



Physicochemical, structural and thermal properties of oxidized, acetylated and dual-modified common bean (*Phaseolus vulgaris* L.) starch

José Pedro WOJECCHOWSKI¹, Geisa Liandra de Andrade de SIQUEIRA¹, Luiz Gustavo LACERDA¹, Egon SCHNITZLER¹, Ivo Mottin DEMIATE^{1*}

Abstract

Common beans are rich in protein and complex carbohydrates that are valuable for the human diet. Starch is the most abundant individual component; however, in its native form it has limited applications and modifications are necessary to overcome technological restrictions. The aim of this study was to evaluate the influence of oxidation, acetylation and dual-modification (oxidation-acetylation) on the physicochemical, structural and thermal properties of common bean starch. The degree of substitution of the acetylated starches was compatible with food use. Fourier transform infrared spectra confirmed the acetylation of the bean starch, with a peak at 1,735cm⁻¹. The granules of the bean starch were oval to spherical in shape, with no differences between the native and modified samples. Typical C-type diffraction of legume starches was found. The modified samples showed a reduced relative crystallinity and lower enthalpy change of gelatinization. The oxidized starch showed the highest peak viscosity, hardness, and gel adhesiveness due to the presence of functional groups. An increase in solubility and swelling power was observed, and the oxidized-acetylated starch presented the highest values. The properties of the modified bean starches made them suitable for application in breaded/battered foods, mainly due to improved textural attributes.

Keywords: Non-conventional starch; rheological properties; FTIR spectroscopy; DSC.

Practical application: Common bean starch is an abundant non-conventional ingredient that was chemically modified in this study in order to become suitable for the food industry. The acetylated, oxidized and dual-modified bean starches produced in this study can be used for applications that require a high degree of adhesion, in breaded foods, for example.

1 Introduction

Common beans (*Phaseolus vulgaris* L.) are important sources of energy and protein in the human diet. On a dry basis, carbohydrates represent more than 60% of the bean seeds, with a predominance of starch (Wang & Ratnayake, 2014). Around 35% of the global production of common beans is from the Americas, and Brazil ranks number two in the world; its average annual production in the period 1993-2014 reached about three million metric tons (Food and Agriculture Organization of the United Nations, 2014).

Native starch is produced and used on a large scale worldwide. The food, pulp and paper, textile, pharmaceutical and chemical industries use native starch as an ingredient or as a raw material. Cereals and tuberous crops, mainly maize, wheat, cassava and potato, are the most common sources of commercial starch (Waterschoot et al., 2015). Modifications of starch are important tools to overcome technological limitations such as syneresis and retrogradation, paste opacity, and instability under conditions of stirred heating. Chemically modified starches, such as oxidized, acetylated and hydroxypropylated starches, can be food-grade but the degree of modification is regulated by legislation.

The acetylation of starch breaks hydrogen bonds inside the granules and results in amphiphilic properties (Hong et al., 2016). Acetylated starch with a low degree of substitution (DS) is used as an emulsifier in films and coatings, and also for adhesion purposes (Chi et al., 2008; Morikawa & Nishinari, 2000).

Oxidized starches are used by the food industry because they have adhesive and coverage properties. They can be applied in battered/breaded coatings to provide adhesion in the coating of foods (Sajilata & Singhal, 2005). According to Wuzrburg (1986), this modification is a conversion of hydroxyl groups, primarily to carbonyl and then to carboxyl. Sodium hypochlorite is an agent that is commonly used for this modification (Garrido et al., 2014).

As well as isolated modifications, multiple methods can also be applied sequentially. As reported elsewhere (Xiao et al., 2012), the use of dual-modification aims to improve the quality of starches. In this study we modified starches from Brazilian common beans by oxidation, acetylation, and oxidation-acetylation. Selected physicochemical, structural and thermal characteristics were evaluated to find applications for these non-conventional modified starches.

Received 10 Feb., 2017

Accepted 21 Dec., 2017

¹Food Science and Technology Graduate Program, Universidade Estadual de Ponta Grossa – UEPG, Ponta Grossa, PR, Brazil

*Corresponding author: demiate@yahoo.com

2 Materials and methods

2.1 Materials and sample preparation

The starch from *Carioca* beans (*Phaseolus vulgaris* L.) was extracted following the procedures described elsewhere (Demiate et al., 2016). The beans were acquired in a local supermarket in the city of Ponta Grossa, Paraná, Brazil. A 10-12% (v v⁻¹) solution of sodium hypochlorite (Cloroquímica, Curitiba, Brazil) was used to oxidize the starch and acetic anhydride (Synth, Diadema, Brazil) was used for the acetylation of the starch. A third sample was produced by dual-modification (oxidation-acetylation) using the same reagents. All the reagents used were of analytical grade.

Oxidation

The bean starch was oxidized according to Wang & Wang (2003), with slight modifications. A 35% (w w⁻¹) starch suspension was prepared and the pH was adjusted to 9.5 with NaOH solution (0.1 mol L⁻¹). Standardized sodium hypochlorite solution (2 g active chlorine 100 g⁻¹ starch) was slowly added, while the pH of the suspension was kept at 9.5. After completing the addition of the NaOCl, the suspension was maintained at the same pH for 50 min. The pH was adjusted to 7.0 and the modified starch was recovered by filtration. The modified starch was washed until the complete removal of the ClO⁻ ions and then dried at 40 °C for 48 h.

Acetylation

The acetylated bean starch was produced following the method of Wang & Wang (2002), slightly modified by (Wani et al., 2012). A 35% starch suspension (w w⁻¹) was prepared with distilled water and the pH was adjusted to 8.0 - 8.5 with NaOH solution (1 mol L⁻¹). The suspension was mechanically stirred (IKA RW20 Digital, Campinas, Brazil) for 30 min at 600 rpm. Acetic anhydride (6 g 100 g⁻¹ starch) was slowly added, maintaining the pH at the same range. The reaction was continued for 60 min before adjusting the pH to 5.5 with HCl solution (1 mol L⁻¹). The suspension was thoroughly washed and then filtered through qualitative paper and air dried at 40 °C for 48 h.

Oxidation-acetylation

The dual-modification was performed by reacting the native starch with sodium hypochlorite, followed by acetylation, as described earlier in this study. The complete modified sample was thoroughly washed, filtered and air dried at 40 °C for 48 h.

2.2 Methods

Carbonyl content

The carbonyl content was determined by the titrimetric method of (Smith, 1967). Four grams of starch sample were suspended in 100 mL of distilled water. The suspension was gelatinized at 95 °C for 20 min, cooled to 40 °C, adjusted to pH 3.2, and then 15 mL of hydroxylamine reagent was added. The flask was capped and maintained in a water bath at 40 °C for 4 h with gentle magnetic stirring. The excess of hydroxylamine

reagent was determined by titrating the reaction mixture to pH 3.2. A blank determination, with only the reagent, was performed in the same way. The carbonyl content was calculated by Equation 1:

$$\% \text{ of carbonyl content} = \frac{\left(\frac{\text{blank} - \text{sample}}{\text{acid normality} \times 0.028 \times 100} \right) \text{ mL} \times \text{sample weight}}{\text{(dry basis) in g}} \quad (1)$$

Carboxyl content

The carboxyl content was determined by the titrimetric method of (Smith, 1967). An amount of 2 g of starch sample was suspended in 25 mL of HCl solution (0.1 mol L⁻¹) and stirred for 30 min. The suspension was filtered and washed with 400 mL of distilled water. The washed starch was transferred into a beaker and the volume was adjusted to 300 mL with distilled water. The starch suspension was heated in boiling water for 15 min to complete gelatinization. The hot starch dispersion was adjusted to 450 mL with distilled water and titrated to pH 8.3 with standardized NaOH solution (0.01 mol L⁻¹). A blank determination with unmodified starch was performed. The carboxyl content was calculated by Equations 2 and 3:

$$\text{milliequivalents of acidity } 100\text{g}^{-1} \text{ starch} = \frac{\left(\frac{\text{sample} - \text{blank}}{\text{normality of NaOH} \times 100} \right) \text{ mL} \times \text{sample weight}}{\text{(dry basis) in g}} \quad (2)$$

$$\text{Percentage of carboxyl} = \left[\frac{\text{milliequivalents of acidity}}{100 \text{ g starch}} \right] \times 0.045 \quad (3)$$

Acetyl content and degree of substitution (DS)

The degree of substitution (DS, %) was determined following the method described by Singh et al. (2004) and used by (Hong et al. 2016). One gram of the sample was placed into a capped flask and 50 mL of 75% (v v⁻¹) ethanol was added. The flask was agitated, heated to 50 °C for 30 min, naturally cooled to 25 °C, and 40 mL of NaOH solution (0.5 mol L⁻¹) was added. The excess of alkali was titrated with standardized HCl solution (0.5 mol L⁻¹). The native starch was used as blank. The content of acetyl and the DS were calculated by Equations 4 and 5:

$$\text{Acetyl}(\%) = \frac{\left(\frac{\text{blank} - \text{sample}}{0.043 \times 100} \right) \times \text{molarity of HCl} \times \text{sample weight}}{\text{(dry basis) in g}} \quad (4)$$

$$\text{DS} = \left[162 \times \text{acetyl}(\%) / 4,300 - (42 \times \text{acetyl}\%) \right] \quad (5)$$

Scanning electron microscopy

The morphology of the starch granules was examined using a scanning electron microscope (Tescan, VEGA 3, Brno, Kohoutovice, Czech Republic). The samples were coated with gold and examined under an acceleration voltage of 25 kV and magnification of 1,000×.

X-ray diffraction

The X-ray diffractometry was performed with a Rigaku Ultima IV (Rigaku, Tokyo, Japan) equipment under the following conditions: X-ray tube CuK α ($\lambda = 1.544 \text{ \AA}$ voltage 40 kV; current 20 mA; the scanning region of the diffraction ranged from 3 to 40° (2 θ angle). The relative crystallinity (RC) of the starch granules was calculated using Equation 6:

$$RC\% = \left[\frac{Ca}{Ca + Aa} \right] \times 100 \quad (6)$$

where Ca and Aa are the crystalline area and the amorphous area, respectively, on the X-ray diffractograms (Song & Jane, 2000).

Mid-infrared spectroscopy (FT-IR)

The FT-IR spectra were produced with a Shimadzu FT-IR 8400 spectrophotometer (Shimadzu, Kyoto, Japan) in the region of 700 - 4,000 cm^{-1} at 4 cm^{-1} resolution. The samples were properly dispersed in KBr pellets.

Thermal properties

The thermal properties of the samples were analyzed using DSC-60A equipment (Shimadzu, Kyoto, Japan). The samples (1.5 mg, dry basis) were weighed in aluminium pans and 4.5 μL of distilled water was added; after sealing the pans with proper lids the moistened starch rested 1 h for equilibration. The samples were scanned from 10 to 100 °C at a constant heating rate (2 °C min^{-1}). The equipment was previously calibrated with indium standard (MP = 156.6 °C, $\Delta H = 28.56 \text{ J g}^{-1}$). An empty, sealed aluminium pan was used as reference. The endothermic curves, with the corresponding gelatinization temperatures (T_o , T_p , T_c), were produced and the transition enthalpy changes (ΔH , J g^{-1}) were calculated.

Pasting properties

The pasting properties of the samples were measured using a Rapid Visco Analyzer (RVA-4, Newport Scientific Pvt. Ltd., Narabeen, Australia). The Standard 2 (STD-2) profile of Thermochline for Windows software was followed for this analysis. A suspension of starch of 8% (w w $^{-1}$) in dry basis and 28 g total weight were employed (Zortéa-Guidolin et al., 2017).

Textural properties

The samples of starch gels (8.0%, dry basis) were prepared in the RVA and stored at 4 °C for 24 h. The gels that formed inside the canisters were evaluated for their textural properties. The texture profile analysis (TPA) was performed using a TA/XT2 texture analyzer (Stable Micro Systems, Surrey, England) (Sandhu & Singh, 2007). Each starch gel was compressed at a speed of 0.5 mm s^{-1} to a distance of 10 mm with a cylindrical probe (diameter = 2 mm). Compressions were made in each sample to generate a force-time curve. The hardness (height of first peak) and adhesiveness (the negative area of the curve during the retraction of the probe) were measured.

Swelling power and solubility

The swelling power and solubility of the samples were determined according to Leach et al. (1959). An amount of 0.4 g of each sample was suspended in 40 mL of distilled water in 50 mL centrifuge tubes. The suspensions were heated for 30 min at different temperatures (60, 70, 80 and 90 °C). The samples were then cooled to 25 °C and centrifuged at 2,000 $\times g$ for 10 min. The soluble fraction quantification was performed by drying the supernatants at 40 °C until constant weight. The solubility was expressed as the percentage of dried solid weight based on the dry sample weight. The swelling power was the ratio between the wet sediment weight and the initial dry sample (deducting the amount of soluble starch).

Statistical analysis

The results are expressed as the mean \pm standard deviation and were analyzed using STATISTICA 12.7 software (Stat Soft Inc., Tulsa, OK, USA). One-way analysis of variance was applied to evaluate the effect of modification on the thermal, textural and pasting properties. Tukey's test was used to determine the differences between the means at 95% confidence level ($p < 0.05$).

3 Results and discussion

3.1 Carbonyl and carboxyl content

The level of starch oxidation is defined by the carbonyl and carboxyl contents. In this study, both these contents increased, confirming that oxidation took place and that the bean starch was modified. There is evidence that a sequential oxidation takes place and hydroxyl groups are converted to carbonyl and then carboxyl groups (Vanier et al., 2012). The intensity of reaction depends on the pH, time of reaction, and the oxidizing agent (Sangseethong et al., 2010). Sodium hypochlorite is the oxidizing agent that is most frequently used to produce oxidized starches for industrial uses (Zhang et al., 2012). The results of the present study are in line with those reported elsewhere Wang & Wang (2003) and Sangseethong et al. (2010), with larger contents of carboxyl than carbonyl groups. The reaction conditions (pH 9.5 and sodium hypochlorite as oxidizing agent) favored the generation of carboxyl groups.

3.2 Acetyl content and degree of substitution

Acetylation was performed by the addition of 0.06 g of acetic anhydride per gram of starch. DS values of 0.064 and of 0.040 were found for the acetylated and the dual-modified starches, respectively. The mechanism of the acetylation reaction involves addition-elimination, with hydroxyl groups being converted to acetyl. The hydroxyl groups present different levels of reactivity. The most reactive group is the hydroxyl of carbon 6, followed by those of carbons 2 and 3 (Wuzrburg, 1986). In the present study, there were differences ($p < 0.05$) between the acetylated and the oxidized-acetylated starches (Table 1). The acetylation reaction is influenced by starch granule morphology, reactant concentration, pH and reaction time (Halal et al., 2015). The lower susceptibility to acetylation of oxidized bean starch, in the dual modified sample may be

Table 1. Thermal and pasting properties; relative crystallinity; carbonyl, carboxyl and acetyl contents; and the degree of substitution of the starch samples.

Parameter	Samples				p-Anova
	N	O	A	OA	
T _o	68.7 ± 0.06a	69.4 ± 0.29a	65.2 ± 1.61b	65.8 ± 1.61b	0.04
T _p	73.8 ± 0.59a	73.5 ± 0.04a	70.6 ± 0.24b	70.5 ± 0.59b	<0.01
T _c	79.1 ± 1.08a	77.4 ± 0.54b	76.0 ± 0.25c	77.6 ± 0.74b	0.04
ΔH	8.1 ± 0.10a	5.4 ± 0.40c	4.9 ± 0.21c	7.2 ± 0.85b	<0.01
PT	77.5 ± 0.56b	79.0 ± 0.16a	79.0 ± 0.23a	77.8 ± 0.25b	0.001
PV	1769.7 ± 152.22a	1910.3 ± 124.24a	1275.0 ± 3.46b	813.7 ± 35.00c	<0.001
FV	2320.0 ± 386.75a	1001.0 ± 71.08b	2162.7 ± 19.63a	504.7 ± 27.06c	<0.001
BD	585.3 ± 88.12b	1138.0 ± 83.14a	241.0 ± 15.59d	430.3 ± 13.80c	<0.001
SB	1135.7 ± 146.56a	228.7 ± 29.69b	1128.7 ± 0.58a	121.3 ± 3.51b	<0.001
RC	32.8 ± 0.2a	29.1 ± 0.5b	28.0 ± 0.5c	28.2 ± 0.5bc	<0.001
COOH	0.001 ± 0.000b	0.170 ± 0.021a	-	0.182 ± 0.024a	<0.01
COH	0.006 ± 0.003b	0.027 ± 0.005a	-	0.012 ± 0.005b	<0.01
Acetyl	-	-	1.661 ± 0.147	1.044 ± 0.130	*0.04
D.S.	-	-	0.064 ± 0.006	0.040 ± 0.005	*0.04
HD	80.9 ± 0.1a	82.7 ± 3.2a	35.3 ± 1.3b	28.5 ± 1.9c	< 0.001
AD	-67.5 ± 3.7a	-95.0 ± 5.1a	-60.0 ± 2.8b	-44.5 ± 1.5c	0.03

N: native bean starch; O: oxidized bean starch; OA: oxidized and acetylated bean starch. **Thermal properties:** T_o, T_p, T_c: Onset, peak and conclusion temperatures of gelatinization, respectively, °C; ΔH: enthalpy change of gelatinization, J g⁻¹. **Pasting properties:** PT: pasting temperature, °C; PV: peak viscosity, cP; FV: final viscosity, cP; BD: breakdown viscosity, cP; SB: setback viscosity, cP. **Textural properties:** HD: hardness, g; AD: adhesiveness, g.s. RC: relative crystallinity, %; COOH: carboxyl, %; COH: carbonyl, %; Acetyl, %. Different letters in the same column represent significant difference according to Fisher's LSD test (p<0.05). *t-Student's test.

attributed to a smaller amount of reactive hydroxyl groups due to their earlier oxidation to carbonyl and carboxyl groups, as reported by Pietrzyk et al. (2014) for corn starch, which was submitted to the same modifications of the present study. The percentage of acetyl groups and the DS of our study were in line with those of (Wani et al., 2012). The aforementioned authors reported a % of acetyl group that ranged from 0.89-2.11 and DS between 0.003 and 0.08 for acetylated starches from Indian kidney beans. Acetylated starches with low DS (0.01-0.2) are used in the food industry as texturizing and stabilizing agents (Colussi et al., 2014). The DS values of the samples from the present study were considered as being safe to be applied in foods (Han et al., 2012).

3.3 Scanning electron microscopy

The size and shape of starch granules are important in relation to their technological properties, including the viscosity of their pastes. The granules of bean starches are oval to spherical in shape and have heterogeneous sizes (Granza et al., 2015), as shown in Figure 1; the spherical granules are larger than the oval shaped granules (Wang & Ratnayake, 2014). The microscopic images from this study did not show differences between the granules of the native and modified starches. Therefore, the chlorine concentration (2% (v v⁻¹)) that was used did not alter the granular integrity. Kuakpetoon & Wang (2001) reported some alterations in relation to a higher concentration of chlorine (5% v v⁻¹). The concentration used during acetylation (6% v v⁻¹) did not result in granular fissures, which differed from a study by Wani et al. (2012) that reported fissures in bean starch granules modified by a higher concentration of acetic anhydride (8% v v⁻¹).

3.4 X-ray diffraction

X-ray diffractometry is used to understand differences between starches in relation to their semi-crystalline behavior (Mbougoung et al., 2012; Zobel et al., 1988). According to models of starch granules, crystalline regions are rich in amylopectin molecules. Amylose is found in the amorphous regions of the starch structure (Mbougoung et al., 2012; Cheetham & Tao, 1998).

Table 1 shows the relative crystallinity (RC) values of the starches. The numbers 1-3 (angle 2θ of 5.5°, 17° and 23°, respectively) represent the peaks detected in the X-ray diffractograms (Figure 2). The C-type was observed, which is typical of pulse starches and is characterized as a mixture between A and B types (Rupollo et al., 2011; Hoover & Ratnayake, 2002). Although the diffractograms were similar, the RC values were lower for the modified samples when compared with the native starch. The RC values were 32.8%, 29.1%, 28.0% and 28.2% for the native, oxidized, acetylated and oxidized-acetylated starches, respectively.

The X-ray diffractograms showed main peaks at 2θ angles 17° and 23°, and a less pronounced peak at 2θ = 5.5°, which was in line with previous studies (Wang & Ratnayake, 2014; Hoover & Ratnayake, 2002). The lower RC of the oxidized starch indicates a certain degree of depolymerization of amylopectin, as reported by (Vanier et al., 2012). The substitution of some hydroxyl groups by acetyl groups reduces inter and intramolecular hydrogen bonds, thereby decreasing crystallinity (Colussi et al., 2014).

3.5 Mid-infrared spectroscopy (FT-IR) analysis

The FTIR spectra of the native, oxidized, acetylated and oxidized-acetylated starches are shown in Figure 3. Peaks between 3,000 - 3,600 cm⁻¹ and at 2,950 cm⁻¹ were detected for the native

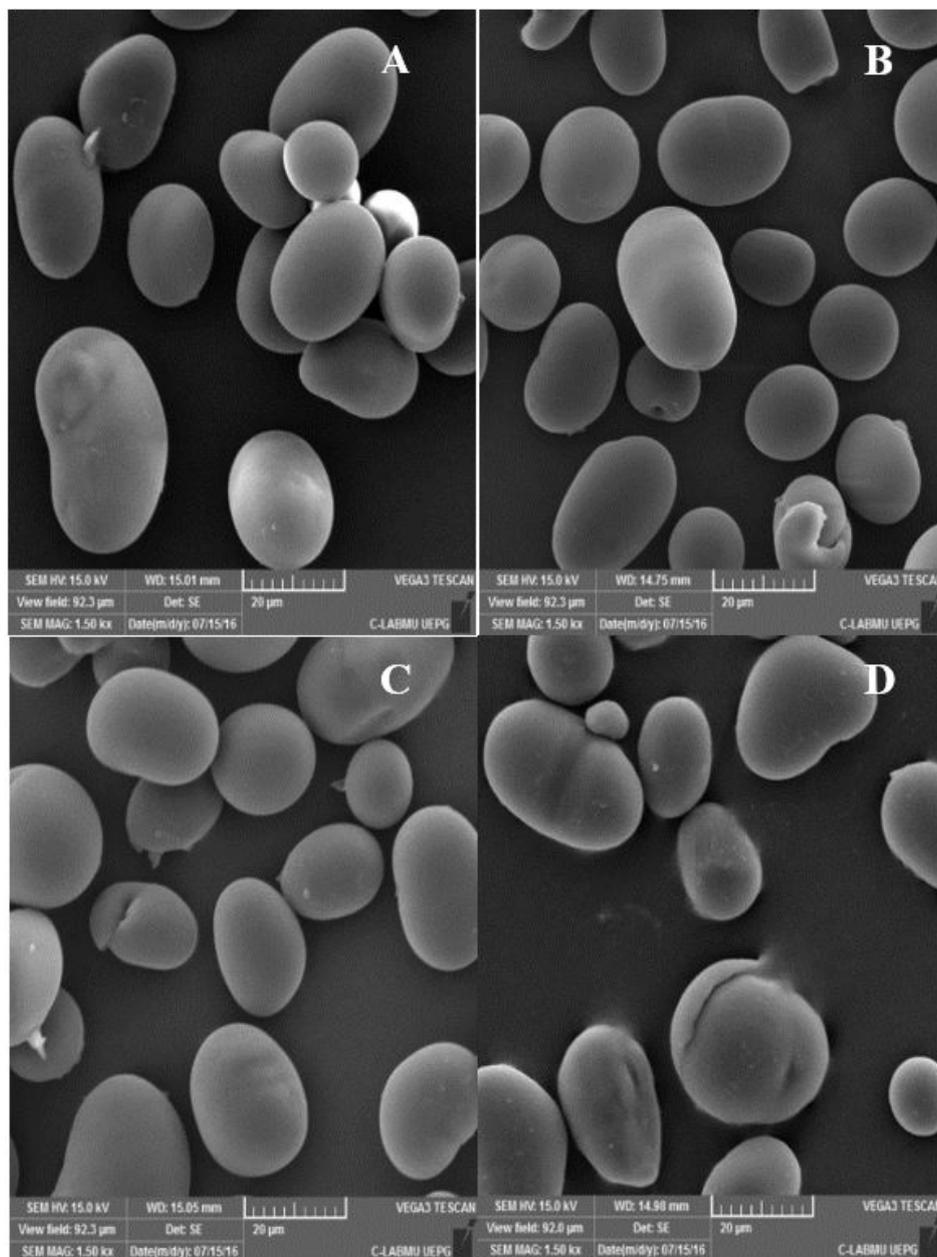


Figure 1. SEM images (1,500 \times) of native starch (A), oxidized starch (B), acetylated starch (C) and oxidized-acetylated starch (D).

starch, which was related to OH and CH elongation, respectively. Peaks at 1,650 cm^{-1} and 1,420 cm^{-1} correspond to OH and CH bending (Halal et al., 2015; Mano et al., 2003).

Starch oxidation includes two steps; firstly, the hydroxyl groups are oxidized to carbonyl and then to carboxyl groups. The oxidizing reaction also produces a partial depolymerization of the starch macromolecules with a rupture of the α -1,4 linkages (Kuakpetoon & Wang, 2008). The reaction mechanisms of the modifications by oxidation and acetylation are different. During acetylation there is addition of acetyl groups to the starch macromolecules due to esterification (Bello-Pérez et al., 2010; Halal et al., 2015). Regarding the FTIR spectra of the native and oxidized starches, no substantial differences were observed.

3.6 Thermal properties

The thermal transitions (T_o , T_p , and T_c) and the enthalpy change (ΔH) of gelatinization of the bean starches are shown in Table 1. The T_o and T_p of the oxidized starch did not differ from the values of its native counterpart. It was expected that the T_o and the ΔH of the oxidized starch would be lower than those of the native starch. However, this is not a rule, and there are reports of the influence of the botanical source, as well as the conditions of the chemical modification (Sangseethong et al., 2009). In the present study, the ΔH of the oxidized sample was lower than that of the native starch, but no difference was found for the T_o . Wang & Wang (2003) reported increased T_o for starches modified with a low concentration of chlorine (active chlorine $\leq 1.25\%$). The aforementioned authors reported

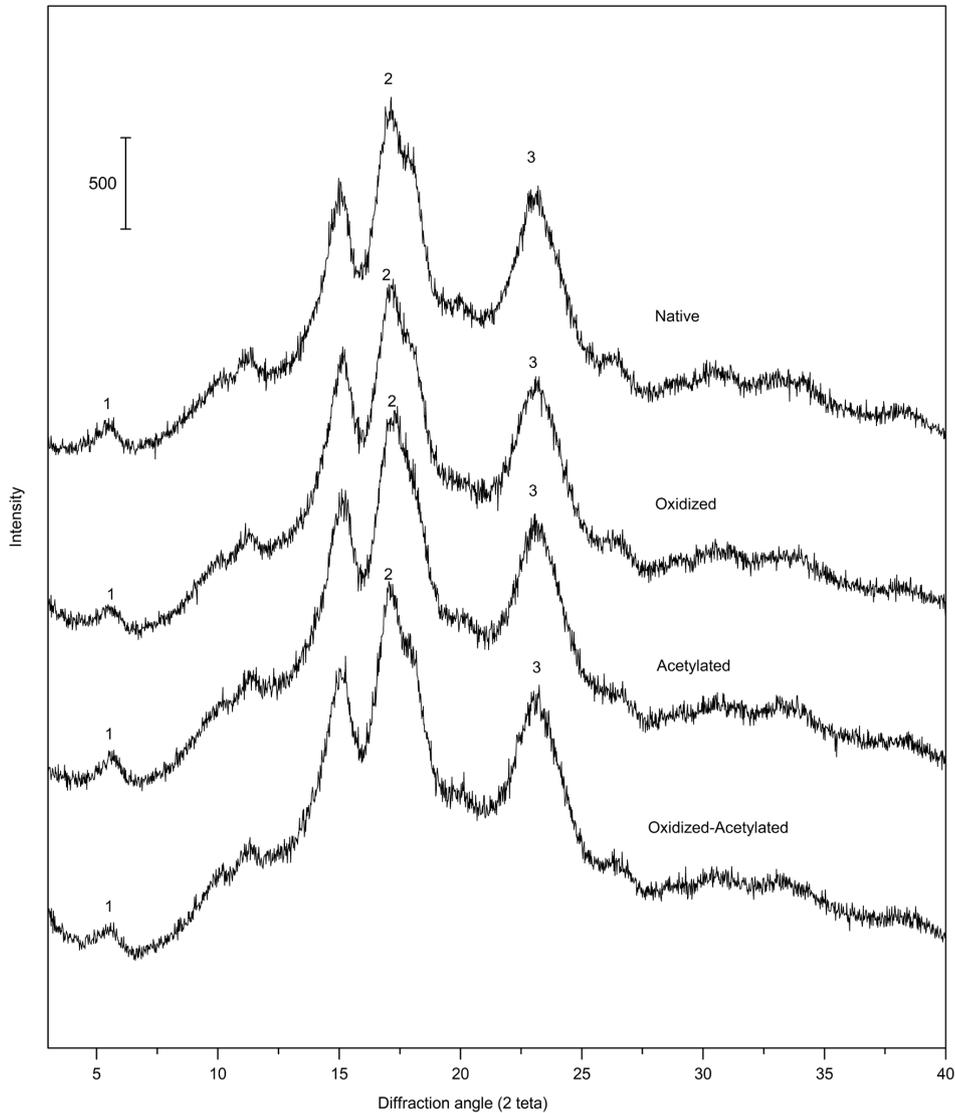


Figure 2. Wide angle X-ray diffraction pattern of native and modified starches.

a decrease in T_0 in relation to higher concentrations of chlorine (active chlorine > 5%), which was explained by the presence of carboxyl groups. The ΔH represents the amount of energy required for the gelatinization process to take place; it provides qualitative and quantitative information about the crystallinity and is considered to be an indicator of the loss of molecular order due to the rupture of hydrogen bonds (Vanier et al., 2012). Compared to the native starch, the ΔH of the modified starches decreased ($p < 0.05$), which can be explained by the weakening of the granular structure. A partial degradation of the crystalline lamellae of the granules takes place and less energy is necessary for gelatinization (Sangseethong et al., 2009). Among the acetylated starches, samples showed lower ΔH and T_0 values compared with the native bean starch, in line with results reported by Singh et al. (2004) for corn and potato starches, and by Halal et al. (2015) for barley starch. The lower the ΔH , the smaller organization is expected and the crystals are less stable (Wani et al., 2010). Our results regarding thermal analysis agree with those that we found for relative crystallinity.

3.7 Pasting properties

The results of the pasting properties are presented in Table 1. The pasting temperatures of the native and dual-modified samples did not differ. However, those values differed from the oxidized and acetylated samples. During the pasting process, starch granules absorb water and swell, increasing the viscosity, which reaches a maximum (peak viscosity). The highest peak viscosity was found for the oxidized starch but it did not differ from that of its native counterpart. The increased viscosity peak of the oxidized starch can be due to the carboxylic groups (Wang & Ratnayake, 2014; Wang & Wang, 2003). On the other hand, the oxidized starch had a lower final viscosity than peak viscosity, which can be attributed to some degree of rupture of the glycosidic linkages (Vanier et al., 2012). Low values of setback viscosity were also found. The presence of bulky carbonyl and carboxyl groups increases the gap between the amylose chains (Vanier et al., 2012), decreasing the retrogradation tendency. The acetylated starch had lower values for peak and breakdown

viscosity, and equal values for final viscosity and setback in comparison with the native starch. The pasting temperature of the acetylated starch was lower than that of its native counterpart. Simsek et al. (2012) also reported lower pasting temperatures for acetylated starches and they attributed that to the introduction of acetyl groups, which weakens hydrogen bonds and the granular structure (Lawal, 2004). The lowest breakdown viscosity was found for the acetylated starch; this reflects the stability of the paste during cooking (Adebowale et al., 2006), which did not

suffer a sharp decrease in viscosity during heating with stirring, as reported by Colussi et al. (2014) for acetylated rice starch. High values for breakdown viscosities suggest a high level of swelling of the granules (Ovando-Martínez et al., 2011), as was found for the oxidized starch, which also had the highest peak viscosity. Setback is associated with the retrogradation tendency, with a high final viscosity and the ability of the starch to form a firm gel.

3.8 Textural properties

The textural properties of gels depend on the source of starch, amylose contents, and the interaction between the phases. Hardness is a measure of texture that corresponds to the force applied to cause deformation of a sample (Dias et al., 2011). As reported by Wani et al. (2012), the hardness and adhesiveness of the acetylated starch gels were lower than those of the native starch. A weaker gel could be attributed to acetyl groups, which prevent the approach of amylose chains, resulting in a lower rate of retrogradation (Singh et al., 2004). The oxidized starch presented the highest level of hardness (82.7 g). Slightly oxidized starches may show an increase in the formation of functional groups, which enhances hydrogen bonds and improves gel resistance, as well as adhesiveness (Dias et al., 2011). The lowest values in terms of hardness and adhesiveness were found for the dual-modified starch. According to Ali & Hasnain (2013), the introduction of functional groups creates steric hindrances and prevents the close entanglement of starch chains, thereby forming a loose gel network.

3.9 Solubility and swelling power

As expected, the solubility of the native and modified starches increased with heating (60 to 90 °C). The order of the increase in solubility with heating was as follows: oxidized-acetylated > oxidized > acetylated > native (Figure 4a). The lowest solubility for the native starch at 60 °C was because its gelatinization started at a temperature close to 70 °C (Table 1). At a temperature above 60 °C amylose leaches from starch granules (Halal et al.,

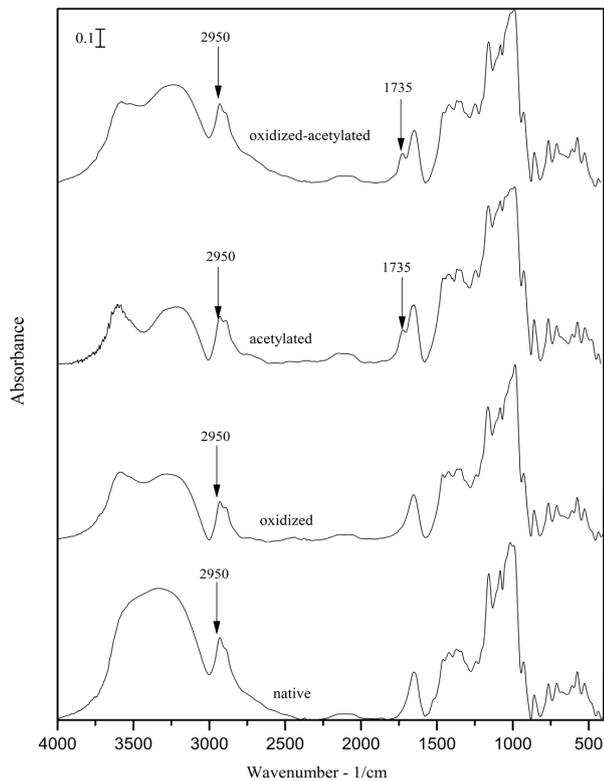


Figure 3. FTIR spectra of native and modified bean starches.

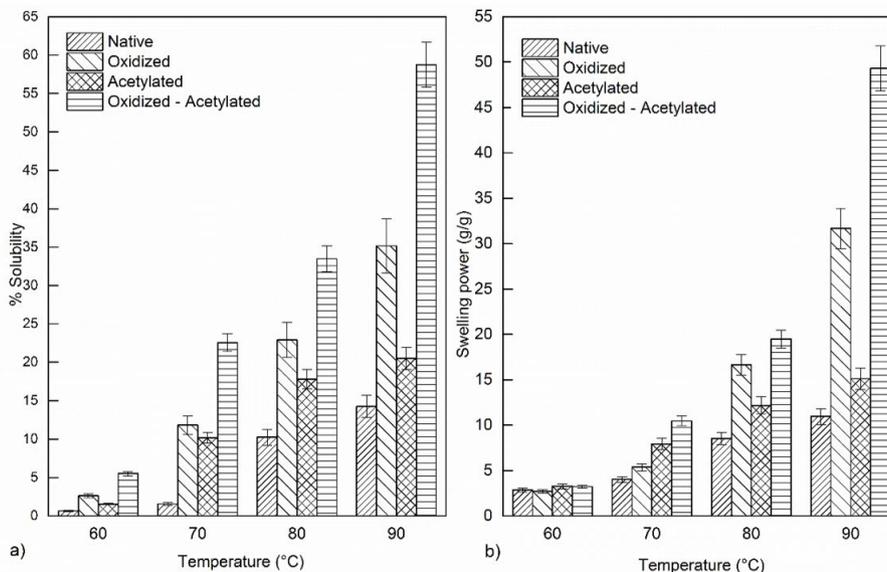


Figure 4. Solubility (a) and Swelling power (b) at different temperatures of the native and modified bean starches.

2015), with more evident increment for the oxidized starch. The partial disruption of the α -1,4glycosidic linkages results in depolymerization and increases granule solubilisation (Sánchez-Rivera et al., 2005). When heated in excess water, hydrogen bonds inside the starch granules, which are responsible for crystal double helices, are broken. Therefore, water is absorbed, causing granular swelling (Mbougoung et al., 2012). The native starch had the lowest solubility values when compared with the modified starches for all the temperatures. On the other hand, the oxidized-acetylated starch presented the highest solubility values. The bulky acetyl groups inside starch granules can cause structural reorganization, disrupting inter and intramolecular hydrogen bonds, leading to an increase in the motional freedom of starch chains in amorphous regions. As a consequence, there are modifications in some properties such as increased solubility (Hong et al., 2016; Han et al., 2012).

The swelling power (SP) of all the samples increased with heating (Figure 4b). The swelling of starch granules is concomitant with the loss of birefringence, and it precedes solubilisation (Sandhu & Singh, 2007; Singh et al., 2004). The SP of the chemically modified samples may be associated with the binding forces inside the starch granules (Simsek et al., 2012; Singh et al., 2007).

During heating there is weakening of the internal starch structure, which results in swelling due to water absorption. The acetylated starches had higher levels of water absorption and swelling (Simsek et al., 2012). This behavior is dependent on the acetyl contents of the modified starches. Singh et al. (2004) also observed, for potato and corn starches, that acetylation increased the swelling power in the same way we found for bean starch. A high degree of acetylation makes starch more hydrophobic and reduces water absorption by the granules (Hong et al., 2016). The lower SP of the native starch granules may be related to the higher degree of crystalline order, which limits hydration (Simsek et al., 2012).

4 Conclusion

Chemical modifications influenced the starch properties. There was an increase in the carbonyl and carboxyl contents that confirmed the modification of the oxidized starch samples. The degree of substitution for the acetylated starches presented values that were compatible with the limits for food use, and the FTIR spectra exhibited peaks at 1,735 - 1,740 cm^{-1} . The granules of bean starches were oval to spherical in shape and no differences between the granules of native and modified starches were detected, which was probably due to the level of modification. All the starches presented a C-type diffraction pattern but the modified starches had a lower RC, which was related to partial depolymerization and the presence of bulky acetyl groups for the oxidized and acetylated samples, respectively. The modified starches had lower ΔH values because of the weakening of the granular structure due to chemical modifications. The highest values regarding peak viscosity, hardness and adhesiveness of the gel were found for the oxidized starch, which was related to the formation of functional groups. The acetylated starch showed the lowest breakdown viscosity, which refers to the cooking stability of the paste. The dual-modified sample showed

the lowest values in relation to textural attributes due to steric hindrance, which prevents the close entanglement of starch chains. An increase in solubility and swelling power, which was associated with heating, was observed and the oxidized-acetylated starch presented the highest values. Modified bean starch, which is a non-conventional starch source, could be utilized in the food industry when lower enthalpy change of gelatinization and setback viscosity, enhanced solubility, swelling power and adhesiveness are important issues.

Acknowledgements

The authors are deeply grateful to the CAPES/Brazilian Ministry of Education and to the CNPq/Brazilian Ministry of Science and Technology for financial support, and to the C-LABMU-UEPG for support regarding the infrastructure.

References

- Adebowale, K. O., Afolabi, T. A., & Olu-Owolabi, B. I. (2006). Functional, physicochemical and retrogradation properties of sword bean (*Canavalia gladiata*) acetylated and oxidized starches. *Carbohydrate Polymers*, 65(1), 93-101. <http://dx.doi.org/10.1016/j.carbpol.2005.12.032>.
- Ali, T. M., & Hasnain, A. (2013). Effect of emulsifiers on complexation, pasting, and gelling properties of native and chemically modified white sorghum (*Sorghum bicolor*) starch. *Stärke*, 65(5-6), 490-498. <http://dx.doi.org/10.1002/star.201200175>.
- Bello-Pérez, L. A., Agama-Acevedo, E., Zamudio-Flores, P. B., Mendez-Montealvo, G., & Rodríguez-Ambríz, S. L. (2010). Effect of low and high acetylation degree in the morphological, physicochemical and structural characteristics of barley starch. *Lebensmittel-Wissenschaft + Technologie*, 43(9), 1434-1440. <http://dx.doi.org/10.1016/j.lwt.2010.04.003>.
- Cheetham, N. W. H., & Tao, L. (1998). Solid state NMR studies on the structural and conformational properties of natural maize starches. *Carbohydrate Polymers*, 36(4), 285-292. [http://dx.doi.org/10.1016/S0144-8617\(98\)00004-6](http://dx.doi.org/10.1016/S0144-8617(98)00004-6).
- Chi, H., Xu, K., Wu, X., Chen, Q., Xue, D., Song, C., Zhang, W., & Wang, P. (2008). Effect of acetylation on the properties of corn starch. *Food Chemistry*, 106(3), 923-928. <http://dx.doi.org/10.1016/j.foodchem.2007.07.002>.
- Colussi, R., Pinto, V. Z., Halal, S. L. M., Vanier, N. L., Villanova, F. A., Silva, R. M., Zavareze, E. R., & Dias, A. R. G. (2014). Structural, morphological, and physicochemical properties of acetylated high-, medium-, and low-amylose rice starches. *Carbohydrate Polymers*, 103, 405-413. PMID:24528747. <http://dx.doi.org/10.1016/j.carbpol.2013.12.070>.
- Demiate, I. M., Figueroa, A. M., Zortéa-Guidolin, M. E. B., Santos, T. P. R., Yangcheng, H., Chang, F., & Jane, J. (2016). Physicochemical characterization of starches from dry beans cultivated in Brazil. *Food Hydrocolloids*, 61, 812-820. <http://dx.doi.org/10.1016/j.foodhyd.2016.07.014>.
- Dias, A. R. G., Zavareze, E. R., Elias, M. C., Helbig, E., Silva, D. O., & Ciacco, C. F. (2011). Pasting, expansion and textural properties of fermented cassava starch oxidised with sodium hypochlorite. *Carbohydrate Polymers*, 84(1), 268-275. <http://dx.doi.org/10.1016/j.carbpol.2010.11.033>.

- Food and Agriculture Organization of the United Nations – FAO. (2014). *Production of commodity*. Retrieved from <http://faostat3.fao.org/browse/Q/QC/E>
- Garrido, L. H., Schnitzler, E., Zortéa, M. E. B., de Souza Rocha, T., & Demiate, I. M. (2014). Physicochemical properties of cassava starch oxidized by sodium hypochlorite. *Journal of Food Science and Technology*, 51(10), 2640-2647. PMID:25328206. <http://dx.doi.org/10.1007/s13197-012-0794-9>.
- Granza, A. G., Travalini, A. P., Farias, F. O., Colman, T. A. D., Schnitzler, E., & Demiate, I. M. (2015). Effects of acetylation and acetylation-hydroxypropylation (dual-modification) on the properties of starch from Carioca bean (*Phaseolus vulgaris* L.). *Journal of Thermal Analysis and Calorimetry*, 119(1), 769-777. <http://dx.doi.org/10.1007/s10973-014-4092-9>.
- Halal, S. L., Colussi, R., Pinto, V. Z., Bartz, J., Radunz, M., Carreño, N. L., Dias, A. R., & Zavareze, E. R. (2015). Structure, morphology and functionality of acetylated and oxidised barley starches. *Food Chemistry*, 168(0), 247-256. PMID:25172707. <http://dx.doi.org/10.1016/j.foodchem.2014.07.046>.
- Han, F., Liu, M., Gong, H., Lü, S., Ni, B., & Zhang, B. (2012). Synthesis, characterization and functional properties of low substituted acetylated corn starch. *International Journal of Biological Macromolecules*, 50(4), 1026-1034. PMID:22402247. <http://dx.doi.org/10.1016/j.ijbiomac.2012.02.030>.
- Hong, J., Chen, R., Zeng, X., & Han, Z. (2016). Effect of pulsed electric fields assisted acetylation on morphological, structural and functional characteristics of potato starch. *Food Chemistry*, 192, 15-24. PMID:26304315. <http://dx.doi.org/10.1016/j.foodchem.2015.06.058>.
- Hoover, R., & Ratnayake, W. S. (2002). Starch characteristics of black bean, chick pea, lentil, navy bean and pinto bean cultivars grown in Canada. *Food Chemistry*, 78(4), 489-498. [http://dx.doi.org/10.1016/S0308-8146\(02\)00163-2](http://dx.doi.org/10.1016/S0308-8146(02)00163-2).
- Kuakpetoon, D., & Wang, Y. J. (2008). Locations of hypochlorite oxidation in corn starches varying in amylose content. *Carbohydrate Research*, 343(1), 90-100. PMID:17950716. <http://dx.doi.org/10.1016/j.carres.2007.10.002>.
- Kuakpetoon, D., & Wang, Y.-J. (2001). Characterization of different starches oxidized by hypochlorite. *Stärke*, 53(5), 211-218. [http://dx.doi.org/10.1002/1521-379X\(200105\)53:5<211::AID-STAR211>3.0.CO;2-M](http://dx.doi.org/10.1002/1521-379X(200105)53:5<211::AID-STAR211>3.0.CO;2-M).
- Lawal, O. S. (2004). Composition, physicochemical properties and retrogradation characteristics of native, oxidised, acetylated and acid-thinned new cocoyam (*Xanthosoma sagittifolium*) starch. *Food Chemistry*, 87(2), 205-218. <http://dx.doi.org/10.1016/j.foodchem.2003.11.013>.
- Leach, H. W., Mc Cowen, L. D., & Schoch, T. J. (1959). Structure of the starch granules - swelling and solubility of various starches. *Cereal Chemistry*, 534-544.
- Mano, J. F., Koniarova, D., & Reis, R. L. (2003). Thermal properties of thermoplastic starch/synthetic polymer blends with potential biomedical applicability. *Journal of Materials Science. Materials in Medicine*, 14(2), 127-135. PMID:15348484. <http://dx.doi.org/10.1023/A:1022015712170>.
- Mbougueng, P. D., Tenin, D., Scher, J., & Tchiégang, C. (2012). Influence of acetylation on physicochemical, functional and thermal properties of potato and cassava starches. *Journal of Food Engineering*, 108(2), 320-326. <http://dx.doi.org/10.1016/j.jfoodeng.2011.08.006>.
- Morikawa, K., & Nishinari, K. (2000). Effects of concentration dependence of retrogradation behaviour of dispersions for native and chemically modified potato starch. *Food Hydrocolloids*, 14(4), 395-401. [http://dx.doi.org/10.1016/S0268-005X\(00\)00021-7](http://dx.doi.org/10.1016/S0268-005X(00)00021-7).
- Ovando-Martínez, M., Bello-Pérez, L. A., Whitney, K., Osorio-Díaz, P., & Simsek, S. (2011). Starch characteristics of bean (*Phaseolus vulgaris* L.) grown in different localities. *Carbohydrate Polymers*, 85(1), 54-64. <http://dx.doi.org/10.1016/j.carbpol.2011.01.043>.
- Pietrzyk, S., Juszczak, L., Fortuna, T., & Ciemnińska, A. (2014). Effect of the oxidation level of corn starch on its acetylation and physicochemical and rheological properties. *Journal of Food Engineering*, 120(0), 50-56. <http://dx.doi.org/10.1016/j.jfoodeng.2013.07.013>.
- Rupollo, G., Vanier, N. L., Zavareze, E. R., Oliveira, M., Pereira, J. M., Paraginski, R. T., Dias, A. R. G., & Elias, M. C. (2011). Pasting, morphological, thermal and crystallinity properties of starch isolated from beans stored under different atmospheric conditions. *Carbohydrate Polymers*, 86(3), 1403-1409. <http://dx.doi.org/10.1016/j.carbpol.2011.06.055>.
- Sajilata, M. G., & Singhal, R. S. (2005). Specialty starches for snack foods. *Carbohydrate Polymers*, 59(2), 131-151. <http://dx.doi.org/10.1016/j.carbpol.2004.08.012>.
- Sánchez-Rivera, M. M., García-Suárez, F. J. L., Valle, M. V., Gutierrez-Meraz, F., & Bello-Pérez, L. A. (2005). Partial characterization of banana starches oxidized by different levels of sodium hypochlorite. *Carbohydrate Polymers*, 62(1), 50-56. <http://dx.doi.org/10.1016/j.carbpol.2005.07.005>.
- Sandhu, K. S., & Singh, N. (2007). Some properties of corn starches II: physicochemical, gelatinization, retrogradation, pasting and gel textural properties. *Food Chemistry*, 101(4), 1499-1507. <http://dx.doi.org/10.1016/j.foodchem.2006.01.060>.
- Sangseethong, K., Lertphanich, S., & Sriroth, K. (2009). Physicochemical properties of oxidized cassava starch prepared under various alkalinity levels. *Stärke*, 61(2), 92-100. <http://dx.doi.org/10.1002/star.200800048>.
- Sangseethong, K., Termvejsayanon, N., & Sriroth, K. (2010). Characterization of physicochemical properties of hypochlorite- and peroxide-oxidized cassava starches. *Carbohydrate Polymers*, 82(2), 446-453. <http://dx.doi.org/10.1016/j.carbpol.2010.05.003>.
- Simsek, S., Ovando-Martínez, M., Whitney, K., & Bello-Pérez, L. A. (2012). Effect of acetylation, oxidation and annealing on physicochemical properties of bean starch. *Food Chemistry*, 134(4), 1796-1803. PMID:23442623. <http://dx.doi.org/10.1016/j.foodchem.2012.03.078>.
- 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), 1-22. <http://dx.doi.org/10.1016/j.foodhyd.2006.02.006>.
- Singh, N., Chawla, D., & Singh, J. (2004). Influence of acetic anhydride on physicochemical, morphological and thermal properties of corn and potato starch. *Food Chemistry*, 86(4), 601-608. <http://dx.doi.org/10.1016/j.foodchem.2003.10.008>.
- Smith, R. J. (1967). Characterization and analysis of starches. In R. L. Whistler & E. F. Paschall (Eds.), *Starch: chemistry and technology* (Vol. II, pp. 569-635). New York and London: Academic Press.
- Song, Y., & Jane, J. (2000). Characterization of barley starches of waxy, normal, and high amylose varieties. *Carbohydrate Polymers*, 41(4), 365-377. [http://dx.doi.org/10.1016/S0144-8617\(99\)00098-3](http://dx.doi.org/10.1016/S0144-8617(99)00098-3).
- Vanier, N. L., da Rosa Zavareze, E., Pinto, V. Z., Klein, B., Botelho, F. T., Dias, A. R. G., & Elias, M. C. (2012). Physicochemical, crystallinity, pasting and morphological properties of bean starch oxidised by different concentrations of sodium hypochlorite. *Food Chemistry*, 131(4), 1255-1262. <http://dx.doi.org/10.1016/j.foodchem.2011.09.114>.
- Wang, H., & Ratnayake, W. S. (2014). Physicochemical and thermal properties of *Phaseolus vulgaris* L. var. Great Northern Bean Starch.

- Journal of Food Science*, 79(3), C295-C300. PMID:24506235. <http://dx.doi.org/10.1111/1750-3841.12357>.
- Wang, Y.J., & Wang, L. (2002). Characterization of acetylated waxy maize starches prepared under catalysis by different alkali and alkaline-earth hydroxides. *Stärke*, 54(1), 25-30. [http://dx.doi.org/10.1002/1521-379X\(200201\)54:1<25::AID-STAR25>3.0.CO;2-T](http://dx.doi.org/10.1002/1521-379X(200201)54:1<25::AID-STAR25>3.0.CO;2-T).
- Wang, Y.J., & Wang, L. (2003). Physicochemical properties of common and waxy corn starches oxidized by different levels of sodium hypochlorite. *Carbohydrate Polymers*, 52(3), 207-217. [http://dx.doi.org/10.1016/S0144-8617\(02\)00304-1](http://dx.doi.org/10.1016/S0144-8617(02)00304-1).
- Wani, I. A., Sogi, D. S., & Gill, B. S. (2012). Physicochemical properties of acetylated starches from some Indian kidney bean (*Phaseolus vulgaris* L.) cultivars. *International Journal of Food Science & Technology*, 47(9), 1993-1999. <http://dx.doi.org/10.1111/j.1365-2621.2012.03062.x>.
- Wani, I. A., Sogi, D. S., Wani, A. A., Gill, B. S., & Shivhare, U. S. (2010). Physico-chemical properties of starches from Indian kidney bean (*Phaseolus vulgaris*) cultivars. *International Journal of Food Science & Technology*, 45(10), 2176-2185. <http://dx.doi.org/10.1111/j.1365-2621.2010.02379.x>.
- Waterschoot, J., Gomand, S. V., Fierens, E., & Delcour, J. A. (2015). Production, structure, physicochemical and functional properties of maize, cassava, wheat, potato and rice starches. *Stärke*, 67(1-2), 14-29. <http://dx.doi.org/10.1002/star.201300238>.
- Wuzrburg, O. B. (1986). Converted starches. In O. B. Wuzrburg (Ed.), *Modified starches: properties and uses* (chap. 2, pp. 18-34). Boca Raton: CRC Press.
- Xiao, H.X., Lin, Q.L., Liu, G.Q., & Yu, F.X. (2012). A Comparative study of the characteristics of cross-linked, oxidized and dual-modified rice starches. *Molecules (Basel, Switzerland)*, 17(9), 10946-10957. PMID:22971580. <http://dx.doi.org/10.3390/molecules170910946>.
- Zhang, Y.R., Wang, X.L., Zhao, G.M., & Wang, Y.Z. (2012). Preparation and properties of oxidized starch with high degree of oxidation. *Carbohydrate Polymers*, 87(4), 2554-2562. <http://dx.doi.org/10.1016/j.carbpol.2011.11.036>.
- Zobel, H. F., Young, S. N., & Rocca, L. A. (1988). Starch gelatinization: an X-ray diffraction study. *Cereal Chemistry*, 65(6), 443-446.
- Zortéa-Guidolin, M. E. B., Demiate, I. M., Godoy, R. C. B., Scheer, A. P., Grewell, D., & Jane, J. L. (2017). Structural and functional characterization of starches from Brazilian pine seeds (*Araucaria angustifolia*). *Food Hydrocolloids*, 63, 19-26. <http://dx.doi.org/10.1016/j.foodhyd.2016.08.022>.