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## Acetylation of rice starch in an aqueous medium for use in food

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## ABSTRACT

The degree of acetylation controls the use of starch acetate, since the FDA (Food and Drug Administration USA) recommends an acetyl groups' percentage below 2.5 g/100g for a food application. The objective of this work was to evaluate the effects of the acetic anhydride concentration, in an aqueous solution, on the degree of acetylation, in physical, thermal, pasting and morphological properties, and enzymatic susceptibility on rice starch acetylated with the acetyl groups' percentage up to 2.5 g/100g. The rice starch was acetylated using different concentrations of acetic anhydride (5 g/100g, 10 g/100g and 20 g/100g, starch db). Acetylation increased the peak viscosity, breakdown, final viscosity, retrogradation and pasting temperature and reduced the hardness of the gels, as well as their adhesiveness and gumminess; however this did not affect the morphology and susceptibility to hydrolysis by the  $\alpha$ -amylase of the starch. The acetylated starches using 10 and 20 g/100g acetic anhydride showed lower swelling power and solubility compared to native starch.

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## 1. Introduction

Starch is the major constituent of rice grains and it is an important ingredient in food preparation (Bao, Kong, Xie, & Xu, 2004; Blazek & Gilbert, 2010). The rice starch has been widely used as an ingredient in various food and industrial products such as desserts, bakery products and alternative fats. Also, it has been used as an ingredient for the production of breakfast cereals, hypoallergenic products, and infant formulas (Puchongkavarin, Varavinit, & Berghaller, 2005), due to the hypoallergenicity of the associated protein. Rice starch with high amylose content has a low glycemic index too (Champagne, 1996).

The starch in its native form has limited applications; therefore, it does not always have the desired properties for certain types of processing; but when modified, it increases its range of use. The chemically modified starch by acetylation has been widely studied (Bello-Pérez, Agama-Acevedo, Zamudio-Flores, Mendez-Montealvo, & Rodriguez-Ambriz, 2010; Chen, Li, Li, & Guo, 2007; Gunaratne & Corke, 2007; Huang, Zhang, Chen, & Li, 2010; Luo & Shi, 2012; Mbougueng et al., 2012; Singh, Chawla, & Singh, 2004; Singh & Nath, 2012). The acetylation may be performed to

improve the physical, chemical and functional properties of starch (Xu, Miladinov, & Hanna, 2004). In the acetylation process, the hydroxyl groups of the glucose monomers are converted to the groups  $\text{CH}_3\text{COO}-$  (Graaf, Broekroelofs, Janssen, & Beenackers, 1995); therefore the acetylation is an esterification of hydroxyl groups in the anhydroglucose unit of the starch molecule.

The starch acetate has applications which are regulated by their characteristics, such as the degree of acetylation or the degree of substitution (DS), and the percentage of acetyl groups (% Ac). These characteristics determine the use of starch acetate, for example, for food application; the FDA (Food and Drug Administration USA) recommends a percentage of acetyl groups of less than 2.5 g/100g.

The changes promoted by acetylation depend on the botanical source, the degree of substitution, the proportion of amylose/amylopectin and the molecular structure of starch. In the acetylation reaction, the number of acetyl groups incorporated in the starch molecule and the efficiency of the reaction depend on the type of reagent, concentration, pH, presence of catalyst, reaction time, botanical origin and the characteristics of the size and structure of the starch granules (Huang, Schols, Jin, Sulmann, & Voragen, 2007; Huber & BeMiller, 2000).

According to Mark and Mehlretter (1972), the starch acetates, in accordance with DS, are classified as low DS (<0.1), medium DS (0.1–1.0) and high DS (>1.0) acetylated. There is a commercial interest in starches with a DS of 0.01–0.20 because of their use based

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on properties to film formation, adhesion, thickening, stabilizing and texturizing (Boutboul, Giampaoli, Feigenbaum, & Ducruet, 2002). The acetylated starch with a low DS is commonly obtained by the esterification of native starch with acetic anhydride, in an aqueous medium, and in the presence of an alkaline catalyst.

The applicability of acetylated starches is completely dependent on the degree of substitution. The introduction of acetyl groups interrupts the ordered structure of native starch and interferes with the reassociation of amylose and amylopectin molecules in the gelatinized starch, leading to a decrease in the gelatinization temperature, an increase or decrease in the swelling power, and solubility along with the storage stability.

Previous studies on acetylated rice starch have been reported by Colussi et al. (2014) and Gonzalez and Perez (2002). Colussi et al. (2014) studied the effect of acetylated rice starch varying the amylose content with the different reaction times. Gonzalez and Perez (2002) evaluated the effect of acetylation in the chemical composition, and the physical, chemical, functional and rheological properties of rice starch. However, to our knowledge, there are no studies in literature on the acetylation of rice starch with an acetylation degree allowed by legislation (FDA) for food applications. Therefore, this work aims to produce rice starch acetylated with a percentage of acetyl groups up to 2.5 g/100g to allow for use in foods and evaluate the influence of the acetic anhydride concentration on the pasting, physical, thermal and morphological properties, and enzymatic susceptibility.

## 2. Materials and methods

### 2.1. Material

Rice starch of a high amylose (32% amylose), cultivar IRGA 417, was extracted according to the method proposed by Wang and Wang (2004).

### 2.2. Synthesis of starch acetates in an aqueous medium

The starches were acetylated according to the method proposed by Phillips, Huijum, Duohai, and Harold (1999). A suspension of 50 g of rice starch in 112 mL of distilled water was subjected to shaking at 1500 rpm (RW 20, IKA, Germany) for 60 min at 25 °C. The pH of the suspension was adjusted to 8.0 with an aqueous 3.0 g/100g NaOH solution, and a slow addition of 5, 10 and 20 g/100g of acetic anhydride (starch db), respectively, maintaining the pH between 8.0 and 8.4 with a 3.0 g/100g NaOH solution. After the complete addition of acetic anhydride, the reaction was continued for 15 min. The reaction was stopped by adjusting the pH to 4.5 with a 0.5 mol equi/L HCl solution. The final suspension was centrifuged for 3 min at 1000 × g and subjected to successive washings with 95 mL/L ethyl alcohol. The starch was dried in an oven with air circulation at 40 °C where the humidity reached about 10 g/100g.

### 2.3. Determination of the acetyl percentage (Ac%) and degree of substitution (DS)

The percentage of acetyl groups (Ac%) and the degree of substitution (DS) of the acetylated starches were determined by the titration method described by Wurzburg (1964). Acetylated starch (1 g) was mixed with 50 mL of 75 mL/L ethanol in distilled water. The 250 mL flask containing the slurry was covered with aluminum foil and placed in a water bath at 50 °C for 30 min. The samples were then cooled and 40 mL of 0.5 mol equi/L KOH were added. The slurry was kept under constant stirring at 200 rpm for 72 h. After this period, the alkali excess was titrated with 0.05 mol equi/L HCl,

using phenolphthalein as an indicator. The solution was left to stand for 2 h and then any additional alkali, which may have leached from the sample, was titrated. A blank, using the original unmodified starch, was also used. The Ac % and the DS were calculated according to Equations (1) and (2) respectively.

$$\text{Ac\%} = \frac{([V_{\text{Blank}} - V_{\text{Sample}}] \times \text{Molarity of HCl} + 0.043 \times 100)}{\text{Sample weight}} \quad (1)$$

Where:  $V_{\text{Blank}}$  is the titration volume used for the blank sample;  $V_{\text{Sample}}$  is titration volume used for each sample, both titration volumes were expressed in mL; the sample weight was expressed in g. DS is defined as the average number of sites per glucose unit that possess a (Whistler & Daniel, 1995).

$$\text{DS} = \frac{(162 \times \text{Acetyl\%})}{(4300 - [42 \times \text{Acetyl\%}])} \quad (2)$$

### 2.4. Fourier Transform Infrared (FTIR) spectroscopy

The infrared spectra of the native and acetylated starches were obtained using a Fourier Transform Infrared (FTIR) spectrometer Prestige-21, Shimadzu, in the region of 4000–400  $\text{cm}^{-1}$ . Pellets were created by mixing the sample with KBr at a ratio of 1:100 (sample:KBr). Ten readings were collected at a resolution of 4  $\text{cm}^{-1}$ .

### 2.5. Pasting properties

The pasting properties of the starch samples (3.0 g, 14 g/100g moisture basis) were determined with a Rapid Visco Analyser (RVA-4; Newport Scientific, Warriewood, Australia). The viscosity was expressed in rapid visco units (RVU). The sample (3.0 g) was held at 50 °C for 1 min, heated to 95 °C in 3.5 min, and then held at 95 °C for 2.5 min. The sample was then cooled to 50 °C in 4 min and held at 50 °C for 2 min. The rotating speed was held at 960 rpm for 10 s and then maintained at 160 rpm during the process. Parameters including pasting temperature, peak viscosity, breakdown, final viscosity and setback were recorded.

### 2.6. Texture profile of gels

The gel texture profile was analyzed with Texture Analyser (TA.XTplus, Stable Micro Systems Ltd., Godalming, UK) according to the method described by Horndok and Noomhorm (2007) with some modifications. After taking the RVA measurement, the gelatinized mixture in the canister was stored at room temperature (20 °C) for 24 h, allowing the formation of a solid gel. The canister was sealed with parafilm to prevent moisture loss during storage. The gel was punctured at 1.0  $\text{mms}^{-1}$  to a distance of 10.0 mm using a stainless steel cylindrical probe (P/20, 20 mm diameter). The peak force measured was reported as the gel hardness (height of the first peak).

### 2.7. Swelling power and solubility

The swelling power and solubility of the starches were determined as described by Leach, McCowen, and Schoch (1959).

### 2.8. Thermal properties

The gelatinization characteristics of starches were determined using differential scanning calorimetry (DSC model 2010, TA Instruments, New Castle, USA). Starch samples (approximately 2.5 mg, dry basis) were weighed directly in an aluminum pan, and

distilled water was added to obtain a starch–water ratio of 1:3 (g/g). The pan was hermetically sealed and allowed to equilibrate for 1 h before analysis. The sample pans were then heated from 20 to 100 °C at a rate of 10 °C min<sup>-1</sup>. An empty pan was used as a reference. The temperature at the onset of gelatinization ( $T_o$ ), the temperature at the peak ( $T_p$ ), the temperature at the end of gelatinization ( $T_c$ ) and the enthalpy ( $\Delta H$ ) of gelatinization were determined. The temperature range of gelatinization was calculated as  $T_c - T_o$ .

### 2.9. Scanning electron microscopy (SEM)

Starch granule morphology was examined using a scanning electron microscope (Shimadzu, SSX-550). Starch samples were initially suspended in acetone at 1 g/100 mL. A small quantity of each sample was spread directly on the surface of the stub and dried in an oven at 32 °C for 24 h. Subsequently, all of the samples were coated with gold and examined using the scanning electron microscope under an acceleration voltage of 15 kV and a magnification of 5000 $\times$ .

### 2.10. Starch hydrolysis

Enzymatic hydrolysis was based on the methodology proposed by Foresti, Williams, Martínez-García, and Vázquez (2014), with some modifications. A sample of 1 g of starch was dispersed in 50 mL of buffer pH 7 kept at 40 °C at 350 rpm. The  $\alpha$ -amylase (Sigma, 500 U) was added and was defined as the beginning of the reaction. Suspensions were kept at a constant temperature and magnetic stirring during 24 h. Samples (1 mL) were withdrawn at reaction time intervals of 1, 2, 4, 6, 8 and 24 h, centrifuged for 5 min at 1300  $\times$  g, and the glucose concentration was quantified by the DNS method using glucose as a standard. The extent of starch hydrolysis (%) achieved at each reaction interval was calculated as the amount (g) of glucose equivalents released per gram of dried starch.

### 2.11. Statistical analysis

Analytical determinations for the samples were performed in triplicate and standard deviations were reported. A comparison of the means was ascertained by Tukey's test to a 5% level of significance using an analysis of the variance (ANOVA).

## 3. Results and discussion

### 3.1. Acetyl percentage (Ac%) and degree of substitution (DS)

The DS and Ac% of acetylated starches ranged from 0.05 to 0.10, and 1.37–2.58 g/100g, respectively. The acetylated starches with the highest concentrations of acetic anhydride (10 and 20 g/100g) presented higher DS and Ac% as compared with starch acetylated with 5 g/100g acetic anhydride (Table 1). The acetylated starches showed lower Ac% than 2.5 g/100g which is percentage of acetyl groups maximum recommended by FDA for foods uses (Code of Federal Regulations, 1994). Vasanthan, Sosulski, and Hoover (1995) in acetylations of 26 g/100g starch slurries reported an Ac% between 1.01 and 2.80 g/100g in several starches (potato, waxy corn, corn, wheat, field pea and lentil) using 5 and 10 g/100g of acetic anhydride. Liu, Ramsden, and Corke (1997) verified an Ac% between 2.71 and 4.22 g/100g and a DS of 0.105–0.165 in acetylated maize starches, using 10 g/100g of acetic anhydride in starch slurry of 31 g/100g. Differences in DS and Ac% can be attributed to the type of reagent, concentration, pH, presence of catalyst, reaction time,

**Table 1**

Degree of substitution (DS) and percentage of acetyl groups rice starch native and acetylated with different concentrations of acetic anhydride.

Starch <sup>a</sup>	Acetic anhydride (g/100g) <sup>b</sup>	Degree of substitution	Ac% (g/100g)
Native	0	–	–
Acetylated	5	0.05b	1.37b
	10	0.08a	2.21a
	20	0.10a	2.58a

<sup>a</sup> The results are the means of three determinations. Values with different online letters in the same column are significantly different ( $p < 0.05$ ).

<sup>b</sup> Concentration of acetic anhydride used in the reaction (starch basis).

botanical origin and characteristics of size and structure of starch granules.

### 3.2. Fourier Transform Infrared (FTIR) spectroscopy

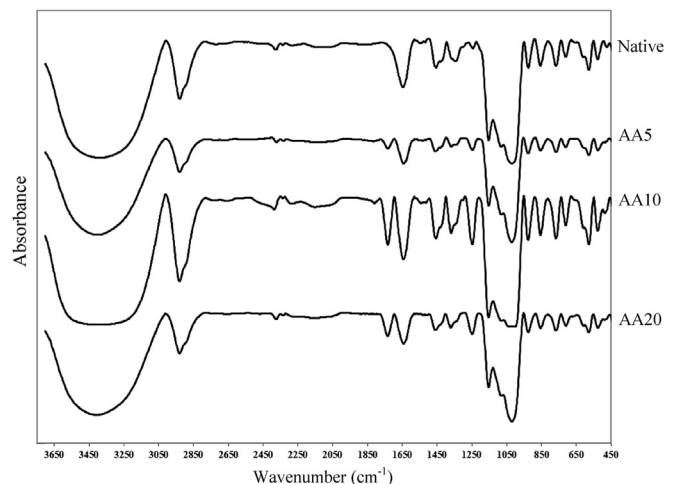
FTIR analysis also confirmed that the addition of acetic anhydride in the acetylation reaction (Fig. 1) favors the insertion of acetyl groups to the starch molecule. The extent of the incorporation of acetyl groups in the starch molecule may be visualized by an increase in the peak intensity of carbonyl C=O (1741.7 cm<sup>-1</sup>) (Fig. 1).

FTIR spectra of different DS of acetylated starches showed some new absorption bands at 1742, 1435, 1375, 1240 cm<sup>-1</sup> assigned to carbonyl C=O, CH<sub>3</sub> antisymmetry deformation vibration, and CH<sub>3</sub> symmetry deformation vibration and carbonyl C–O stretch vibration, respectively. These new absorptions suggest that the acetylated starch products were formed during the esterification process (Chi et al., 2008).

The absence of the peak in the region 1850–1760 cm<sup>-1</sup> implied that the product was free of unreacted acetic anhydride, and the absence of absorption in the area of 1700 cm<sup>-1</sup> for the carboxylic group indicated that the product was also free of acetic acid byproduct; the same observation was reported by Diop, Li, Xie, and Shi (2011).

### 3.3. Pasting properties

The pasting properties of native and acetylated starches with different concentrations of acetic anhydride are shown in Table 2. Acetylation in the highest concentration of acetic anhydride (20 g/100g) increased the peak viscosity of the starch when compared to



**Fig. 1.** FTIR spectra of Native and acetylated rice starches. (AA5) 5 g/100g; (AA10): 10 g/100g; (AA20): 20 g/100g of anhydride acetic used in the reaction (starch basis).

**Table 2**

Pasting properties rice starches native and acetylated with different concentrations of acetic anhydride.

Starch <sup>a</sup>	Acetic anhydride (g/100g) <sup>b</sup>	Peak viscosity (RVU) <sup>b</sup>	Breakdown (RVU)	Final viscosity (RVU)	Setback (RVU)	Pasting temperature (°C)
Native	0	261.66b	30.46b	349.54c	118.33d	75.42a
Acetylated	5	269.16b	53.08a	421.80b	190.37c	69.71b
	10	267.29b	50.79a	484.62a	268.12a	69.15b
	20	306.47a	53.33a	485.25a	233.87b	65.03c

<sup>a</sup> The results are the means of three determinations. Values with different online letters in the same column are significantly different ( $p < 0.05$ ).

<sup>b</sup> Concentration of acetic anhydride used in the reaction (starch basis).

native starch. The final viscosity of acetylated starches was higher than in the native one; however, there were no differences between the acetylated starches with different degrees of substitution. Gonzalez and Perez (2002), in a study on the effect of acetylation, in low levels, on the properties of rice starch, found similar results to this study. In contrast to these results, Saartrat, Puttanlek, Rungsardthong, and Uttapap (2005) found that the viscosities of acetylated canna starches with high degrees of substitution were lower than those of native starches. Reduction in paste viscosity may be attributed to the depolymerization of the starch chains during acetylation. These results suggest that the viscosity of the acetylated starch is highly dependent on the degree of substitution, since this influences the depolymerization of the molecule and consequently the peak viscosity of the starch.

Acetylation increased the breakdown of the rice starch, reducing its thermal and mechanical stability and increasing the tendency of retrogradation. However, this increase was not significant for acetylated starches with different degrees of substitution. The increase in retrogradation on acetylated starch is due to the introduction of acetyl groups. The hydrophobic character of acetyl groups and the reapproximation of amylose molecules promote an organization of hydrogen bonds and water expelled from the gel system.

The pasting temperature of acetylated starch decreased when the intensity of acetylation was increased. According to Saartrat et al. (2005), the acetylation reduces pasting temperature as it increases the percentage of acetyl groups in the starch molecule. The reduction in the pasting temperature is an advantage of acetylation because it allows the use of acetylated starches in cases where a thickening agent must gelatinize at a lower temperature, or also to reduce energy costs during processing of products where these starches are used.

### 3.4. Texture profile of gels

The textural properties of starch gels are very important parameters to evaluate the performance of a starch in the food system, thus favoring optimization of industrial processes and developing new products (Sandhu & Singh, 2007). The texture

**Table 3**

Profile gel texture of rice starch native and acetylated with different concentrations of acetic anhydride.

Starch <sup>a</sup>	Acetic anhydride (g/100g) <sup>b</sup>	Hardness (g)	Adhesiveness (gs <sup>-1</sup> )	Cohesiveness (ratio)	Gumminess (g)
Native	0	678.15a	-533.51b	0.51a	348.92a
Acetylated	5	182.68b	-561.03b	0.56a	102.83b
	10	213.45b	-816.28a	0.54a	117.03b
	20	182.74b	-796.07a	0.53a	97.00b

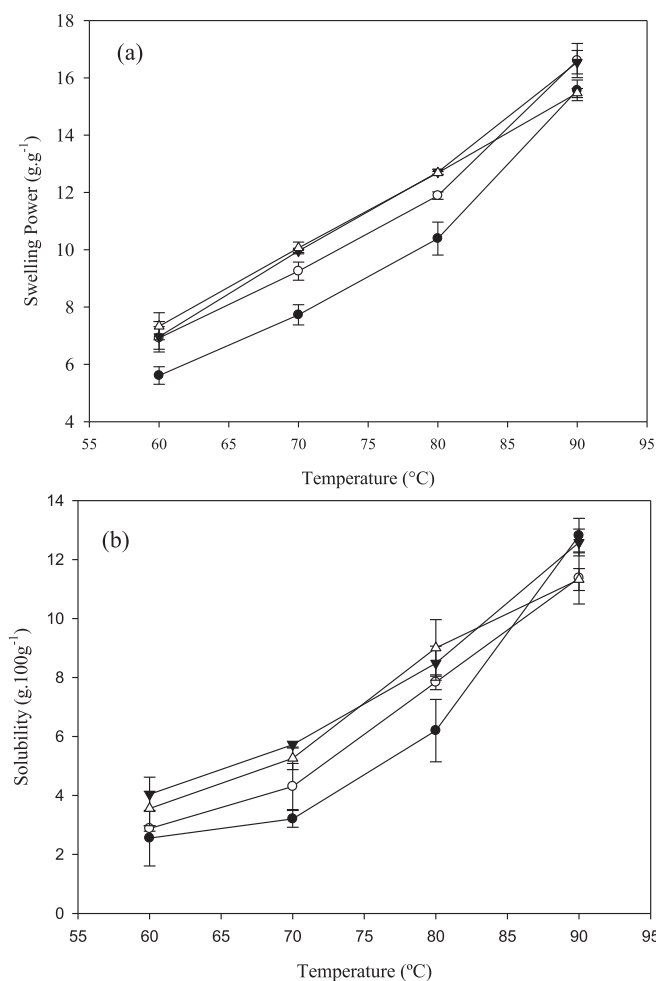
<sup>a</sup> The results are the means of three determinations. Values with different online letters in the same column are significantly different ( $p < 0.05$ ).

<sup>b</sup> Concentration of acetic anhydride used in the reaction (starch basis).

profiles of acetylated rice starch that have gels with different concentrations of acetic anhydride are presented in Table 3.

Acetylation reduced the gels hardness of starch; however, it was not affected by the increase in the intensity of acetylation. The gel hardness depends on factors, such as volume and deformation of the granules, retrogradation in combination with gel syneresis and crystallization of amylopectin. These factors, in turn, depend on the content of the amylose, amylopectin structure and insertion of acetyl groups.

The introduction of acetyl groups increases the space between the starch molecules, which reduces the retrogradation. However, in this work, there was an increase in retrogradation that indicates an increase in a reassociation of amylose molecules. It could be the reason for gel hardness increasing. However, the acetylation

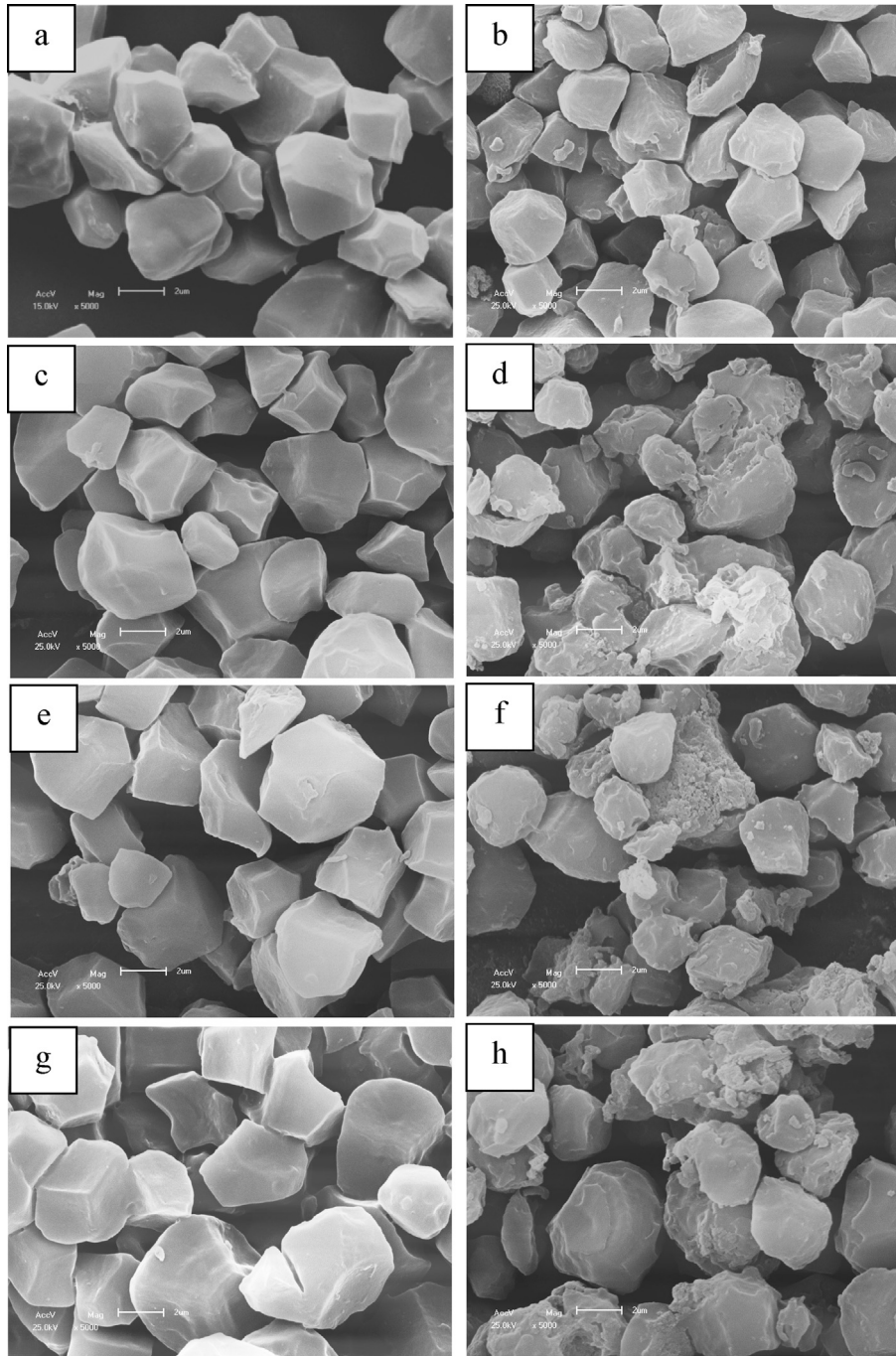


**Fig. 2.** Swelling power and solubility of (—●—) native and acetylated rice starches. (—○—): 5 g/100g; (—▲—): 10g/100g; (—△—): 20 g/100g of anhydride acetic used in the reaction (starch basis).

reduced the hardness of starch gels. Based on this, we suggest that the low level of acetylation was not enough to interspace the molecules and reduce the molecular reassociation. The reduction in the hardness of the acetylated starch gel may be due to a slight depolymerization of the starch molecules by the acetic anhydride reaction; even though more amylose molecules are reassociated, the depolymerization can cause a weakening of the gel. Acetylation did not cause differences in cohesiveness, however, but it increased gumminess and reduced adhesiveness of starch gels.

### 3.5. Swelling power and solubility

The swelling power and solubility of native and acetylated starches are presented in Fig. 2. Acetylation with the highest concentrations of acetic anhydride (10 and 20 g/100g) reduced the swelling power of rice starch. According to Gonzalez and Perez (2002) the introduction of acetyl groups of rice starch can facilitate access of water to the amorphous regions due to structural disruptions caused by intragranular steric effects and the



**Fig. 3.** Scanning electron micrographs of rice starches: native starch (a), hydrolyzed native starch (b), starch modified with 5 g/100g acetic anhydride (c), starch modified with 5 g/100g acetic anhydride hydrolyzed (d), starch modified with 10 g/100g acetic anhydride (e), starch modified with 10 g/100g acetic anhydride hydrolyzed (f), starch modified with 20 g/100g acetic anhydride (g), starch modified with 20 g/100g acetic anhydride hydrolyzed (h).

**Table 4**

Thermal properties of rice starch native and acetylated with different concentrations of acetic anhydride.

Starch	Acetic anhydride (g/100g) <sup>a</sup>	To (°C)	Tp (°C)	Tc (°C)	Tc – To (°C)	ΔH (J/g)
Native	0	59.30	65.70	71.90	12.60	9.33
Acetylated	5	54.12	62.00	68.69	14.57	8.78
	10	52.93	60.81	67.73	14.80	7.71
	20	42.33	61.16	67.59	25.26	7.49

<sup>a</sup> Concentration of acetic anhydride used in the reaction (starch basis).

disruption of hydrogen bonds of starch molecules. In studies using rice (Colussi et al. 2014; Gonzalez & Perez, 2002; Liu & Corke, 1999), sword bean (Adebowale, Afolabi, & Olu-Owolabi, 2006) and cassava (Osundahunsi, Seidu, & Mueller, 2014) starches, the authors also reported an increase in water absorption, swelling power and solubility of starches promoted by acetylation.

The acetylated starches showed lower solubility compared to native starch (Fig. 2). This decreased solubility of acetylated starch can be attributed to the introduction of bulky acetyl groups in the starch molecules, which impart greater hydrophobicity than the hydroxyl groups in the starch molecule, due to the lower binding affinity with the water.

### 3.6. Thermal properties

The thermal properties of the acetylated rice starches using different acetic anhydride concentrations were determined by differential scanning calorimetry, and are presented in Table 4. Acetylation reduced To, Tp and Tc, and the highest concentration of acetic anhydride has the lowest gelatinization temperature. Starch gelatinization is controlled by the molecular structure of amylopectin, and the granule structure. The decrease in the gelatinization temperature is due to a premature opening of the amylopectin double helices and fusion of the crystalline lamellae of the starch, induced by acetylation.

Luo and Shi (2012) and Singh et al. (2004) who acetylated the corn and potato starches, respectively, reported a significant decrease in the gelatinization temperature of the starch after acetylation. They also reported that the introduction of acetyl groups in the polymer chains resulted in a disruption of the

granular structure, thereby causing a decrease in gelatinization temperature.

The acetylation decreased the ΔH of the acetylated rice starches (Table 4). The ΔH mainly reflects the loss of the order of double helices, and the loss of crystallinity within the granule. The highest degree of substitution of the starch (Table 1) promotes the greatest reduction in ΔH. The decrease in the ΔH of acetylated starch is a result of the double helices, present in crystalline and non-crystalline regions of the granule that were broken during the acetylation reaction. The lowest ΔH of acetylated starches suggests a lower percentage of organized or less stable crystal structures.

### 3.7. Starch hydrolysis and morphology

The percentage of starch hydrolysis was evaluated with the objective to verify the susceptibility of native and acetylated rice starches to the α-amylase enzyme from a porcine pancreas. The kinetic hydrolysis curves are shown in Fig. 4, which show the effect of the degree of substitution on the enzyme susceptibility of native and acetylated rice starches with different concentrations of acetic anhydride. The different levels of acetylation did not change significantly the hydrolysis of starch subjected to the α-amylase action (Fig. 4). Other authors (Kim, Park, & Lim, 2008; Wang & Wang, 2004) also studied the enzymatic activity of the α-amylase in the different sources of starches and reported that amorphous structures are preferentially hydrolyzed during the starch hydrolysis. The enzymatic hydrolysis of starch is influenced by a variety of factors which greatly affect the rate and efficiency of hydrolysis. Among these factors it is possible to highlight the botanical origin, the physical form in which the starch is subjected to the attack of the enzyme (such as native granules, gelatinized starch), the concentration of the starch solution, the type of enzyme, the enzyme concentration, thermal stability, starch state (in solution or in immobilized form), pH and the temperature of the reaction (Foresti et al. 2014).

The morphology of native and acetylated starch granules was analyzed by a scanning electron microscopy (SEM) and the micrographs are shown in Fig. 3. The images of rice starches showed the presence of small polyhedral granules (2–5 μm). The acetylation did not aggregate the granules and they did not show changes in the shape.

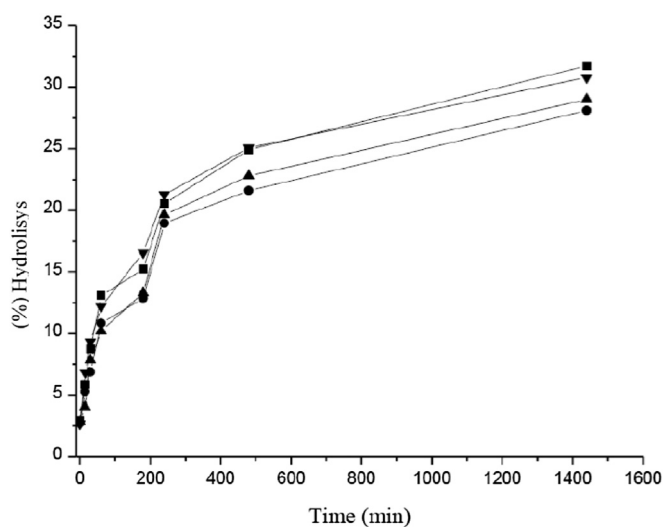
Sodhi and Singh (2005) reported that SEM revealed no significant differences between the external morphology of native and acetylated rice starches.

Sha et al. (2012) reported that the surface of acetylated starch granules was less smooth than those of the native starch. However, the granules still retained their particle structure relatively complete after modification. These authors explained that the increase in the intensity of acetylation damaged the hydrogen intermolecular bonds and promoted the disruption of some starch granules. These authors reported that the crystalline regions were also involved in the reaction, but without the occurrence of some granular structure loss.

According to the micrographs of starch granules after 24 h of hydrolysis (Fig. 3) it was observed that the hydrolysis of acetylated starch granules showed higher disintegration as compared to native starch. It was also found that certain granules appeared pasted to each other, after the α-amylase hydrolysis, which was explained by the effect of some amylose leached out that acted as a gluing material.

## 4. Conclusions

In this study it was possible to obtain acetylated rice starches for use in food according to FDA standards, with the maximum



**Fig. 4.** Enzymatic hydrolysis of rice starch (●) native and acetylated. (○): 5 g/100g; (▲): 10g/100g; (△): 20 g/100g of anhydride acetic used in the reaction (starch basis).

percentage of acetyl groups of 2.5. Increasing the concentration of acetic anhydride in the acetylation reaction increased the degree of substitution of starch by changing its application properties, but did not affect the morphological structure of the granules. The acetylated starches showed higher final viscosity when compared to native starches and can be used in products that require a thickening power after preparation by heating, such as the production of dehydrated soups, instant soups, broths, and sauces. Based on the characteristics of acetylated starch gels compared to the native starch, such as lower hardness, adhesiveness and gumminess, the acetylated starches could be applied in canned food, such as pastes, sauces, mustard and ketchup. The acetylated starches also showed lower swelling power and solubility, and that they could be applied in higher concentrations than in native starch, providing a product with higher concentrations of solids. The fact that acetylation decreased gelatinization temperatures and enthalpy is very important to the industry due to the need of low-power applied to the process, thereby reducing processing costs.

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### 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, 93–101.
- Bao, J., Kong, X., Xie, J., & Xu, L. (2004). Analysis of genotypic and environmental effects on rice starch. 1. Apparent amylose content, pasting viscosity, and gel texture. *Journal of Agriculture and Food Chemistry*, 52, 6010–6016.
- 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. *LWT - Food Science and Technology*, 43, 1434–1440.
- Blazek, J., & Gilbert, E. P. (2010). Effect of enzymatic hydrolysis on native starch granule structure. *Biomacromolecules*, 11, 3275–3289.
- Boutboul, A., Giampaoli, P., Feigenbaum, A., & Ducruet, V. (2002). Influence of the nature and treatment of starch on aroma retention. *Carbohydrate Polymers*, 47, 73–82.
- Champagne, E. T. (1996). Rice starch composition and characteristics. *Cereal Foods World*, 41(11), 833–838.
- Chen, L., Li, X., Li, L., & Guo, S. (2007). Acetylated starch-based biodegradable materials with potential biomedical application as drug delivery systems. *Current Applied Physics*, 7, 90–93.
- Chi, H., Xu, K., Wua, X., Chen, Q., Xue, D., Song, C., et al. (2008). Effect of acetylation on the properties of corn starch. *Food Chemistry*, 106, 923–928.
- Code of Federal Regulations. (1994). *Food additives permitted in food for human consumption*. Washington, DC, USA: U.S. Government Printing Office. Title 21, Chap. 1. Part 172, Section 172.892, Food Starch.
- Colussi, R., Pinto, V. Z., Halal, S. L. M., Vanier, N. L., Villanova, F. A., Silva, R. M., et al. (2014). Structural, morphological, and physicochemical properties of acetylated high-, medium-, and low-amylose rice starches. *Carbohydrate Polymers*, 103, 405–413.
- Diop, C. I. K., Li, H. L., Xie, B. J., & Shi, J. (2011). Impact of the catalytic activity of iodine on the granule morphology, crystalline structure, thermal properties and water solubility of acetylated corn (*Zea mays*) starch synthesized under microwave assistance. *Industrial Crops and Products Journal*, 33, 302–309.
- Foresti, M. L., Williams, M. P., Martínez-García, R., & Vázquez, A. (2014). Analysis of a preferential action of  $\alpha$ -amylase from *B. licheniformis* towards amorphous regions of waxy maize starch. *Carbohydrate Polymers*, 104, 80–87.
- Gonzalez, Z., & Perez, E. (2002). Effect of acetylation on some properties of rice starch. *Starch/Stärke*, 54, 148–154.
- Graaf, R. A., Broekroelofs, G. A., Janssen, L. P. B. M., & Beenackers, A. A. C. M. (1995). The kinetics of the acetylation of gelatinized potato starch. *Carbohydrate Polymers*, 28, 137–144.
- Gunaratne, A., & Corke, H. (2007). Influence of prior acid treatment on acetylation of wheat, potato and maize starches. *Food Chemistry*, 105, 917–925.
- Horndok, R., & Noomhorm, A. (2007). Hydrothermal treatments of rice starch for improvement of rice noodle quality. *Lebensmittel Wissenschaft und Technologie, Oxford*, 40, 1723–1731.
- Huang, J., Schols, H., Jin, Z., Sulmann, E., & Voragen, A. G. J. (2007). Pasting properties and (chemical) fine structure of acetylated yellow pea starch is affected by acetylation reagent type and granule size. *Carbohydrate Polymers*, 68, 397–406.
- Huang, J., Zhang, P., Chen, Z., & Li, H. (2010). Characterization of remaining granules of acetylated starch after chemical surface gelatinization. *Carbohydrate Polymers*, 80, 215–221.
- Huber, K. C., & BeMiller, J. N. (2000). Channels of maize and sorghum starch granules. *Carbohydrate Polymers*, 41, 269–276.
- Kim, J.-Y., Park, D.-J., & Lim, S.-T. (2008). Fragmentation of waxy rice starch granules by enzymatic hydrolysis. *Cereal Chemistry*, 85, 182–187.
- Leach, H. W., McCowen, L. D., & Schoch, T. J. (1959). Structure of the starch granule. I. Swelling and solubility patterns of various starches. *Cereal Chemistry*, 36, 534–544.
- Liu, H., & Corke, H. (1999). Physical properties of cross-linked and acetylated normal and waxy rice starch. *Starch/Stärke*, 51, 249–252.
- Liu, H., Ramsden, L., & Corke, H. (1997). Physical properties and enzymatic digestibility of acetylated ae, wx, and normal maize starch. *Carbohydrate Polymers*, 34, 283–289.
- Luo, Z. G., & Shi, Y. C. (2012). Preparation of acetylated waxy, normal, and high-amylose maize starches with intermediate degrees of substitution in aqueous solution and their properties. *Journal of Agricultural and Food Chemistry*, 60, 9468–9475.
- Mark, A. M., & Mehlretter, C. L. (1972). Facile preparation of starch triacetates. *Starch/Stärke*, 24, 73–76.
- Mbougoung, 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, 320–326.
- Osundahunsi, O. F., Seidu, K. T., & Mueller, R. (2014). Effect of presence of sulphur dioxide on acetylation and sorption isotherm of acetylated starches from cultivars of cassava. *Food Chemistry*, 151, 168–174.
- Phillips, D. L., Huijium, L., Duohai, P., & Harold, C. (1999). General application of Raman spectroscopy for the determination of level of acetylation in modified starches. *Cereal Chemistry*, 76, 439–443.
- Puchongkavarin, H., Varavinit, S., & Bergthaller, W. (2005). Comparative study of pilot scale rice starch production by an alkaline and an enzymatic process. *Starch/Stärke*, 57, 134–144.
- Saartrat, S., Puttanlek, C., Rungsardthong, V., & Uttapap, D. (2005). Paste and gel properties of low-substituted acetylated canna starches. *Carbohydrate Polymers*, 61, 211–221.
- Sandhu, K. S., & Singh, N. (2007). Some properties of corn starches II: physicochemical, gelatinization, retrogradation, pasting and textural properties. *Food Chemistry*, 101, 1499–1507.
- 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, 601–608.
- Sha, X. S., Xiang, Z. J., Bin, L., Jing, L., Bin, Z., Jiao, Y. J., et al. (2012). Preparation and physical characteristics of resistant starch (type 4) in acetylated indica rice. *Food Chemistry*, 134(2012), 149–154.
- Singh, A. V., & Nath, L. K. (2012). Evaluation of acetylated moth bean starch as a carrier for controlled drug delivery. *International Journal of Biological Macromolecules*, 50, 362–368.
- Sodhi, N. S., & Singh, N. (2005). Characteristics of acetylated starches prepared using starches from different rice cultivars. *Journal of Food Engineering*, 70, 117–127.
- Vasanthan, T., Sosulski, F. W., & Hoover, R. (1995). The reactivity of native and autoclaved starches from different origins towards acetylation and cationization. *Starch/Stärke*, 4, 135–143.
- Wang, L., & Wang, Y. J. (2004). Rice starch isolation by neutral protease and high-intensity ultrasound. *Journal of Cereal Science*, 39, 291–296.
- Whistler, R. L., & Daniel, J. R. (1995). Carbohydrates. In O. R. Fennema (Ed.), *Food chemistry* (pp. 69–137). New York: Marcel Dekker.
- Wurzburg, O. B. (1964). Acetylation. In R. L. Whistler (Ed.), *Methods in carbohydrate chemistry*. Boca Raton, FL: Academic Press (p. 240).
- Xu, Y., Miladinov, V., & Hanna, M. A. (2004). Synthesis and characterization of starch acetates with high substitution. *Cereal Chemistry*, 81, 735–740.