

**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

Potato starch modification using the ozone technology

Nanci Castanha da Silva

Dissertation presented to obtain the degree of Master
in Science. Area: Food Science and Technology

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2016**

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1. Amido de batata 2. Modificação de amido 3. Oxidação de amido 4. Ozônio 5. Tecnologias emergentes 6. Propriedades dos alimentos I. Título

DEDICATION

This work is dedicated to Zé, Lia, Manda, Fezão and my little friend Carlota, for all their patience and support, and to Caio, for all his love and constant motivation.

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RESUMO

Modificação de amido de batata utilizando a tecnologia do ozônio

Modificação de amidos é uma técnica amplamente empregada e muito importante, permitindo a obtenção de ingredientes com diferentes características, ampliando a gama de aplicação dos amidos e trazendo vantagens competitivas para diferentes indústrias. Apesar da produção de resíduos tóxicos, e apesar da crescente preocupação com as questões ambientais, o uso de agentes químicos na modificação de amidos ainda é muito popular. O ozônio pode ser uma alternativa para mudar este cenário, uma vez que é um poderoso agente oxidante que pode ser rapidamente decomposto em oxigênio, cujos resíduos vão de encontro com a demanda global por sustentabilidade. Portanto, esta pode ser uma alternativa mais segura tanto para os consumidores quanto para o meio ambiente. Neste trabalho avaliou-se o uso de ozônio em solução aquosa como método de modificação de amido. Foram investigados os efeitos de diferentes tempos de ozonização na estrutura e nas propriedades do amido de batata. Com o aumento do tempo de ozonização, foram observados aumento nos teores de carbonila, carboxila e açúcares redutores e diminuição no pH, no teor de amilose aparente e no tamanho molecular, demonstrando que o amido de batata processado com ozônio foi modificado a nível molecular. Além disso, análises de difração de raio-X não mostraram alterações significativas na cristalinidade relativa dos grânulos, enquanto nas análises de microscopia foram observadas algumas alterações na morfologia dos grânulos. Todas estas alterações afetaram diretamente as propriedades do amido modificado, resultando em diferenças significativas nas propriedades de pasta, textura de gel e claridade de pasta. Os resultados obtidos são bastante promissores, uma vez que provam a eficácia de se utilizar o ozônio, um método sustentável e ainda pouco explorado, como um eficaz agente oxidante para a modificação de amido.

Palavras-chave: Amido de batata; Modificação de amido; Oxidação de amido; Ozônio; Tecnologias emergentes; Propriedades dos alimentos

ABSTRACT

Potato starch modification using the ozone technology

Starch modification is a widely employed and important technique, allowing to obtain ingredients with different characteristics, expanding the range of applications of starches and bringing competitive advantages for different industries. Despite the production of toxic residues, and despite the increasing concern about environmental issues, the use of chemical agents in starch modification by oxidation is still very popular. Ozone can be a solution to change it, since is a very powerful oxidising agent that can be quickly decomposed into oxygen, whose residues meet the global demand for sustainability. Therefore, it can be a safer alternative for both consumers and environment. In this work the use of ozone in aqueous solution as a starch modification method was evaluated. The effects of the different ozonation times on the structure and properties of the potato starch were investigated. With increasing the ozonation time, it was observed an increase in the carbonyl, carboxyl and reducing sugar contents, as well as a decrease in the pH, apparent amylose content and molecular size, demonstrating that the potato starch processed with ozone was modified in its molecular level. Further, X-ray diffraction analysis showed no significant changes in the relative crystallinity of the granules, while in the microscopy analysis it was observed some alterations in the granules morphology. All these changes directly affected the modified starch properties, resulting in significant differences in the pasting properties, gel texture and paste clarity. The obtained results are promising, since they can prove the effectiveness of using ozone, a still little explored and sustainable method, as an efficient oxidizing agent for starch modification.

Keywords: Potato starch; Starch modification; Starch oxidation; Ozone; Emerging technologies; Food properties

RESUMEN

Modificación de almidón de papa utilizando la tecnología de ozono

La modificación de almidón es una técnica importante ampliamente utilizada, permitiendo la obtención de ingredientes con diferentes características, ampliando el rango de la aplicación de almidones y brindando ventajas competitivas para diferentes industrias. A pesar de la producción de residuos tóxicos y del incremento de la preocupación por cuestiones ambientales, el uso de agentes químicos en la modificación de almidones es aún muy común. El ozono puede ser una alternativa, debido a que es un poderoso agente oxidante que puede ser rápidamente descompuesto en oxígeno, cuyos residuos estarían de acuerdo a la demanda global por la sustentabilidad. Por lo tanto, este puede ser una alternativa segura para el consumidor y para el ambiente. En este trabajo fue evaluado el ozono en solución acuosa como método de modificación de almidón. Los efectos de diferentes tiempos de ozonización en la estructura y propiedades del almidón de papa fueron investigados. Con el incremento del tiempo de ozonización, fue observado un incremento en el contenido de carbonilos, carboxilos y reducción de contenido de azúcar, así como también una disminución en el pH, contenido aparente de amilosa y tamaño molecular, demostrando que el almidón de papa procesado con ozono fue modificado en su nivel molecular. Por otro lado, análisis de difracción de rayos X mostraron cambios no significativos en la cristalinidad relativa de los gránulos, mientras que en los análisis de microscopia se observó algunas alteraciones en la morfología de los gránulos. Todos estos cambios afectaron directamente las propiedades del almidón modificado, resultando en diferencias significativas en términos de propiedades de pasta, textura de gel y claridad de pasta. Los resultados obtenidos son promisorios desde que estos comprobaron la efectividad del uso del ozono, un método poco explorado y sustentable, como un agente oxidante eficiente para modificar almidón.

Palabras clave: Almidón de papa; Modificación de almidón; Oxidación de almidón; Ozono; Tecnologías emergentes; Propiedades de los alimentos

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ABBREVIATION LIST

BD	Breakdown*
BV	Blue Value
FAV	Final Apparent Viscosity*
GPC	Gel Permeation Chromatography
LM	Light Microscopy
OGT	Ozone Generation Time
PAV	Peak Apparent Viscosity*
PT	Pasting Temperature*
RBD	Relative Breakdown*
RC	Relative Crystallinity
RSB	Relative Setback*
RVA	Rapid Viscometer Analyser
SB	Setback*
SEM	Scanning Electronic Microscopy
TAV	Trough Apparent Viscosity*
TCH	Total Carbohydrates

*** These parameters are better described in APPENDIX B.**

1. INTRODUCTION

Starch is an important energy source in human diet and a major component of many plants. Although being formed mainly by α -D-glucopyranosyl units, starch is a very complex material, being presented as a water insoluble granule with a double-helical, semi-crystalline and birefringent structure, formed by two main polysaccharides: amylose and amylopectin (Liu, 2005; Miles, Morris, Orford, & Ring, 1985). Furthermore, it is an important component for many industrial applications, as it is an abundant, renewable, cheap and biodegradable resource, as well as generally regarded as safe and environmentally acceptable (Kochkar, Morawietz, & Hölderich, 2001; Zhang, Zhang, Wang, & Wang, 2009). On the other hand, the naturally available starches have limited properties, being necessary the modification in order to provide further properties for the different industries requirements.

The modified starches present some characteristics that the native starches does not have, thus allowing further uses from a specific source. In fact, the modified starches can bring competitive advantages to some products, with many new functional and added value properties (Kaur, Ariffin, Bhat, & Karim, 2012).

Oxidation is a chemical method of starch modification, being performed by a reaction with an oxidant agent under controlled conditions (Wang & Wang, 2003). The main reagents used for starch oxidation are sodium hypochlorite and hydrogen peroxide. However, by using these chemical agents, toxic wastes can be generated (Chan et al., 2011).

In contrast, if compared to other chemical oxidants, the ozone is considered a “green” and “environmentally friendly” technology, since, by being quickly decomposed to oxygen, it does not leave any residues in food or in environment (Çatal & İbanoğlu, 2014). In fact, it meets the global demand for sustainability being safer for both consumer and environment. The ozone is a very powerful oxidizing agent, with a relatively high ($E^0 = +2.075 \text{ V}$) electrochemical potential (Mahapatra, Muthukumarappan, & Julson, 2005). It can be generated by the exposure of oxygen/air to a high-energy source, which convert molecules of oxygen (O_2) to ozone (O_3) (Khadre, Yousef, & Kim, 2001).

Potato (*Solanum tuberosum* L.) is a worldwide known tuber, cropped under a wide range of climatic and soil conditions, and contains an unique starch, presenting a

large granule size, long amylose and amylopectin chain lengths and the presence of phosphate ester groups (Vasanthan, Bergthaller, Driedger, Yeung, & Sporns, 1999). The phosphorus content in potato starch is relatively high when compared with other sources, and seems to be contained mostly in the branched (amylopectin) fraction in the form of covalently bonded phosphate groups (Posternak, 1950), which also affect the starch properties.

In fact, the ozone has already been used for starch modification in a wide range of starch sources, such as corn, sago, cassava, rice, wheat and potato, among others (An & King, 2009; Çatal & İbanoğlu, 2012a, 2013, 2014; Chan et al., 2011; Chan, Bhat, & Karim, 2009; Klein et al., 2014). However, it is still a fairly studied process. For example, Çatal and İbanoğlu (2012a; 2012b) studied the effects of processing the starches with ozone in aqueous solution for a determined time (1 hour for both studies). In their first work (2012a), corn and potato starch suspensions were processed and the effects on the structural (only the granule morphology), thermal and pasting properties were evaluated. The second study (2012b) evaluated the effect of the ozonation on the structure (only the granule morphology), physic-chemical (pH and color) and microbiological (total bacteria and mold/ yeast) properties of wheat, corn, potato and rice starches. Both works discuss the functional properties of the processed starches. However, they slightly discussed the mechanisms of ozone action and how it acts over the process. In addition, the structural changes are described only through the granule morphology, without further molecular information - chemical and structural modifications of starches, i.e. the possible changes in the crystallinity of the starch granules or carbonyl and carboxyl group contents, were not demonstrated. Finally, only one experimental condition was evaluated, which limits the understanding of the involved mechanisms.

The present work, therefore, aims to study the potato starch modification by ozone, providing more information about the molecular and granule modification and degradation by ozone, correlating it with the resulted changes on functional and technological properties. Therefore, it proposes a better understanding about the process-structure-properties relationships.

In fact, this work is accepted for publication on Food Hydrocolloids (APPENDIX D; DOI: [10.1016/j.foodhyd.2016.12.001](https://doi.org/10.1016/j.foodhyd.2016.12.001)).

2. OBJECTIVES

2.1. General Objectives

The general objective of the present work was to modify potato starch using the ozone technology.

2.2. Specific Objectives

The specific objectives of the present work can be described as follows:

- Increasing the possibilities of using potato starch, by meeting the growing industrial demand for specific functional characteristics and considering the environmental claim;
- Evaluating and describing the structural and functional properties of the modified starch, aiming to better understand the degree of the oxidation in each processing time;
- Correlating and describing the process-structure-properties relationships.

3. LITERATURE REVIEW

3.1. Starch

Starch is an important energy source in human diet and a major component of many plants. Although being formed mainly by α -D-glucopyranosyl units, starch is a very complex material, being presented as a water insoluble granule with a double-helical, semi-crystalline and birefringent structure, formed by two main polysaccharides: amylose and amylopectin (Figure 1). The ratio of these macromolecules varies according to some factors, as botanical source and harvest period (Liu, 2005; Miles et al., 1985; Svegmarm et al., 2002; Tester et al., 2004).

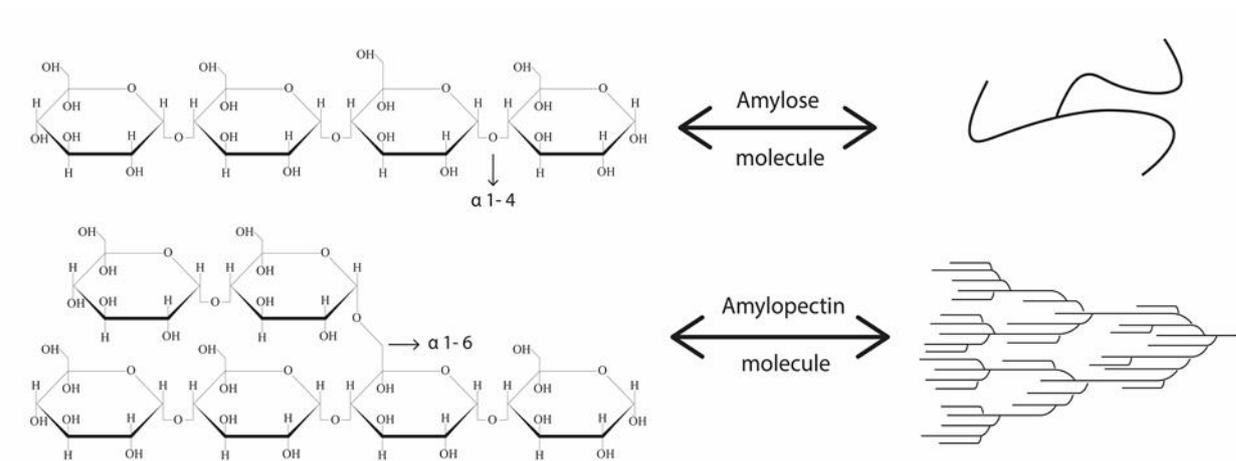


Figure 1 – Schematic representation of amylose and amylopectin molecules

Amylose molecules are essentially linear, composed by (1→4)-linked α -D-glucopyranosyl, presenting a small amount of branches, and it is believed to be randomly mixed with other materials in the amorphous domain of the starch granule (Hizukuri et al., 2006; Tester et al., 2004). The addition of such factors as linearity, mobility and hydroxyl groups give to the amylose molecules a parallel conformation and thus the capacity of stay closely enough and form hydrogen bonds between hydroxyls and adjacent molecules. Such phenomenon of intermolecular association of amyloses is known as retrogradation (Wurzburg, 1986b).

Amylopectin is a highly branched molecule composed by short chains of (1→4)-linked α -D-glucopyranosyl units with (1→6)-linked branches, with a tree-like

arrangement of double helices, being the main component of the crystalline domains in the starch granule (Hizukuri et al., 2006; Tester et al., 2004). The fact of having large size and highly branched molecules decreases the capacity of the amylopectin to form intermolecular hydrogen bonds, since they have a reduced mobility and cannot be oriented close enough to permit bonding. Consequently, amylopectin result in clear and stable aqueous solutions, becoming less harder and not opaque on aging (Wurzburg, 1986b).

Starch granule is normally round or oval shaped, formed by an hilum at the center surrounded by amylopectin molecules, arranged radially in a cluster structure, and amylose molecules dispersed among them, being more concentrated at the periphery of the starch granule (Jane & Shen, 1993).

When heated in the presence of a sufficient amount of water, the starch granules swell irreversibly, becoming much larger than the original size. This is known as starch gelatinization, and occurs at temperatures usually between 60-70°C. During this phenomenon the molecular conformation of the interior of the granule is lost, as well as the granule birefringence and its crystallinity. If this swollen starch remains in the absence of mechanical shear and at temperatures below 100°C, the granule integrity is maintained and temporary networks are formed in the swollen granules. This allows the formation of an opaque and firm gel in room temperature (Miles et al., 1985).

As an abundant, renewable, cheap and biodegradable resource, and due to that being generally regarded as safe and environmentally acceptable, starch has attracted much academic and industrial interest (Kochkar et al., 2001; Zhang et al., 2009). In food and non-food industries, like pharmaceuticals, textiles, alcohol-based fuels, paper, mining and adhesives, starches has wide application, affecting products homogeneity, texture, viscosity, adhesion, moisture retention etc., being also used in the production of biodegradable packaging materials, thin films and thermoplastic materials, among others (Biliaderis, 1982; Kaur et al., 2012).

However, the starch of each botanical source has specific properties, since the amylose-amylopectin ratio and their conformation vary widely within the starch granules. Consequently, the selection of a specific starch source is necessary in order to provide the necessary properties that are desirable for the different industries.

3.2. Potato Starch

Potato (*Solanum tuberosum* L.) is a worldwide known tuber, cropped under a wide range of climatic and soil conditions. Starch is a major component of potato, corresponding up to 85% of its dry matter, and considerable amounts of potato are used for starch production every year (Vasanthan et al., 1999).

Compared to other commercially available starches, especially from cereals, potato starch is unique, presenting a large granule size, purity, long amylose and amylopectin chain lengths, presence of phosphate ester groups on amylopectin and ability to form thick viscoelastic gels upon heating and subsequent cooling (Vasanthan et al., 1999). Besides, potato starches has the capacity to form highly viscous and clear gels when gelatinized, due to the phosphate ester groups and relative absence of lipid (Mishra & Rai, 2006).

The different properties of potato starches have been reported to be affected by environmental factors during growth and harvest, storage temperature, genotypes and cultural practices (Singh et al., 2008), potato cultivars and growth time and starch properties variation during potato growth (Liu et al., 2003).

The phosphorus content in potato starch is relatively high (about 1 atom of phosphorus per 300 glucose units) when compared with other sources, and seems to be contained in the branched (amylopectin) fraction in the form of covalently bonded phosphate groups (Posternak, 1950). It is considered to be an important factor affecting the rheological, thermal, structural and nutritional properties of potato starches and starch gels (Lu et al., 2012; Svegmarm et al., 2002).

The properties of native potato starch, however, may not be desirable for all applications. As an alternative, many modification methods can be used to achieve characteristics that better suits the industrial applications.

3.3. Starch Modification

Some characteristics, or the lack of them, limits the application of native starches. Starch modification can improve these characteristics, bringing competitive advantages to some products, with many new functional and added value properties, by improving product aesthetics, lowering recipes and production costs, ensuring product consistency and extending shelf-life (Kaur et al., 2012; Wurzburg, 1986c).

Modification of starches are widely used in many food and non-food applications, affecting the reactivity, functionality and applicability of the modified starches. According to Kaur et al. (2012), there are basically four kinds of starch modifications: chemical, physical, enzymatical and genetical.

Physical modifications, such as microwave, radiation, extrusion, deep freezing, ultrasound, among others, has been used to produce modified starch with specific functional properties, with the positive aspect of not involving any chemical agents. Enzymatic modification is based on starch modification using different enzymes, mainly the hydrolyzing ones. Genetic, or biotechnology, modification is a transgene technology with potential to produce novel starches, such as amylose-free (waxy) starch, high-amylose starch, higher or lower phosphate content starches, among others (Kaur et al., 2012). These methods have the advantage of not generating chemical wastes in high concentrations.

In contrast, the chemical modification methods, such as derivatization (etherification, esterification and crosslinking), oxidation, cationization and grafting, are recently rising issues concerning consumers and the environment, since they have as a consequence a chemical waste generation and high costs, which leads to a search for new modification processes (Kaur et al., 2002; Liu et al., 2003). Still, the chemical methods of starch modification are widely employed, considering the facility of application and obtainment and also considering it is a relatively simple methods which impart unique characteristics to the starches.

Oxidation, a chemical method of starch modification, is mainly performed by a reaction of starch with an oxidant agent under controlled conditions (Wurzburg, 1986c). It is a commonly used method for obtaining modified starches with low viscosity at high solids concentration (Parovuori et al., 1995), as well as forming starches with good binding capacity and film forming properties (Chan et al., 2012).

In oxidation, two main reactions take place simultaneously. Firstly, the hydroxyl groups of the starch molecules are oxidised to carbonyl groups, followed by carboxyl groups, primarily at C-2, C-3, and C-6, as illustrated in Figure 2. Secondly, the starch molecules are depolymerized by cleaving α -(1 \rightarrow 4)-glucosidic linkages. The number of carbonyl and carboxyl contents indicate the extent of oxidation (Wang & Wang, 2003; Wurzburg, 1986c).

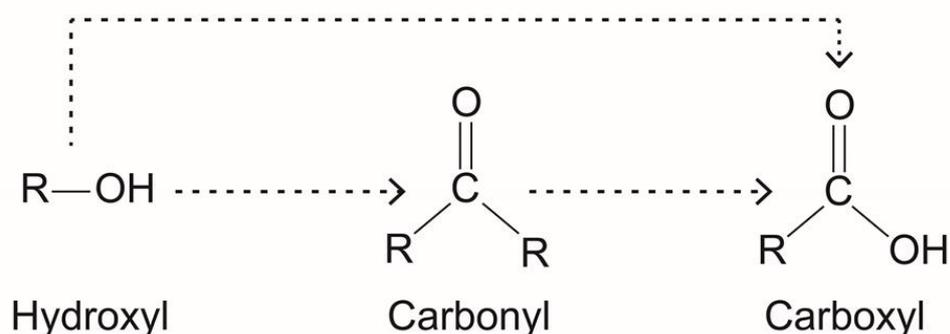


Figure 2 – Hydroxyl groups oxidation into Carbonyl and/or Carboxyl groups

The main reagents used for starch oxidation are sodium hypochlorite and hydrogen peroxide, but ammonium persulfate, sodium bromate and sodium and potassium permanganate have also been utilized (Dias et al., 2007; Klein et al., 2014; Wang & Wang, 2003; Zhang, Zhang, Wang, Chen, & Wang, 2009).

Starch oxidation using chemical agents, however, have some drawbacks. The starch breakdown produces small molecules that lower the process yield (Wing & Willett, 1997). Furthermore, and mostly important, a large amount of wastewater containing high concentrations of salts or toxic wastes can be generated (Chan et al., 2011).

In this context, is necessary to find alternatives to these oxidation agents. Ozone, a high oxidative gas, is currently being used for this purpose.

3.4. Ozone

Ozone, a natural substance found in our atmosphere, is a form that includes three atoms of oxygen (O_3) compared to the standard two (O_2) in a molecule of oxygen. It is a blue gas, partially soluble in water, has a pungent and very characteristic odour and is relatively unstable at normal temperatures and pressures. Ozone is a very powerful oxidizing agent, with a relatively high ($E^0 = +2.075$ V) electrochemical potential (Mahapatra et al., 2005).

Ozone was discovered by C. F. Schonbein in 1839, being its first commercial use attributed to a water supply treatment in 1907 (Kogelschatz, 1998). However, the Food and Drug Administration (FDA) of the U. S. only approved the ozone as generally recognized as safe in 1997, and its use as a direct contact food sanitizing agent was

allowed only in 2001 (Graham, 1997; Mahapatra et al., 2005). Since then, food industry has shown great interest in using ozone for several applications.

Ozone can be generated synthetically, usually by the exposure of air or another gas containing normal oxygen, to a high-energy source, which convert molecules of oxygen into molecules of ozone. It must be manufactured on site immediately before use, since it is unstable. In aqueous solutions it decomposes continuously and slowly to oxygen (Khadre et al., 2001).

In water solution, the ozone penetrates easier the starch than in gas form (Tiwari, Muthukumarappan, O'Donnell, Chenchiah, & Cullen, 2008). Still, it may react better in water due to different pathways: direct reaction with the sample or decomposition into radicals, and the radicals react with the sample, as described by Khadre et al. (2001). Some external factors that affect the ozone solubilization in water, its reactivity and antimicrobial efficacy are: the flow rate of ozone, contact time of the gas with the substances, turbulence of bubbles, design of reactors, purity, pH and temperature of the water and presence of ozone-consuming compounds in the solution (Khadre et al., 2001).

Although ozone is an excellent oxidizing agent, the oxidation of starches using ozone are not completely understood, and new researches still can be done.

3.5. Starch modification by ozonation

The new challenges of starch modifications require safer methods for both consumer and environment. Ozone, if compared to other chemical oxidants, is considered as a green and environmentally friendly technology, since it does not leave toxic residues in food or in environment, being quickly decomposed to oxygen (Çatal & İbanoğlu, 2014; Klein et al., 2014).

Ozone as a gas or in aqueous solution has been used in a wide range of starch sources, such as corn, sago, tapioca, cassava, rice, wheat and potato, among others (Çatal & İbanoğlu, 2012a, 2013, 2014; Chan et al., 2011; Chan et al., 2009; Klein et al., 2014; Oladebeye et al., 2013).

The main observed results in these studies were the variation in molecular weight (Chan et al., 2011), decreasing in starch apparent viscosity (Çatal & İbanoğlu, 2012a, 2014; Chan et al., 2011; Chan et al., 2009; Klein et al., 2014) , no changes in the granule surface morphology or in the birefringence of starch under polarized light

microscopy (Çatal & İbanoğlu, 2012a, 2013; Klein et al., 2014), carboxyl and carbonyl contents increasing (Chan et al., 2009). The extent of starch oxidation and main differences in the results depends on the botanical sources of starches (Chan et al., 2011; Chan et al., 2009) and the process conditions (amount of ozone, time, temperature).

Although some studies involving potato starch and ozone have already been published (Çatal & İbanoğlu, 2012a, 2012b), the mechanisms of action of the ozone in the potato starch was slightly discussed. Based on this information, this work intends to better discuss the ozone action mechanisms in the potato starch and the process-structure-properties relationship.

3.6. Potato starch oxidation: general hypotheses

To subsidize the evaluation and the discussion of the results in the present work, it is important to point out some assumptions and information that explain or at least elucidate the characteristics and the structure of starches and some hypothesis about its modification by oxidation. The most important information that should be enlightened is described in the topics listed below.

- I. Ozonation is an oxidation method, and it is considered a process of starch chemical modification (Kaur et al., 2012);
- II. Two main reactions may take place during the starch processing by an oxidizing agent: oxidation and hydrolysis. The first one consists on the oxidation of the hydroxyl groups (C-OH) of the starch molecules to carbonyl (C=O) and carboxyl (HO-C=O) groups, primarily at the carbons 2, 3 and 6 (Figure 2). In the second reaction, the starch molecules are depolymerized by cleaving glycosidic bonds (Boruch, 1985; Forssell et al., 1995; Wurzburg, 1986a). Further details are presented in the Section 5.3 – Summarizing: Proposed Mechanisms of the present work.
- III. The starch granules, a highly organized structure, is composed essentially by amorphous and crystalline domains. This conformation is ruled by genetic and environmental factors, being the structure affected by intermolecular bonding between amylose and amylopectin molecules (Liu, 2005). A possible representation of the starch granule structure is shown

in Figure 3. Figure 3 was inspired by the illustrations presented in the works of Gallant, Bouchet, & Baldwin (1997); Pérez, Baldwin, & Gallant (2009); and Tang, Mitsunaga, & Kawamura (2006); and refined by the theories and details presented in the works of Eliasson & Gudmundsson (2006); Gallant et al. (1992); Hizukuri, Abe & Hanashiro (2006); Liu, 2005; and Tester, Karkalas & Qi (2004). This scheme is important to indicate the parts in the starch granule where the oxidation possibly occurred.

- IV. Acids and enzymes attacks initially and rapidly the amorphous region of the starch granules (Figure 3d), which is also more susceptible to be rapidly penetrated by water (Hizukuri et al., 2006; Robyt, 2009), due to its higher accessibility. Then, if the attack is long/ strong enough, a second slowly stage may occur on the more crystalline domains (Liu, 2005). Thus, it is reasonable to expect that the oxidation by ozone in aqueous solution occur in the same way. Furthermore, the amylose molecules and some of the branching points of the amylopectin molecules are believed to be mainly in the amorphous phase of the granules (Figure 3d), and these regions, as the periphery of the crystalline rings, are supposed to be attacked first (Eliasson & Gudmundsson, 2006).
- V. Potato starch, unlike starches from other sources, has a high phosphorus content in its composition, which is in the form of covalently bond phosphate groups, mainly associated with the amylopectin molecules (Takeda & Hizukuri, 1982). This phosphate groups are believed to be located far from the reducing groups and from the branching points in the potato starch molecules (Posternak, 1950). The phosphorus content can affect several properties of the starch, such as rheological, thermal and structural properties, depending, however, of an delicate balance between phosphorus and amylose contents (Lu, Donner, Yada, & Liu, 2012). This is due to the high electronegativity of the phosphate group when ionised, which is repelled by the electronegative oxygen of the starch molecules, causing a higher dispersion of the amylopectin molecules (Craig et al., 1989; Nutting, 1951).

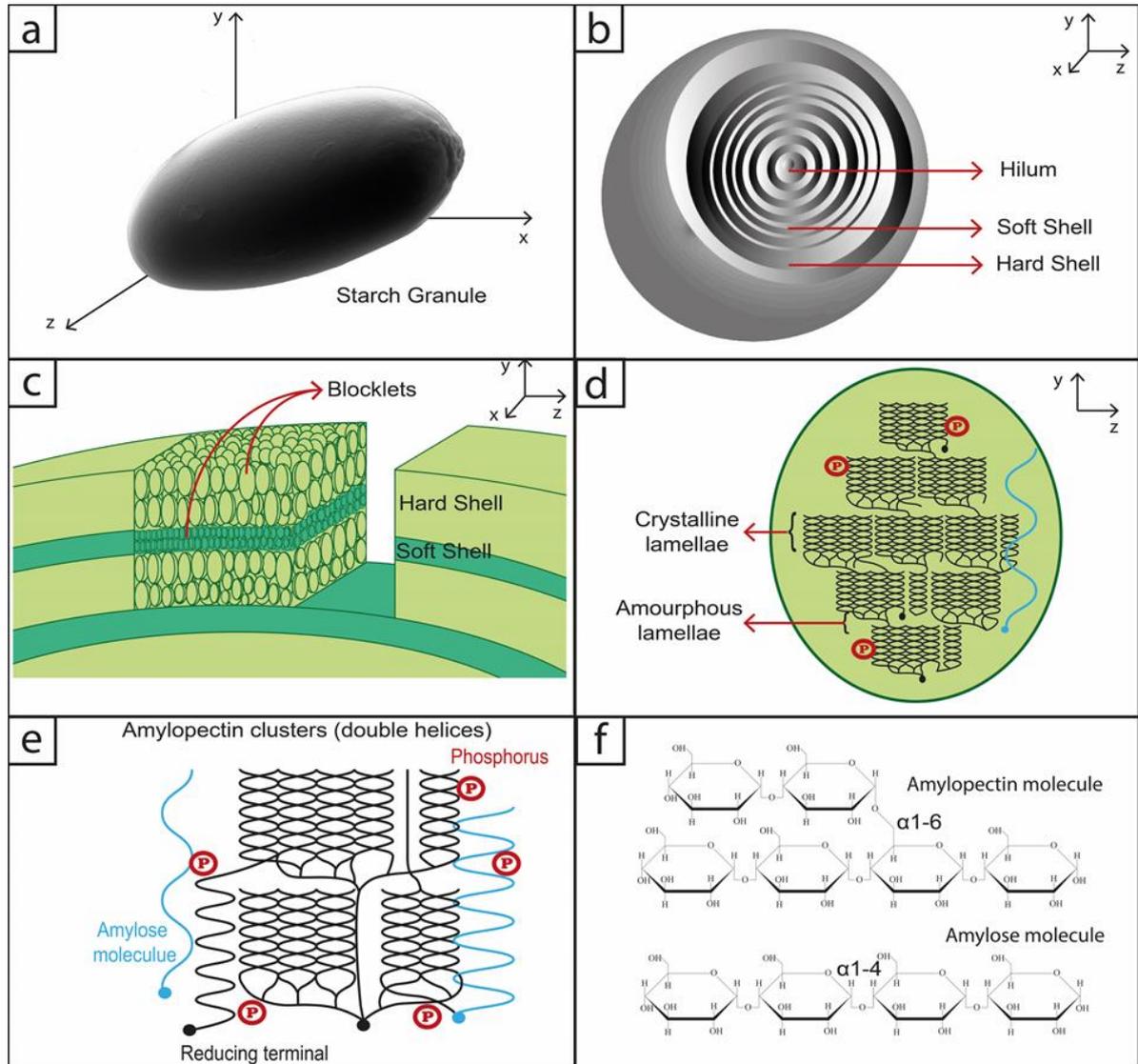


Figure 3 - Schematic representation of potato starch structure. a. Image of a potato starch granule (obtained by scanning electronic microscopy); b. Representation of the interior of a starch granule, emphasizing the alternation between the hard and the soft shells; c. Spatial conformation of the blocklets in the shells; d. Details of a blocklet; e. Spatial conformation of amylose and amylopectin molecules and phosphate groups in the blocklets; f. Details of amylose and amylopectin molecules. This figure was inspired by the illustrations presented in the works of Gallant, Bouchet, and Baldwin (1997); Pérez, Baldwin, and Gallant (2009); and Tang, Mitsunaga, and Kawamura (2006); and refined by the theories and details presented in the works of Eliasson and Gudmundsson (2006); Gallant et al. (1992); Hizukuri, Abe and Hanashiro (2006); Liu, 2005; and Tester, Karkalas and Qi (2004).

4. MATERIAL AND METHODS

The ozonation was carried out by dissolving the ozone into the starch suspension in water, by considering that the ozone in aqueous solution penetrates easier the starch than in gas form (Tiwari et al., 2008), as well as, it may react better in water due to different pathways: direct reaction with the sample or decomposition into radicals, and the radicals react with the sample, as described by Khadre et al. (2001). Furthermore, it is the feasible way of industrially processing the starch, justifying the selection. Consequently, the evaluated process, although in a laboratorial scale, was conducted as closer as possible to the industrial process, facilitating further scale up studies.

4.1. Materials

The starch used in this study was extracted from native potatoes (*Solanum tuberosum* L., Monalisa cultivar) purchased in markets in Hortolândia (São Paulo, Brazil). The starch extraction was performed immediately after the obtainment of the fresh samples. All the chemicals were in analytical grade, being used without further purification.

4.2. Starch extraction

Potato tubers (moisture $84.4 \pm 0.2\%$; in dry basis, % in w/w: lipids $0.34 \pm 0.00\%$; proteins $10.18 \pm 0.04\%$; ashes $5.92 \pm 0.00\%$) were washed, brushed and all the bruises were removed. The potatoes were cut into slices of 4-5 cm of thick, which were immediately soaked in a solution of sodium bisulphite 0.4% to avoid enzymatic browning and blended in a laboratorial scale blender (maximum capacity = 10 L) for 5 minutes. The obtained suspension was passed through a 250 μm sieve. The retained was mixed again in distilled water and passed through the same sieve. The filtrate was then sieved (63 μm) and the obtained starch slurry was allowed to sediment for about 1 hour. The supernatant liquid was discarded and the starch layer was suspended in distilled water and allowed to settle again. This was repeated 4-5 times until the supernatant liquid becomes clear. The obtained starch was dried in an air circulation oven at 35°C until moisture content of approximately 12%. The dried starch was ground

in a mortar, sieved (250 μm), packed in glass containers and stored at room temperature until further use. The obtained starch presented $28.4 \pm 0.2\%$ of apparent amylose content, $790 \pm 20 \text{ mg}\cdot\text{kg}^{-1}$ of phosphorus content and approximately 99.5% of purity (% in w/w: lipids $0.05 \pm 0.00\%$; proteins $0.10 \pm 0.01\%$; ashes $0.33 \pm 0.05\%$).

4.3. Potato and potato starch chemical composition

The proximate composition was carried out using the methods described in AOAC (2006): nitrogen content was determined by micro-Kjeldahl method using the 6.25 factor for the crude protein content; the lipid content was evaluated in Soxhlet device using hexane as a solvent; and the ash content was determined in muffle furnace at 550°C until complete calcination.

For the phosphorus content determination an acidic digestion of the samples was performed in a microwave oven (Provecto Analitica, Jundiai, São Paulo, Brazil), using 3 mL of concentrated HNO_3 and 2 mL of H_2O_2 . The following program was used: 320 W (hold for 2 minutes), 0 W (hold for 1 minute), 400 W (hold for 5 minutes), 790 W (hold for 1 minute) and finally 0 W (hold for 1 minute). Subsequently, the digested samples were cooled and transferred to 50 mL volumetric flasks, and diluted with deionized water. The solutions obtained were analysed by Optical Emission Spectrometry (OES) with an Inductively Coupled Plasma (ICP) using a Spectrometer (Optima 8300 ICP-OES, PerkinElmer, Norwalk, CT, USA) with 213.617 nm of emission wavelengths, calibrated with a standard curve with concentrations in the range of 0.1 to $5 \text{ mg}\cdot\text{L}^{-1}$.

4.4. Ozone processing

The laboratorial scale plant of ozonation is schematized in Figure 4.

The ozone was produced in an ozone generator unit (Ozone & Life, O&G Model RM 3.0, São José dos Campos, Brazil) by the coronal-discharge method from industrial oxygen (95% purity). After passing through the ozone generator, the ozone-rich gas current was directed to a cylindrical glass reactor (2 L capacity, 56 cm of height, 6 cm of internal diameter) by a glass tube, which ends in a porous gas disperser. The starch suspension was placed inside the reactor, and the gas current was dispersed as small bubbles to increase the ozone diffusion in the suspension. After passing through the sample, the gas leaves the reactor, being directed to an ozone destructor, which

thermally destroys the residual ozone, releasing oxygen. The gas flow in the reactor was maintained constant at $0.5 \text{ L} \cdot \text{min}^{-1}$, and the ozone concentration in the gas current was kept at $47 \text{ mg O}_3 \cdot \text{L}^{-1}$.

The starch (10% w/w in dry basis) suspension was prepared in distillate water, being 700 mL placed in the main reactor and processed for 15, 30, 45 and 60 minutes, under constant stirring using a magnetic stirrer. After each desirable processing times, the samples were maintained in the reactor for further 10 min receiving oxygen only, in order to eliminate the residual ozone. After processing, the suspensions were allowed to settle and the supernatant liquid was discarded. The starch was dried in an air circulation oven at 35°C until moisture content of approximately 12%. The dried starch was ground in a mortar, sieved ($250 \mu\text{m}$) and stored for further analysis in glass containers. A control sample (unprocessed starch) was used for comparison. Four replicates for each processing condition were prepared.

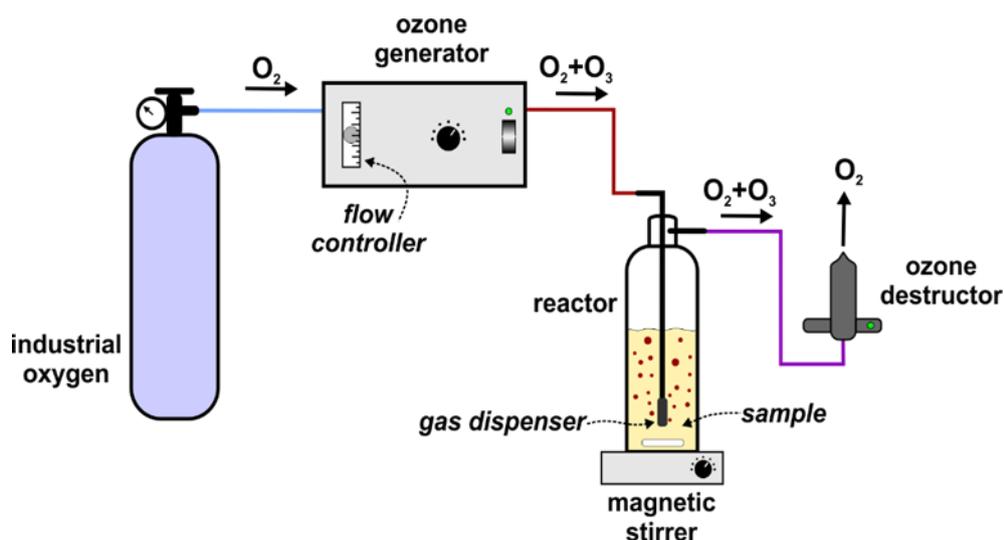


Figure 4 - General aspect of the starch ozonation system

4.5. Starch structure evaluation

4.5.1. Carbonyl and carboxyl group contents

The carboxyl content was determined using the methodology described by Chattopadhyay, Singhai and Kulkarni (1997) with modifications. A slurry containing 3 g of starch and 25 mL of 0.1 M HCl was stirred for 30 min, being then vacuum filtered

using qualitative filter paper (205 µm), and washed with 400 mL of distilled water. The starch obtained was suspended in 300 mL of distilled water and brought to boiling in water bath for 15 minutes to gelatinize. The sample was then titrated with 0.1 M NaOH still warm with the aid of an automatic titrator (Plus Titrimo 848, Metrohm, Herisau, Switzerland) until pH 8.3. A starch native sample was analysed similarly and regarded as the analysis blank. The result was expressed in quantity of carboxyl groups per 100 glucose units (COOH/ 100GU) according to Equation 1 (where V_s is the volume of NaOH required for the ozonated samples, V_b is the volume of NaOH used in the blank, M is molarity of NaOH and W is the sample mass in dry basis).

$$\text{Equation 1: } \frac{COOH}{100GU} = \frac{(V_s - V_b) \cdot M \cdot 0.045 \cdot 100}{W}$$

The carbonyl content was determined according to the procedure of Smith (1967), with modifications. A suspension of 4 g of starch in 100 mL of distilled water was led to a boiling water bath for 30 minutes for complete gelatinization. The obtained gel was cooled to 40°C and had its pH adjusted to 3.2 with HCl 0.1 M. 15 mL of previously prepared hydroxylamine solution (made with 10 g of hydroxylamine hydrochloride, 40 ml of 0.5 M NaOH solution and distilled water until the solution reach 200 mL) were added to the sample, which remained in a 40°C water bath for 4 h. After this time, the excess of hydroxylamine was titrate with 0.1 M HCl until pH 3.2. A blank was prepared with all the reagents, without a starch sample. The result was expressed in quantity of carbonyl groups per 100 glucose units (CO/ 100GU), as described in Equation 2 (where V_b is the volume of HCl used in the blank, V_s is the volume of HCl required for the sample, M is molarity of HCl and W is the sample mass in dry basis).

$$\text{Equation 2: } \frac{CO}{100GU} = \frac{(V_b - V_s) \cdot M \cdot 0.028 \cdot 100}{W}$$

4.5.2. pH

The pH was determined in a calibrated potentiometer (Tecnal, TEC-5 mode, Piracicaba, Brazil), using a 10% starch slurry under constant stirring using a magnetic stirrer, as specified in Adolfo Lutz Institute (2008).

4.5.3. Apparent amylose content

Apparent amylose content was determined according to ISO methodology (1987). Starch grains were dispersed with ethanol and gelled with sodium hydroxide in a boiling water bath for 10 minutes. An aliquot of 18 mL was transferred to a tube with 2 mL of NaOH 0.09 M. Then, an aliquot of 5 mL of this new solution was transferred to a volumetric flask of 100 mL with acetic acid $1 \text{ mol}\cdot\text{L}^{-1}$ and iodine, forming a complex of blue colour, which was quantified by spectrophotometry at 620 nm wavelength (spectrometer Femto, Model 600S, São Paulo, Brazil). A reference curve containing standards amylopectin (A8515) and amylose (A0512 type III) from Sigma-Aldrich Pty Ltd. (Castle Hill, NSW, Australia) was used.

4.5.4. Reducing sugar content

The reducing sugar content was determined by the Somogyi-Nelson colorimetric method (Nelson, 1944; Somogyi, 1945). 2 g of starch (dry basis) were diluted in distillate water in a 200 mL volumetric flask. 1 mL of the starch dispersion was then transferred to a 10 mL tube and 1 mL of alkaline copper reagent (prepared as described in Somogyi (1945)) was added, being the tube stirred and subsequently heated for 10 min in a boiling water bath. The tubes were then cooled in cold water and 1 mL of the arsenomolybdate reagent, prepared as described in Nelson (1944), was added. The solution was then diluted in distilled water until reaching 10 mL, vigorously stirred and finally quantified in a spectrometer at 535 nm wavelength (spectrometer Femto, Model 600S, São Paulo, Brazil). The blank was prepared using 1 mL of distillate water and the Somogyi-Nelson reagents. Also, a standard curve was previously prepared using 5 solutions at different glucose concentrations (ranging from 0 to 100 μg of glucose per 1 ml).

4.5.5. Gel permeation chromatography

The molecular mass distribution profiles of the starch samples were determined by gel permeation chromatography (GPC) according to Song and Jane (2000), with modifications. A GE XK 26/70 column (2.6 cm diameter and 70 cm high) packed with Sepharose CL-2B gel (Sigma, Sweden) was used. 10 mL of dimethylsulfoxide (DMSO; 90%, Labsynth, Brazil) was added to 0.1 g of starch and heated in boiling water bath

for 1 h, then remaining for 24 h at 25°C under constant stirring. An aliquot of 3 mL (30 mg of starch) was then mixed with 10 mL of absolute ethanol to precipitate the starch, being the suspension centrifuged for 30 min at 3000 g. The precipitated starch was dissolved in 9 mL of boiling distilled water and put in boiling water bath for 30 min. An aliquot of 4 mL was then eluted in the chromatographic column upwardly. A solution containing 25 mmol·L⁻¹ of NaCl and 1 mmol·L⁻¹ of NaOH was used as eluent at a rate of 60 mL·h⁻¹. Fractions of 4 mL were collected by a fraction collector (Gilson, model FC203B, Middleton, England) and analysed for total carbohydrate content at 490 nm by the phenol sulfuric method (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956) and blue value at 620 nm (Juliano, 1971), using a microplate reader (Asys Expert plus, Biochron, England). A detector substance (glucose, in this case) was used to mark the end of the elution of the sample.

4.5.6. X-ray diffraction and relative crystallinity

Samples of starch were firstly equilibrated in a desiccator containing saturated BaCl₂ solution (25°C, $a_w = 0.900$) for 10 days. Then, the samples were subjected to an X-ray diffractometer (Rigaku Miniflex 600 RXD, Tokyo, Japan) with copper radiation at an angle 2θ ranging from 4 to 36°, using the working conditions: scan rate of 2°·min⁻¹, 40 kV and 15 mA. The relative crystallinity (RC) of the starch granules was calculated as described by Nara e Komiya (1983), using the Origin software version 9.1 (Microcal Inc., Northampton, MA, USA). The graphics were plotted between 2θ angles from 3 to 36° and the curves were smoothed using the software tools.

4.5.7. Scanning electronic microscopy

Images of the starch granules, showing their morphology and general appearance, were observed by a scanning electronic microscopy (LEO 435 VP, Leo Electron Microscopy Ltd., Cambridge, England), operating at an acceleration voltage of 20 kV. Dry starch was sprinkled into circular stubs, with double sticky tape, using a brush to get a uniform layer of sparsely scattered powder particles. The stubs were coated with a 30-nm gold layer and then evaluated in the microscope.

4.5.8. Light microscopy

The shape of the surface and of the edges of the starch granules were observed using a light microscope (model L1000, Bioval, Curitiba, Brazil), with a 20 W halogen lamp. Starch (0.1 g) were dispersed in 10 mL of distillate water with 50 μ L of Lugol. Then, a drop of this dispersion was placed on a glass slide covered by a glass cover slip. The magnification used was of 100x and one portable camera of 8 megapixels was used to obtain the images.

4.6. Starch properties evaluation

4.6.1. Pasting properties

The pasting properties were determined using RVA-4 (Newport Scientific Pvt. Ltd., Australia, with the Thermocline for Windows software, version 3.0). A suspension of 3 g (correct to 14% moisture basis) starch in 25 g of distilled water was analysed according to a programmed heating, retention and cooling cycle under constant shear. The suspension was first held at 50°C for 1 min, then heated to 95°C at a rate of 6°C·min⁻¹, being then kept at 95°C for 5 min, followed by cooling to 50°C at a rate of 6°C·min⁻¹, and finally holding it at 50°C for 2 min.

4.6.2. Instrumental texture of starch gels

The firmness of the potato starch gels was determined by instrumental texture, using a Texture Analyser (TA.XT Plus, Stable Micro Systems Ltd., Surrey, UK) with a load cell of 50 kgf (490.3 N). The gel obtained after the RVA determination was stored in a 40 x 20 mm (diameter x height) plastic cup for 24 h to stay solid before evaluation. To ensure uniform moisture of the samples, they were held in a desiccator with water at the bottom. A 0.5 cm cylindrical probe (P/0.5R) was used to compress the samples until the distance of 4 mm at 1 mm·s⁻¹. The force measured by the equipment as a function of the penetration depth was then used to evaluate the gel rigidity.

4.6.3. Paste clarity

Paste clarity was evaluated by the transmittance measurement (T%) of the samples, as described by Craig et al. (1989) and modified by Aplevicz and Demiate

(2007). Samples were prepared mixing 0.2 g of starch in 20 mL of distilled water in test tubes with screw cap. The tubes were then brought to thermal bath with boiling water for 30 min and stirred individually 5 times every 5 minutes, being subsequently cooled at room temperature and evaluated in glass cuvettes at 650 nm wavelength in a spectrometer (Femto, Model 600S, São Paulo, Brazil).

4.7. Statistical analysis

Each process condition was performed 4 times, and the analysis was performed at least in duplicate. The average values was calculated and, when appropriate, the Tukey's multiple comparisons was used through Statistica 13.0 (StatSoft, USA) software. A significance level of 5% was considered.

5. RESULTS AND DISCUSSION

The goal of this work was not only to obtain starches with different characteristics, but also to understand how the oxidation occurred at the molecular level under the conditions studied, thus describing and correlating the process-structure-properties relationships. Therefore, after presenting and discussing the obtained results, a section with the proposed mechanisms of the ozone action is presented. The further discussion is subsidized by the information presented in the Section 3.6 of the present work.

5.1. Effect of the ozonation on the potato starch structure

5.1.1. Changes in functional groups: pH and carbonyl and carboxyl contents

Starch molecules are rich in hydroxyl groups (C-OH). As pointed earlier, the substitution of these groups by carbonyl (C=O) and carboxyl (HO-C=O) groups is expected during the oxidative process, and the higher the value of these new groups, the higher the degree of substitution due to oxidation. In fact, the carbonyl and carboxyl groups content increased with the increasing in the processing time, as shown in Figure 5. The carboxyl groups, present in carboxylic acids, increases the acidity of the samples. In fact, for the processed samples the pH decreased with increasing in the ozonation process in the presence of water (Figure 5), and in accordance with the carboxyl content.

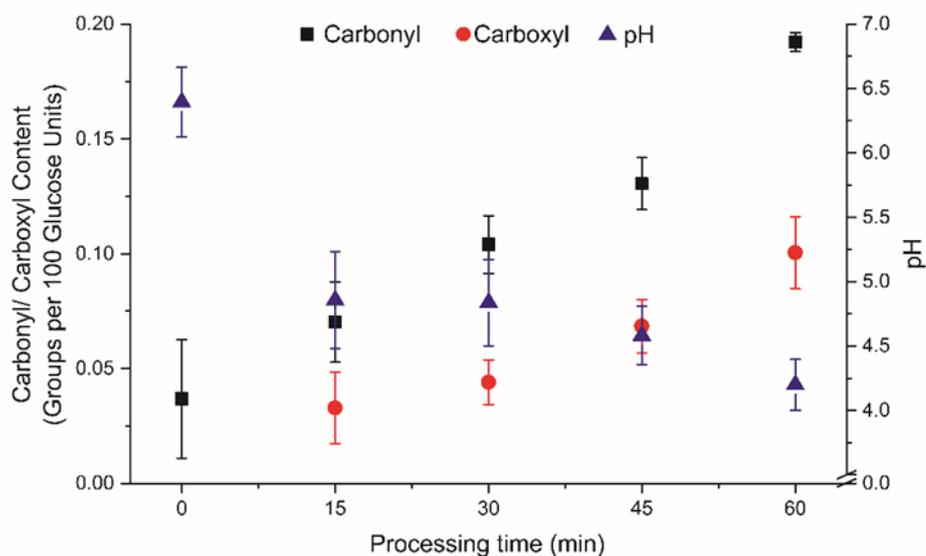


Figure 5 - Carbonyl and carboxyl groups content and pH of the native and the ozone processed potato starch. Carboxyl groups in native starches are considered negligible, and for this reason the value of carboxyl content for the unprocessed starch was considered zero. The dots are the mean values and the vertical bars are the standard deviation.

It was expected that both carbonyl and carboxyl groups were introduced simultaneously in the molecules. Still, as the carbonyl groups are an intermediate state in the oxidation of the hydroxyl to carboxyl groups, it was expected that there would be a larger amount of carboxyl groups, since they are the final version of the reaction, especially in the samples oxidized for longer times. However, there was a more significant amount of carbonyl groups than carboxyl groups after ozonation in all the processed samples. Chan and co-workers (2009) found similar results, and concluded that the major value for carbonyl groups were due to the considered mild ozone treatment used. However, this may not be the only factor. Wurzburg (1986a) suggested that the reaction conditions influence the relative amount of the groups formed in the oxidation, so the type of the chemical reagent used and its affinity with the starch molecule can interfere in these values. Moreover, it is expected that with the cleavage of the glycosidic bonds the number of carbonyl and carboxyl groups increases. In fact, there was a higher cleavage of the glycosidic bonds (discussed in the next section).

The obtained results are compatible with the values obtained in other works of starch modification with ozone, using different starches and process conditions, all of them observing significant increases in the carbonyl and carboxyl contents through ozonation (Chan et al., 2009; Chan et al., 2012; Klein et al., 2014; Oladebeye, Oshodi, Amoo, & Karim, 2013).

Carboxylic groups are also known to be electronegative charged and bulkier than the hydroxyl groups, and due to that it causes an electrostatic repulsion between the molecules, being responsible for expanding their chain conformation, making difficult their intermolecular associations, and increasing their solubility. This leads to starches with higher paste clarity and stability (Xie, Liu, & Cui, 2005) – as described further.

The data reported here supports the assumption that the potato starch processed with ozone in the conditions described in this study was oxidized, being modified in the molecular level.

5.1.2. Molecular characterization: apparent amylose and reducing sugar contents and molecular size distribution

The oxidation of the starch granules can also be demonstrated by evidencing the hydrolysis of the glycosidic linkages (the covalent bonds between the α -D-glucopyranosyl units). This hydrolysis results in the depolymerisation of parts of the amylose and amylopectin molecules, leading to a formation of smaller size chains. As discussed in the hypotheses III and IV of the previous Section 3.6, it is reasonable to expect a higher oxidation of the amylose molecules and of the branched parts of the amylopectin molecules present in the amorphous domains of the granule. To demonstrate the molecular size distribution of the samples over the processing, a size exclusion chromatography were performed. The gel permeation chromatography (GPC) results for the native and ozonated potato starch are presented in Figure 6, with total carbohydrates (TCH) results, and in Figure 7, with the blue value (BV) results.

The gel permeation chromatography (GPC) is a size-exclusion separation and identification technique, where the molecules are separated basically by their sizes. The gel that fills the column has a known porosity. The higher molecular weight fractions are firstly eluted, since they rarely penetrate the pores, while the lower molecular weight fractions are longer retained in the column (Hizukuri et al., 2006). Therefore, it is supposed that the first fraction that elutes from the column is composed mainly by the large size and highly branched molecules of amylopectin, and the following portion is composed mainly by the linear amylose and other small size fractions. However, a small amount of large and slightly branched amylose molecules

can elute first, as well as small sized molecules of amylopectin can elute with the second fraction.

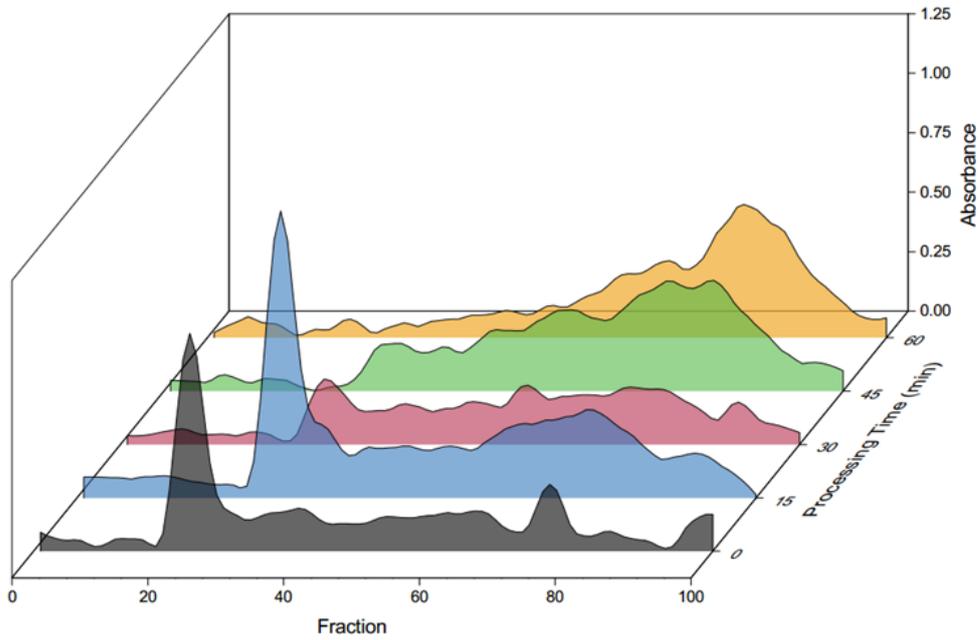


Figure 6 - Total carbohydrates (TCH) results of the gel permeation chromatography (GPC) analysis for native and ozonated samples.

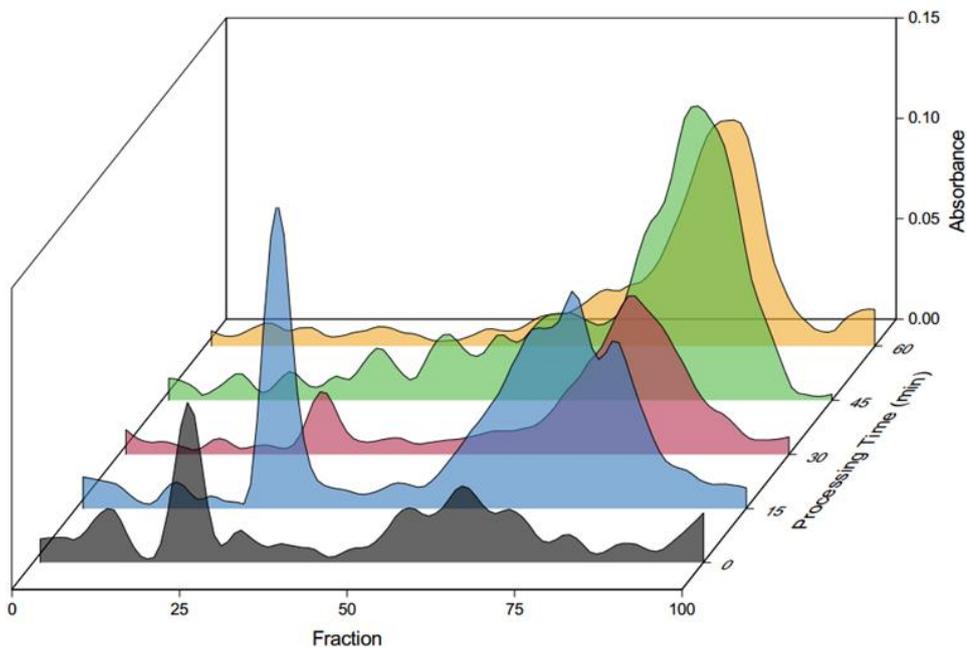


Figure 7 - Blue value (BV) results of the gel permeation chromatography (GPC) analysis for native and ozonated samples.

In the TCH results, it is clear that the fraction that was eluting first in the native sample (0 min) was eluting later and/ or decreasing its size with increasing ozonation time, being followed by a gradual increase in the following fraction. It indicates that the higher molecular weight molecules were broken and the smaller size fractions were rising in quantity. In fact, after 60 minutes of ozonation the peaks in the first fraction were no longer distinguishable, while the peaks in the second fraction reached higher values.

The BV results also presented the first fraction peaks eluting later and/ or decreasing their sizes. In the 15-min sample, there was an increase in the BV of both first and second fractions, and their peaks moved to the right, if compared with the native starch sample, indicating, besides an increase in the smaller sized molecules, an increase of molecular fractions that can form iodine complexes. After processing for 30 minutes, both fractions started to decrease; after 45 minutes, a decrease in the peaks in the first fraction and an increase in the peaks from the second one can be seen; and after 60 minutes, the first fraction peaks are negligible, while the second fraction decreased its size if compared to the 45-min sample. It is important to point out that BV is the measure of the absorbance of the starch-iodine complex. It is a specific and easy way to estimate amylose content, since this molecule presents a helical structure and the iodine can fill in the central cavity, resulting in a blue coloured complex. However, the obtained value must be considered "apparent" because the amylose content can be over or underestimated, depending on some characteristics of the molecules. For example, long linear chains of amylopectin can be interpreted as amylose chains, while the presence of short-chain-length amylose can draw in a reduced absorption of iodine, and then result in a smaller amylose value than the actual (Liu, 2005; Shannon, Garwood, & Boyer, 2009). Even though small portions of the amylopectin molecules can form iodine-complex, in this work we will assume that the results of the BV analysis are only related to the amylose molecules. Therefore, the obtained results indicate that the molecules that can form iodine complexes decrease in size to the point that the chains become so small that reduces the absorption of iodine, giving a smaller BV.

Karin et al. (1992) and Forssell et al. (1995) also observed a decrease in the molecular weight of potato and barley starches due to the hypochlorite oxidation, when the higher the oxidation was, the smaller the molecules became.

Summarizing, the GPC data here presented proved the hypothesis that the oxidation results in a molecular depolymerisation of the starch, since a gradual reduction of the high molecular-weight portions, with increasing processing time, was observed. Further, assuming that only amylose molecules can form-iodine complexes, it can be considered that the amylose molecules were vastly affected, since the BV results presented a clear decrease in these molecules size.

Two analysis were used to confirm the molecular depolymerisation of the starch: apparent amylose and reducing sugar contents, whose results are presented in Figure 8.

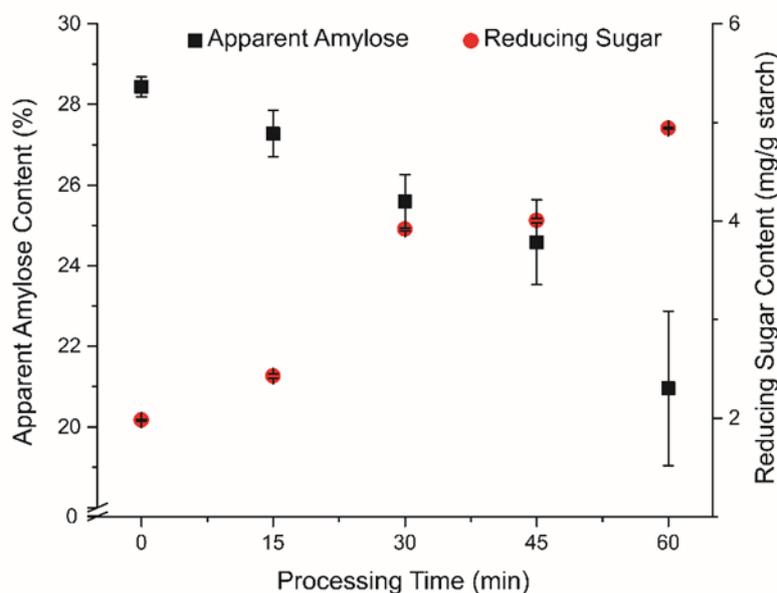


Figure 8 - Apparent amylose and reducing sugar contents of the native and ozonated potato starch samples. The dots are the mean values, the vertical bars are the standard deviation.

The considerable increasing in the samples reducing sugar content with increasing ozonation time (Figure 8) indicates the formation of new reducing groups in the molecules, presumably formed by the hydrolysis of the glycosidic linkages of the starch molecules, as mentioned. Further, the apparent amylose content decreased with increasing processing time, also in accordance with the GPC results. In fact, the apparent amylose content analysis is similar to the BV observed in the GPC, since both are based on colorimetric methods of amylose-iodine complex.

Although the influence of the starch oxidation on the amylose content is contradictory in the literature, varying with the starch source and processing conditions, the reduction on the apparent amylose content is frequently observed. As the amylose presents a random arrangement and a linear structure, being mainly detected in the

amorphous phase of the granule, some authors assume that these characteristics make this molecule more susceptible to oxidation (Sandhu, Kaur, Singh, & Lim, 2008). For example, Zhu e Bertoft (1997) evaluated the apparent amylose content of potato starch oxidised by chemical agents. The apparent amylose content decreased from 20.6 to 6-8% when using sodium hypochlorite or hydrogen peroxide. Also, Sandhu et al. (2008), observed a significant reduction of apparent amylose content in corn starch oxidized with hypochlorite.

Taken together the GPC, the apparent amylose content, and the reducing sugar content analysis, the obtained results indicates that the starch molecules suffered a depolymerisation, showing the effectiveness of the oxidative process.

5.1.3. Starch granule structure: crystalline and amorphous regions

As already discussed (Section 3.6), the starch granule is composed by amorphous and crystalline domains, thus presenting a heterogeneous structure. Crystalline domains are a more structured phase, with strong and regular hydrogen bonds, believed to be composed essentially by amylopectin chains oriented in double helices conformation. This conformation is very compact, not allowing water or smaller molecules from reaching its center, being less susceptible to chemical attacks (Hizukuri et al., 2006; Liu, 2005). Amorphous phases, or noncrystalline domains, are believed to compose the major part of the starch granule, being constituted by different materials, among which the free amylose and the branched portion of the amylopectin stands out, all in a random conformation, with weaker bonds if compared to the crystalline domains (Hizukuri et al., 2006).

X-rays, unlike visible light, has a quite small wavelength, being able to be diffracted by crystalline or sufficiently regular planes. The intensity of the X-ray beam diffraction will depend on the structure and atomic disposal of the crystal, resulting in patterns related to each type of crystal, being able to give information about its molecular structure (Wang & Cui, 2005). In an X-ray diffraction equipment, the material is generally scattered by an X-ray beam from 0 to 90 degrees, in an incident θ angle, and is diffracted by a 2θ angle. Each of these angles will correspond to a different atomic spacing ("d-spacing"), and can be converted into peaks by graphing the X-ray intensity by the angles in 2θ degrees. In a typical starch diffraction result, peaks from

the crystalline phase and backgrounds from the amorphous phase shall be found, allowing to obtain the starch Relative Crystallinity (RC). The RC is the ratio between the total scattering of the crystalline phase versus the total scattering of the sample (sum of the crystalline and amorphous domains).

The X-ray diffraction patterns of the native and the ozone-oxidised potato starches, as well as their RC, are presented in Figure 9. In X-ray diffraction analysis, each type of starch generates a different type of diffraction patterns. In general, the A-type patterns are characteristic of cereal grain starches, and the B-type characteristic of tubers and high amylose starches. A third pattern, known as C-type, is a combination between the other two (Hizukuri et al., 2006; Tester et al., 2004). The X-ray diffraction patterns of the oxidised potato starches were similar to the presented for the native potato starch, exhibiting the most significant peaks at angles close to the values of 5.7° , 17.2° (maximum value), 19.7° , 22.2° and 24.0° (Figure 9). Therefore, as expected, it reveals a characteristic B-type pattern, typical of tubers (Liu, Weber, Currie, & Yada, 2003).

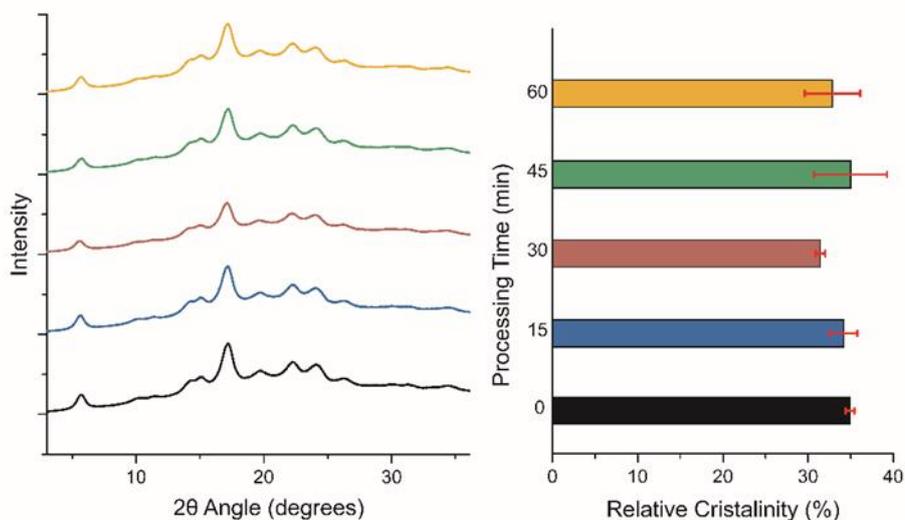


Figure 9 - X-Ray diffraction patterns (in arbitrary units) of the native and ozonated starch samples and their correspondent relative crystallinity (RC).

The value of the relative crystallinity (RC) for the native potato starch ($34.9 \pm 0.5\%$) was slightly higher than the observed by other authors. Vansanathan et al (1999) describe RC ranging from 21.4% to 32.4% for starches extracted from six different potato varieties. Alvani et al. (2011) evaluated starches extracted from 10 different potato cultivars and found RC with the highest value of 31.2%, while the lowest value

was about 29.3%. It is important to note that the crystals of the starch molecule are very small and are often interspersed with amorphous phases without a clear division. Thus, the degree of crystallinity of the sample cannot be rigorously stated (French, 1984). Moreover, RC values can vary significantly, depending on starch source and the methods used for calculating the crystallinity (Liu, 2005).

The relative crystallinity (RC) presented no significant ($p < 0.05$) difference between the native and the ozonated starches (Figure 9). Therefore, the similarities in the X-ray diffraction patterns, combined with the values obtained in the RC, indicates that the crystalline domain of the starch granules was not affected by the potato starch ozonation. This result is in accordance with the theory discussed by Xie et al. (2005), who stated that oxidised starches are affected mainly in their amorphous phases, indicating little or no change in the crystalline region of the granule.

Even so, Klein et al (2014) reported a reduction in the RC after the ozonation of cassava starch in aqueous solution, and concluded that the semi-crystalline domain of the processed starches was affected by the ozonation. However, the difference among their results are smaller than those obtained in the present work, where no statistical difference was observed. This information, combined with the fact that they did not show any statistics, difficulty the comparison of the results. Also, they related the decrease in the RC to the depolymerisation of the amylopectin chains. However, as demonstrated in this present work, it is possible to depolymerize the amylopectin chains without changing the crystalline structure of the granule. Zhou et al. (2015) processed potato starch with different chlorine concentrations, and observed that at lower concentrations the RC was higher if compared to the native starch, but the RC decreased with increasing chlorine concentration. They concluded that with higher concentrations the crystalline chains were destroyed. Similar results were described by Kuakpetoon and Wang (2001) while processing corn starch with active chlorine.

5.1.4. Starch granules structure: morphology

Having defined the modifications in the structure of the potato starch after ozone processing (changes in functional groups, the hydrolysis of glycosidic bonds and the extent of the oxidation in the granule crystalline and amorphous domains), two different

types of microscopy were performed, allowing the evaluation of the morphology of the granules after processing.

The Figure 10 illustrates the morphology of the potato starch granules through its observation by scanning electronic microscopy (SEM) and light microscopy (LM).

The granule surface of the native sample was smooth and relatively free from imperfections, as observed in native starch granules (Pérez, Baldwin, & Gallant, 2009). The processed samples, in contrast, presented some roughness and became more heterogeneous with increasing processing time, as indicated by the red arrows in the Figure 10. After 30 minutes of ozonation, the granules with irregular shapes were more frequent and was even possible to see some fissures and pores in the granules surface.

Similar SEM observations were obtained by Çatal and Ibanoglu (2012b) in potato and corn starches submitted to ozone oxidation. The native starch samples presented surfaces that were smooth and regular, while after ozonation the surfaces became rough and fibrous, especially in the potato starch. Using a polarized light microscope, the same authors observed that the Maltese crosses of the processed starches were maintained, presenting slight differences if compared to the control sample, suggesting that the crystalline structure of the granule was little affected (also agreeing with the results of the present work). Zhou et al. (2015) observed potato starch in its native form and after being processed with sodium hypochlorite. The SEM results revealed native granules with oval and/or spherical shapes with smooth surfaces, and irregular granular shapes and pores in the surface of the processed granules. The authors observed that the starch particles cracked with higher oxidant concentration, and they suggested that it is due to the starch crystalline structure weakening.

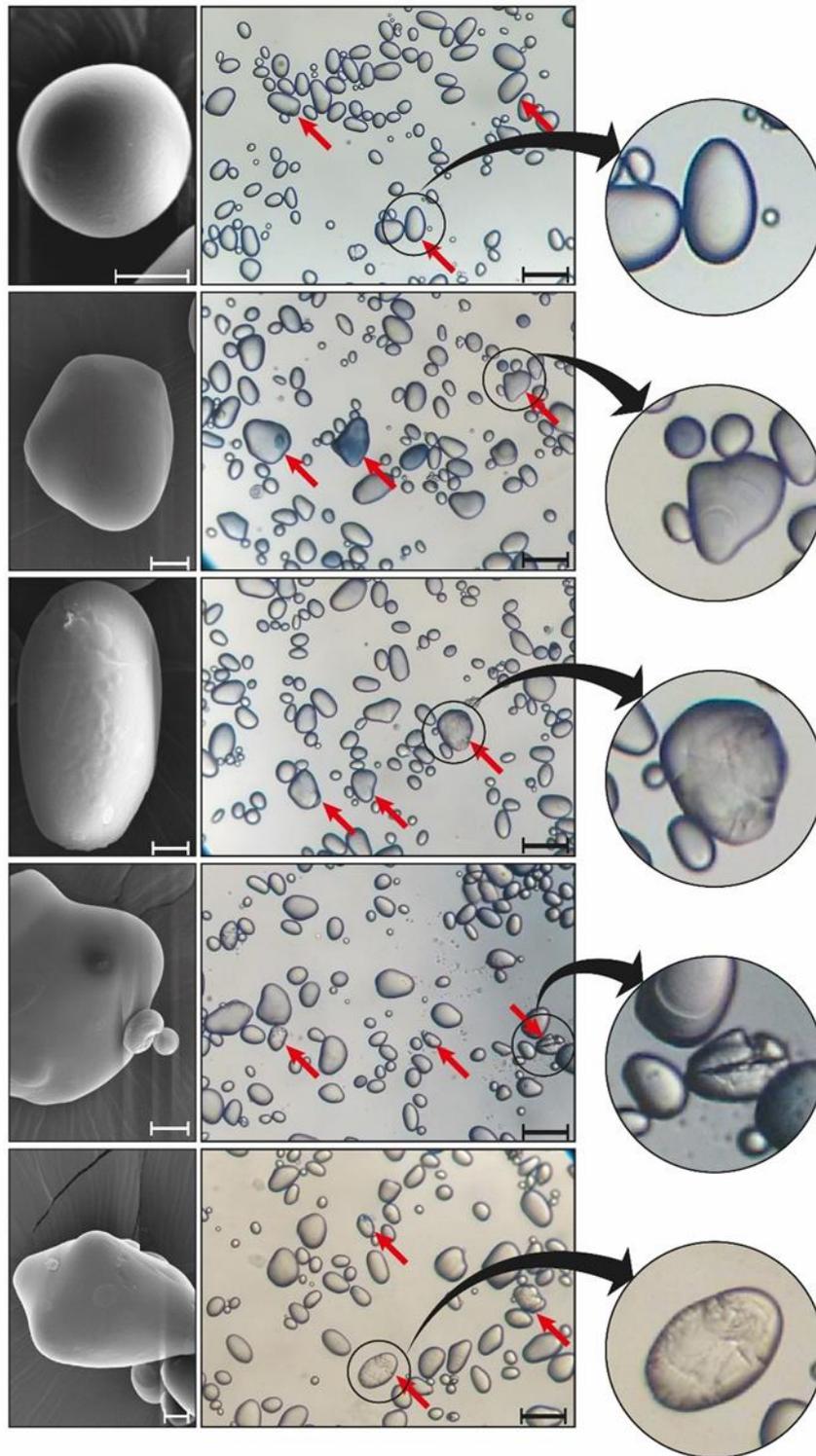


Figure 10 - Microscopic images of the potato starch. From top to bottom: 0, 15, 30, 45 and 60 minutes processing time. Images from the first column are scanning electronic microscopy (SEM) images (white line = 10 μm). The second column shows images from light microscopy (LM) (black line = 100 μm). Further details are shown on the third column (without scale).

Our results indicate that, besides the ozonation in the conditions studied was not sufficient to promote alterations in the crystalline domains of the starch granules, their surfaces revealed marks of the oxidative process they were submitted. It is probably due to the granule surface conformation and composition. It has been reported that in the granules surface a large amount of nonreducing end-groups from amylopectin and amylose molecules are established, but how the molecules are organized in the surface is still uncertain (Eliasson & Gudmundsson, 2006). Thus, it is possible that the surface of the starch granules are more susceptible to oxidative attack than its crystalline regions, possibly due to its higher accessibility.

5.2. Effect of the ozonation on potato starch functional properties

So far, this work have focused on discussing and demonstrating how the ozonation affected the structural properties of the potato starch. The next steps will focus on describing the effect of these changes on the functional-technological properties of the obtained modified starches.

5.2.1. Pasting properties

Starch gelatinization consists basically in its heating above its pasting temperature in the presence of sufficient amount of water. During this phenomenon, the hydrogen bonds between the hydroxyl groups (which are mainly responsible for maintaining the native starch granules integrity) suffers a natural weakening, allowing the granules to absorb water, becoming thinner and swelling (Wurzburg, 1986b). If the gelatinization is continued, the starch reaches the “pasting state”, in which a two-phase system is obtained, being composed by swollen granules and leached molecules (especially amylose). In specific conditions (i.e. cooling and storage), both amylose and amylopectin can suffer an intermolecular re-association, resulting in a strong gel. This phenomenon is known as retrogradation (Liu, 2005).

The RVA equipment can measure the apparent viscosity variation of a starch solution over a predetermined process, in which in a specific time the sample is heated above its gelatinization temperature, and then cooled. Through this equipment, it is possible to observe specific moments of the starch gelatinization, which are described by some parameters: Peak Apparent Viscosity (PAV), Trough Apparent Viscosity

(TAV), Breakdown (BD), Final Apparent Viscosity (FAV), Setback (SB) and Pasting Temperature (PT). These parameters are illustrated in the APPENDIX B. The pasting properties are important not only to characterize a native starch sample, but also to demonstrate how a modification process affected the starch characteristics. The pasting properties of native and 15, 30, 45 and 60 minutes processed potato starch samples are given in Table 1, and its related curves are represented in Figure 11.

Table 1 - RVA parameters for aqueous suspensions of the native and processed potato starch samples. Peak Apparent Viscosity (PAV), Trough Apparent Viscosity (TAV), Relative Breakdown (RBD), Final Apparent Viscosity (FAV), Relative Setback (RSB) and Pasting Temperature (PT).

Processing time (min)	PAV (mPa.s)*	TAV (mPa.s)	RBD (%)	FAV (mPa.s)	RSB (%)	PT (°C)
0	12498 ± 242 a	3699 ± 78 a	70.4 ± 0.5 d	4522 ± 140 a	18.1 ± 2.5 c	65.7 ± 0.6 c
15	10800 ± 372 b	560 ± 119 b	94.8 ± 0.9 c	898 ± 161 b	37.9 ± 2.3 a	65.8 ± 0.2 c
30	11006 ± 466 b	191 ± 29 c	98.3 ± 0.2 b	310 ± 61 c	38.0 ± 2.8 a	66.7 ± 0.5 b
45	9716 ± 358 c	88 ± 16 d	99.1 ± 0.2 a	133 ± 26 d	33.6 ± 1.5 b	67.5 ± 0.2 a
60	8266 ± 165 d	45 ± 4 d	99.5 ± 0.0 a	66 ± 5 d	32.1 ± 1.1 b	67.9 ± 0.5 a

*Average ± standard deviations (n = 7); variations followed by the same small letters within a column do not differ significantly (p < 0.05).

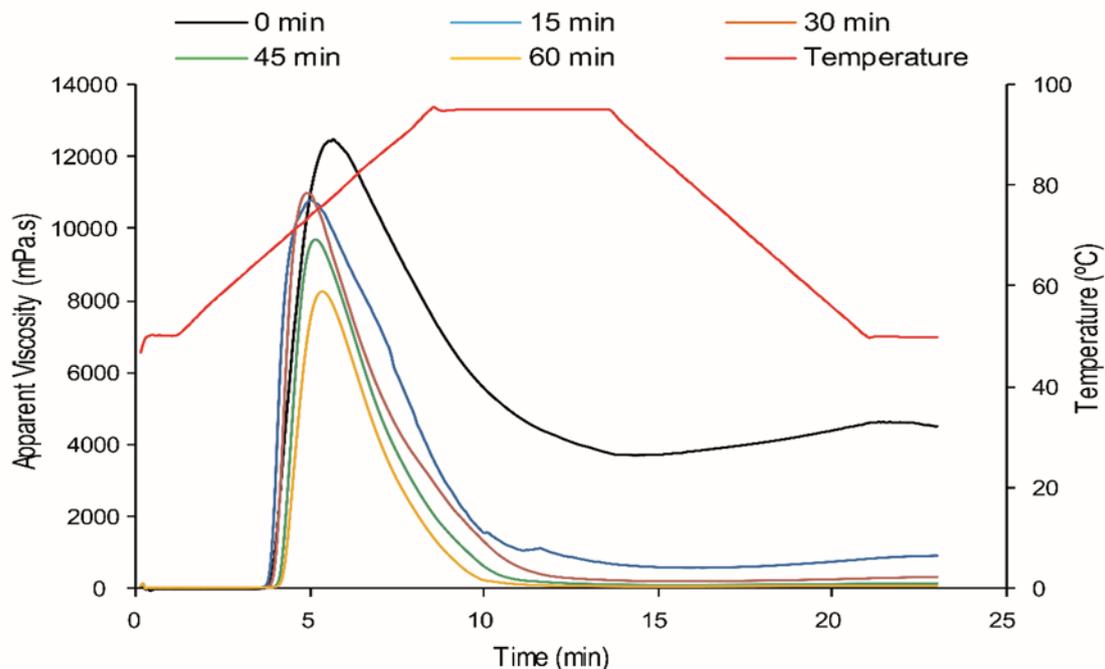


Figure 11 - RVA curves for the native and processed potato starch samples

The PAV occurs at the exact point between the maximum swelling of the granules and the granule rupture. Before the maximum swelling, a fast apparent viscosity increase is reached, and after the rupture, a subsequent leaching of polymers from the granules and its alignment occurs, which decreases the apparent viscosity of the paste (Liu, 2005). As observed from the data (Table 1), the PAV decreased with increasing processing time, which indicates that, under stirring and heating, the modified starch granules present a reduced capacity to maintain its integrity, being ruptured in a shorter time if compared to the native starch. This granular “weakening” can be attributed to different changes that have occurred in the granule, which are directly related to its integrity and had already been established on the Sections 5.1.1 and 5.1.2: the replacement of part of the hydroxyl groups by carbonyl and carboxyl groups and the partial cleavage of the glycosidic bonds.

The replacement of the hydroxyl groups had as a consequence the decrease of the hydrogen bonds between the molecules of the modified starches. As these bonds are mainly responsible for maintaining the native starch granules integrity, this integrity is reduced. The carbonyl and carboxyl groups formation can also be suggested as responsible for the granular weakening. Lu et al. (2012) hold the view that a potato starch granule with high phosphorus content may present a less rigid structure, being more easily ruptured, due to the Coulombic repulsion between the phosphate groups in the amylopectin molecules. This discussion appear to support the assumption that the ozonated starches probably presented weaker granule structure also due to the electronegative carbonyl and carboxyl groups formed in the molecules. Ultimately, the partial cleavage of the glycosidic bonds leads to degraded molecules and consequently to a partially degraded network between them, leading to granules with lower resistance to shear forces.

Several authors reported similar results (PAV decreasing with increasing process time/ ozone concentration) (Çatal & İbanoğlu, 2012a, 2014; Klein et al., 2014; Oladebeye et al., 2013). All of them attributed the results to the partial cleavage of the glycosidic bonds, although some of them have not demonstrated it with analysis. Chan et al. (2009), on the other hand, observed a PAV increase for sago starch processed for 5 and 10 minutes of ozone generation time (OGT) and tapioca starch processed for 1 and 3 minutes OGT. The authors attributed the PAV increase to a possible cross-linking effect and to the increase of carboxylic groups in some samples, which,

supposedly, would be responsible for preventing molecular association and in this way would allow the granules to uptake more water during heating. However, their findings must be interpreted with caution, because the PAV results are more related to the granule integrity, and so, as discussed in the present work, the carboxylic groups would not contribute to a higher swelling and consequently to a higher PAV, but rather to a decrease in its integrity.

After the granules rupture, an alignment of the leached polymers occurs. This molecular alignment and consequent decrease in the apparent viscosity is described by the BD parameter (Liu, 2005). BD is calculated as the difference between the PAV and the TAV, another parameter obtained in the RVA analysis, which represents the minimum apparent viscosity reached by the starch paste before cooling.

However, the fact that the BD represents the absolute difference between the PAV and the TAV should receive some attention, since both PAV and TAV values of the samples are changing at the same time, leading to a misinterpretation of the BD values. Thus, another parameter was defined to better understand the apparent viscosity variation of the samples: the Relative Breakdown (RBD), being calculated as the ratio between the BD value and the PAV value.

The data reported in the Table 1 shows that the longer the processing time, the lower the TAV and the higher the RBD, indicating that the higher the samples oxidation, the easier the disruption of the starch granules. This results means, therefore, that the ozonation interfered with the intermolecular association ability of polymers, probably due to the carboxylic bonds cleavage and to the reduction of hydroxyl groups and the consequent formation of carbonyl and carboxyl groups in the molecules (as discussed for the PAV results).

In the sequence, the RVA system is cooled and a re-association of the starch molecules (especially amylose) occurs, increasing the apparent viscosity until reaching the FAV. This “cooling region” is described by the SB parameter, which also represents the retrogradation tendency of the starch molecules, being calculated as the difference between the FAV and the TAV values (Liu, 2005). Again, this difference may not be the best form to compare the apparent viscosity variation among the samples, since both FAV and TAV values changes simultaneously. To represent this variation, another parameter was calculated: the Relative Setback (RSB), being the ratio between the SB and the TAV values.

The molecular re-association tendency (retrogradation) in the starch granules can be determined by the affinity of hydroxyl groups of one molecule to another, especially in the amylose molecules. The amylose has a bigger capacity to stay closely enough to form hydrogen bonds between hydroxyls and adjacent molecules, due to the addition of such factors as linearity, mobility and high amount of hydroxyl groups. The fact of having large size and highly branched molecules decreases the capacity of the amylopectin to form intermolecular hydrogen bonds, since they have a reduced mobility and may not be oriented close enough to permit bonding (Wurzburg, 1986b).

As observed in the Table 1, the RSB of the ozonated potato starches were higher than the native starch, besides having a lower amount of hydroxyl groups if compared to the native sample. Further, the 15 and 30 minutes samples presented even higher values than the 45 and 60 minutes samples (Table 1). This is explained by the molecular depolymerisation (formation of smaller molecular portions, or dextrans - stated in the previous Section 5.1.2), since smaller molecular chains present a more suitable size for re-association due to its higher mobility. The decrease in RSB values after 45 minutes of process, however, may indicate an even higher molecular depolymerisation, especially of the amylose molecules, presenting a too small size to permit molecular interaction (the apparent amylose content decreased with increasing processing time, as showed in Figure 8 in the Section 5.1.2 of this present work). This scenario is aggravated by the fact that the hydroxyl groups (main responsible for the intermolecular bonds) have suffered even greater oxidation in samples processed for longer times. These findings are in accordance with Mishra & Rai (2006) who, studying potato starch samples, discussed that a higher degree of the amylose molecules polymerization may lead to a lower setback, despite the high content of this polymer in the sample, indicating that the retrogradation tendency can be affected not only by the amylose content, but also by its conditions in the granule (i.e. state of dispersion and length of the molecules).

Chan et al. (2009), Çatal and İbanoğlu (2014) and Oladebeye et al. (2013) observed a SB viscosity decreasing with the increasing on the oxidation level in almost all processed samples. The authors suggested that the replacement of the hydroxyl groups by carboxyl and carbonyl groups can limit the formation of binding forces and limit the molecular interaction, reducing the tendency to retrogradation. However, none

of the authors considered using the relative SB value, making it difficult to compare the results.

The PT indicates the minimum temperature necessary to start the starch gelatinization. The pasting temperatures of the ozone-oxidised potato starch samples slightly increased with increasing processing time (Table 1). This increase in the PT may be related to the molecular depolymerisation, since smaller size molecules require more energy to gelatinize than larger sized molecules.

This results are in accordance with the observed by Çatal & İbanoğlu (2012a) after processing potato and corn starches with ozone, also observing an increase in the PT. The authors also suggested that this increasing occurred due to the starch depolymerisation (formation of dextrans) in the oxidation, which demand a higher temperature to gelatinize if compared to longer polymers chains. On the other hand, other authors observed reductions in the PT of oxidised samples, such as Chan et al. (2009) for ozone-oxidised sago starch, Kuakpetoon and Wang (2001) for potato, rice and corn starches oxidized by hypochlorite, Oladebeye et al. (2013) after processing white and red cocoyam starches with ozone and Çatal and İbanoğlu (2014) for ozone-oxidised wheat starch, showing that the botanic source and the processes conditions can significantly affect the characteristics of the modified starch.

5.2.2. Texture properties of the potato starch gels

The firmness of the potato starch gel samples and their correspondent required energy (area below the curve force versus strain) to be deformed are shown in Figure 12. After the gel obtainment, it was stored for 24h until further use. It was necessary for the natural cooling of the gel until room temperature. After this time, the gel suffered a consequent “stabilization” and became more rigid, what can be explained by the re-association of the starch molecules (especially amylose), associated to the retrogradation tendency of the starch gel, as discussed for the RVA analysis – Relative Setback parameter.

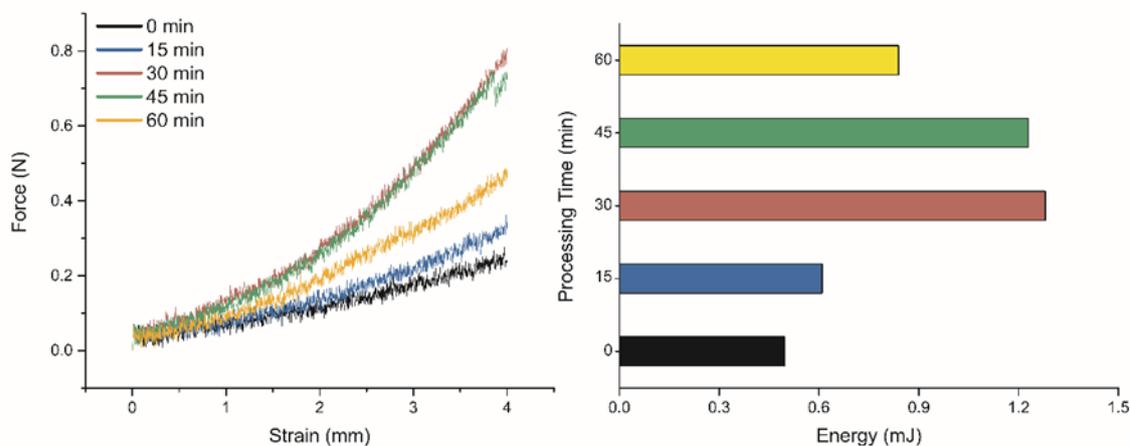


Figure 12 - Firmness of the potato starch gels measured by Instrumental Texture and their correspondent required energy.

The gel firmness increased with the increasing in the ozonation time, except for the 60-min processed sample, which presented a gel firmness higher than the control and 15-min samples, but lower than the 30 and 45-min samples.

In fact, the results obtained in the texture analysis are consistent with the observed in the RVA analysis, which leads to a similar conclusion: the molecular depolymerisation after the ozonation increased the mobility of the amylose molecules, leaving them with a more suitable size for re-association, and thus increasing its firmness. Still, the decrease in the firmness of the sample processed for 60 minutes is also consisted with the RVA results, indicating that this sample, by suffering an even higher molecular depolymerisation, had its re-association tendency impaired by its too small molecular size and also by its higher oxidation of the hydroxyl groups.

Chan et al. (2011) oxidized corn starch with ozone, and noticed a notably higher gel firmness after processing. The authors related their results with the lower molecular weight of the processed starches. Zhou et al. (2015) also observed an increase in gel firmness after potato starch oxidation with lower levels of hypochlorite. However, with increasing oxidant level they observed a decrease in the gel firmness. Their justification is based on the amylose molecules degradation: with intermediate weights (that occurs in milder oxidation) these molecules have a higher capacity to form gels; however, in a more severe oxidation, the amylose is more degraded, being less

capable of form gels. In fact, it is in accordance with the discussions of the present work.

5.2.3. Paste Clarity

The clarity of starch pastes was assessed by the percentage of transmittance (%T) at 650 nm, and the results are shown in Figure 13.

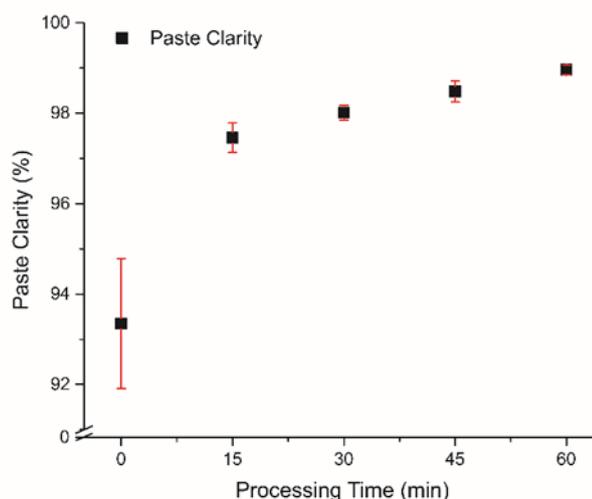


Figure 13 - Paste Clarity of the native and processed potato starch samples. The dots are the mean values, the vertical bars are the standard deviation.

According to Craig et al. (1989), some statements can be done about paste clarity: (a) the less intra and intermolecular bonds exist between starch molecules, the more they will remain hydrated, promoting light transmittance; (b) electronegative repulsion provides a high paste clarity (by preventing starch molecules from hydrogen bonding to each other); (c) the presence of reducing sugar can increase the paste clarity (by forming hydrogen bonding with starch molecules, interfering in the process of molecular association).

As explained in the statement V from the Section 3.6, the covalently bonded phosphate groups present in the potato starch can affect several of its properties, due to its high electronegativity when ionised, being repelled by the electronegative oxygen of the starch molecules, which contributes to a higher dispersion of the amylopectin molecules in solution (Craig et al., 1989; Nutting, 1951). Thus, the native potato starch has a natural clear paste – when compared with other sources.

The oxidised potato starches presented an even more clear paste (Figure 13). This result is due to the formation of the carboxylic acids in the starch oxidation as showed in the Figure 5. The carboxylic groups, as discussed, are electronegative, presenting the same characteristic repulsion of the phosphate groups in the starch molecule. Besides, as discussed by Craig et al. (1989), the reducing sugar may increase the paste clarity too. In fact, the reducing sugar content increased with increasing processing time (Figure 8). Therefore, once again, the results are in accordance.

However, it is important to highlight that, as discussed by Craig et al. (1989), the higher the intermolecular bonds, the lower the paste clarity. As evidenced in the items 5.2.1 and 5.2.2, the association between the amylose molecules were higher after the ozonation. Despite that, in this analysis, the concentration of the starch in solution is quite small (around 1%), hindering the interaction between the molecules, explaining the higher paste clarity.

From the industrial viewpoint, these are very promising results, since oxidized starches are widely used in textile and paper industries, being also applicable in the food industry, and a high paste clarity is highly desirable for many applications.

5.3. Summarizing: Proposed mechanisms

The data reported in the present work supports some statements about how the potato starch was affected by the ozonation in the evaluated conditions, as well as illustrate how these changes affected the starch properties. Firstly, it was observed through the carbonyl and carboxyl content that the hydroxyl groups of the starch molecules were highly affected. Also, as demonstrated by the GPC, the apparent amylose content, and the reducing sugar content evaluations, the glycosidic bonds of the potato starch were hydrolysed, especially from the amylose molecules. These data are sufficient to state that the starch suffered a modification in the molecular level, showing the effectiveness of the oxidative process.

By the X-ray diffraction pattern and the relative crystallinity data, it was observed that the crystalline domain of the starch granules was not significantly affected by the potato starch ozonation. It confirms the theory that the oxidation occurs mainly in the amorphous region of the granule. As observed by the microscopy analysis, the potato

starch surface revealed marks of the oxidative process. Based on these results, we can affirm that, although the ozonation process has not been enough to cause changes in the crystalline domains of the granules, their surfaces have been modified in some extent, probably due to the granule surface conformation, composition and/or accessibility.

Thereafter, the potato starch properties were evaluated. By the RVA analysis, it was possible to observe that the pasting properties were highly affected due to the starch modification (replacement of part of the hydroxyl groups by carbonyl and carboxyl groups and the partial cleavage of the glycosidic bonds). The Peak Apparent Viscosity decreased with increasing processing time, indicating that the ozonated granules were less resistant to shear under heating, becoming “weaker”. Also, the Relative Breakdown indicated that the higher the samples oxidation, the easier the disruption of the starch granules, confirming the previous results. The Relative Setback of the ozonated samples were higher than the native starch, which can be explained by the molecular depolymerisation, and indicates a higher retrogradation tendency. And finally, the Pasting Temperature slightly increased with increasing ozonation time, probably due to the formation of dextrans in the oxidation.

The texture and the paste clarity properties of the modified starches were also evaluated, showing that, after the ozonation, the gel firmness increased, as well as the paste clarity, being both results related to the starch modification at the molecular level.

The mechanisms of action of the ozone in the starch molecules proposed in this work can be summarized in the schematic representation in Figure 14.

Finally, by comparing the obtained results with previous works, it can be observed that the botanic source and the processes conditions can significantly affect the characteristics of the modified starch.

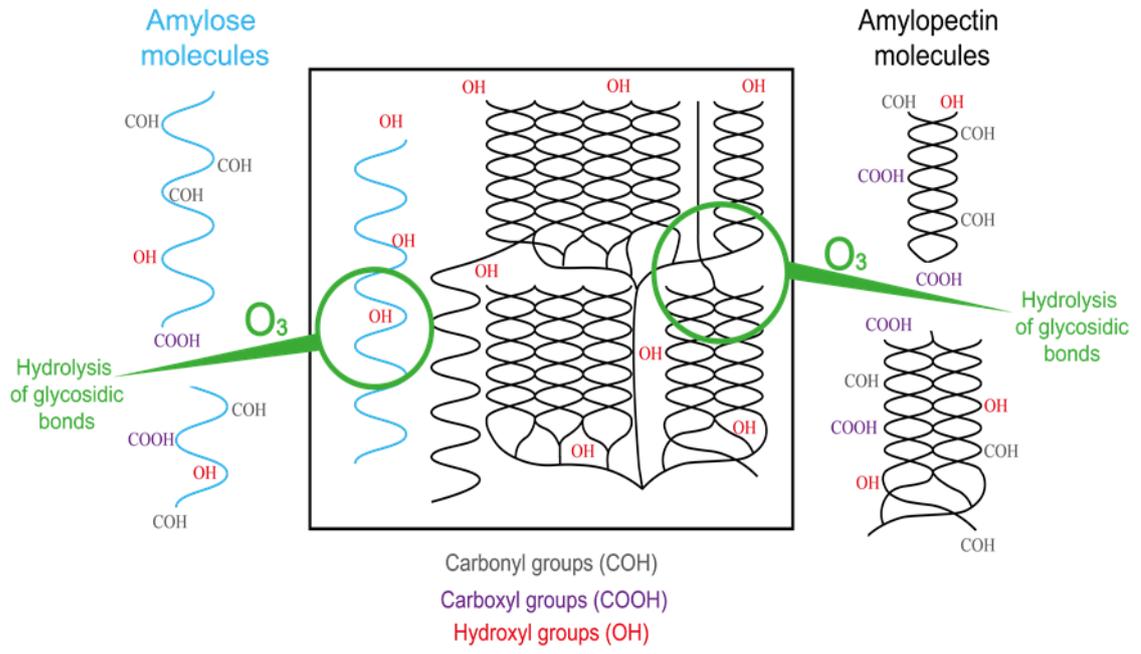


Figure 14 - Mechanisms of action of the ozone in the starch molecules

6. CONCLUSIONS

Starch modification, an important and widely employed technique, brings innumerable advantages to native starches. In oxidative processes, the use of chemical agents is rising concerns about security and environmental issues. Ozone has as an advantage the fact of being safe for consumers and for the environment, and in this present work, potato starch was successfully oxidized by ozone in aqueous solution, proving its efficacy as an alternative oxidative agent for food and non-food industrial applications. Further, as the correlation between the process-structure-properties could be evaluated, the action mechanisms of the ozone in the starch could be stated.

Firstly, with increasing ozonation time, it was observed an increase in the carbonyl and carboxyl contents, what permitted to state that the hydroxyl groups of the starch molecules were oxidized and highly affected. Also, the molecular size distribution (demonstrated by the GPC) and the apparent amylose content decreased, in contrast with the reducing sugar content, which increased. These evaluations proved the hydrolysis of the glycosidic bonds of the potato starch, especially from the amylose molecules. These results data are sufficient to state that the starch suffered a modification in the molecular level.

The X-ray diffraction pattern and the relative crystallinity of the modified starches presented no significant differences, proving the theory that the ozonation affects preferably the amorphous regions.

Further, the effect of these changes in the starch properties were evaluated. The pasting properties were highly affected, as well as the texture and the paste clarity properties, showing that the ozone successfully modified the potato starch and its properties.

The results obtained with the starch functional and structural properties allows the statement that the potato starch was successfully oxidized by ozone in aqueous solution in this work conditions, being possible to understand and to correlate the process-structure-properties relationship. Thus, ozone can be stated as an alternative for oxidative processes of starch modification for food and non-food industrial applications, having as an advantage the fact of being safe for consumers and for the environment.

7. SUGGESTIONS FOR FUTURE RESEARCH

In future research works, the effect of the ozone technology as a modification method to starches from different botanical sources can be evaluated. By comparison with the results obtained in the present work, the effect of some properties as the presence of phosphate groups or the size of the starch granules could be evaluated, determining how it affects the starch properties. Further, the effect of the ozonation on the starch digestibility could be evaluated, as well as the possible applications for the obtained ozonated starches. Moreover, it can be studied the modification of starches using the ozone technology in combination with other methods, such as the ultrasound technology, high hydrostatic pressures, irradiation, etc.

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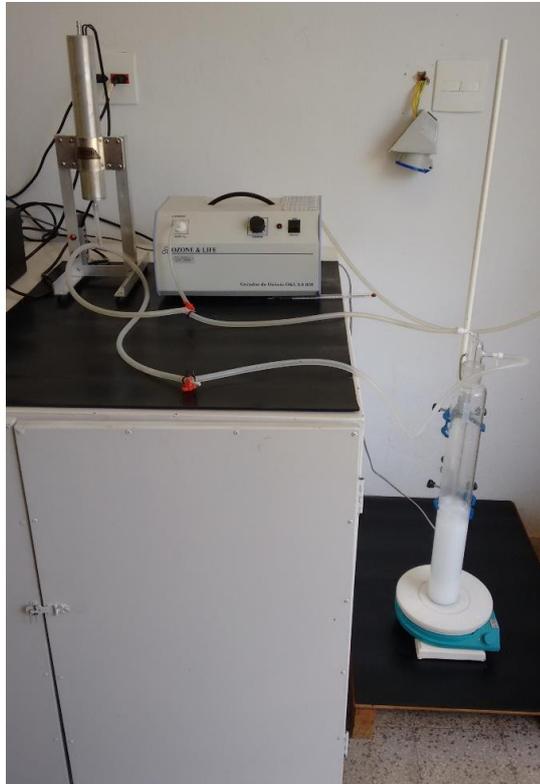
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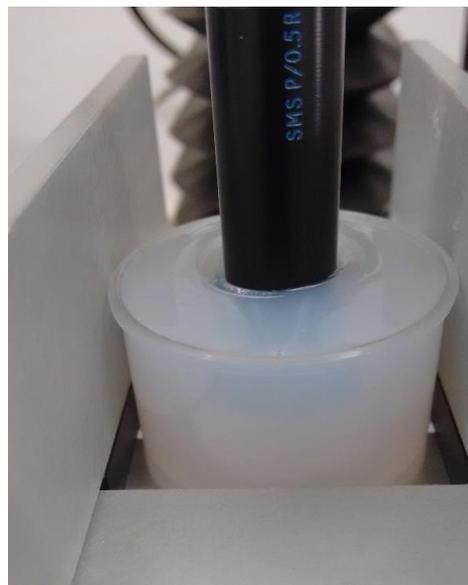
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APPENDIX A.

Actual process images



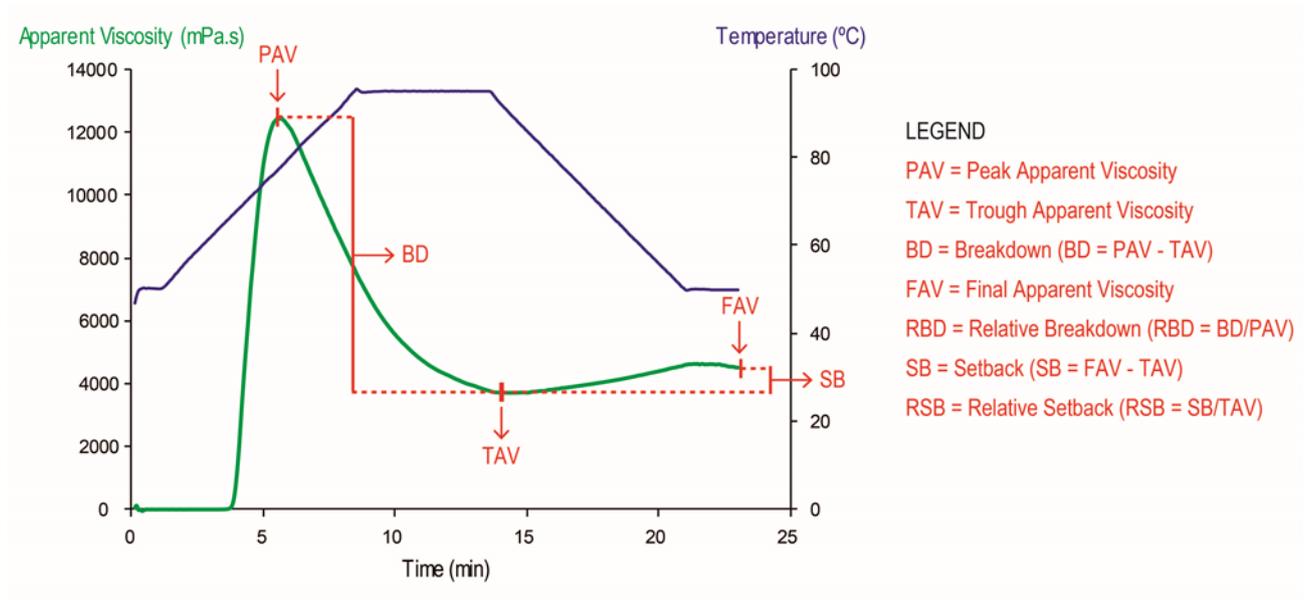
1: Ozonation system



2: Texture analysis

APPENDIX B.

RVA parameters indicated on a RVA profile graph of native potato starch



APPENDIX C.

Simple Abstract

English

The modification of native starches is a very important and widely used technique in the industry, allowing to obtain starches with desirable characteristics for some applications. The modification can be done in several ways, but modification by chemical methods is still widely employed, even generating toxic waste. With the growing concern about environmental issues, new techniques for starch modification is gaining space. Ozone is a gas present in our atmosphere and that decomposes in oxygen without leaving residues. In addition, it is highly oxidative and can be used to replace chemical agents in the starch modification. In this work, potato starch was processed with ozone, and the resulting changes were investigated by evaluating the characteristics of the obtained starch. Through this evaluation, it can be verified that the ozone modified the potato starch, and thus it can be used as an alternative for the chemical agents currently used. In addition, through other analyzes, it was possible to understand how this modification occurred.

Keywords: 1. Potato starch 2. Starch modification 3. Starch oxidation 4. Ozone 5. Emerging Technologies 6. Food properties

Português

A modificação de amidos nativos é uma técnica bastante importante e muito empregada na indústria, pois permite a obtenção de amidos com características desejáveis para algumas aplicações. A modificação pode ser realizada de diversas maneiras, porém a modificação por métodos químicos ainda é muito empregada, mesmo gerando resíduos tóxicos. Com a crescente preocupação com o meio ambiente, novas técnicas para modificar amido tem ganhado espaço. O ozônio é um gás presente na nossa atmosfera e que se decompõe em oxigênio, sem deixar resíduos. Além disso, ele é bastante oxidante, podendo ser utilizado no lugar de um agente químico para modificar amidos. Neste trabalho, amido de batata foi processado com ozônio, e as alterações resultantes foram investigadas através da avaliação das características do novo amido obtido. Através desta avaliação, pode-se comprovar que o ozônio modificou o amido de batata, podendo ser utilizado como alternativa para os agentes químicos atualmente utilizados. Além disso, através de outras análises, conseguiu-se entender como esta modificação ocorreu.

Palavras-chave: 1. Amido de batata 2. Modificação de amido 3. Oxidação de amido 4. Ozônio 5. Tecnologias emergentes 6. Propriedades dos alimentos

Español

La modificación de los almidones nativos es una técnica muy importante y utilizada en la industria, ya que permite obtener almidones con características deseables para algunas aplicaciones. La modificación se puede lograr de varias maneras, pero la modificación por métodos químicos todavía se utiliza, a pesar de la generación de residuos tóxicos. Con la creciente preocupación por el medio ambiente, las nuevas técnicas para modificar el almidón han ganado terreno. El ozono es un gas presente en nuestra atmósfera y se descompone en oxígeno, sin dejar residuos. Por otra parte, es muy oxidante, y se puede utilizar en lugar de agentes químicos para modificar el almidón. En este trabajo, almidón de papa se procesó con ozono y los cambios resultantes se investigaron mediante la evaluación de las características del nuevo almidón obtenido. A través de esta evaluación, podemos probar que el ozono modificó el almidón de papa y por lo tanto se puede utilizar como una alternativa a los agentes químicos utilizados actualmente. Además, por otro análisis, fue posible comprender cómo se ha producido este cambio.

Palabras clave: 1. Almidón de papa 2. Modificación de almidón 3. Oxidación de almidón 4. Ozono 5. Tecnologías emergentes 6. Propiedades de los alimentos

APPENDIX D.

Accepted Article

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Potato starch modification using the ozone technology

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ABSTRACT

Ozone is a very powerful oxidizing agent and it can be quickly decomposed into oxygen, leaving no residues and meeting the global demand for sustainability. The effects of the different ozonation times on the structure and properties of the potato starch were investigated. With increasing ozonation time, it was observed an increase in the carbonyl, carboxyl and reducing sugar contents, as well as a decrease in the pH, apparent amylose content and molecular size, demonstrating that the potato starch processed with ozone was modified in its molecular level. Further, X-ray diffraction showed no significant changes in the relative crystallinity, while it was possible to observe alterations in the granules morphology. All these changes directly affected the modified starch properties, resulting in significant differences in terms of pasting properties, gel texture and paste clarity, proving the effectiveness of using ozone as an efficient oxidizing agent for starch modification.

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1. Introduction

Starch is an important energy source in human diet and a major component of many plants. Furthermore, it is an important component for many industrial applications, including different industries such as food (human and animal), pharmaceutical, cosmetic, chemical, petrochemical, textile, etc. Although being formed mainly by α -D-glucopyranosyl units, starch is a very complex material, being presented as a water insoluble granule with a double-helical, semi-crystalline and birefringent structure, formed by two main polysaccharides: amylose and amylopectin (Liu, 2005; Miles, Morris, Orford, & Ring, 1985). Amylopectin is a highly branched molecule composed by short chains of (1 \rightarrow 4)-linked α -D-glucopyranosyl units with (1 \rightarrow 6)-linked branches, with a tree-like arrangement of double helices, being the main component of the crystalline domains in the starch granule. Amylose is essentially linear, composed by (1 \rightarrow 4)-linked α -D-glucopyranosyl, presenting a small amount of branches, and it is believed to be randomly mixed with other materials in the amorphous domain of the starch granule (Hizukuri, Abe, & Hanashiro, 2006; Tester, Karkalas, & Qi, 2004).

Starch has attracted much academic and industrial interest as it is an abundant, renewable, cheap and biodegradable resource, as well as generally regarded as safe and environmentally acceptable (Kochkar, Morawietz, & Hölderich, 2001; Zhang, Zhang, Wang, & Wang, 2009). On the other hand, the naturally available starches have limited properties, being necessary the modification in order to provide further properties for the different industries requirements.

The modified starches present some characteristics that the native starches does not have, thus allowing further uses from a specific source. In fact, the modified starches can bring competitive advantages to some products, with many new functional and added value properties (Kaur, Ariffin, Bhat, & Karim, 2012). The starch modification can be carried out by chemical, physical and/or enzymatic processes, as well as by genetic improvement.

Oxidation is a chemical method of starch modification, being performed by a reaction with an oxidant agent under controlled conditions (Wang & Wang, 2003). The main reagents used for starch oxidation are sodium hypochlorite and hydrogen peroxide. However, by using these chemical agents, toxic wastes are generated (Chan et al., 2011).

In contrast, if compared to other chemical oxidants, the ozone is considered a "green" and "environmentally friendly" technology, since, by being quickly decomposed to oxygen, it does not leave any residues in food or in environment (Çatal & İbanoğlu, 2014). In fact, it meets the global demand for sustainability being safer for both consumer and environment. The ozone is a very powerful oxidizing agent, with a relatively high ($E^0 = +2.075$ V) electrochemical potential (Mahapatra, Muthukumarappan, & Julson, 2005). It can be generated by the exposure of oxygen/air to a high-energy source, which convert molecules of oxygen (O_2) to ozone (O_3) (Khadre, Yousef, & Kim, 2001).

In fact, the ozone is already being used for starch modification in a wide range of starch sources, such as corn, sago, cassava, rice, wheat and potato, among others (Çatal & İbanoğlu, 2012a, 2013, 2014; An & King, 2009; Chan, Bhat, & Karim, 2009; Chan et al., 2011; Klein et al., 2014). However, it is a still fairly studied process. For example, Çatal and İbanoğlu (2012a; 2012b) studied the effects of processing starches with ozone in aqueous solution for a determined time (1 h for both studies). In their first work (2012a), corn

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