UNIVERSIDADE TECNOLÓGICA FEDERAL DO PARANÁ
Programa de Pós-Graduação em Tecnologia de Alimentos

TANIA MENEGOL

OXIDATION OF CASSAVA STARCH WITH OZONE AND HYDROGEN PEROXIDE: EFFECT ON SOME FUNCTIONAL PROPERTIES

DISSERTAÇÃO

Medianeira
2013

OXIDATION OF CASSAVA STARCH WITH OZONE AND HYDROGEN PEROXIDE: EFFECT ON SOME FUNCTIONAL PROPERTIES

TANIA MENEGOL

Medianeira
2013

TANIA MENEGOL

OXIDATION OF CASSAVA STARCH WITH OZONE AND HYDROGEN PEROXIDE: EFFECT ON SOME FUNCTIONAL PROPERTIES

Medianeira
2013

AGRADECIMENTO

BIOGRAFIA

Eu Tania Menegol, nascida em Medianeira, Estado do Paraná, Brasil, ingressei, no ano de 1996 no antigo Centro Federal de Educação Tecnológica do Paraná no Curso de Técnico em Alimentos, tive como principal trabalho o desenvolvimento de novo produto "Pizza Vegetariana". Graduada em Tecnologia de Alimentos – Enfâse Industrialização de Carnes pelo Centro Federal de Educação Tecnológica do Paraná (2004), e meu trabalho de conclusão de curso foi sobre desenvolvimento de um novo produto "Embutido de Frango tipo Salame", foi publicado na Higiene de Alimentos em agosto de 2006. Pós-Graduação nível especialização em Microbiologia Aplicada pela Universidade Estadual do Oeste do Paraná (2008) trabalhei no tema sobre contaminação de águas subterrâneas (poços rasos). Atualmente sou discente de mestrado acadêmico em Tecnologia de Alimentos pela Universidade Tecnológica Federal do Paraná, estou trabalhando com Modificação de Amido. Possuo experiência profissional na área de gestão de laboratório e da qualidade, atuando principalmente nos seguintes temas: microbiologia de alimentos, tecnologia de alimentos, análises laboratoriais e no momento atuando como Analista de Laboratório, no Laboratório de Biogás no Centro Internacional de Energias Renováveis com Ênfase em Biogás na Fundação Parque Tecnológico Itaipu.

GENERAL ABSTRACT

INTRODUCTION AND OBJECTIVES: Modified starches are obtained by the treatment of starch suspensions with an oxidizing agent to convert hydroxyl groups to carbonyl groups and carboxyl groups, providing starch with interesting properties in the field of food manufacturing, such as neutral taste, adhesion to surfaces, low viscosity binding, transparency gels and low tendency to retrogradation. Different oxidizing treatments have been applied to obtain cassava starch with good expansion properties. In the present study, the changes that occurred in cassava starch subjected to ozone/hydrogen peroxide and the effect of oxidation on the functional properties and expansion of modified starch with the objective of get through this treatment, starches free of metal waste and with favorable expansion properties, clarity and low viscosity are of particular interest in food applications.

METHODS: To study the effect of the independent variables, i.e. ozone dosage (1, 3 and 5 g) and hydrogen peroxide content (0.25, 0.5 and 0.75%), on the amylose content, specific volume of biscuits, sum of carbonyl and carboxyl groups, paste viscosity nd intrinsic viscosity, we used the method of experimental research following a 2^2 composite rotational design with four axial testing conditions, four factorial trials and two repetitions at the central point, for a total of eleven randomized trials.

MAIN RESULTS: The modified starches showed different properties compared to the native starch, as evidenced mainly by greater reducing power and swelling. Chemical oxidation was evidenced by an increase in the carbonyl content, as well as reduced solubility and intrinsic viscosity of amylose.

DISCUSSION AND CONCLUSION: The observed differences in the physicochemical properties of native starch and modified starches using experimental research design were attributed to the differences are more specific material evaluated in this experiment, since oxidation occurred mainly in amorphous starch granules. The comparative analysis between the modified starches and native starch showed that chemical oxidation increased the content of carbonyl and carboxyl groups, increased solubility and reduced the intrinsic viscosity of amylose and decreased swelling. However, the paste viscosity and the gelation temperature were higher in the oxidized starches compared to the native starch. This was evidenced by the expansion property increase nd the specific volume of biscuits when compared to the standard. Response surface analysis showed that the combination of ozone and hydrogen peroxide had an antagonistic effect and only ozone influenced the functional properties of starch.

Keywords: Starch, Oxidation, Expansion, Ozone, Hydrogen Peroxide.

RESUMO GERAL

INTRODUÇÃO E OBJETIVOS: Os amidos oxidados são obtidos através do tratamento de suspensões de amidos com um agente oxidante, que transformam seus grupos hidroxilas em grupos carbonilas e carboxilas, conferindo-lhes propriedades de interesse na área de industrialização de alimentos, tais como sabor neutro, aderência a superfícies, baixa viscosidade de pasta, géis transparentes e baixa tendência a retrogradar. Diferentes tratamentos oxidantes têm sido aplicados para a obtenção de amido de mandioca com propriedade de expansão. No presente estudo discute-se a modificação ocorrida no amido de mandioca submetido ao par ozônio/peróxido de hidrogênio e o efeito da oxidação nas propriedades funcionais e de expansão do amido modificado, com o objetivo de obter através desse tratamento, amidos isentos de resíduos metálicos e com propriedade de expansão, claridade de pasta e baixa viscosidade, de interesse em aplicações alimentícias específicas.

MÉTODOS: Para estudar o efeito das variáveis independentes, dosagem de ozônio (1, 3 e 5g) e teor de peróxido de hidrogênio (0,25, 0,5 e 0,75%) sobre propriedade de pasta, claridade de pasta, teor de amilose, volume específico dos biscoitos, somatório de carbonila e carboxila, poder de inchamento e solubilidade e viscosidade intrínseca usou-se o método de pesquisa experimental, seguindo delineamento composto rotacional 2^2, com 4 ensaios nas condições axiais, 4 ensaios fatoriais e 2 repetições no ponto central, no total de 11 ensaios distribuídos aleatoriamente.

PRINCIPAIS RESULTADOS: Os amidos modificados obtidos apresentaram diferentes propriedades em relação ao amido nativo, evidenciadas, principalmente, pela maior poder redutor e inchamento. A oxidação química foi evidenciada pelo aumento do teor carbonilas, solubilidade e redução na viscosidade intrínseca e no teor de amilose.

DISCUSSÃO E CONCLUSÃO: As diferenças observadas nas propriedades físico-químicas do amido nativo e dos amidos oxidados conforme delineamentos da pesquisa experimental são atribuídos às diferenças mais específicas do material avaliado no experimento, visto que a oxidação ocorreu principalmente na região amorfa do grânulo de amido. Na análise comparativa entre os ensaios e o amido nativo, observou-se a oxidação química pelo aumento no teor de carbonilas e carboxilas, de solubilidade e de redução da viscosidade intrínseca e amilose, e diminuição do inchaço. No entanto, a viscosidade de pasta e a temperatura de gelatinização foram maiores nos amidos oxidados em comparação com amido nativo. Evidenciou-se a propriedade de expansão pelo aumento no volume específico (16,03mLg-1) dos biscoitos quando comparada ao padrão. A análise de superfície de resposta indica que a combinação de ozônio e peróxido de hidrogénio tem efeito antagônico e que somente o ozônio influencia nas propriedades funcionais do amido.

Palavras chaves: Amido, Oxidação, Expansão, Ozônio, Peróxido de Hidrogênio.

APRESENTAÇÃO

Esta dissertação é composta por um artigo de autoria de Tânia Menegol, Manuel Salvador Vicente Plata Oviedo, Elizabeth Harumi Nabeshima sob o título de *Oxidação do amido de mandioca com ozônio e peróxido de hidrogênio: efeito sobre as propriedades funcionais e propriedade de expansão*, submetido para publicação na revista Food Chemistry.

Oxidation of cassava starch with ozone and hydrogen peroxide: effect on some functional properties

Tania Menegol[*], [2]Manuel Salvador Vicente Plata-Oviedo, [3]Elizabeth Harumi Nabeshima

[*]Corresponding author: Department of Food Technology, Federal Technological University of Paraná.
Email: <tmenegol@hotmail.com>.
[2]Department of Food Technology, Federal Technological University of Paraná. E-mail: <mapaov@utfpr.edu.br>
[3]Instititut of Food Technology: Email: <nabeshima@ital.sp.gov.br>.

Abstract

Different oxidizing treatments with different concentrations of hydrogen peroxide and ozone were used to study the effect of oxidation on the functional properties and the expansion of cassava starch. Response surface methodology was used to study the effect of the independent variables, i.e. the amount of ozone (1, 3 and 5 g) and the amount of hydrogen peroxide (0.25, 0.50 and 0.75%) on the amylose content, carboxyl and carbonyl groups, Intrinsic viscosity, pasting properties, clarity of starch, swelling power and solubility and specific volume. The comparative analysis between the samples and native starch provided evidence of chemical oxidation by an increased content of carbonyl and carboxyl groups; this increased solubility and reduced the intrinsic viscosity, amylose content and swelling. However, the paste viscosity and the gelation temperature were higher in the oxidized starches compared to the native starch. Excellent expansion properties (16.03 mL.g^{-1}) were found, assessed by the specific volume of biscuits when compared to the standard starch. Response surface analysis showed that the combination of ozone and hydrogen peroxide had an antagonistic effect and only ozone influenced the functional properties of starch.

Keywords: Starch, Oxidation, Expansion, Ozone, Hydrogen Peroxide.

1 Introduction

Starch is a homopolysaccharide composed of glucose units formed by two similar polysaccharides, amylose and amylopectin, in proportions determined according to the botanical species of origin, variations within the same species and the degree of maturity of the plant (Bemiller, 1997). This polymer is an important industrial ingredient due to its low cost, wide availability and different functional properties as a food and non-food, as it is used by the food, textile, papermaking and plastic industries in either native or modified form (Bertolini, 2010).

In the food industry, there is growing demand for starches that possess specific rheological properties (Cereda, 2003), since the use of native starches presents undesirable characteristics such as low shear stress resistance and solubility as well as high retrogradation and syneresis, which can be overcome by modifying the starch (Spier, 2010; Singh, Kaur & McCarthy, 2007; Sandhu, 2011). These modifications to the structure of the starch polymer can be performed by physical, chemical or enzymatic processes (Dias, Zavareze, Helbig, Moura, Galarza & Ciacco, 2011; Beninca, Colman, Lacerda, Carvalho Filho, Bannach & Schnitzler, 2013).

In the process of chemical modification by oxidizing, commercial starch is obtained by the reaction between a starch suspension and an oxidizing agent solution (Hu, Chen & Gao 2009) in which the hydroxyl groups of the glucopyranosyl

units located at carbons C-6, C-3, C-2 and C-4 are converted into carboxyl groups and carbonyl groups. This transformation is probably linked to the modification of the properties of oxidized starches (Wurzburg, 1986). The modification of the physical-chemical characteristics of native starch (clarity, low viscosity and retrogradation) favors the application of oxidized starches in food production in order to increase adhesion coating in prepared products or as additives in the food industry, such as in puddings and creams (Singh, Kaur & McCarthy, 2007; Pietrzyk, Juszczak, Fortuna, Labanowska, Bidzinska & Bloniarczyk, 2011).

There are several chemical reagents used as starch oxidants, such as sodium hypochlorite, hydrogen peroxide, peracetic acid, potassium permanganate and ozone (Cereda, 2003). Oxidizing agents whose residues are easily destroyed without affecting the taste of food and without generating environmental problems (Chan, Bhat & Karim, 2009) are suitable for starch modification. The ozone/hydrogen peroxide pair meets these requirements, as ozone is not retained in the product and is easily destroyed by neutralization before release into the atmosphere. Residual hydrogen peroxide is neutralized with catalase and a synergistic effect between these two reagents could be used in the oxidation of starches, with the possibility of reducing the levels of ozone and hydrogen peroxide required, which would lower the cost of production of oxidized starches.

Cassava starch modified by oxidation presents intriguing functional properties, which has motivated investigations into different oxidizing treatments and increased the industrial use of oxidized starch. Cassava starch is considered an excellent starting material for modification into specialty products (Breuninger, Piyachomkwan & Klanarong, 2009). The industrial use of cassava starch stems from its features such as lower amounts of undesirable components such as fat, protein and ash as well as its high molecular weight amylose and amylopectin (Breuninger, Piyachomkwan & Klanarong, 2009). This has enabled the food industry to preserve the desired characteristics of food produced with lower amounts of aromas and flavorings (Cereda, Vilpoux & Demiate, 2003).

In Brazil, sour cassava starch is obtained by a process of anaerobic fermentation of the cassava starch and drying in the sun. Its main characteristic is its expansion properties, which allows it to be used in baked goods without the addition of yeast (Plata-Oviedo, 1998). The expansion properties of starch, commonly observed by an increase in the specific volume of baked biscuits, has been linked to the formation of carboxyl groups (Takizawa, Silva, Konkel & Demiate, 2004; Shirai, Haas, Ferreira, Marsuguma, Franco & Demiate, 2007).

Within the scientific literature, various oxidizing treatments have been applied for tapioca starch to improve the expansion property. Among the various treatments, using hydrogen peroxide/Fe^{2+} stands out (Plata-Oviedo, 1998; Demiate, Dupuy, Huvenne, Cereda & Wosiacki, 2000; Shirai et al., 2007; Dias et al., 2011). Moreover, sodium hypochlorite (Dias, 2001; Silva, Ferreira, Shirai, Haas, Scherer, Franco & Demiate, 2008) or potassium permanganate (Takizawa et al., 2004; Silva et al., 2008) are used in the presence of organic acids in order to produce starch with expansion properties similar to a sour native starch, i.e. fermented cassava dried in the sun. Starches derived from oxidation with sodium hypochlorite or the use of heavy metals (iron, copper and tungsten) as catalysts for oxidation reactions contain a high amount of inorganic residues (Tolvanen, Maki-Arvela, Sorokin, Salma & Murzina 2009).

However, there have been few publications on the effect of oxidation in the development of the functional properties of cassava starch subjected to the process

of ozone/hydrogen peroxide modification. Therefore, this study aimed to submit cassava starch to a modification process with the ozone/hydrogen peroxide pair and to study the effect of oxidation on the functional properties of oxidized starch and its expansion properties.

2 Materials and Methods

2.1 Experimental design and statistical analysis

A 2^2 central composite design with four factors, four axial points and two repetitions in the center with a total of eleven tests (Table 1) was used to study the effect of the independent variables, i.e. the amount of ozone (1, 3 and 5 g) and the amount of hydrogen peroxide (32% w/v; 0.25, 0.50 and 0.75% on a 40% dry starch basis) on the properties of oxidized starch. The limits were established for each ingredient based on previous tests. The dependent variables were amylose content, carboxyl and carbonyl groups, intrinsic viscosity, pasting properties, clarity of starch, swelling power and solubility, thermal properties and specific volume.

The effect of process variables on each response and mathematical models fitted to each response were statistically analyzed by analysis of variance (ANOVA). By analysis using the variance (F-test), the significance of the effect of the main factors and the interaction of two factors could be determined. If the variance from the observed response (F-value) for a given parameter was less than F (tabulated) in the range of 95%, we could conclude that the effect of the particular parameter was not significant. The determination coefficient was also determined by ANOVA (adjusted R^2) and the predictive model when the value of R^2 was set above 0.70%. Pearson correlation analysis was used to evaluate the interactions between variables. Evaluate statistically of the variables that had no significant effect was also applied Tukey test 5%.

Data processing and calculations were performed using Statistica 7.0 (StatSoft, Inc.)

2.2 Materials and modification of starch

The cassava starch was donated by company producing starches, located in the central-western of Paraná, Brazil. All reagents were of analytical grade.

Dispersions were prepared from 40% w/w starch (dry basis) in a sufficient amount of distilled water to make the mass to 1 kg. The mixture was homogenized and hydrogen peroxide (H_2O_2) was added according to the experimental design (Table 1). The dispersion was then transferred into a reaction column.

Oxidation was performed with an ozone generator (Ozone & Life® model L3.0) with a flow of ozone (1.0 g/h) sufficient to reach the dosage according to the experimental design. The residual ozone, upon leaving the column, was neutralized with 3% potassium iodide (Mahmoud, 2006). After each oxidation with ozone, the samples were washed with distilled water until a negative test with iodine solution was found, then samples were dried in an oven with air circulation (CIENLAB brand) at a temperature of 45°C for 12 h. After milling, the starches were stored in plastic bags (polyethylene) until further analysis.

2.3 Characterization of the modified starches

2.3.1 Amylose content and carboxyl and carbonyl groups

The amylose content of the native and oxidized starches was determined by a colorimetric method according to the standard ISO 6647.

To determine the amount of carboxyl and carbonyl groups, Smith's method (1967) used and the calculations were performed according to Eq. (1) and Eq. (2), respectively.

(1)

$$\%carboxyl = \frac{(Vb-Vs)x\,M\,x\,0.45\,x\,100}{W}$$

where Vs is the volume of NaOH required for the sample (mL), Vb is the volume of NaOH used to test the blank (mL), M is the molarity of NaOH and W is the sample weight (dry basis).

(2)

$$\%carbonyl = \frac{(Vb - Vs)\,x\,M\,x\,0.028\,x\,100}{W}$$

where Vb is the volume of HCl used for the blank (mL), Vs is the volume of HCl required for the sample (mL), M is the molarity of HCl and W is the sample weight (dry basis).

2.3.2 Intrinsic viscosity

The Leach method (1963) was used with modifications at the concentracions (0,1, 0,2, 0,3 and 0,4%) to determine the intrinsic viscosity obtained by extrapolation of the reduced and inherent viscosity equal to a concentration of zero.

2.3.3 Pasting properties and clarity of starch

The characteristics such as minimum and maximum viscosity (RVU), setback (value obtained by the difference between the final viscosity and the minimum viscosity), breakdown (value obtained by the difference between the maximum and minimum viscosity) and final viscosity (50°C).

In the evaluation of the pasting properties, a Rapid Visco Analyser (RVA-4500 model Perten Instuments, Warriewood, Australia) were evaluated by *Thermocline for Windows version 3*. Each sample was assessed in triplicate. In the procedure, 2.5 g of the sample was weighed out (corrected to 14% moisture) and 25 mL of water was added to the aluminum cup. The sample was dispersed with an acrylic stirrer coupled to the cup/apparatus/stirrer assembly. The test program used was Standard 1, as proposed by Chan, Bhat & Karim (2009) with modifications.

Clarity was determined by the pulp method of Nishinari & Morikawa (2000) with modifications, using starch dispersion at 1% (w/v).

2.3.4 Swelling power and solubility

The swelling power (SP) and solubility of the starch at temperatures from 60 to 90°C by the method of Jeng-Yune & AN-I (2001). After centrifugation, two phases were separated: the liquid phase was used for the determination of solubility and the gel phase was used to determine the SP.

2.3.5 Thermal properties

In determining the temperature and enthalpy of gelatinization, a differential scanning calorimeter (DSC TA Instruments model Q20) was used with the method of Lawal, Adebowale, Ogunsanwo, Beard & Ilo (2005), with adaptations. Analyses were performed in a hermetically sealed aluminum capsule containing 4.0 mg of the sample and 20.0 µL of distilled water. After 2 h of rest, thermal analysis was performed using the samples within the temperature range of 30 to 160°C with a heating rate of 5°C/min. As standard, an empty aluminum capsule was used. Based on the weight of dry starch, the onset temperature (To), peak temperature (Tp), completion temperature (Tc) and gelatinization enthalpy (J/g) were calculated from the graphs built with the aid of *Universal Software* version 4.5 A.

2.3.6 Preparation of biscuits and expansion properties

For the analysis of the specific volume, biscuits were prepared according of the method of Plata-Oviedo (1998) with the following formulation: 50 g starch, 10 g hydrogenated vegetable fat, 1,5 g salt and 25 g of water.

The displacement method was used to measure out millet seeds in a beaker (2000 mL) and for determining the specific volume (mL/g) of the biscuits.

2.3.7 Morphology of the granules: scanning electron microscopy and light microscopy

For scanning electron microscopy (SEM), a Shimadzu SS-550 microscope was used under an accelerating voltage of 10 kV. Each sample was observed and photographed at a magnification of 3000x.

A simple analysis of the morphology of the starch granule was performed with the aid of an Olympus light microscope (model BX51-P) coupled to an analytical system (Image-Pro Plus Cybernetics Average), at a magnification of 200x. In preparing the samples for staining, methylene blue was used according to the method of Schoch & Maywald (1968).

3. Results and discussion

3.1. Carboxyl and carbonyl content

The analysis of the average values of the carbonyl and carboxyl content of the treated starches (Table 2) showed that sample 6 (5.82 g ozone and 0.5% H_2O_2) contained 0.18% carbonyl + carboxyl as the highest amount of functional groups resulting from ozone/H_2O_2 oxidation. Similar results can be found in the literature. Oxidation of cassava starch with ozone for 5 minutes resulted in 0.14% carbonyl + carboxyl content, which decreased to 0.05% after 10 minutes of treatment (Chan, Bhat & Karim, 2009).

The carbonyl and carboxyl content increased following the oxidation reaction, indicating that oxidation had occurred. In the oxidation reaction, the hydroxyl groups of the starch molecules are transformed into carbonyl groups and then to carboxyl groups (Kuakpetoon & Wang, 2006; Pietrzyk et al., 2011; Dias et al., 2011).

In the summation of the carbonyl and carboxyl levels (p<0.05), there are terms for the linear concentration of the ozone/H_2O_2 pair and a quadratic term for the concentration of H_2O_2 (p<0.10).

The analysis of variance validated the model because the coefficient of determination (adjusted R^2 value) was equal to 0.73 The F test ($p<0.05$) was highly significant. An appropriate model to describe the results using the response surface was obtained as a second-order model shown in table 1.

In the response surface analysis (Fig. 3), it was found that when the ozone concentration was maximal (1.41), the values of the sum of the carboxyl and carbonyl content increased, which remained around 0.18%, indicating a high degree of oxidation. However, when the concentration of H_2O_2 varied between, at the upper and lower levels, an antagonistic effect occurred, resulting in a decrease in the values of the sum of the carbonyl and carboxyl content, which remained around 0.10%.

3.2 Amylose content

In examining the effects of oxidation on the amylose content of the samples (Table 2), the smallest value (13.73%) was found for sample 7 (3 g ozone and 0.15% H_2O_2) and the highest (18.43%) was found in the standard sample (a value consistent with that found by Hoover, 2001). The decrease in the amylose content in oxidized starches was found because the oxidation reaction principally occurs in the amorphous region of the granule, where amylose is more vulnerable to oxidation (Kuakpetoon & Wang, 2001). The amount of amylose is crucial to verifying the efficiency of the oxidation of starch (Sangseethong, Lertphanich & Sriroth 2009).

In the analysis of the amylose content, a significant ($p<0.05$) effect was observed for the linear concentration of ozone and H_2O_2 and the quadratic concentration of H_2O_2.

The analysis of variance validated the model, as the adjusted coefficient of determination (R^2) for the amylose content was 0.76. The F test ($p<0.05$) was highly significant and the model was adequate to describe the response surface analysis, obtaining a second-order model shown in table 1.

In the response surface analysis (Fig. 3), it was found that when the ozone concentration was maximal (+1.41) and the H_2O_2 minimal level (-1.41), the amylose values decreased, remaining around 13%, indicating a high degree of oxidadion. When the concentration of H_2O_2 increased to a higher level, the response surface analysis showed an antagonistic effect, with an increased amylose content (around 16%), indicating a weak influence of H_2O_2.

3.3 Intrinsic viscosity

In intrinsic viscosity was found to be significant ($p<0.05$) for the linear terms for the concentration of ozone and H_2O_2 and the quadratic term for H_2O_2 significance level ($p<0.10$). The analysis of variance validated the model, with an adjusted coefficient of determination (R^2) equal to 91.64%. The F test ($p<0.05$) was significant, indicating an adequate model to describe the results through a response surface, and providing a second-order model as shown in table 1.

In the response surface analysis (Fig. 4), it was found that when the ozone concentration was high (+1.41) and the H_2O_2 concentration was low (-1.41), the intrinsic viscosity decreased and remained close to 0.40 dL/g. But, if the H_2O_2 concentration was high (+1.41) antagonism was seen, along with increased intrinsic viscosity. Native starch had an intrinsic viscosity 2.66 dL/g, close to the published value of 2.56 dL/g (Plata-Oviedo, 1991) but different from the values of 2.36 dL/g for cassava starch at 30°C (Ferrini, 2006).

The intrinsic viscosity decreased from 2.66 dL/g in native starch to 0.40 dL/g in oxidized starches. The results were similar to those reported in the literature after ozone oxidation (3, 5 and 10 min) of tapioca starch in the solid state. Oxidation for 10 minutes led to an intrinsic viscosity of 0.26 dL/g at 35°C (Chan, Bhat & Karim, 2009). This decrease may be associated with partial depolymerization of starch polymers, thus reducing its molecular weight (Pietrzyk et al., 2011), as indicated by the positive Pearson correlation between intrinsic viscosity and the amylose content (Chan, Bhat & Karim, 2009). Following oxidation with sodium hypochlorite and H_2O_2, the viscosity may decrease due to the increased reaction time. It is likely that the fission of starch chains occurs during this time interval, which may happen regardless of the formation of functional groups (Sangseethong, Termvejsayanon & Sriroth, 2010).

3.4 Pasting properties

The pasting properties measure intensity the effect of modifying in the starch. The effect depends on the botanical source of the starch, temperature, mechanical shearing and chemical reagents used in the modification (Bemiller, 1997; Dias et al., 2011). In the analysis of the viscosities (Table 3), it was found that various concentrations of ozone and H_2O_2 had no significant effect on the peak viscosity, which prevented the presentation of a regression model for this variable. In terms of viscosity breakdown, the linear and quadratic terms of ozone concentration were significant (significance level of 5%), but the analysis of variance indicated the impossibility of a presentation a mathematical model for response surface analysis because only part of the variability was explained by the model (adjusted R^2 value equal to 61.65%).

The effect of the variable content of ozone, as a linear term was negative, but positive as a quadratic term for 95°C viscosity, final viscosity and retrogradation. The analysis of variance and F test validated the models for the responses and the response surfaces (Fig. 4). The equations of the second order equations for 95ºC viscosity, final viscosity and retrogradation shown in table 1.

For the values of peak viscosity (Table 3), a significant increase in the viscosity of the starch was found when compared to the standard sample except for samples 6 (5.82 g ozone and 0.5% H_2O_2) and 9 (3 g ozone and 0.5% H_2O_2) which showed a significant decrease. This increase in the maximum viscosity has been reported in the literature, and is thought to be due to chemical bonding between the polymers (Chan, Bhat & Karim, 2009) and is attributed to chemical cross-linking, because these connections reinforce the starch granule (Wongsagonsup et al., 2005). The decrease in the viscosity peak in the oxidized starches may be associated with degradation of the molecules, resulting in a decrease in the molecular weight (Chan, Bhat & Karim, 2009; Sangseethong, Lertphanich & Sriroth, 2009); this is supported by the decrease in intrinsic viscosity.

The results on starch viscosity at 95°C and the final viscosity (Table 3) demonstrated that there were weak connections in the structure of the granule due to the large decrease in the final viscosity (Plata-Oviedo & Camargo, 1995), this indicates that oxidized starches have high sensitivity mechanical agitation and high temperatures. The viscosity, setback and retrogradation of the oxidized starches (Table 2) indicate that these starches are resistant to retrogradation, due to the low values between (6.81 - 35.42%) of the samples (1 -11) modified starches except sample 5 compared to the native starch (92.70%). A weak tendency for retrogradation is linked to the formation of carboxyl and carbonyl groups, which

minimize the reassociation of starch chains (Silva, Ferreira, Shirai, Hass, Scherer, Franco & Demiate, 2008; Sangseethong, Lertphanich & Sriroth, 2009), as demonstrated by the significant negative correlation of -0.78 between the carboxyl content and retrogradation.

Tabela 1. *Regression models for response variables*

RESPONSE VARIABLE	REGRESSION MODEL[a]	R^2
Sum of the carboxyl and carbonyl	$0.15 + 0.03X_1 - 0.01X_2 - 0.02X_2^2$	0.73
Amylose content	$15.76 - 0.69X_1 + 0.61X_2 - 0.47X_2^2$	0.76
Intrinsic viscosity	$1.00 - 0.39 X_1 + 0.09 X_2 - 0.16 X_2^2$	0.91
Viscosity at 95ºC	$22.28 - 61.55 X_1 + 40.60X_1^2$	0.97
Final viscosity	$37.24 - 87.05 X_1 + 57.66 X_1^2$	0.94
Setback	$15.05 - 25.49 X_1 + 17.00 X_1^2$	0.82
Paste clarity	$85.30 + 17.64X_1 - 11.13X_1^2 - 3.62X_2 + 4.46X_1X_2$	0.99
swelling power 60°C	$37,94 - 2,28X_1 - 10,64X_1^2 + 1,21X_2 - 5,75X_2^2$	0.98
swelling power 80°C	$22.53 - 15.30X_1$	0.79
Specific volume	$14.02 + 2.22 X_1 - 2.30X_1^2$	0.75

[a] X_1= Ozone; X_2= Hidrogen Peroxide.

3.5 Clarity of the starch paste

Pastes of the native and modified starches (Table 2) became clearer with increasing concentrations of the oxidants, reaching the highest value (90.54%) in sample 4 (5 g of ozone and 0.75% H2O2) (ambient temperature). This result was expected, since oxidized starches have a clear appearance and are more stable when heated/cooled; this treatment introduces carboxyl groups, which have a negative charge that prevents and minimizes the reassociation of linear molecules, with lower retrogradation (Huber & Bemiller, 2010; Wurzburg, 1986; Sangseethong, Lertphanich & Sriroth, 2009).There was a positive correlation (0.78 and 0.60) between the clarity of the paste and the introduced functional groups (carbonyl and carboxyl).

The values of paste clarity, the linear and quadratic terms for ozone concentration and the linear term for H2O2 were significant (p<0.05). The interaction between the concentrations of ozone and H2O2 was significant. The model was validated by analysis of variance, with significant F tests (p<0.05) enabling presenting the results using a response surface. Equations shown in table 1 present the mathematical model and regression coefficient obtained by the transmittance percentage (650 nm) in the oxidized starches.

The response surface analysis for paste clarity (Fig. 3) revealed that an increased ozone concentration resulted in a paste with higher clarity, with little influence of the amount of H2O2.

3.6. Swelling power and solubility

For each sample, the SP and solubility of the starches oxidized by the ozone/H_2O_2 pair were assessed at different temperatures (60ºC, 70ºC, 80ºC and 90ºC). The results (Fig. 1) show a reduction in the swelling power in all samples, except for sample 5 (0.18 g ozone and 0.50% H_2O_2) analyzed at temperatures of 80

and 90ºC. The largest reduction was observed in sample 2 (5 g ozone and 0.25% H_2O_2). There have been reports showing reduced swelling power following acid and oxidative modification of maize starch, which was assigned to the disintegration of the structure of the starch granules during the modification process (Lawal et al., 2005).

The higher solubility at temperatures of 80 and 90ºC, which may be associated with the depolymerization of amylose (Pietrzyk et al., 2011). At temperatures of 60 to 70°C, an increase in the binding affinity for water was observed in the oxidized starches compared to native starch, which may have resulted from the formation of carboxyl groups that weakened the internal chemical bonds and increased the capacity for water absorption by the starch (Pietrzyk et al., 2011).

In the analysis, the effect of ozone on swelling power was significant. The linear and quadratic terms were studied at all temperatures, but only the variable H_2O_2 at a temperature of 60ºC was significant ($p < 0.05$). The models temperatures of 60 to 80°C was validated by analysis of variance, with significant F tests ($p < 0.05$) enabling presenting the results using a response surface (Fig. 3) . At temperatures of 70 to 90°C, the analysis of variance showed a lack of fit and the model was invalidated.

The modified starches had greater solubility than native starch at all temperatures (Fig. 2). The ozone and hydrogen peroxide concentration had no significant effect on the solubilitys , do not present a regression model for this variable.

Variations in the structure of the granule and amylose and amylopectin concentrations interfere with the swelling power and solubility of starch (Perez, Gilbert, Rolland-Sabaté, Segovia, Sánchez, Reynes & Dufour, 2011). The depolymerization of amylose can contribute to an increase in the solubility of starches oxidized with ozone, but the correlation analysis indicated a negative correlation between solubility (60, 70, 80 and 90ºC) and amylose (-0.64, -0.65, -0.64 and -0.61). This was attributed to the increased mobility of the starch molecules, driven by higher temperatures that facilitate leaching and increase water solubility and swelling of the starch (Lawal et al., 2005). The swelling power and solubility provide relevant information about the type of linkages inside the starch granule (Singh, Kaur & McCarthy, 2007).

Fig 1. Swelling power of the modified starches.
*Mean values in the same column with different letters are significantly different (P < 0.05).

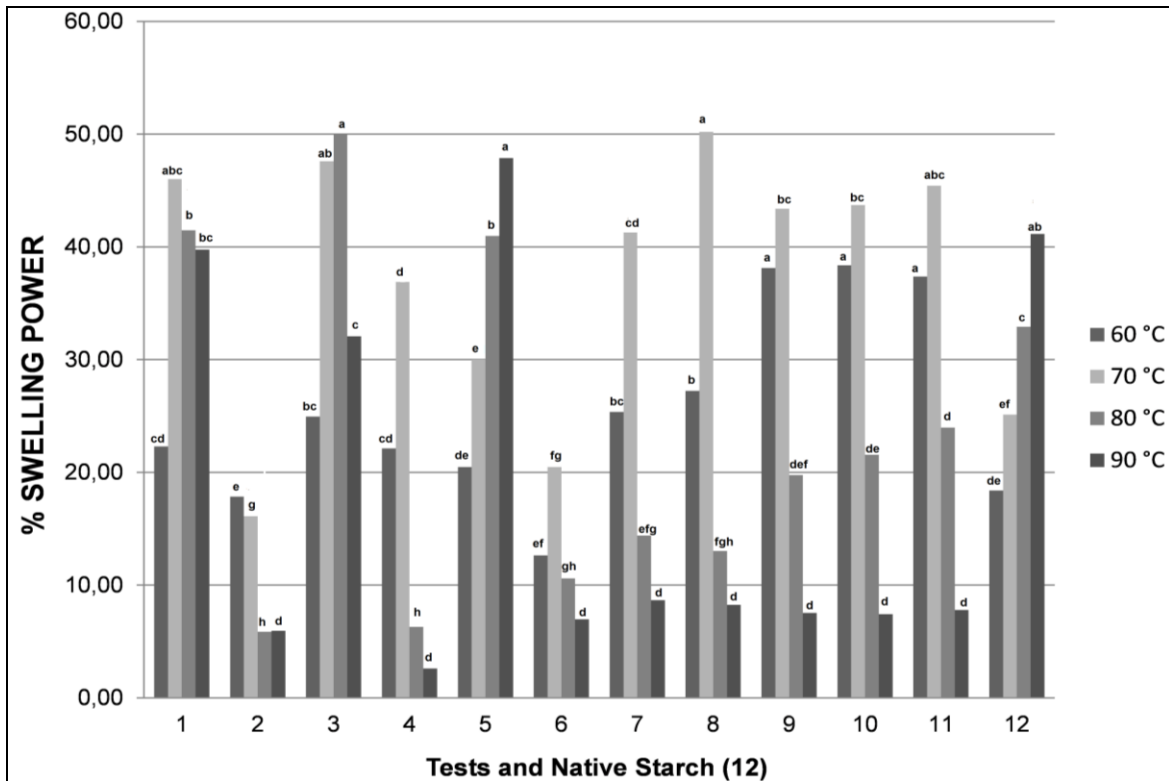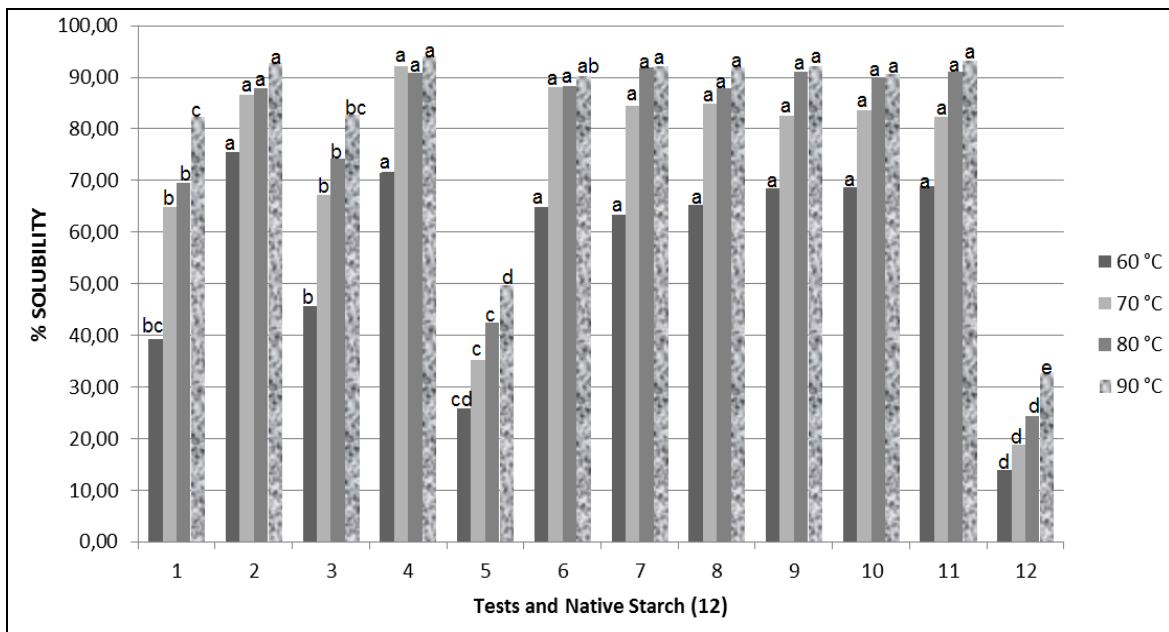**Fig 2**. Solubility of the modified starches.
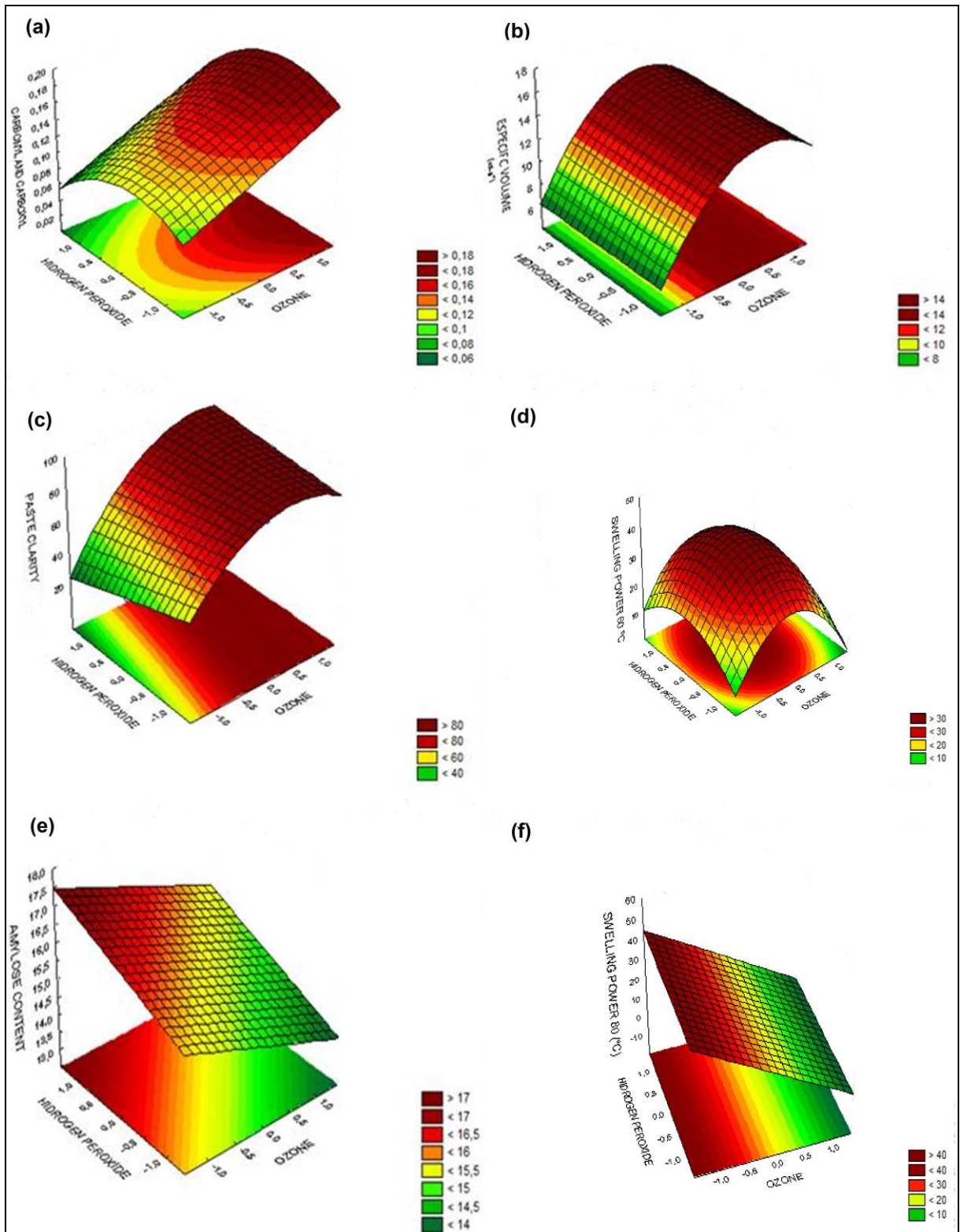*Mean values in the same column with different letters are significantly different (P < 0.05).

Fig 3. (a) Carbonyl and carboxyl content, (b) specific volume, (c) paste clarity, (d) swelling power (60°C), (e) amylose content and (f) swelling power (80°C).

3.8 Thermal properties

The initial gelatinization temperature in all tests (Table 3) was greater when compared to native starch, but not statistically different among the samples. The ozone and hydrogen peroxide concentration had no significant effect on the thermal properties , do not present a regression model for this variable. A similar increase was observed following the ozone oxidation of different starches; this was attributed to the weak influence of oxidative treatment on the crystalline structure of the starch granules (Chan, Bhat & Karim, 2009).

The gelatinization of starch is influenced by the glass transition of the amorphous regions, because the granular amorphous material is responsible for the effect on the and region crystalline melting temperature (Biliaderis, 2009; Sangseethong, Termvejsayanon & Sriroth, 2010). The gelatinization temperature increase observed in this study may be associated with the degradation of the amorphous region of the starch granule, i.e. the depolymerization of amylose.

When compared to standard starch, the gelatinization enthalpy of the modified starches was lower (table 3). This decrease may be due to the oxidation process of the starch, causing breakage of the glycosidic linkages (Lawal et al., 2005), weakening of the granules and, consequently, a decline in the energy required for gelation (Sangseethong, Termvejsayanon & Sriroth, 2010).
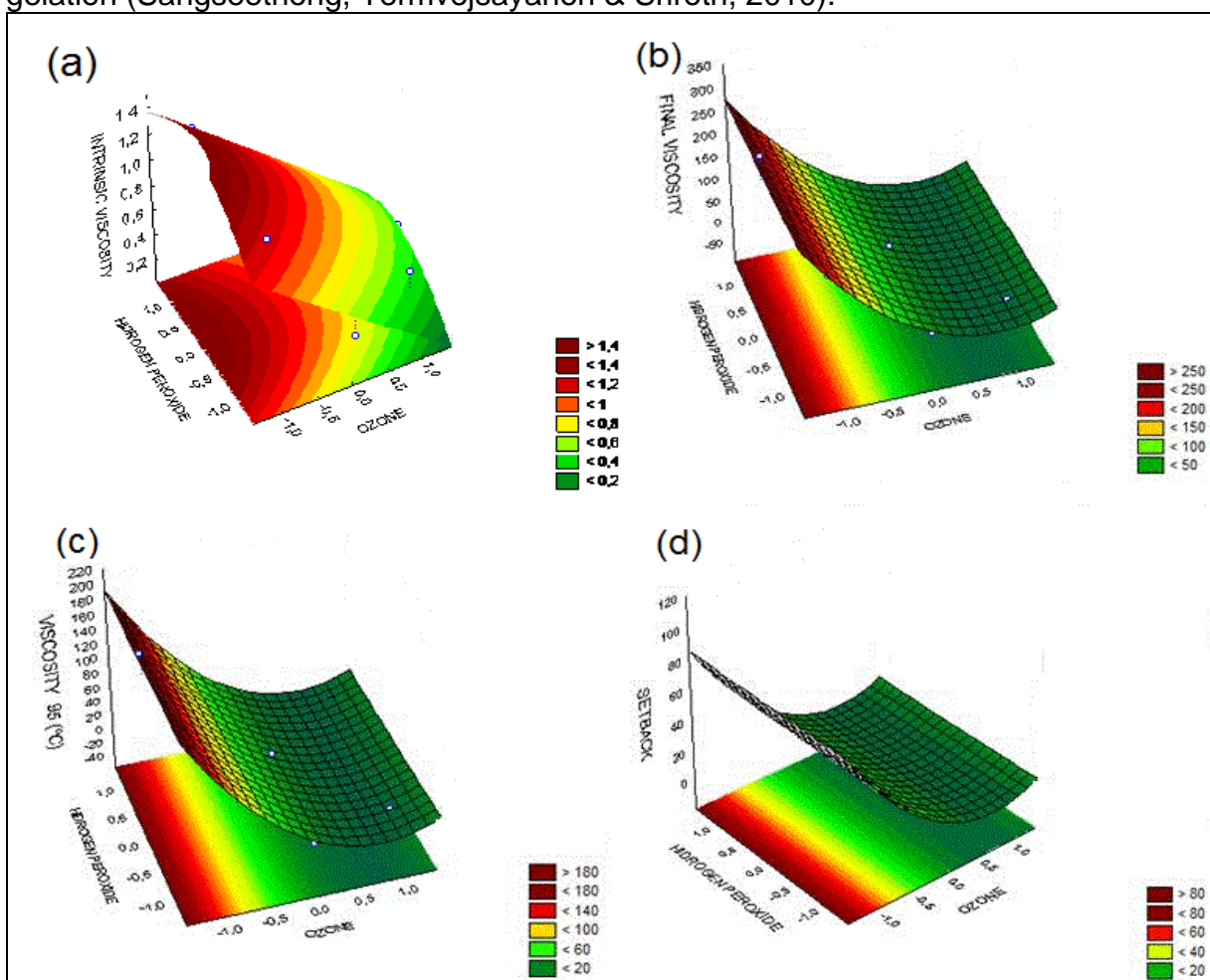

Fig 4. (a) Response surfaces for intrinsic viscosity, (b) minimum viscosity, (c) final viscosity, (d) setback.

3.9 Expansion properties

By the analysis of the volume of biscuits (Table 1), the expansion property of the modified starches was assessed (volumes between 5.49 and 16.03 mL/g). This characteristic is typical of sour cassava starch treated by natural fermentation for 20 to 60 days and drying in the sun (Plata-Oviedo, 1991). The expansion property was highlighted by sample 7 (3 g ozone and 0.15% H_2O_2) with a volume of 16.03 mL/g. Starches with similar properties to sample 7 can replace sour cassava starch (Maeda & Cereda, 2001) without presenting a risk of contamination during fermentation/drying (Tolvanen, Maki-Arvela, Sorokin, Salmi & Murzina, 2009).

The volume values were similar to those mentioned in the literature. Sour cassava starch used in commercial dough biscuits showed specific volumes 11 and 15 mL/g (Plata-Oviedo & Camargo, 1995). After photochemical modification of cassava starch and sweet potato, a corresponding expansion of 11.5 and 2.4 mL/g (Leonel, Garcia & Reis, 2004) was found.

In the mathematical model of the second order equation obtained for the specific volume of the modified starches shown in table 1, it was found that only variations in ozone, both linear and quadratic, were significant ($p < 0.05$). The analysis of variance showed an adjusted coefficient of determination (R^2) of 0.75, and the F test ($p < 0.05$) was highly significant, indicating an appropriate model to study the behavior of the specific volume with a variation in the ozone concentration (Fig. 3).

3.10. Morphology of the granules

The optical micrographs with methylene blue staining and SEM images of the cassava starch (Fig. 1). The analysis of the optical microscopy and SEM results revealed that the shape of the cassava starches did not change with oxidation; an oval shape was maintained, with truncated sides and flat smooth surfaces and diameters characteristic of native starch (Sánchez, Salcedo, Ceballos, Defour et al., 2009; Hoover, 2001). In the analysis, no changes were observed on the surface of oxidized starch granules in comparison with native starch granules. In the literature, there is mention of a similar result in the oxidation of corn starch with 0.8 and 2% sodium hypochlorite (Kuakpetoon & Wang, 2001).

In the optical micrographs with methylene blue staining, only traces of the dye were observed in the oxidized starches. This is explained by the amount of carboxylic groups present in starches which, in this study, was not sufficient to provide the intense blue color seen in native starch.
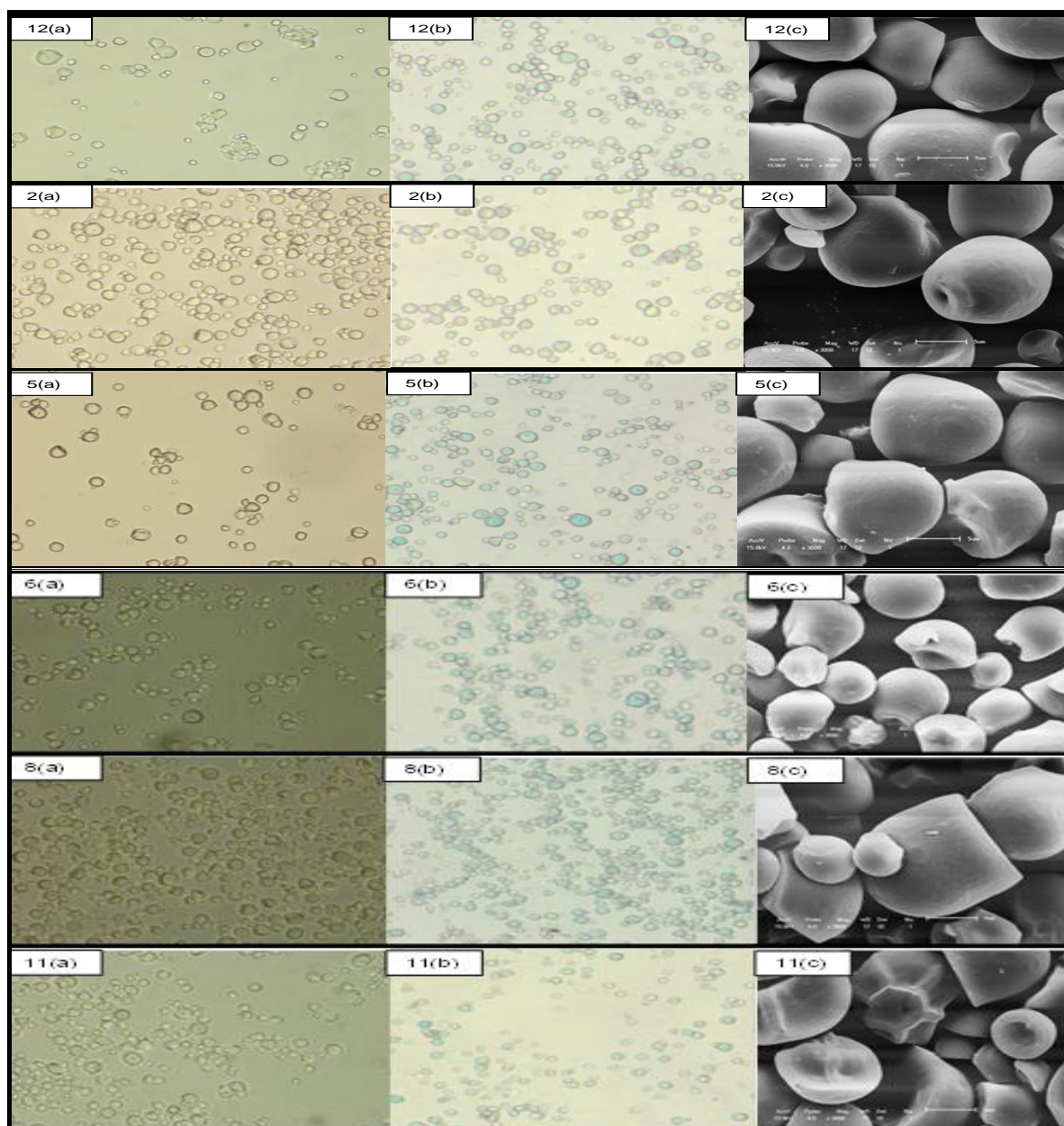
Fig 5. Optical microscopic images (a) with methylene blue staining (b) and SEM images (c) of native starch and samples: 2 (5 g of ozone and 0.25% H_2O_2), 5 (0.18 g of ozone and 0.5% H_2O_2), 6 (5.82 g of ozone and 0.5% H_2O_2), 8 (3 g of ozone and 0.85% H_2O_2) and 11 (3 g of ozone and 0.5% H_2O_2).

3 Conclusion

Compared to native starch, the modified starches showed different properties, primarily demonstrated as increased susceptibility to swelling. Chemical oxidation with hydrogen peroxide and ozone led to higher percentages of carboxyl and carbonyl groups, increased solubility and a reduction in the intrinsic viscosity and amount of amylose. The gelatinization temperature and viscosity showed higher peaks in the oxidized starches. Setback was decreased in the oxidized starches, evidenced by the lower setback values.

The combination of ozonation and hydrogen peroxide showed antagonistic effects, i.e., hydrogen peroxide showed a negative effect, while ozone had a positive effect on most of the studied responses. This can be attributed to the slow reaction of hydrogen peroxide, explaining why no effect was seen in most of the assessments. However, the oxidized starches obtained in this study may be used to develop new starch ingredients with various features, such as starches with high brightness, high solubility in hot water and low swelling power, which can be explored in foods that need of these characteristics. These starches with good expansion properties can also be used as substitutes for traditional sour cassava starch.

5 Acknowledgments

6 References

Bemiller, J. N. (1997) Starch modification: challenges and prospects. *Starch-Stärke*, 49(4), 127-131.

Beninca, C., Colman, T. A. D, Lacerda, L. G., Carvalho Filho, M. A. S., Bannach, G., & Schnitzler, G. (2013). The thermal, rheological and structural properties of cassava starch granules modified with hydrochloric acid at different temperatures. *Thermochimica Acta*, 552, 65-69

Bertolini, A. (2010) Trends in starch applications. Starches, Characterization, Properties, and aplications. New York, U. S.: Ed. Taylor & Francis Group. Ch. 1: 1-19 and Ch. 8, 145-205.

Biliaderis, C. G. (2009) Structural transitions and related physical properties of starch: production and use. In J. BeMiller, & R. Whistler. *Starch: chemistry and technology.* Food Science and Technology International Series. New York, U.S.: Third Ed, Ch. 8, 293-359.

Breuninger, W. F., Piyachomkwan, K., & Klanarong S. K. (2009). Tapioca/cassava starch: production and use. In J. N. BeMiller, & R. L. Whistler. *Starch: chemistry and technology*. Food Science and Technology International Series. New York, U. S.: Third Ed, Ch. 12, 541-567.

Cereda, M. P. (2003). Processing of cassava as a detoxification mechanism. In M. P. Cereda, & O. F. Vilpoux. *Technology, uses and potential starchy tuberous Latin America*. Sao Paulo, Brazil: Cargill Foundation, v. 3. Ch. 1, 147-80.

Cereda, M. P., Vilpoux, O., & Demiate, I. M. (2003). Modified starches. In M. P. Cereda, & O. F. Vilpoux. *Technology, uses and potential starchy tuberous Latin America*. Sao Paulo, Brazil: Cargill Fundation, v. 3, Ch.12, 246-332.

Chan, H. T., Bhat, R., & Karim, A. A. (2009). Physicochemical and functional properties of ozone-oxidized starch. *Journal of Agricultural and Food* Chemistry. 57(13), 5965-5970.

Dias, A. R. G. (2001). Effect of oxidants, organic acids and water-soluble fraction in the expansion property of fermented cassava starch. Thesis (Ph.D. in Food Technology). Campinas, Sao Paulo, Brazil: Faculty of Food Engineering, State University of Campinas.

Dias, A. R. G., Zavareze, E., Helbig, E., Moura, D. A., Galarza, V. C., & Ciacco, C. F. (2011). Oxidation of fermented cassava starch using hydrogen peroxide. *Carbohydrate Polymers,* 86, 185-191.

Demiate, I. M.; Dupuy, N. Huvenne, J. P.; Cereda, M. P. & Wosiacki, G. (2000). Relationship between baking behavior of modified cassava starches and starch chemical structure determined by FTIR spectroscopy. *Carbohydrate Polymers*, v. 42,n. 2, 149-158.

Ferrini, L. M. K. (2006). Effect of acid-methanol modification in the structural and physicochemical corn starch and cassava. Dissertation (Masters in Engineering and Food Science). State University Paulista Júlio de Mesquita Filho. Sao José do Rio Preto, Sao Paulo, Brazil.

Hoover, R. (2001). Composition, molecular structure, and physicochemical properties of tuber and root starches: a review. *Carbohydrate Polymers.* 45, 253-267.

Huber, K. C. & Bemiller, J. N. (2010) Modified starch Chemistry and properties. Bertolini, A., *Starches: Characterization, Properties, and Applications.* New York, U.S.: Taylor and Francis Group, Ch. 8,145-183.

Hu, G., Chen, J., & Gao, J. (2009). Preparation and characteristics of oxidized potato starch films. *Carbohydr Polym*, 76(2), 291-298.

International Organization for Standardization. (1987) ISO 6647. International Standard: Determination of amylose content. Geneve.

Jeng-Yune, L., & An-I, Y. (2001). Relationships between thermal, rheological characteristics and swelling power for various starches. *Journal of Foode Engineering*, 50(3), 141-146.

Kuakpetoon, D., Wang, Y-J., & Wang, L. (2001). Characterization of different starches oxidized by hypochlorite. *Starch-Stärke,* 53, 211-218.

Kuakpetoon, D., & Wang, Y-J. (2006). Structural characteristics and physicochemical properties of oxidized corn starches varying in amylose content. *Carbohydrate Research,* 341(11),1896-1915.

Lawal, O. S., Adebowale, K. O., Ogunsanwo, B. M., Barba, L. L., & Ilo, N. S. (2005). Oxidized and acid thinned starch derivatives of hybrid maize: functional characteristics, wide-angle Xray diffractometry and thermal properties. *International Journal of BiologicalMacromolecules*, (35)1-2, 71-79.

Leach, H. W. (1963). Determination of intrinsic viscosity of straches. *Cereal Chemistry,* (40)6, 593-600.

Leonel, M., Garcia, A. C. B., & Reis, M. M. (2004). Characterization physico-chemical and microscopic starch sweet potato, biri, cassava and cocoyam and expansion properties after photochemical modification. *Brazilian Journal of Food Technology*, (7)2, 129-137.

Maeda, K. C., & Cereda, M. P. (2001). Evaluation of two methods of expanding the oven sour. *Journal of Food Science and Technology* 21(2), 139-143.

Mahmoud, A. (2006). Evaluation of emergent methods aiming at to increase the efficiency of ozone in the mineralization of the azodye Remazol Black B. 2006. Dissertation (Masters in Chemistry). São Paulo, Brazil, University of São Paulo.

Morikawa, K., & Nishinari, K. (2000). Effects of concentration dependence of retrogradation behaviour os dispersions for native and chemically modified potato starch. *Food Hydrocolloids*, 14, 395-401.

Perez, E., Gilbert, O., Rolland-Sabaté, A., Segovia, X., Sánchez, T., Revnes, M., Dufour, D. (2011). Evaluation of the functional properties of promising *Dioscorea trifida L.* waxy starches for food innovation. *International Journal of Carbohydrate Chemistry*, 59, 263–273.

Pietrzyk, S., Juszczak, L., Fortuna, T., Łabanowska, M., Bidzińska, E., & Błoniarczyk, K. (2011). The influence of Cu(II) ions on physicochemical properties of potato starch oxidised by hydrogen peroxide. *Starch-Stärke* 64, 272-280.

Plata-Oviedo, M., & Camargo, C. R. O. (1995). Determination of physico-chemical and functional properties of two cassava starch fermented (sour cassava starch). *Science and Food Technology*, 15(1), 59-65.

Plata-Oviedo, M. S. V. (1991). The effect of acid treatment on the physical, chemical and functional cassava starch. Thesis (Masters in Food Technology). Faculty of Food Engineering, UNICAMP, Campinas, São Paulo, Brazil.

Plata-Oviedo, M. S. V. (1998). Drying the fermented cassava starch: chemical modification related to expansion property and physicochemical characteristics. Thesis (Ph.D. in Food Technology). Faculty of Food Engineering, UNICAMP, Campinas, São Paulo, Brazil.

Sánchez, T., Salcedo, E., Ceballos, H., Defour, D., & et al. (2009). Screening of Starch Quality Traits in Cassava (*Manihot esculenta* Crantz). *Starch-Stärke*, (61)1-5, 12–19.

Sandhu, R. P. S. (2011) *Effect of ozone on wheat flour functionality and bread making quality*. Dissertation (Ph.D. in Food Science). North Dakota State University, Fardo, North Dakota, U.S.

Sangseethong, K., Termvejsayanon, N., & Sriroth, K. (2010). Characterization of physicochemical properties of hypochlorite- and peroxide-oxidized cassava starches. *Carbohydrate Polymers,* 82, 446–453.

Sangseethong, K.; Lertphanich, S.; & Sriroth, K. (2009). Physicochemical properties of oxidized cassava starch prepared under various alkalinity levels. *Starch-Stärke*, 61, 92-100.

Schoch, T. J, & Maywald, E. C. (1956). Microscopic examination of modified starches. *Chemistry Analytical*, 28(3), 382-87.

Shirai, M. A., Haas, A., Ferreira, G. F., Marsuguma, L. S., Franco, C. M. L., & Demiate, I. M. (2007). Physico-chemical and food use of starch modified by oxidative treatment. *Sciences and Food Technology*, Campinas, São Paulo, Brazil, 27(2), 239-247.

Silva, R. M., Ferreira, G. G., Shirai, M. A., Haas, A., Scherer, M. L., Franco, C. M. L., & Demiate, I. M. (2008). Characterization physico-chemical properties of starches modified with potassium permanganate/lactic acid and sodium hypochlorite/lactic. *Sciences and Food Technology,* Campinas, Sao Paulo, Brazil, 28(1), 66-77.

Singh, J., Kaur, L., & McCarthy, O. J. (2007). Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications – a review. *Food Hydrocolloids*, 21,1-22.

Smith, R. J. Characterization and analysis of starches. (1967). In R. L. Whistler, E. F., Paschall. *Starch: chemistry and technology*, v. 2. New York, U.S.: Academic Press.

Spier, F. (2010). Effect of alkali treatment, acid and oxidative properties of corn starch. Dissertation (Masters in Science and Technology Agroindustrial). Eliseu Maciel School of Agronomy. Pelotas, Rio Grande do Sul, Brazil: UFP.

Takizawa, F. F. Silva, G. de O. da.; Konkel, F. E.; Demiate, I. M. (2004). Characterization of tropical starches modified with potassium permanganate and lactic acid. *Brazilian Archives of Biology and Technology*, v. 47, n. 6, Curitiba, Paraná, Brazil.

Tolvanen, P., Mäki-arvela, P., Sorokin, A. B., Salmi, T., & Murzina, D. Y. (2009) Kinetics of starch oxidation using hydrogen peroxide as an environmentally friendly oxidant and an iron complex as a catalyst. *Chemical Engineering Journal*, 154, 52-59.

Wongsagonsup, R.; Shobsngob, S.; Oonkhanond, B.; Varavinit, S. (2005). Zeta potential (ζ) and pasting properties of phosphorylated or crosslinked rice starches. *Starch-Stärke*, 57(1), 32-37.

Wurzburg, O. B. (1986). *Modified starches*: properties and uses. Boca Raton, Florida, U.S.: CRC-Press, Inc.

7 Appendix

Table 2. *Mean values of responses in samples and native cassava starch with different levels of modification by oxidation with ozone and hydrogen peroxide*

Tests	Independent Variables		Dependent Variables						
	X_1 Ozone (g)	X_2 Hydrogen peroxide (%)	% Carbonyl	% Carboxyl	%Carbonyl + %Carboxyl	% Content of Amylose	% Paste Clarity	Intrinsic Viscosity $(dL.g^{-1})$	Specífic Volume (mLg^{-1})
1	(−) 1	(−) 0.25	0.06±0.00	0.03±0.00	0.09	16.10±0.40	65.37±1.60	1.20±0.03	11.06±0.54
2	(+) 5	(−) 0.25	0.11±0.00	0.05±0.00	0.16	14.32±0.25	90.29±1.22	0.55±0.13	11.62±0.34
3	(−) 1	(+) 0.75	0.03±0.00	0.04±0.00	0.07	16.53±0.27	47.72±1.96	1.32±0.06	8.90±0.42
4	(+) 5	(+) 0.75	0.10±0.01	0.05±0.00	0.15	14.99±0.21	90.50±0.54	0.49±0.18	14.05±0.63
5	(-1.41) 0.18	(0) 0.50	0.08±0.04	0.02±0.00	0.10	17.09±0.37	38.11±1.27	1.63±0.05	5.49±0.57
6	(+1.41) 5.82	(0) 0.50	0.13±0.00	0.05±0.00	0.18	15.54±0.14	89.88±0.10	0.46±0.19	13.98±0.36
7	(0) 3	(-1.41) 0.15	0.12±0.00	0.03±0.00	0.15	13.73±0.27	90.45±0.10	0.40±0.01	16.03±0.79
8	(0) 3	(+1.41) 0.85	0.07±0.01	0.03±0.00	0.10	16.41±0.06	82.33±1.08	0.88±0.03	14.03±0.44
9	(0) 3	(0) 0.50	0.12±0.01	0.05±0.00	0.17	16.00±0.04	85.52±0.43	0.91±0.01	14.65±0.33
10	(0) 3	(0) 0.50	0.11±0.00	0.05±0.00	0.16	15.84±0.07	85.77±0.36	0.94±0.13	12.87±0.43
11	(0) 3	(0) 0.50	0.11±0.00	0.04±000	0.15	15.44±0.23	84.59±0.98	1.00±0.04	13.14±0.43
12	Native starch		0.00±0.00	0.01±000	0.01	18.43±0.10	29.43±0.48	2.66±0.65	4.89±0.47

Table 3. *Mean values of paste viscosity, pasting temperature and Thermal properties of samples and native cassava starch with different levels of modification by oxidation with ozone and hydrogen peroxide.*

Sample	Independent Variables		Dependet Variables									
	X₁ Ozone (g)	X₂ Hydrogen Peroxide (%)	Pasting Temp	Peak Viscosity	Viscosity minimun at 95ºC	Breakdown Viscosity	Final Viscosity	Setback	Temperature Onset (To)°C	Temperature Peak (Tp)°C	Temperrature Conclusion (Tc)°C	Enthalpy J/g
1	(−) 1	(−) 0.25	66.42±0.42[de]	833.14±5.81[ab]	117.86±0.56[c]	715.28±5.27[efg]	153.28±5.44[c]	35.42±7.37[bc]	56.86±0.02[a]	65.06±0.09[a]	70.28±008[e]	8.13±0.82[c]
2	(+) 5	(−) 0.25	69.22± 0.49[a]	777.33±5.78[bc]	6.17±0.09[g]	771.17±5.86[cde]	13.70±0.29[g]	7.53±0.35[d]	56.94±1.25[a]	64.58±1.01[a]	72.50±0.01[ab]	8.45±0.02[c]
3	(−) 1	(+) 0.75	66.07±0.06[ef]	756.17±15.30[bc]	112.39± 7.10[c]	643.78±15.75[g]	160.72±5.57[c]	48.33±9.90[b]	56.20±0.07[a]	64.25±1.48[a]	72.50±0.01[ab]	8.43±0.01[c]
4	(+) 5	(+) 0.75	68.37±0.49[abc]	898.83±31.06[a]	9.75±0.08[fg]	889.08±31.07[a]	20.03±0.54[fg]	10.28±0.47[d]	57.67±0.36[a]	65.43±0.72[a]	71.75±0.01[bc]	8.08±0.01[c]
5	(-1.41) 0.18	(0) 0.50	65.15±0.03[f]	722.80±12.55[cd]	201.64±1.47[b]	521.17±11.09[h]	307.83±10.61[b]	102.86±12.08[a]	57.87±0.58[a]	64.93±0.07[a]	70.75±0.36[de]	6.97±0.01[cd]
6	(+1.41) 5.82	(0) 0.50	69.17±0.35[ab]	670.25±0.53[d]	6.25± 0.00[g]	662.83±0.35[fg]	13.03± 0.02[g]	6.81±0.02[d]	57.07±0.54[a]	65.75±0.89[a]	73.12±0.01[a]	8.49±0.01[c]
7	(0) 3	(-1.41) 0.15	67.98±0.54[bc]	861.09±35.37[a]	18.86±0.67[e]	842.25±36.00[abc]	34.61±1.63[e]	15.78±0.59[d]	56.50±0.07[a]	65.67±0.09[a]	72.02±0.65[bc]	10.66±0.01[b]
8	(0) 3	(+1.41) 0.85	67.25±0.47[cde]	772.56±63.00[bc]	31.90± 0.72[d]	740.89±63.25[def]	56.25± 0.63[d]	24.58±0.30[cd]	57.10±0.24[a]	64.72±1.04[a]	71.33±0.01[cd]	7.71±0.01[cd]
9	(0) 3	(0) 0.50	68.13±0.49[abc]	717.42±27.87[cd]	13.67±0.00[efg]	703.97±27.49[efg]	22.97±0.25[fg]	9.53±0.71[d]	56.45±0.48[a]	65.44±0.36[a]	71.45±0.02[cd]	5.93±0.10[d]
10	(0) 3	(0) 0.50	67.55±0.48[cd]	818.34±41.81[ab]	18.58± 0.25[e]	799.78±42.04[bcd]	36.86± 0.92[e]	18.31±0.06[cd]	57.07±0.11[a]	64.64±1.24[a]	71.49±0.01[cd]	8.53±0.54[c]
11	(0) 3	(0) 0.50	68.08±0.50[abc]	885.42±25.11[a]	31.30± 0.50[d]	854.11±24.62[ab]	51.80±0.48[d]	20.50±0.30[cd]	56.97±0.30[a]	64.80±1.36[a]	72.53±0.01[ab]	8.59±0.82[c]
12	Native Starch		65.17±0.15[f]	735.92±5.26[cd]	225.53± 6.64[a]	507.06±13.31[h]	319.89± 8.50[a]	92.70±21.39[a]	55.97±0.54[a]	66.92±0.53[a]	73.27±0.09[a]	17.33±1.1[a]

Mean values in the same column with different letters are significantly different ($P < 0.05$).

Table 4. ANOVA of the quadratic model for (%) Carbonyl + (%) Carboxyl

Source of variation	Sum of squares	Degrees of frefom	Mean squares	F Tabulated	F Test[a]	p value
Regression	0,0046	1	0,0046	9,01	5,12	0,0149
Residual	0,0046	9	0,0005			
Total	0,0092	10				

Table 5. ANOVA of the quadratic model for specific volume (mLg^{-1})

Source of variation	Sum of squares	Degrees of frefom	Mean squares	F Tabulated	F Test[a]	p value
Regression	71,84	3	23,94	9,67	4,34	0,0069
Residual	17,33	7	2,47			
Total	89,17	10				

Table 6. ANOVA of the quadratic model for paste clarity (%)

Source of variation	Sum of squares	Degrees of frefom	Mean squares	F Tabulated	F Test[a]	p value
Regression	3430,49	5	686,09	232,04	5,05	<0,0001
Residual	14,78	5	2,95			
Total	3445,27	10				

Table 7. ANOVA of the quadratic model for amylose content (%)

Source of variation	Sum of squares	Degrees of frefom	Mean squares	F Tabulated	F Test[a]	p value
Regression	6,77	2	3,38	9,19	4,46	0,00846
Residual	2,94	8	0,37			
Total	9,72	10				

Table 8. ANOVA of the quadratic model for intrinsic viscosity (dL.g^{-1})

Source of variation	Sum of squares	Degrees of frefom	Mean squares	F Tabulated	F Test[a]	p value
Regression	1,38	3	0,45	20,51	4,34	0,00076
Residual	0,15	7	0,02			
Total	1,53	10				

Table 9. ANOVA of the quadratic model for 95ºC viscosity (RVU)

Source of variation	Sum of squares	Degrees of frefom	Mean squares	F Tabulated	F Test[a]	p value
Regression	40314,82	2	20157,41	198,67	4,45	1,517E-07
Residual	811,69	8	101,46			
Total	41126,51	10				

Table 10. ANOVA of the quadratic model for final viscosity (RVU)

Source of variation	Sum of squares	Degrees of frefom	Mean squares	F Tabulated	F Test[a]	p value
Regression	80798,19	2	40399,09	896,21	4,45	3,89E-10
Residual	360,62	8	45,07			
Total	1283,63	10				

Table 11. ANOVA of the quadratic model for setbank (RVU)

Source of variation	Sum of squares	Degrees of frefom	Mean squares	F Tabulated	F Test[a]	p value
Regression	6953,97	2	3476,99	23,87	4,45	0,00042
Residual	1165,34	8	145,67			
Total	8119,32	10				

Table 12. ANOVA of the quadratic model for swelling power (60 ºC)

Source of variation	Sum of squares	Degrees of frefom	Mean squares	F Tabulated	F Test[a]	p value
Regression	730,94	4	182,73	186,40	4,53	2,023E-06
Residual	5,88	6	0,98			
Total	736,82	10				

Table 13. ANOVA of the quadratic model for swelling power (80 ºC)

Source of variation	Sum of squares	Degrees of frefom	Mean squares	F Tabulated	F Test[a]	p value
Regression	1869,47	1	1869,47	38,83	5,11	0,00015
Residual	433,23	9	48,13			
Total	2302,70	10				