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STUDIES OF CONFORMATIONAL CHANGES, CRYSTALLINE AND GRANULAR STRUCTURES, AND IN VITRO DIGESTIBILITY OF CROSS-LINKED AND METHYLATED CORN STARCHES

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SUMMARY

Starch granular ultrastructure is an important determinant of its functional properties. Its knowledge, control and application should help to produce a wide range of food products. The goal of the study was to measure the modifications produced by cross-linking and methylation on the conformation and the granular and crystalline structures of corn starch, as well as the effects on the in vitro enzymatic digestibility of starch by α-amylase. Cross-linked and methylated starches were produced from commercial corn starch by low degree substitution (DS) methods. Both native and modified starches were analyzed employing NMR, SEM and X-ray diffraction. The morphologic characteristics, crystalline structure and susceptibility to hydrolysis by α-amylase were dramatically affected by the methylation process, while cross-linked corn starch showed non-significant variations that did not change the type A pattern of the native one. However, the 13C CP-MAS NMR spectrum of the cross-linked starch is suggesting a change from a type A to a type B crystalline pattern, while the X-ray diffraction pattern of the methylated starch was completely different from those reported in the literature for any starches. No changes in granular shape were observed (SEM) in the cross-linked starch, while the methylated starch showed larger chunks without granular integrity and with a rough surface due to exo-erosion. It can be presumed that, due to the presence of methyl groups inside the granules, the enzyme-substrate interaction is hindered by steric effects.

Introduction

Corn (Zea mays L.) grows on a wide variety of soil types, from loamy sands to clays to organic soils. It is the main cereal source for many countries around the world. Due to its high starch content it is used, together with cassava, as the main sources of raw material for starch extraction (Thomas and Atwell, 1999).

In turn, starch is an important renewable raw material used in the food, pharmaceuticals and paper industries (Van der Burgt et al., 1999). The properties of native starches can be altered by a diversity of physical, as well as, chemical treatments such as oxidation, substitution and cross-linking, among others. These alterations are carried out in order to produce modified starches with the needed properties for industrial uses. Detailed information on the distribution of the substitutions performed can help understand the relation between molecular structure and functional properties (Van der Burgt et al., 2000a). Moreover, the study of the effects of the modifications is of interest, as it could give additional experimental information on the structural variety of amylose and amylpectin, and could help elucidate general factors that determine the structure of these carbohydrates. Knowledge of the molecular structure is especially critical for understanding the properties of polysaccharides (Van der Burgt et al., 1999, Jhanson et al., 2007).

In most chemical modifications of starch, usually referred to as chemical derivatization, the granular form is maintained and hydroxyl groups are partially substituted, yielding ether and starch esters, as well as anionic and cationic starches (Van der Burgt et al., 2000b). The number, location and distribution of the substitutions are not expected to occur randomly, in view of the different levels of organization within the starch granule, and they determine the properties of these starch derivatives (Van der Burgt et al., 1999).

Usually, the modification treatments inhibit retrogradation (Tovar et al., 1999a, b) and the formulated products maintain uniformity and appearance for a long time. These modified starches offer advantages over the native starch by diminishing its consistency and increasing its shelf life. Van der Burgt et al. (1999) reported that the crystalline linear side-chains of the amylpectin in methylated starches, which play an important role in the retrogradation of gelatinized starches, contain fewer substituents, than the amorphous branched parts and that they are almost randomly distributed. The same authors (Van der Burgt et al., 2000b) also demonstrated that the methylation process does not have any preference for substitution at either branched or linearly linked glucose residues, taking into account the inherently lower amount of substituted sites at branched residues. In order to learn about the relationship between structure and function, it is important to determine the changes in the starch molecules after modification by the derivatization processes and to measure their susceptibility to hydrolysis by α-amylase.
effects on the functional properties and digestibility of the modified starches.

Nuclear magnetic resonance (NMR) is a well-established tool for studying the molecular structure and dynamics of disordered solids such as polymers and bio-materials. The versatility of the technique is such that, new refinements are constantly being developed, resulting in spectra with higher signal-to-noise ratios, better resolution and increased information content (LeBoplane et al., 1998; Li et al., 1996). Van der Burgt et al. (2000a) have studied the structure of methylated starch using NMR techniques.

X-ray diffraction analysis has been used to reveal the presence and characteristics of the crystalline structure of the starch granule. In native starch, crystal forming zones can be evidenced at the crystalline lamellae. The technique allows defining the types of crystal adjustments, depending on the position of the peak on the diffraction line. For example, an A-type crystal has a major peak at around d-spacing (20 angle), a doublet at 17° and 18°, and a single peak at 23° (Thitiprapaphunkul et al., 2003). Indeed, crystalline and non-crystalline structures, and the relationship between them, are factors in determining starch properties and have been studied using X-ray diffraction techniques (Matos and Pérez, 2003).

Variations of the in vivo and in vitro digestibility of starch granules depend on the botanical source (granular size, structure or core, and amylose/amilopectin relationship) and other factors such as location of the granules in the cells, external treatment conditions, modifying agents and conditions of the food processing and storage (Biliaderis, 1991; Ring et al., 1998; Tovar et al., 1999a).

The goal of this study was to obtain structural and morphometric information about native and chemically modified starches by means of nuclear magnetic resonance, scanning electron microscopy and X-ray diffraction techniques, and to evaluate the susceptibility of the native and modified corn starches to α-amylase digestion.

Materials and Methods

Starch materials

Commercial raw material was provided by INDELMA C.A., Cagua, estado Aragua, Venezuela. Chemical modification of the commercial native starch was carried out at the laboratory.

Moisture content

The moisture content of the native and modified starches was measured following the methods described by Whistler and Paschall (1964).
Starch modifications

Phosphate starch. Corn starch was modified by cross-linking using the method described by Whistler and Paschall (1964). The native starch was phosphated with Na3(PO)4 at alkaline pH, using low degree substitution (DS), so that the modified starch contained about 0.35% of bound phosphorus (DS=0.02). The DS for the modified starch samples was determined using the equation for monosodium esters

\[ DS = \frac{162P}{3100-102P} \]

where P is the difference in phosphorous content (dry basis) between the chemically modified and the native starch, expressed as percentage (Matos and Pérez, 2003).

Methylated starch. Granular native corn starch was methylated in aqueous suspension with dimethyl sulfate to DS values up to 2.0, using the method of partially methylated starch described by Whistler and Paschall (1964) modified as follows.

Native corn starch (300g) was shaken in 800mL of distilled water, in a water bath (28 ±2°C) to form a smooth paste. The paste was transferred to a vessel containing a vigorously stirred solution of 330g of crystalline barium hydroxide in 800mL of hot water. The mixture was heated to boiling and 94.7mL of 50% NaOH in 800mL of hot water was added drop by drop while the needles were kept at 45ºC and stirred continuously. The mixture was kept as a suspension with dimethyl sulfate for 24h and then centrifuged twice with 100mL of chloroform and the chloroform extracts evaporated to dryness under reduced pressure.

This procedure left a residue which was powdered and dried at 50°C under reduced pressure.

Nuclear magnetic resonance (NMR)

All NMR spectra were obtained with a Bruker AM300 (Bruker Instrument, Mountain View, CA, USA) equipped with a 7mm rotor, to CP/MAS. The acquisition parameters used were spin velocity 3500Hz, temperature 300ºK, contact time 2ms, a 4sec 90º pulse and scanning number 128. All spectra were replicated at least twice (Choi and Kerr, 2003).

Scanning electron microscopy (SEM)

Starch samples were sprayed on a metal plate previously covered with double-sided adhesive tape and shadowed under vacuum with gold-palladium. The starch granules were examined using a scanning electron microscope (Hitachi S-2400) at 20kV accelerating voltage (Matos and Pérez, 2003).

X-ray diffraction

X-ray diffraction patterns were obtained with a Philips diffractometer using monochromatic cobalt radiation, 31kV, 26mA, 4sec time constant and 1cm/min chart speed. Diffractograms were recorded at 2\( \theta \) = 4-30° at a scan rate of 1°/min (Zobel, 1988). The samples were measured on wet basis, moisture contents being given below.

Starch \( \alpha \)-amyloylasis

The degree of hydrolysis of both native and modified starches was assessed following the method described by Holm et al. (1985) and modified by Tovar et al. (1992), using type B pancreatic \( \alpha \)-amylase. Wheat starch was used as a reference. The starches (native and modified) were not gelatinized previous to the hydrolysis.

Statistical analysis

Mean and standard deviation were calculated, using the statistical package SPSS version 8.0 (1997).

Results and Discussion

Starch moisture content

The moisture content was 11.47% in the case of the native starch, while for the phosphorylated and methylated starches it was 6.87% and 6.71%, respectively.

Nuclear magnetic resonance (NMR)

NMR spectra of native, cross-linked and methylated corn starches (Figure 1) showed signals between 50 and 110ppm. In native starch (Figure 1a) the signal at 59-61ppm corresponds to that of C6, as indicated by Atichokudomchai et al. (2004), who reported a signal at 58-65ppm for C6. The strong signals at 72-80ppm match the resonance signals of the C2, C3, and C4 internal carbons of the glucose chain. C2, C3, and C4 are the reactive carbons from the anhydroglucose unit. The C1 resonance appeared as a weak peak at 82ppm. Finally, the region around 100ppm corresponds to the anomeric carbons C1(\( \alpha \)) according to Li et al. (1996) and Atichokudomchai et al. (2004). The crystalline structure of starch has been demonstrated by the shape of the C1 resonance line (Li et al., 1996). Morgan et al. (1995) reported that the C1 carbon atoms show chemical shifts characteristic for each of the three types of crystalline conformation. The C1 resonances of the native starch are triplets (~102, 101 and 100ppm) and relate to the double helices symmetry, because the repeated unit is a maltotriose unit, in the A form (Buléon et al., 1998). In Figure 1a the C1 carbon atom appears as an incipient cluster of three peaks. It corresponds with the X-ray pattern of conformation A, in harmony with reports by Morgan et al. (1995), Li et al. (1996) and Buléon et al. (1998).

Figure 1b shows the NMR spectrum of cross-linked corn starch. As can be noted in the figure, the changes are most evident in regions from 80 to near 110ppm, which correspond to C1 internal carbons of the glucose chain, and to C2 or
anomeric carbons. The C₄ resonance in the region ~82ppm of the cross-linked starch is higher than that of the native counterpart, probably due to changes in crystalline conformation produced by the thermal treatment.

The C₁ resonance in the region ~100ppm is characterized as a doublet splitting, as the B form crystalline structure, and is also higher than the native counterpart. No substitution was observed over the branched regions, as expected. An increment was also evident in the height of the signals from C₆ and the C₂, C₃, and C₅ internal carbon regions of the glucose chain.

The ¹³C CP-MAS NMR spectrum of the methylated starch (Figure 1c) shows an incipient signal at 61ppm, which indicates the presence of the methyl (-OCH₃) groups in the anhydroglucose chain branched region. It is also observed that the region above 100ppm is narrower and lower than in the spectra of the other two samples, which could be due to the change at its anomeric carbon. The intensity of the signal in the region from 80 to 82ppm, corresponding to the C₁ carbon, is higher and more conspicuous than in the native starch. Also, the branched region (60-40ppm) is altered, probably due to a preference for the substitution sites at branched glucose residues (Van der Burgt et al., 2000b).

On the other hand, the changes in resonance reflect the structural transition from crystalline to amorphous state due to the temperature (Li et al., 1996). The resonance from the amorphous domain appeared between the C₁ and C₄ regions. The crystalline regions are narrower than amorphous regions.

Scanning electron microscopy (SEM)

The appearance of the starch granules before and after modification is shown in Figure 2. That of native corn starch is similar to that reported in the literature (Thomas and Atwell, 1999). Both native and cross-linked starches show granules of various types, round and polyhedral shaped. These findings suggest that there are no changes in the shape of corn starch granules due to modification by cross-linking, because the treatment did not produce gelatinization of the starch. Similar results were reported by Matos and Pérez (2003) for cassava starch. The surfaces of the native and phosphate granules are smooth, with some surface pores. In contrast, the methylated starch shows larger chunks without granular integrity, as compared to the native and cross-linked counterparts and, also, a rough surface due to exo-erosion.

X-ray diffraction pattern

Figure 3 presents the X-ray diffraction patterns of native and modified starches. Native starch (in a) shows a type A pattern, which is characteristic of cereal starches (Thomas and Atwell, 1999). The cross-linked starch (in b) maintains the crystalline type A-pattern, with insignificant variations that do not change the original pattern. These variations could explain the result found by NMR, which suggests a B type crystalline conformation. The small variations could be due to the introduction of phosphate groups inside the crystalline lamellae. On the other hand, the marked change observed after modification by methylation (Figure 3c) represents a different structure, not found in the reviewed literature.

Starch digestibility

The degree of hydrolysis of native and modified starches by α-amylase, expressed as the percentage of starch that was hydrolyzed, is shown in Figure 4. It can be seen that the degree of hydrolysis in vitro of the native and cross-linked corn starch is similar to that shown by the native wheat starch used as a reference up to 60min. A plateau is reached at less than 40% hydrolysis after 30min of digestion. These results agree with those reported by several authors, who maintain that these types of modification do not af-
fect the hydrolysis significantly (Wooton and Chaundry, 1979, Ostergrad et al., 1988; Hung and Morita, 2005). As pointed out before, these starches were not gelatinized previou-

s to the hydrolysis, a fact contrib-
uting to a lower degree of hydrolys-
sis than that found in gelatinized corn starch. Of 66.6% at 15min and 73.7% at 60min (Laurentín et al., 2003). Except for methylated starch, all the starches were rap-

didly hydrolyzed in the initial 5min and thereafter the rate of hydrolysis decreased sig-

ificantly.

However, it can be noted in Figure 4 that the modifica-
tions alter the degree of hydrolysis of the starches and also that the methyl starch is severely affected as compared with cross-linked corn starch, which is affect-
ed in a minor proportion. It can be presumed that the low degree of hydrolysis shown by the methylated starch could be due to the presence of methyl groups within the granules, hinder-
ing the enzyme-substrate interaction by steric effects.

Conclusion

Changes in the conformation of corn starches were demonstrated by the

$^{13}$C CP-MAS NMR spectra. The changes are indicative of the degree of substitution and the effect of temperature modification on the crystalline confor-
mation. The $^{13}$C CP-MAS NMR spectrum of native starch is compatible with a crystalline type A-pattern, as is reported for cereals. However, the crossed-linking starch C, resonance in the spectrum region ∼100ppm is characterized as a split doublet, similar to the B form of the crystalline structure reported for non-
cereal starches. Despite of this, the X-ray diffraction pattern for both native and cross-linked starches reveal them as type A starch. There are more evident changes in the methylated starch spectrum, where the presence of $^{13}$C resonance near the $C_6$ terminal can be clearly observed. The cross-linking modification does not alter the granular structure of the starch, but methylation does alter it, the granular integrity being lost. The cross-linked corn starch shows a similar degree of hydrolysis by $\alpha$-amylose to that of the native corn starch. As a result, they should have similar digestibilities when cooked and ready to eat. In turn, methylation reduces the starch bio-availability by hindering enzymatic diges-
tion. All of these characteristics must be considered when using these modified corn starches.

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