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Production of Esterified Starches with Increased Resistant Starch Content by an α-Hydroxy Acid-Catalyzed Route

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Resistant starch (RS) refers to the portion of starch that is not broken down by human enzymes in the small intestine, and thus reaches the large bowel of healthy individuals. In the large bowel RS is fermented by colonic microflora producing short chain fatty acids (SCFA) – predominantly acetate, propionate, and butyrate – that are known to contribute substantially to large bowel health. Currently, there is an increasing interest in obtaining RS by esterification of starch with target SCFA, due to its capacity to deliver significant quantities of the particular esterified SCFA to the colon. In the current contribution an α-hydroxy acid-catalyzed methodology, which has recently proven useful for esterifying starch, was used to produce acetylated, propionylated, and butyrylated corn starch samples with varying degree of substitution (DS) (0.05–0.75). Results showed the suitability of the route to produce the RS4 starch type, with RS contents that increased with DS as a consequence of a larger steric hindrance effect caused by a higher number of ester groups introduced. Data also indicated that the DS conferred to starch was the key factor conditioning its resistance, whereas RS content showed to be independent of the resulting esterified starch structure and the type of ester group introduced.

1. Introduction

Starch is the main digestible carbohydrate and the most important source of energy for human nutrition. In general, starch is digested and absorbed progressively as free D-glucose in the small intestine.[1] However, there is a portion of starch, defined as resistant starch (RS), which is not broken down by human enzymes in the small intestine and reaches the large bowel of healthy individuals. There, RS becomes a source of carbon for colonic microflora through fermentation producing important metabolites.[2,3] The major end products of bacterial metabolism in the human large intestine are short chain fatty acids (SCFA) predominantly acetate, propionate, and butyrate.[4–7] Diverse studies have shown that these SCFA contribute substantially to large bowel health, playing a major role in preventing the production and absorption of potential carcinogens.[8–10] Then, beneficial effects may be obtained from the consumption of resistant starch because of the high yield of SCFAs when RS is fermented by human gut microbiota.[11]

Resistant starch has been classified (based on structure or source) into four main types, namely RS1, RS2, RS3, and RS4. RS1 refers to starch that is physically inaccessible to digestive enzymes due to the presence of intact cell walls in grains, seeds, tubers, and legumes.[5,12] RS2 refers to resistant starch that occurs in its natural granular form, such as that found in raw potato, unripe banana, some legumes, and in high-amylose starches. The compact structure of the raw starch granule limits the accessibility of digestive enzymes.[13] RS3 comprises retrograded starches. This type of RS can be found in starches or starch-based foods that have been cooked and subsequently cooled. In this process amylose segments align into condensed double helices leading to crystal formation, which hinders the accessibility of digestive enzymes to α-1,4 glucosidic linkages.[11,13] RS4 is produced by chemical modification, such as conversion, substitution (e.g., etherification and esterification), or cross-linking. The substituted groups may sterically block enzymatic attack because of the formation of atypical linkages upon the chemical modification.[5,7,14]

Among RS sources, RS4 starches resulting from the esterification of starch with acetate, propionate, and butyrate

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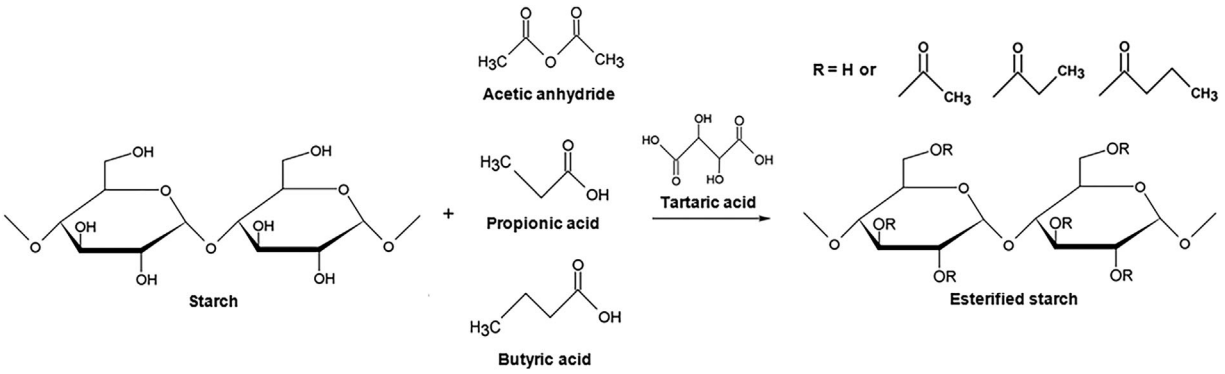

Scheme 1. Esterification of starch with acetic anhydride, propionic acid, and butyric acid catalyzed by tartaric acid.

groups are of particular interest, since it has been demonstrated that they have the potential to deliver significant quantities of the *specific esterified SCFA* to the large bowel for therapeutic, clinical, and public health applications.[15–17] Following the same strategy, other acids recognized for their chemopreventive activity on colon cancer (e.g., ferulic acid), have also been esterified to starch as a mean to deliver the target acid safely to the colon.[18,19] In a study of the effects of the starch type and the esterification methodology on the indices of large bowel fermentation using acetylated starch as a model delivery vehicle, it was confirmed that the greatest rise was in acetate, i.e., the SCFA esterified to the starch.[20] Similar conclusions were drawn for starch esterified with butyrate, which was shown to be able to significantly increase luminal free butyrate amounts far in excess of those produced by the unmodified counterpart.[21] Clarke et al.[22] also concluded that cooked butyrylated starch delivered esterified butyrate to the human colon effectively. In a study covering all target SCFA, Annison et al.[15] acylated maize starch with acetic, propionic, and butyric anhydride in hot dimethyl sulfoxide to a DS in the 0.16–0.20 interval, and fed the esterified starches to rats. Their analysis demonstrated that the produced starches were effective in raising total SCFA concentrations and pools throughout the large bowel, but the greatest increase was in the acid that had been esterified to starch. Highest colonic concentrations of the esterified SCFA are associated with the action of ubiquitous bacterial esterases and lipases which release the esterified SCFA in the colon. Fermentation of the residual starch further contributes to the increase in total SCFA levels.[23]

In the current study, based on their importance as RS4 sources, and particularly on their capability to deliver the specific SCFA to the colon, the suitability of a non-conventional sustainable esterification route to produce acetylated, propionylated, and butyrylated starches with increased resistant starch content was analyzed. Attention was particularly focused on the synthesis of starches esterified with propionate groups, since this specific SCFA has been reported to stimulate gluconeogenesis by functioning as a gluconeogenic substrate.[24] In spite of the previous, contributions on starch propionylation are scarce. Among them, propionylation methodologies catalyzed by 1-methylimidazole reaching a degree of substitution (DS) of 0.16,[15] sodium hydroxide leading to DS values of up to 0.94,[25,26] or pyridine with DS up to 2.51[27–29] have been proposed. Esterification of starch with acetate and butyrate groups have mainly relayed on the same routes. However, all of those catalysts have some limitations, related to their handling, toxicity, wastewater treatment, or required preactivation steps. Alternatively, in a recent contribution of our group propionylation of starch catalyzed by L-tartaric acid (which is a naturally occurring α-hydroxy acid found in grapes, apricots, and tamarinds) was proposed.[30] Acetylation and butyrylation of corn starch catalyzed by L-tartaric acid have also been reported recently.[31]

In the current contribution, the proposed methodology was adapted to esterify corn starch under pre-optimized conditions (**Scheme 1**) and assay the suitability of the methodology to produce resistant starch for specific delivery of target SCFA. With this aim, starches esterified with acetate, propionate, and butyrate groups with varying DS were prepared, and the effects of derivatization extent, starch crystallinity (native vs. pregelatinized starches were assayed), and the type of ester group introduced on the resulting resistant starch content were all evaluated. To get insight on the effects of derivatization on starch structure, and its concomitant impact on the resulting resistant fraction, chemical structure (solid state ^{13}C CP/MAS-NMR and FTIR), morphology (SEM), and crystallinity (XRD) of the esterified starches as a function of DS were also analyzed.

2. Experimental Section

2.1. Materials

Native and pregelatinized corn starches were generously provided by Ingredion, Argentina (commercial products Buffalo 4301 (28% amylose, 72% amylopectin, 0.05% lipids) and Amidex G 2100, respectively). Pancreatic α-amylase from porcine pancreas (Pancreatin, 3 Ceralpha Units/mg) and amyloglucosidase from *Aspergillus niger* (3300 U mL^{-1} on soluble starch at pH 4.5 and 40 °C) were provided by Megazyme (Granotec, Argentina). Reagents used in starch esterification and DS quantification were propionic acid (94.5%, Cicarelli), acetic anhydride (≥97.0%, Cicarelli), butyric acid (≥99.0%, Sigma–Aldrich), L-tartaric acid (≥99.0%, Biopack), hydrochloric acid (36.5–38.0%, Anedra), sodium hydroxide (98.0%, Biopack), potassium hydrogen phthalate (99.5%, Cicarelli), and sodium carbonate anhydrous (Mallinckrodt). Maleic acid (≥99.0%, Sigma–Aldrich), calcium chloride dehydrate (Stanton), sodium

azide (Stanton), potassium hydroxide (Anedra), and ethyl alcohol (Stanton) used in RS determination were all of analytical grade.

2.2. α-Hydroxy Acid-Catalyzed Esterification of Starch

Propionylation of corn starch was performed using propionic acid as acylant and L-tartaric acid as catalyst, as an adaptation of the methodology previously reported by our group.[30] Briefly, dried (105 °C, 2 h) corn starches (2 g, 12.3 mmol AGU), L-tartaric acid (6.7 mmol), and propionic acid (160 mmol) were mixed in an oven-dried 100 mL glass flask equipped with a magnetic stir bar and a reflux condenser. The mixture was then heated to 130 °C with continuous agitation in a thermostatized oil bath. When the mixture reached the chosen temperature, the α-hydroxy acid was completely dissolved in the acylant, and that was considered as the beginning of reaction. Reaction was then run for different time intervals within the 1–5 h range. In order to inspect the effect of the ester group introduced in the RS content of esterified starches, chosen acetylation and butyrylation assays were also carried out. The syntheses were performed using acetic anhydride and butyric acid as acylants and tartaric acid (6.7 mmol) as catalyst, and operating in similar conditions as those described for starch propionylation.[31,32] After the chosen reaction time, 20 mL of ethanol were added into the vial, and the solid product was then separated by vacuum filtration in a Buchner funnel. Several washings (six) with ethanol to guarantee catalyst and unreacted acid removal were performed prior to final drying at 40–50 °C overnight. Assays were run in duplicate. The efficiency of catalyst and acylant removal was checked out by ^{13}C solid state RMN and FTIR (i.e., signals typical of carboxylic acids, see Section 3.1) and determination of free acidity by use of the protocol recommended in ASTM D871 for cellulose acetate (Ref. [33], adapted). Briefly, carefully dried esterified samples (0.5 g) were shaken in stoppered 50 mL Erlenmeyer flasks with 15 mL of freshly boiled cold water during 3 h. After this time, the samples were recovered by filtration and washed with the same freshly boiled cold water. The combined filtrate and washings were titrated with 0.01 N NaOH solution using phenolphthalein indicator solution. A blank determination on boiled cold water using the same total volume used for extracting the sample was also run.

2.3. Determination of Substitution Degree

The degree of substitution (DS) of esterified starches was determined by heterogeneous saponification and back titration with HCl as described previously.[31] Briefly, 0.1 g (dry basis) of esterified starch was contacted with 20 mL of ethyl alcohol (75%) in 100 mL Erlenmeyer flasks which were heated loosely stoppered during 30 min at 50 °C. Afterwards, 2–3 drops of 0.1 N NaOH were added to bring the suspensions to slightly basic pH, using phenolphthalein solution (1% w/w) as end-point indicator. Subsequently, 20 mL of 0.1 N NaOH were added to each flask, which were heated again at 50 °C for 15 min. The flasks were finally allowed to stand tightly stoppered at room temperature for 48 h, after which the excess of NaOH was back titrated with 0.1 N HCl. Blank determinations (unmodified native and pregelatinized corn starches) were carried through the complete procedure. NaOH and HCl solutions were standardized using previously dried standard potassium hydrogen phthalate and sodium carbonate, respectively. The acyl content was then calculated by:

$$\text{Acyl}(\%) = \left[(V_B - V_S) \times N_{HCl} \times M_{acyl} \times 10^{-1}\right]/W \quad (1)$$

where V_B (mL) is the volume of HCl required for titration of the blank; V_S (mL) is the volume of HCl required to titrate the sample; N_{HCl} is the normality of the HCl solution; M_{acyl} is the molecular weight of the acyl group (43 g mol^{-1} for acetyl, 57 g mol^{-1} for propionyl, and 71 g mol^{-1} for butyryl); and W (g) is the mass of sample used. The degree of substitution of esterified starches (DS) was then calculated according to Eq. (2):

$$DS = 162 \times \text{Acyl } \% / \left[M_{acyl} \times 100 - \left((M_{acyl} - 1) \times \text{Acyl } \%\right)\right] \quad (2)$$

2.4. Resistant Starch Determination

Samples (100 mg) were incubated in a shaking water bath with pancreatic α-amylase (4 mL, 10 mg mL^{-1}) containing amyloglucosidase (3 U mL^{-1}) at 37 °C for 16 h, in order to solubilize and hydrolyze non-resistant starch to D-glucose, as described in the AOAC Method 2002.02 (Megazyme). The reaction was terminated by the addition of an equal volume of absolute ethanol, and the RS was recovered as a pellet by centrifugation (3000 rpm, 10 min). The supernatants were decanted and the pellets were resuspended in 8 mL of 50% ethanol and further washed twice, followed by centrifugation (3000 rpm, 10 min). The supernatants were decanted and the pellets were dried at 45 °C till constant weight; this value was taken as a measure of resistant starch content. Systematic check out of the RS content of a control provided by Megazyme (Resistant starch control, RS = 44% dwb, Resistant starch kit, Megazyme), always showed error values ≤2.5%.

2.5. Characterization of Starch Esters

2.5.1. Solid-State ^{13}C CP/MAS-NMR Spectroscopy

High-resolution ^{13}C solid-state spectra of grinded samples were obtained using the ramp $\{^1H\} \rightarrow \{^{13}C\}$ CP/MAS pulse sequence (cross-polarization and magic angle spinning) with proton decoupling. All experiments were recorded at room temperature in a Bruker Avance II-300 spectrometer equipped with a 4-mm MAS probe. The operating frequency for protons and carbons was 300.13 and 75.46 MHz, respectively. Glycine was used as an external reference for the ^{13}C spectra and to set the Hartmann–Hahn matching condition in the cross-polarization experiments. The recycling time varied from 5 to 6 s according to the sample. The contact time during CP was 2 ms for all of them. The SPINAL64 sequence (small phase incremental alternation with 64 steps) was used for heteronuclear decoupling during acquisition with a proton field H1H

satisfying $\omega 1H/2\pi = \gamma HH1H = 62$ kHz. The spinning rate for all the samples was 10 kHz.

2.5.2. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectra of native and esterified starch samples were acquired on an IRAffinity-1 Shimadzu Fourier transform infrared spectrophotometer in absorption mode. Dried (105 °C, 2 h) powdered samples were mixed with previously dried KBr in a 1:20 ratio. Spectra of KBr pellets were obtained using 40 scans in the range of 4000–650 cm^{-1} with a resolution of 4 cm^{-1}. Finally, spectra were normalized using the signal at 1025 cm^{-1}, corresponding to the stretching vibration of the acetal of the anhydroglucose unit.[34]

2.5.3. Scanning Electron Microscopy (SEM)

Drops of neat and esterified starch suspensions were mounted on microscope glasses and dried at 40 °C for 15 min. Samples were then coated with gold using an ion sputter coater, and observed in a scanning electron microscope Zeiss Supra 40 with field emission gun at magnification of 500× and 2000× at 3 kV.

2.5.4. X-Ray Diffraction (XRD) Measurements

The structure of dried samples (105 °C, 2 h) was analyzed with a D/Max-C Rigaku automated wide-angle powder X-ray diffractometer. The operating conditions were 40 kV and 30 mA, with Cu/Kα radiation ($\lambda = 0.154$ nm). XRD diagrams were recorded in a 2θ angle range of 10–45° with a step of 0.02° at 0.6° min^{-1}.

3. Results and Discussion

3.1. Preparation and Characterization of Starch Esters

Aiming to produce propionylated starches with varying DS, esterification of corn starch with propionic acid catalyzed by L-tartaric acid was carried out during varying reaction times (i.e., 1–5 h, see Section 2.2 for details on other reaction conditions). The effect of the initial structure of corn starch on the DS values attained was explicitly evaluated by assaying native corn starch versus pregelatinized corn starch, both commercially available. The results obtained as a function of time are shown in **Figure 1**.

As it is shown in Figure 1, for both corn starches the increase in reaction time within the chosen interval resulted in a continuous rise in the derivatization extent achieved, reaching in 5 h DS values of 0.46 and 0.69 for native and pregelatinized corn starch, respectively. The higher susceptibility of pregelatinized starch over native starch was evident for all time intervals assayed, a phenomenon associated with a higher accessibility of the acylant and the dissolved catalyst to regions of low crystalline order. Increased DS values resulting from partial gelatinization of the starch prior to esterification have previously been reported.[23]

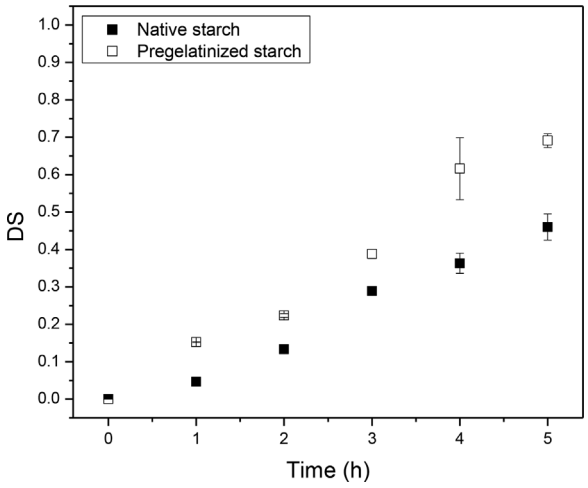

Figure 1. Effect of the reaction time on the organocatalytic propionylation of native and pregelatinized corn starches using propionic acid as acylant and L-tartaric acid as catalyst, 130 °C.

Propionylation of native and pregelatinized corn starches was further confirmed by Nuclear magnetic resonance and Fourier transform infrared spectroscopies. The ^{13}C CP/MAS-NMR spectra of chosen propionylated starches (i.e., samples from 4 h of reaction) in comparison with the spectra of both unmodified starches are shown in **Figure 2**. Neat starches spectra showed signals corresponding to carbons resonances characteristic of starch, namely C-1 site in the 90–107 ppm range, C-4 in the 79–87 ppm interval, C-2,3,5 in the highly overlapped 65–80 ppm region, and C-6 in the 56–65 ppm range. Signal intensity between 93 and 101 ppm observed in all spectra is attributed to the contribution of amorphous material.[35] In the case of semicrystalline native corn starch, the C-1 peak appeared as a triplet with peaks at 102.4, 101.1 (maximum), and 99.7 ppm, indicating a double helical A-type crystalline polymorph typical

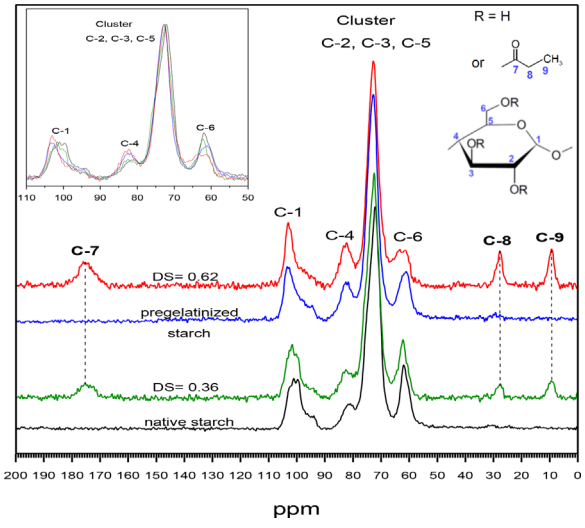

Figure 2. Solid-state ^{13}C CP/MAS-NMR spectra of unmodified and propionylated corn starches (4 h).

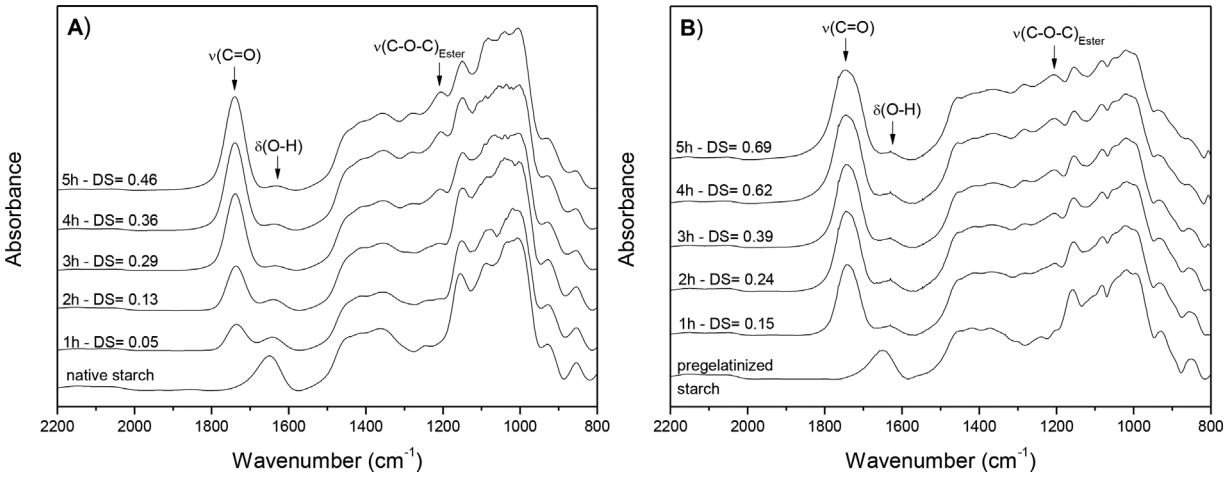

Figure 3. FTIR spectra of neat and propionylated corn starch samples obtained from native starch (A) and pregelatinized starch (B).

of cereal starch.[35,36] On the other hand, in neat pregelatinized starch the C-1 resonance appeared as a single peak at 103.2 ppm, which can result either from V-type structures which give rise to signals at 103–104 ppm, or from amorphous materials which have substantial C-1 signal intensity in the same range. Corn starch, as all other amylose-containing cereal starches contain a small (<1.2%) but significant percentage of lipids, which readily associate with the amylose component of starch to form complexes known as V-type structures. Similar structures are formed in the presence of a wide variety of other complexing agents such as iodine, alcohols, ketones, etc.[35,37] Compared with native corn starch, pregelatinized starch evidenced a higher intensity at the C-4 site, which is associated with a higher contribution of amorphous material or single helical V-type structures, which both give rise to signals centered at 82–84 ppm (Figure 2, inset). The increase in the relative intensity of the peaks at 82 and 103.3 ppm upon gelatinization of starch is well established.[38,39] Pregelatinized corn starch also evidenced a relative decrease in the intensity of the C-6 site, which has been associated with a higher rotational mobility of this side group in gelatinized starch, which makes the cross-polarisation from the ^{13}C to the protons connected to it, less efficient.[39]

After starch propionylation, new chemical shifts were observed in Figure 2 for both native and pregelatinized starch, i.e., a peak at 173 ppm assignable to the resonance of the carbonyl ester peak of propionate, and two additional peaks at 27 and 9 ppm attributable to the CH_2 and CH_3 of propionate groups, respectively. These observations confirmed successful propionylation of both corn starches, and discarded the presence of residual free propionic acid (the corresponding C═O signal would appear centered at 180 ppm).

Esterification was also confirmed by FTIR. A zoom of the region between 2200 and 800 cm^{-1} of the FTIR spectra of propionylated starch samples with varying DS is shown in **Figure 3**. Neat starch samples showed bands characteristic of the polysaccharide, namely stretching of hydrogen bonded O—H groups (3700–3000 cm^{-1}), C—H stretching modes (3000–2800 cm^{-1}), O—H bending vibration of absorbed water molecules (1642 cm^{-1}), C—O—H bending, CH_2 twisting, CH_2 bending and C—O—O stretching (1500–1300 cm^{-1}), CH_2OH (side chain) related mode/C—O—H deformation mode (1240 cm^{-1}), coupling mode of C—O and C—C stretching (1158 cm^{-1}), C—O—H bending mode (1084 cm^{-1}), C—O stretching (1055 cm^{-1}), skeletal mode vibrations of the α-1,4 glycosidic linkage (923 cm^{-1}), C—H and CH_2 deformation (857 cm^{-1}), and C—C stretching (763 cm^{-1}).[40–44] IR spectra of both propionylated starches clearly evidenced the esterification reaction that took place by the appearance of new signals associated with propionate groups vibrations. The most significant new absorption appeared at ≈1740 cm^{-1} (1739 cm^{-1} for propionylated native starch samples, Figure 3a; and 1745 cm^{-1} for propionylated pregelatinized starch samples, Figure 3b); which corresponds to the stretching vibration of the carbonyl group C═O of the ester group introduced. The intensity of this new signal strengthened with the increment of DS. The esterification reaction was also confirmed by the appearance of an additional signal at 1205 cm^{-1}, attributed to the C—O—C stretching vibration of the ester groups introduced.[26] The increase of the mentioned band with DS was especially noticeable for propionylated starches obtained from native starch. The absence of overlapping absorbances at 1710 cm^{-1} further confirmed the removal of residual propionic acid.

Figure 4 collects crystallinity and morphology results from the effect of L-tartaric acid-catalyzed propionylation on native and pregelatinized corn starch as a function of DS. Native corn starch showed strong diffraction peaks at $2\theta = 15$, 17.0, 17.8, 19.8, and 22.7°, which in line with the corresponding ^{13}C CP/MAS-NMR spectrum (Figure 2), indicated an A-type pattern, characteristic of cereal starches.[45] As shown in Figure 4a, the intensity of the crystalline peaks of native corn starch diminished with the esterification extent. The phenomenon was particularly evident for DS values ≥0.13, in accordance with SEM micrographs which evidenced a progressive loss of the original poliedric shape of the granules. The changes in the diffraction patterns observed for increasing DS values indicated that the intermolecular hydrogen bonding interaction was progressively damaged, which can be attributed to the introduction of bulky ester groups which can disrupt the internal structure and weaken the granule.[30,41]

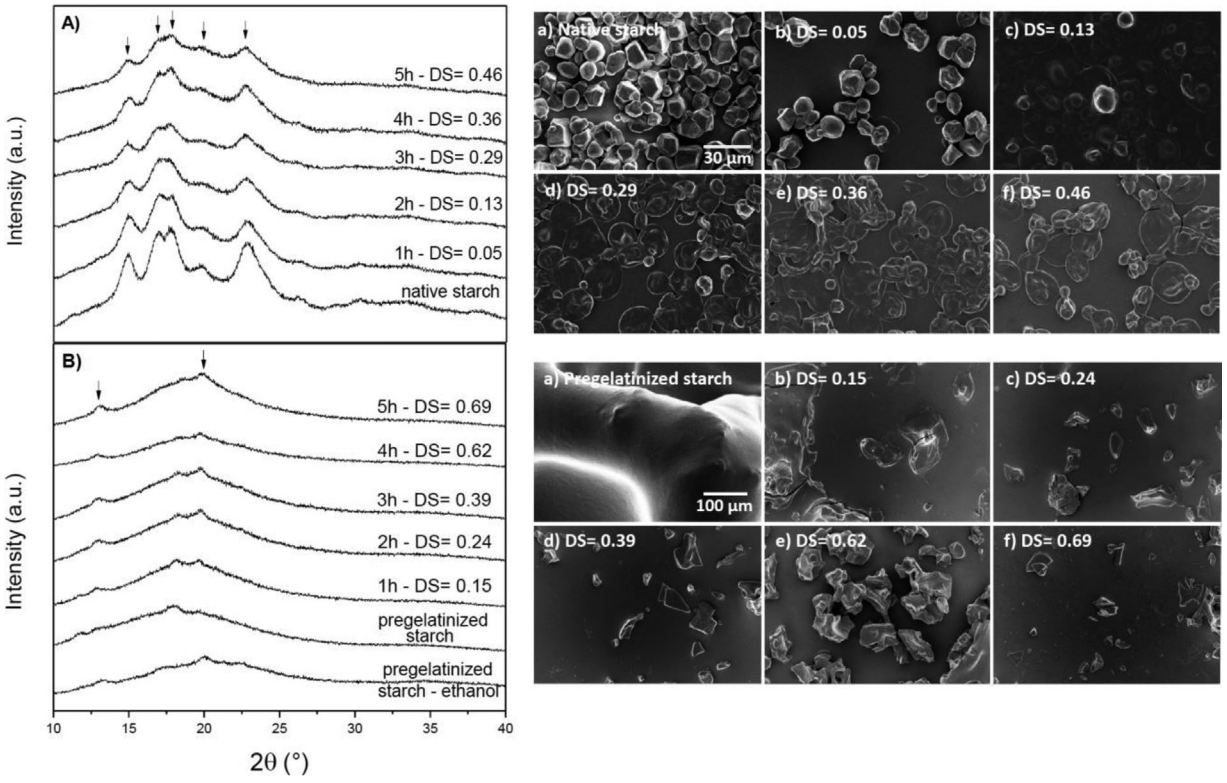

Figure 4. Left: X-ray diffractograms of propionylated starches obtained from (A) native and (B) pregelatinized corn starch. Right: SEM micrographies of propionylated starches with increasing DS.

Figure 4b collects XRD data from neat and esterified pregelatinized starch. Commercial pregelatinized starch is generally obtained by a roll drying procedure where the gelatinized starch is dried quickly. The process of gelatinization causes substantial changes in granular starch due to the rearrangement of intra and intermolecular hydrogen bonding between the water and starch molecules, resulting in the collapse or disruption of molecular orders (dissociation of the double helices) within the starch granule.[46] Accordingly, pregelatinized corn starch yielded a highly amorphous pattern in which only very weak reflections at $2\theta = 12.0$, 12.9, and 18° were detected (Figure 4b). X-ray diffraction patterns of propionylated samples evidenced a slight increase in the reflection at 12.9°, as well as the appearance of a weak diffraction peak at 19.7°; both indicating the contribution of single helical V-type structures resulting from the formation of amylose complexes in gelatinized starches.[35,47,48] Besides the amylose–lipids complexes originally present in the unmodified pregelatinized starch, amylose–ethanol complexes formed during recovery operations might have also contributed to the weak typical V-type reflections shown in propionylated starch XRD patterns (notice the appearance of the mentioned reflection at 19.7° in commercial pregelatinized corn starch when gelatinized and precipitated with ethanol, Figure 4b lowest diffractogram "pregelatinized starch-ethanol"). In terms of morphology, the absence of intact starch granules in the unmodified pregelatinized starch sample indicated the fully gelatinization during heating and drying over the drum driers. After reaction and upon ethanol addition, starch esters were recovered as aggregates with no particular morphology.

Previous studies on the effect of amylose complexes on the enzyme susceptibility of different starches concluded that the complex formation reduces the digestibility of starch as a consequence of the lower swelling of starch granules and the steric hindrance exerted by the complexes.[37,49,50] In fact, in a recent contribution, Birt et al.[2] named the amylose complex as RS5, claiming that starch binding and cleavage by amylase are prevented when the linear starch chain is in a helical-complex structure.

3.2. Resistant Starch Content of Propionylated Starches

Amylose content, granule size, architecture, crystalline pattern, degree of crystallinity, presence of surface pores or channels, degree of polymerization, and nonstarch components of unmodified starch, all influence its digestibility.[51,52] Moreover, chemical modification of starch is known to be able to significantly affect the rate and extent of starch digestibility in the small intestine, with its effect depending on starch source, the type and degree of modification, and the extent of starch gelatinization/granules integrity.[53–55] In the current section, the resistant starch content of propionylated corn starch samples with varying degree of substitution and with previously demonstrated differences in terms of crystallinity was evaluated.

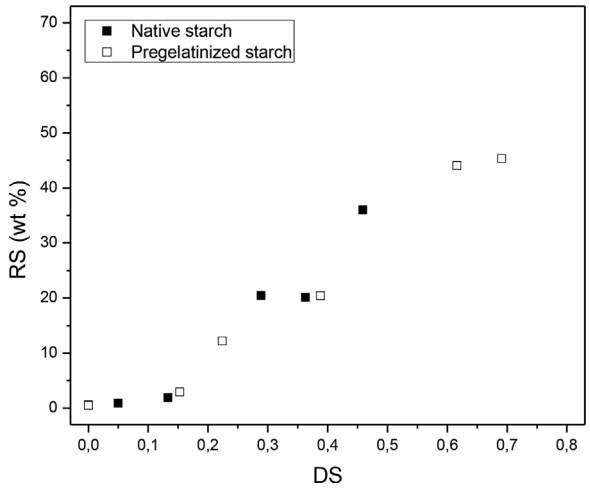

Figure 5. Resistant starch (RS) of propionylated starch samples as a function of their degree of substitution (DS).

Figure 5 shows the RS content determined for unmodified starches (0.62 and 0.48% for native and pregelatinized corn starch, respectively), and L-tartaric acid-catalyzed propionylated starch samples obtained in Section 3.1 as a function of their substitution degree. The analysis of RS versus DS data leads to several conclusions. In the first place, data confirmed that under the reaction conditions chosen, tartaric acid-catalyzed propionylation of corn starches could significantly rise their RS content, with RS values of up to 45 wt.%. Increased RS content of esterified starches is associated with a sterically hindered attack of digestive enzymes caused by the substituents which prevent the formation of the enzyme–substrate complex making neighboring bonds resistant to degradation.[56] In the colon, the esterified acids will be liberated by ubiquitous bacterial esterases and lipases, leaving the residual starch backbone available for subsequent fermentation, further increasing total luminal SFCA levels.[9,15–17]

Secondly, data included in Figure 5 indicate that for propionylated starches (both native and pregelatinized) with DS $\geq$ 0.1, an strong positive correlation between RS and DS was found, suggesting that manipulation of reaction conditions to modulate DS may also allow tailoring the RS content of the resulting esterified starches. A greater stereohindrance effect as a higher number of ester groups is introduced justifies the increase of RS with DS, as previously observed by other authors dealing with starch esterification by other routes.[53,57]

Lastly, Figure 5 suggests that even if the initial crystallinity of starch played a key role in its susceptibility to organocatalytic propionylation (Figure 1), samples esterified to a similar DS had equal RS content irrespectively of their crystallinity and corresponding granules integrity (Figure 4). Previous contributions have pointed out the importance of starch structure on its digestibility, with crystalline regions of the polysaccharide being more resistant to amylase hydrolysis than amorphous regions.[58,59] Actually, the digestion of gelatinized starch molecules is recognized to be much faster than that of raw starch granules, due to an increased accessibility of enzymes upon destruction of the structure of the starch granule.[52,60] In esterified starches, the importance of starch structure on RS content was also raised by Sha et al.[53] who claimed that despite the hindering effect of ester groups, the increase in DS could result in the crystalline region of the granules being disrupted or collapsed, leading to the reduction of their resistance to enzymatic degradation. However, with the protocol used herein, and despite the very different structure of the products shown in Figure 4 (and maybe also a different amylose/amylopectin ratio resulting from gelatinization), the DS of the propionylated corn starch samples showed to be the only key factor to determine their resistant starch content.

3.3. Resistant Starch Content of Starch Acylated with SCFA

As previously introduced, besides being a source of resistant starch, starches esterified with acetate, propionate, or butyrate groups have the additional benefit of being a tool to deliver SCFA specifically.[23] In the colon, the target-esterified acids are liberated by ubiquitous bacterial esterases and lipases, which is of particular importance for individuals whose large bowel microflora is unable to ferment certain types of RS. Although chemically similar, short chain fatty acids are metabolized differently and have been reported to exert very different effects on host physiology, i.e., acetate is a substrate for hepatic de novo lipogenesis and cholesterol biosynthesis, propionate is a substrate for hepatic gluconeogenesis, and butyrate acts as an energy substrate for enterocytes lining the colon.[24] Given the results obtained in the previous section in the propionylation of corn starch, the L-tartaric acid-catalyzed esterification protocol was herein extended to the production of acetylated and butyrylated corn starches with increased RS content. **Figure 6** summarizes RS results as a function of DS and the SCFA introduced.

Results included in Figure 6a clearly evidenced that no matter the target SCFA introduced by the proposed route, for an equal starch esterification extent the resulting RS content was similar. The behavior observed thus suggests that acetate, propionate and butyrate all exerted an analogous blockade effect, sterically hindering the formation of the enzyme-substrate complex and thus increasing the RS fraction to a similar extent. Moreover, results confirmed that at least for $0.1 \leq DS \leq 0.8$ (Figure 6a), the RS content of corn starch esterified with SCFA by the proposed methodology was positively correlated with DS (actually, a clearer linear correlation than in Figure 5 was observed), again highlighting its importance to modulate the resistant starch content. On the other hand, Figure 6b (corn starch samples acetylated to greater DS values have been intentionally included), illustrates that for higher DS values (DS $\geq$ 1.5) the linear RS–DS relationship was lost, and acetylated corn starch samples showed constant RS contents of ≈90 wt.%. Constant RS values attained at sufficiently high DS may be associated with a minimum number of ester groups required to block most digestive enzyme–starch complexes formation, and thus maximize RS content. Figure 6b suggests that for acetylated starch this value is close to a DS of 1.0–1.5, indicating that in average not more than ≈one half of the hydroxyls group of starch need to be acetylated to make 90 wt.% of the ingested starch resistant to digestion. Further studies on the RS content of highly esterified starches would be of help to get more basic insight on this issue.

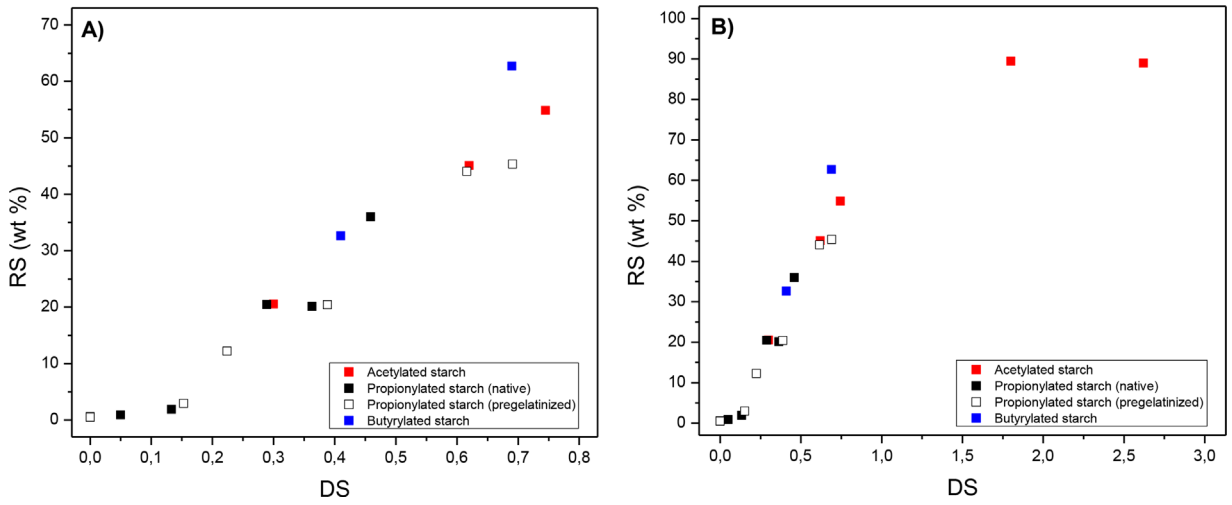

Figure 6. Resistant starch (RS) content as a function of DS of native corn starch esterified with selected SCFA.

4. Conclusions

Bacterial fermentation of undigested resistant starch in the colon of healthy humans is known to produce SCFA (principally acetate, propionate, and butyrate), all recognized for their collective action to maintain the normal physiologic function of the large bowel. Among resistant starch sources, starch acylated with the target SCFA is of especial interest based on its proved capacity to deliver and specifically release the esterified SCFA. Fermentation of the residual starch further contributes to raising total SCFA concentration in the large bowel.

In the current contribution, a non-conventional esterification protocol involving a naturally occurring α-hydroxy acid as catalyst was used to acylate corn starch with acetate, propionate and butyrate groups at varying derivatization extents. The results obtained in terms of the resistant starch content of propionylated starch samples of varying DS, clearly indicated that tartaric acid-catalyzed esterification could certainly produce starch with increased RS content, and with its value showing a linear correlation with DS (valid at least for DS $\leq$ 0.8). Moreover, the measured RS values showed not to be dependent on the very different structures of raw and pregelatinized propionylated corn starches; but only on the extent of derivatization achieved. Finally, the extension of the esterification route to the acylation of corn starch with acetate and butyrate groups suggested that for a definite DS the corresponding RS content was also independent of the nature of the ester group introduced. Overall, the current basic study highlighted the possibility of easily preparing target SCFA-acylated starches with tailored RS by proper manipulation of the esterification level conferred to the polysaccharide.

Further studies devoted to assaying the suitability of the tartaric acid-catalyzed route for obtaining RS4 starch from other botanical sources (e.g., starches with higher intrinsic RS content, whose esterification within DS values allowed by the FDA could be of interest) are currently in progress. Moreover, the contribution to RS content of potential crosslinking of starch with tartaric acid is also under analysis, mainly in view of slight acidity values determined for propionylated samples (data not shown) which highlighted the possibility of tartaric acid been esterified to starch (at least by one side).

Abbreviations

DS, degree of substitution; RS, resistant starch; SCFA, short chain fatty acids.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

α-hydroxy acids, esterification, resistant starch, starch

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