microscope digital camera of 1.3 megapixels was used to obtain the images after verifying being representative.

2.3.5. Gel strength

The strength of the potato starch gels was determined by a puncture assay using a Texture Analyser (TA.XT Plus, Stable Micro Systems Ltd., United Kingdom) with a load cell of 50 kgf (490.3 N). The gel obtained in the Rapid Visco Analyser (RVA) (after the analysis was performed at 65, 70, 85 and 95 °C) was stored in a 40 × 20 mm (diameter × height) plastic cup for 7 days in a refrigerator (5 ± 2 °C) and was retained inside a desiccator with water to ensure uniform moisture of the samples. A 0.5 cm cylindrical probe (P/0.5R) was used to penetrate the samples until a distance of 6 mm at 1 mm·s^{-1}. To ensure a constant temperature during the analysis, the samples were removed from the refrigerator and analysed immediately. The force measured by the equipment as a function of the penetration depth was then used to evaluate the sample strength. The total energy required for penetration was then calculated through the area below the "force versus penetration" curve.

2.3.6. In vitro digestibility

The *in vitro* digestibility of the starch samples was determined according to the method described by Englyst, Kingman, and Cummings (1992), considering the modifications proposed by Zhang, Mei, Chen, and Chen (2017). The enzymatic solution used was composed by α-amylase and amyloglucosidase at a ratio of 120 U/mL:80 U/mL.

After preparing them (according to the aforementioned methods), the samples were incubated at 37 °C under stirring (170 RPM), thus simulating their digestion. Then, 5 mL of each sample was collected at different time intervals (20 and 120 min), mixed with 4 mL of ethanol solution (80%) to inactivate the enzymes and analysed using two different methods: glucose content and reducing sugar content.

The glucose content was measured by the glucose reagent kit based on the GOD/POD reaction method (Ref. number 133, Glicose liquiform, Brazil). The reducing sugar content was determined by the Somogyi-Nelson colorimetric method (Nelson, 1944; Somogyi, 1945).

Following the Englyst et al. (1992) terminology, the percentages of glucose and reducing sugar found in each digestion phase were converted to Rapidly Digestible Starch (RDS - Eq. 5), Slowly Digestible Starch (SDS - Eq. 6) and Resistant Starch (RS - Eq. 7) values. G20 and G120 correspond, respectively, to the glucose/reducing sugar contents after 20 and 120 min of digestion, TS is the Total Starch content, and FG is the Free Glucose (glucose content of the sample before the analysis).

$$\text{RDS}\ (\%) = \frac{(\text{G20} - \text{FG})}{\text{TS}} \cdot 100 \cdot 0.9 \tag{5}$$

$$\text{SDS}\ (\%) = \frac{(\text{G120} - \text{G20})}{\text{TS}} \cdot 100 \cdot 0.9 \tag{6}$$

$$\text{RS}\ (\%) = \frac{(\text{TS} - \text{RDS} - \text{SDS})}{\text{TS}} \cdot 100 \tag{7}$$

2.4. Experimental design and statistical analysis

Each ozone process was performed four times. The analyses were repeated at least two times for each sample. The average values were calculated and, when appropriate, the Tukey's multiple comparisons were adopted using Statistica 13.0 (StatSoft, USA) software. A significance level of 5% was considered.

3. Results and discussion

In general, our first paper (Castanha et al., 2017) demonstrated that the ozonation process changed the potato starch structure by increasing the contents of carbonyl, carboxyl and reducing sugar (increased ~2.5× after 60 min) and by decreasing the apparent amylose content (from 28.4 to 21.0 after 60 min), the pH value (from 6.4 to 4.2 after 60 min) and the molecular size (proved by gel permeation chromatography). The crystallinity of the starch granules was not changed. Analysing all the results concerning the starch structure, a mechanism of action of the ozone in the starch was proposed. Furthermore, the morphology of the granules was changed as the ozonated samples presented cracks and fissures. All these changes affected some of the starch properties, as pasting properties (evaluated under a standard protocol of analysis), gel texture and paste clarity. Further properties are now evaluated, focusing on possible applications.

3.1. Properties of the potato starch granule: particle size distribution (PSD) and zeta potential (ζ)

Fig. 1 presents the PSD and the ζ of the native and ozonated potato starches.

The native sample presented a higher D[4,3] (Fig. 1A) if compared to the ozonated samples, while the D[3,2] values were very similar for all the samples (Fig. 1B). The volume-based mean particle diameter (D[4,3]) is more influenced by large particles, while the area-based (D[3,2]) is more influenced by smaller ones (Bengtsson & Tornberg, 2011; Lopez-Sanchez et al., 2011). Therefore, the results indicate that the ozonation process led to a decrease in the diameter of the larger starch granules, having less influence on the smaller ones. This behaviour may be due to the greater accessibility of the ozone to the larger granules during the processing. On the other hand, the ozonated samples presented similar mean particle sizes among them (Fig. 1C).

The ζ (Fig. 1D) of the starch granules was measured and characterized at different pH conditions. At pH 3.0, the samples were beginning to present negative values of ζ. In all the other pH values, however, the ζ of the samples was negative, including in the pH 5.5, presented by the native potato starch sample with no NaOH or HCl addition. The phosphate groups naturally found in the potato starch samples contributed to this behaviour as they are negatively charged (Szymońska, Molenda, & Wieczorek, 2015). It is important to note that, even within the same sample, the variation of the ζ values was high. This was probably due to the potato starch granular size, which is too large to accurately determine the ζ values.

The combination of both particle size and ζ gives an indication of the stabilization capacity of emulsions and suspensions, as well as the particle aggregation. However, the changes in the PSD and in the ζ are more significant in the case of starch nanoparticles (size < 100 nm, according to Jiang, Mashayekhi, and Xing (2009)), which are much smaller than the granules analysed in this paper. In addition, the observed changes on the potato starch granule PSD and ζ were small in the conditions applied in the present work.

3.2. Pasting properties

The RVA was selected to evaluate the properties of the starch samples under non-standard protocols as this analysis measures the temperature at which the swelling of the starch granules starts to affect

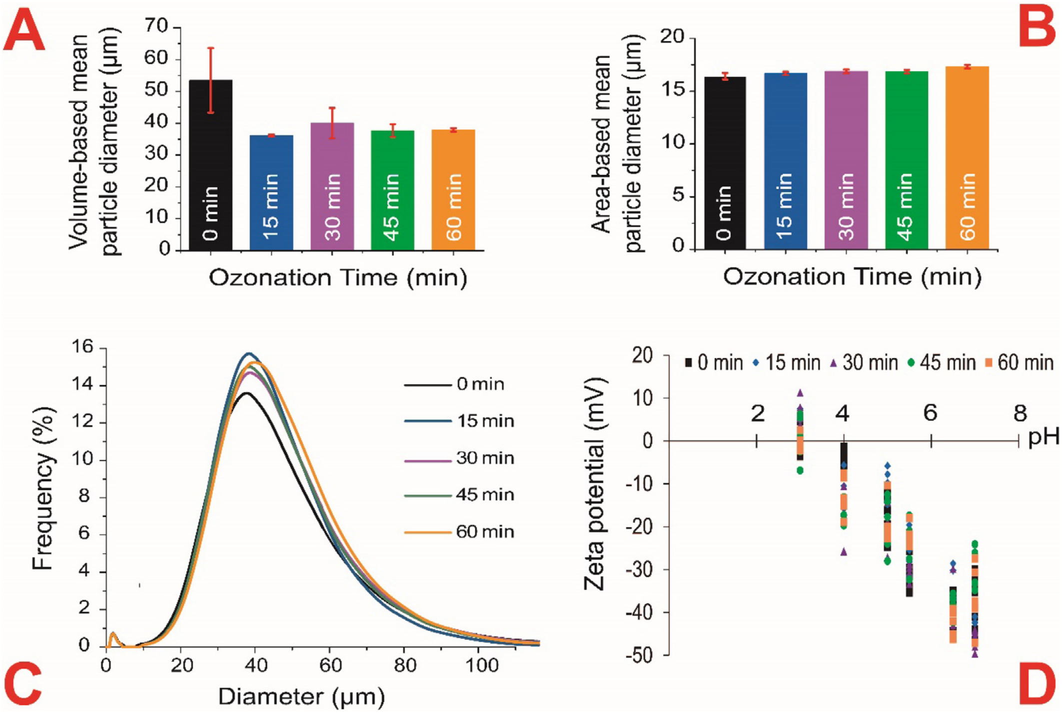

Fig. 1. (A) Volume-based mean particle diameter (D[4,3]) of the native and ozonated potato starches. (B) Area-based mean particle diameter (D[3,2]) of the native and ozonated potato starches. (C) Effect of the ozonation time (0–60 min) on the particle size distribution of the potato starch (based on volume). (D) Zeta potential of the starch granules: all points measured in the analysis are shown in the graphic. Vertical red bars represent the standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the apparent viscosity of the system, under heating and shear (Liu, 2005; Yu & Christie, 2001), thus better reproducing some industrial conditions. Therefore, the RVA analysis was performed using an initial and final temperature of 40 °C, and maximum temperatures ranging from 55 °C (lower than the Onset temperature of gelatinization for all samples, according to the DSC analysis – Table S1, Supplementary Material) to 95 °C (higher than the Endset temperatures measured for all the samples, according to the DSC analysis – Table S1, Supplementary material). The RVA curves are shown in Fig. 2.

The first interesting point that can be observed in Fig. 2 is the shape of the native starch curves, which is different from the commonly observed curves for potato starch. This shape is due to the water/sample proportion used in this study (3.6%, instead of the standard 10.7%). By changing the proportion, the shape of the native starch curve was completely different from the "standard" curve, without the well-known peak apparent viscosity and breakdown regions. This behaviour was probably due to the lower interaction between the swelled granules and the continuous phase, as usually occurs at low concentrated solutions (Biliaderis, 2009; Booth & Bason, 2007).

By analysing the sample's behaviour at different temperatures (Fig. 2), it can be observed that no increase in the apparent viscosity was observed at 55 and 60 °C for all the samples.

However, the most remarkable results were observed at 65 and 70 °C. At these temperatures, the native starch presented only a small increase in its apparent viscosity, while the 15-min ozonated sample presented the highest apparent viscosity among all the treatments. At 70 °C, the apparent viscosity of the 15-min treatment was the highest obtained by this sample (even higher than at 85 or 90 °C). It suggests that the ozonation process, at mild conditions, can be a good alternative to obtain starches that form viscous pastes at moderate temperatures (65–70 °C).

On the other hand, the native starch sample started to present an apparent viscosity higher than that of the 15-min treatment at 85 °C reaching an even higher value at the end of the 95 °C analysis. In contrast, at higher temperatures, the ozonated samples presented lower apparent viscosity values if compared with their results at 70 °C.

3.2.1. *Microscopic evaluation of the starch pastes*

The starch samples were collected at the end of each RVA analysis and evaluated using a light microscope (Fig. 3). This helped to explain this phenomenon, by visually demonstrating the changes observed in the RVA results. A comprehensive scheme (Fig. 4) representing the general steps of the starch gelatinization is also available and is a reference to analyse the microscopic images, thus determining at which stage of the gelatinization process the samples are. The scheme was based on the definitions found in studies by Atwell, Hood, Lineback, Varriano-Marston, and Zobel (1988), Liu (2005) and Xie et al. (2007).

After the RVA analysis at 55 °C, where no apparent viscosity variation was observed, no particular changes in the granule morphology were observed (Fig. 3), indicating that the majority of the granule population did not absorb enough energy to swell at this temperature.

At the RVA analysis at 60 °C, no apparent viscosity variation was observed either (Fig. 2), however some of the starch granules swelled slightly in all the starch samples (Fig. 3). This can be related to the fact that at lower temperatures, there is less swelling and less leaching of starch molecules into the continuous phase and, consequently, lower interactions among the granules. Thus, despite the fact that some of the granules are irreversibly changed, the apparent viscosity of the system was not measurable by the equipment.

At this point, it is important to mention that each particular granule has a specific behaviour and may not perform in the same way the majority granule population does under the same conditions (Huang et al., 2017; Lund & Lorenz, 1984; Tao et al., 2018). The DSC and the RVA results provide a general view of the granular population, while the granules can be observed through the microscope individually. Therefore, this result demonstrates that the ozonation process can change the distribution of gelatinization behaviour among the granules, even though it does not change the average behaviour.

After the RVA analysis at 65 °C, the native starch granules were swollen and their granular integrity (shape and surface) were

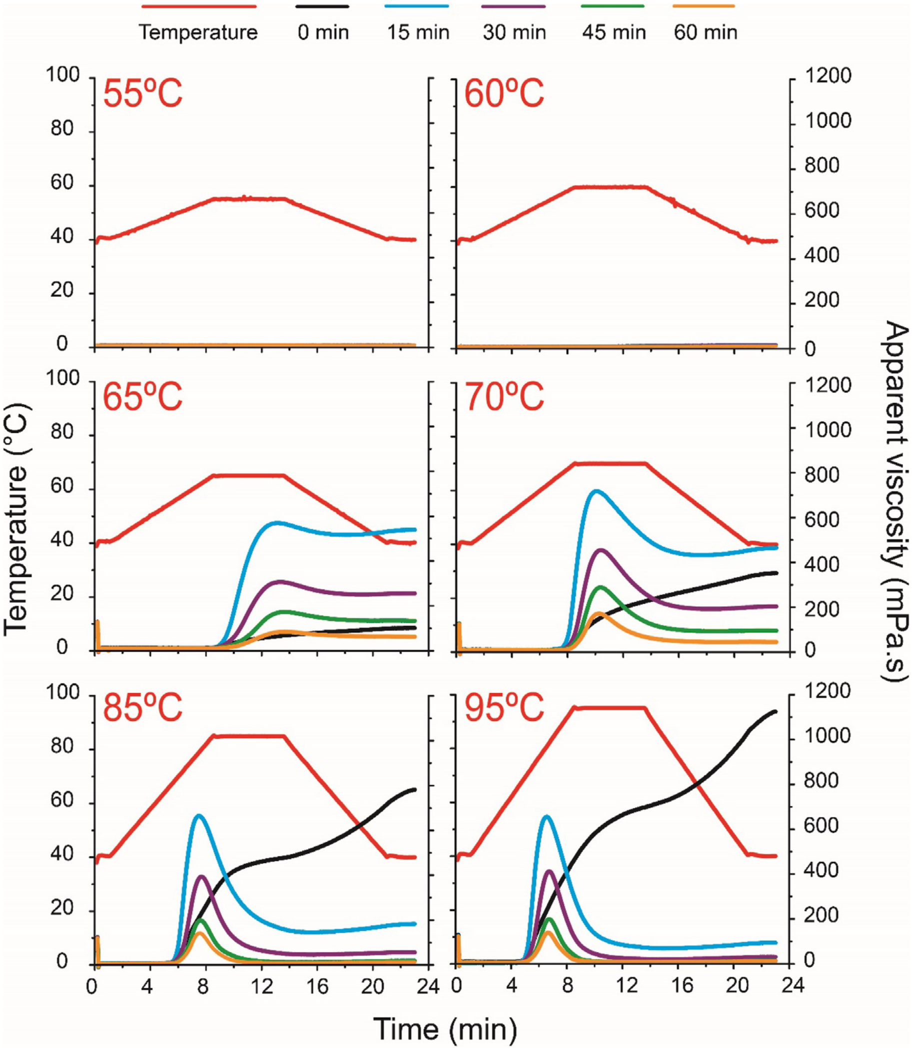

Fig. 2. Rapid Visco Analyser (RVA) curves of the native and ozone-oxidized potato starches analysed under different maximum temperatures.

maintained (Fig. 3). On the other hand, the 15 and 30-min samples also presented whole granules, but their surface was not so marked. In addition, their granules were apparently bigger in size than the native ones, which could explain their higher apparent viscosity. The 45 and 60-min samples also presented highly swollen granules, but their granular surfaces were less distinguishable, as if they were disassembled, indicating that the ozonation at more severe conditions make the starch granules more "fragile".

At 70 °C, all samples presented gelatinized granules (Fig. 3). However, the higher the ozonation time, the less distinguishable the surfaces of the ozonated samples. This indicates that their granular structure no longer withstand these analysis conditions (temperature and shear). Apparently, the largest granules are those of the 15-min sample, which can explain the higher apparent viscosity peak reached by this sample at these conditions (Fig. 2).

In the RVA analyses at 85 and 95 °C, the native starch sample began to exhibit a higher apparent viscosity than the oxidized ones (Fig. 2). By analysing their granules, it is possible to see (Fig. 3) that all the samples started to present granular rupture and higher amylose leaching with increasing temperatures. At 85 °C, the native and the 15-min samples presented distinguishable dispersed granules, while the 30-min and, especially, the 45 and 60-min ozonated samples only presented fragments of the granules dispersed within the system. Similar behaviour occurred after the 95 °C analysis, except that, in this case, the samples of 0 and 15 min were more degraded, while the more ozonated samples were completely disintegrated.

3.2.2. Discussion

Our theory to explain this behaviour is related with the starch structure, which undergoes considerable changes after the ozonation process. Considering the native starch, whose granules carry intact molecules (*i.e.*, non-oxidized), higher temperatures are required to disrupt the bonds of the crystalline region of the granules and lead to a granular swelling, until reaching the point of changing the apparent viscosity of the system. On the other hand, in the oxidized samples, the hydroxyl groups were partially replaced by carbonyl and carboxyl groups, which are electronegative and increased with increasing ozonation time (Castanha et al., 2017). These groups can cause an electrostatic and steric repulsion, resulting in bulkier molecules. Moreover, the cleavage of the glycosidic bonds of the ozonated starch molecules resulted in smaller molecules (Castanha et al., 2017). Both factors probably contributed to the crystalline double helice weakening, thus promoting the flow of water into the granule of the less ozonated samples (15 and 30 min) at lower temperatures if compared to the native sample.

It is important to notice, however, that the same factors that cooperate with the water uptake and granular swelling in the less oxidized samples were responsible for impairing the granule swelling of the highest oxidized samples (45 and 60 min). This occurs since they were developed in an intensified way: there are less hydroxyl groups

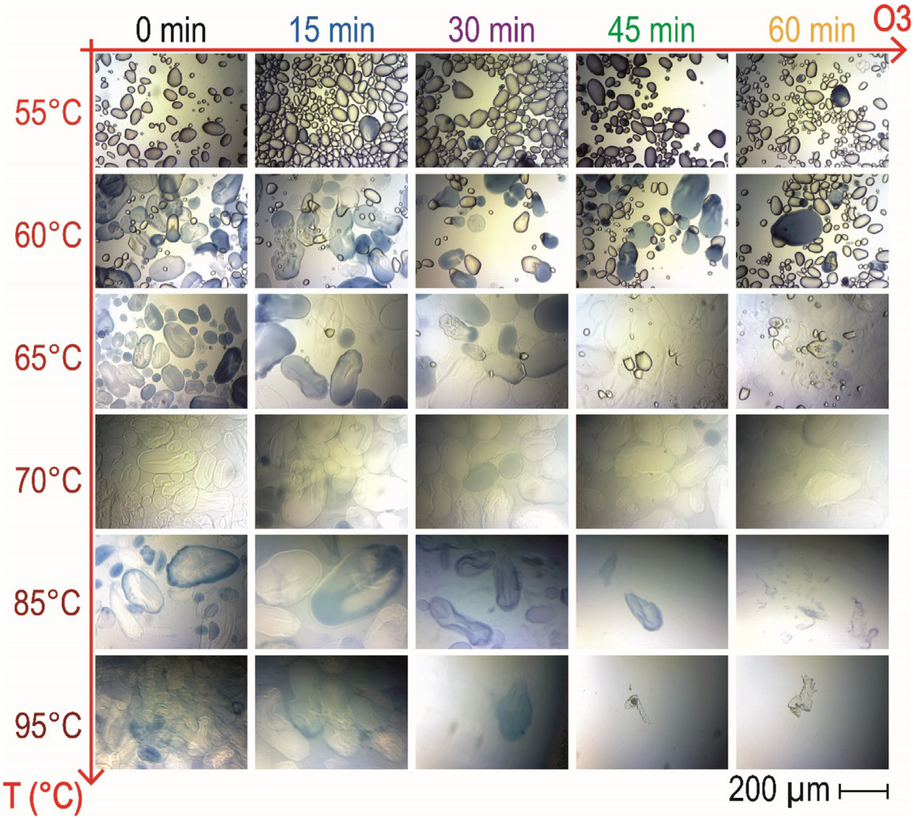

Fig. 3. Light microscopy images of the native and the ozonated potato starch evaluated after the Rapid Visco Analyser (RVA) assays. The black line in the lower right corner measures 200 µm. Lugol solution was used to better visualize the starch granules.

available to form hydrogen bonds with the water molecules and, as these granules are "damaged" (due to the molecular breakdown), they are less capable of maintaining their integrity under stirring and heating.

To sum up, the RVA results (Fig. 2) showed that, in general, the native starch demanded higher temperatures (above 85 °C) to present a more significant apparent viscosity variation compared to the 15 and 30-min ozonated samples. On the other hand, the 15 and 30-min samples presented higher apparent viscosity results at milder temperatures of gelatinization (65 and 70 °C), if compared to the native sample in the same conditions. The 45 and 60-min samples were too degraded to present a good performance in the RVA assays, showing a negligible apparent viscosity in all evaluated conditions. Consequently, focusing on possible industrial applications, it is necessary to understand the capacity of these samples to interact with water (absorption and solubility), as well as the properties of the obtained gel and the starch digestibility.

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

The WSI and WAI of the starch samples are presented in Fig. 5

In general, considering the increase in both ozonation time and temperature (Fig. 5A), the solubility of the starch fractions in water (WSI) increases. Considering only the effect of the temperature on the WSI, the results show that the solubility of the ozonated samples rises more significantly with increasing temperatures above 70 °C, when compared to the variation at mild temperatures. This behaviour is due to the starch gelatinization, in which one of the consequences is leaching of the amylose molecules (which are soluble). Besides, as the amylopectin molecules are the main component of the granules, their solubilization (due to the weakening of the crystalline part during the gelatinization phenomenon) also influences these results. On the other

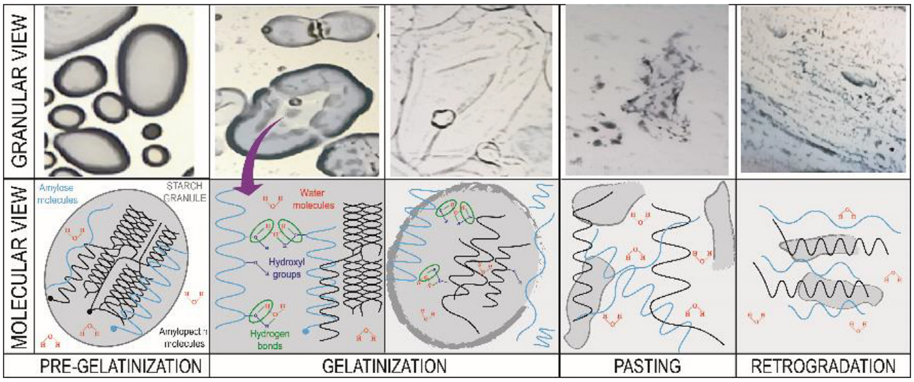

Fig. 4. Graphic representation of the starch pre-gelatinization, gelatinization, pasting and retrogradation phenomena. Top line: granular view (actual images from potato starch). Bottom line: molecular view (representation). Based on the theories presented in the works of Atwell et al. (1988), Liu (2005) and Xie et al. (2007).

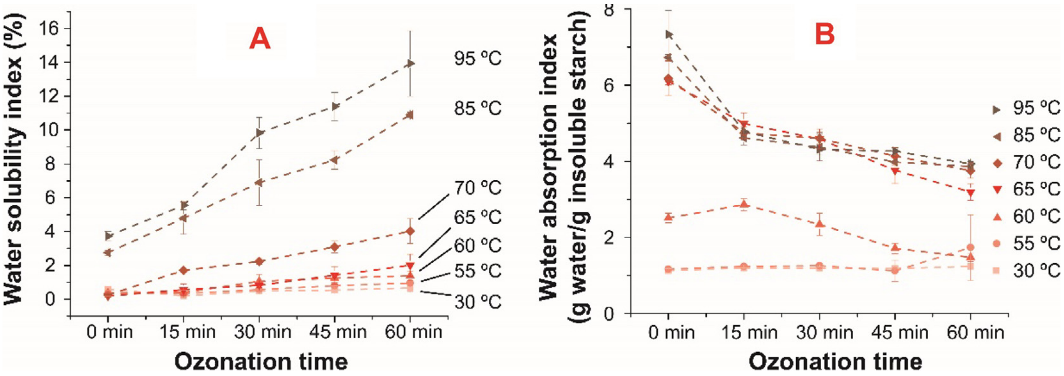

Fig. 5. (A) Water Solubility Index and (B) Water Absorption Index of the native and ozonated samples at different temperatures (30, 55, 60, 65, 70, 85 and 95 °C). Vertical bars represent the standard deviation. The curves are traced to facilitate interpretation.

hand, for the native (0 min) sample, at 70 °C, the WSI value was similar to those observed at lower temperatures, and were only significant above 80 °C. As discussed previously (for RVA analysis), this behaviour is probably due to the fact that the native starch granules are intact: the glycosidic bonds of the molecules are undamaged and their hydroxyl groups were not replaced by electronegative groups, bonding strongly to each other. Thus, their molecules are more tightly bonded, which hinders their solubility in water if compared to the ozonated starches.

Similarly, considering only the effect of the ozonation time on the WSI, it can be observed that the longer the ozonation time, the higher the granule solubility (Fig. 5A). These results are in accordance with the previous discussions and results: the ozonation led to a molecular cleavage and to an increase in electronegative groups (Castanha et al., 2017), and were both responsible for easily disrupting the starch granules, especially at higher temperatures. Thus, the longer the ozonation time, the higher the molecular breakdown and, therefore, the higher the solubility of the granules.

On the whole, the WAI rose when the temperature was increased, but decreased with increasing ozonation time (Fig. 5B). It is important to remember that the WAI represents the amount of water that the starch can absorb and retain, being a property of high importance in the food industry. Considering the effect of the temperature on the WAI, it is clear that the higher the temperature, the higher the water absorption. As in the WSI, the most significant increase occurred at temperatures above 70 °C.

On the other hand, the effect of the ozonation time on the WAI behaviour was more complex to understand. In general, it followed the same pattern observed in the RVA analysis: the 15-min sample presented a slightly higher water absorption capacity at 60 °C. This indicates that the structural changes shown by this sample after the ozonation process allowed the uptake and retention of water in the granules, without using elevated temperatures. Considering the increasing temperatures, however, the native (0 min) starch granules started to be able to absorb and retain more water than the ozonated samples, probably due to their non-damaged structure, which was capable of retaining more water at higher temperatures. Oladebeye et al. (2013) also observed a decrease in the WAI with increasing ozonation time in starches from white and red cocoyam, attributing the results to structural disintegration.

Therefore, the WAI results suggest that the ozonation process can be an alternative to obtain starches with good properties of water absorption and retention if the gelatinization process is conducted at moderate temperatures (above 60 °C). At higher processing temperatures, however, the absorption capacity of the ozonated starches are lower than that of native starch. On the other hand, they presented a higher solubility, which can be interesting for products such as chocolate powder or instant soups, which require easy solubilization.

3.4. Gel strength

The samples obtained after the RVA analysis were stored for 7 days under refrigeration (5 ± 2 °C) before the analysis. After this period, their strength was evaluated through instrumental texture (penetration test). The results obtained are shown in Fig. 6. The samples obtained after the RVA analysis at 55 and 60 °C were not evaluated since they could not form gel, presenting a separation of phases.

The 45 and 60-min ozonated samples did not form consistent gels after RVA analysis (Fig. 6), probably due to the fact that their molecules were too small and with many electronegative groups to permit a strong molecular association in these analysis conditions.

Similar gel characteristics (weak gels) were observed for the native (0 min) starch samples after the RVA analysis at mild temperatures (65 and 70 °C), but for another reason: as explained in previous discussions, the mild temperatures were not sufficient to induce a complete gelatinization of the granules. Therefore, the molecules were not able to reassociate and form firm gels after cooling as they were still in a granular structure. In fact, the strength of the native starch gels gradually grew with increasing processing temperature, achieving its maximum value in the gel obtained after the RVA analysis at 95 °C (0.86 mJ).

On the other hand, the 15-min ozonated sample showed opposing behaviour: a maximum strength was reached at 65 °C, with decreasing gel strength and increasing processing temperature. Molecules with intermediate chain lengths are more likely to reassociate and bind than larger molecules, explaining the gel strength of this sample. As the temperature increased, however, this sample (which was more fragile than the control sample) was more degraded and did not form a consistent gel.

Interestingly, the 15-min sample reached a gel strength value at 65 °C (0.82 mJ) and 70 °C (0.80 mJ) that was similar to the gel strength presented by the native sample at 95 °C (0.86 mJ). This result is highly relevant because the 15-min ozonated starch sample can be used in products where the strength of the gel after storage is more interesting than the pasting properties of the starch, with energy gain (since less heat is needed to paste this starch). This is the case, for example, of meat products, where both water retention and texture are common demands for starch application.

Considering the 30-min ozonated sample, its higher gel strength value was obtained at 65 °C (0.41 mJ), and was half of the force value reached by the 15-min sample in the same conditions. When the processing temperature was increased, the 30-min sample, similarly to the 15-min sample, presented weaker gels.

To visually demonstrate the effect of ozonation on the potato starch gels, a video illustrating the flow of the starch samples gelatinized at 70 °C after 24 h and after 7 days of refrigerated storage was made, and is available at: https://www.youtube.com/watch?v=WtYxreS1-78.

In fact, the results obtained in the texture analysis are consistent with those observed in the other analyses. In the native sample, mild

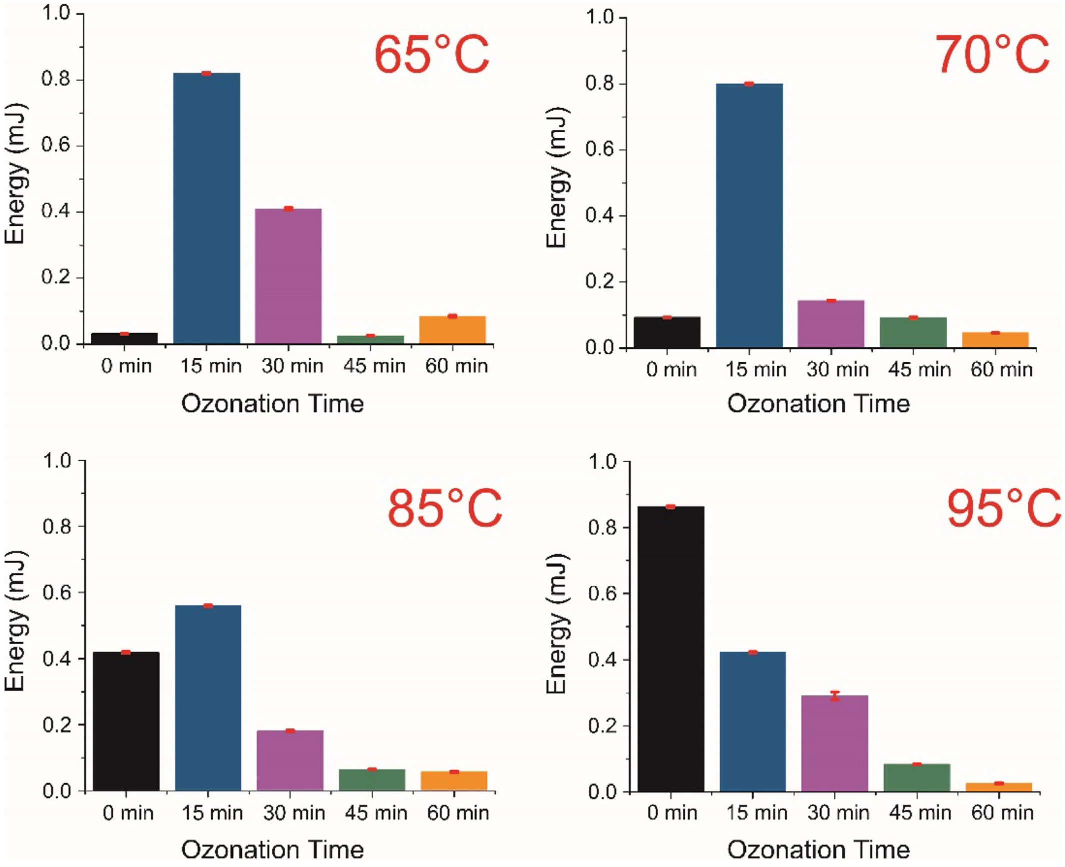

Fig. 6. Strength of the potato starch gels (required energy for penetration of the gels in a puncture test) with different ozonation processes. The gels were obtained after RVA assays at different temperatures (65, 70, 85 and 95 °C). Vertical red bars represent the standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

temperatures were not sufficient to permit a gel formation, but the gel strength increased when the temperature rose. On the other hand, in the 15-min sample, the mild temperatures were sufficient to allow the gelatinization of the starch and consequent gel formation after cooling. The 30-min sample presented similar results, but with lower intensity of gel strength. And the highly-ozonated samples (45 and 60-min) did not form consistent gels (they also did not present viscous pastes in the RVA analysis – Fig. 2) under any condition.

3.5. *In vitro* digestibility

The RDS fraction of the potato starch samples is shown in Fig. 7. The remaining value is basically composed by the SDS fraction, whereas the RS fraction was practically negligible for the ozonated samples, presenting a small percentage (5.3 ± 2.4%, through the glucose analysis approach) in the native sample.

Fig. 7 shows that concerning the reducing sugar content, there is no statistical difference ($p < .05$) between the native and the ozonated samples up to 45 min. Furthermore, no significant difference was observed between the ozonated samples. Only the native and the 60-min sample can be considered different and the reducing sugar content of the 60-min sample is higher than the native sample. In fact, virtually all the 60-min sample was converted into reducing sugar after 20 min of digestion.

Considering the glucose method, the RDS content of the native potato starch sample was lower if compared to the ozonated samples (~73%, while the ozonated samples presented values above 84%). On the other hand, the 15, 30 and 45-min samples can be considered significant equals ($p > .05$) regarding their glucose content, as well as the 30, 45 and 60-min samples.

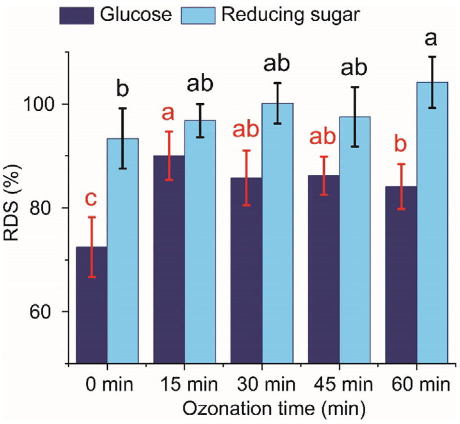

Fig. 7. Rapidly digestible starch (RDS) content of the potato starch samples after *in vitro* digestibility analysis based on both glucose and reducing sugar contents. Vertical red and black bars represent the standard deviation; different small letters indicate significant differences ($p < .05$) among the samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Since the reducing sugar content analysis is based on the evaluation of reducing terminals of the molecules, this analysis is able to detect both the oligosaccharide and glucose molecules. The glucose content analysis, on the other hand, only detects glucose molecules. Considering this information, the RDS result presented in the Fig. 7 is coherent, since the RDS observed through the glucose content was lower than that observed using the reducing sugar content.

In the literature, the RDS fraction of gelatinized potato starch varied from ~73% (Villas-Boas & Franco, 2016) to ~85% (Dupuis, Tsao, Yada, & Liu, 2017), considering studies using methodologies similar to those used in this paper (glucose value after 20 min of digestion), showing a similarity with the reported results (Fig. 7).

The digestibility of the potato starch samples observed in Fig. 7 are also in accordance with the other results presented in this work. The water easily permeates the ozonated starches, which presented lower chain-length molecules. Therefore, it is reasonable to assume that their molecules are more susceptible to an enzymatic degradation. Furthermore, the electronegative nature of carbonyl and carboxyl groups can somehow affect the interaction of the molecules with the enzyme, thus affecting the starch digestibility.

These results may not be considered desirable if considering the trend for products with higher levels of resistant starches as they are continuously related to health benefits for the consumers (Asp & Björck, 1992; Magallanes-Cruz, Flores-Silva, & Bello-Perez, 2017). However, the ozonation can be considered as a good method to increase the enzymatic susceptibility of the starches and can be used for other industrial purposes (as pointed out in the "Technological applications" section of this study). Furthermore, should be mentioned that this result only evaluated the gelatinized starch (which was boiled in water for 20 min). Different results are expected if gelatinization is conducted at mild temperatures, leading to different applications. It is worth mentioning that more studies are needed to evaluate this subject.

Even so, it is important to highlight that we used the methodology proposed by Englyst et al. (1992), which classifies the starch in three fractions of digestibility rates: rapidly digestible starches (RDS), slowly digestible starches (SDS) and resistant starches (RD). Although it is a good approach to evaluate the digestion tendency of isolated starches (without being included in a food matrix, as in this paper), this method cannot be considered a reliable reproduction of the human organism. Thus, a digestibility curve based on the enzymes kinetics would be a better way to evaluate the samples.

4. Final remarks

Considering all the properties presented by the ozonated starch samples, some suggestions for future applications can be made. Regarding the pasting properties, which indicated that the ozonation process can be a good alternative to obtain starches that can form viscous pastes at moderate temperatures, a direct application would be in some conventional products, such as instant soups.

The WAI results suggested that the ozonation process can be a good alternative to obtain starches with good properties of water absorption and retention by conducting the gelatinization process at moderate temperatures. This is the case of many industrial products, especially food, such as meat products for example.

Additionally, in both cases, the ozonation process can be an alternative to obtain hydrocolloids for nursing-care foods, especially dysphagia foods (Funami, 2011) as the gelatinization must be formed at small/moderate temperatures and since syneresis must be avoided due to the risk of aspiration. It is worth mentioning that more studies are needed to evaluate this subject.

Considering the high enzymatic susceptibility presented by the ozonated samples, starch modification using the ozone technology could be applied as pre-treatment for enzymatic processes.

5. Conclusions

The results reported in this paper open up the possibilities of applications for the modified potato starch using the ozone technology. For instance, our results demonstrated that potato starch submitted to mild ozone processing can retain more water after gelatinization at mild temperatures than the native one, resulting in higher apparent viscosity and gel strength. Besides, the *in vitro* digestibility indicated that the ozonated starches presented a higher enzymatic susceptibility if compared to the native sample. On the other hand, the higher ozonated samples were able to completely gelatinize at small temperatures and were also more soluble if compared to the native and less ozonated samples.

All these observed properties can be useful for different technological applications, especially regarding processes/products that demand specific characteristics, such as viscous pastes or higher water retention at moderate temperatures (between 60 and 70 °C).

Consequently, ozone processing can be an alternative to obtain starches with new and potentially useful properties for different industrial applications, with the extra advantage of being obtained through a safe and environmentally friendly technology (ozone).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2018.09.064.

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Declarations of interest

None

Contributors

N. Castanha designed and performed the experiments, evaluated the data and wrote the manuscript. D.N. Santos performed the *in vitro* digestibility experiments and also evaluated and discussed the data. R.L. Cunha evaluated the data and the manuscript. P.E.D. Augusto designed the experiments, evaluated the data, wrote the manuscript and directed and managed the team. All the authors discussed and approved the manuscript.

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