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Level and position of substituents in cross-linked and hydroxypropylated sweet potato starches using nuclear magnetic resonance spectroscopy

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ABSTRACT

Sweet potato starch was cross-linked using sodium trimetaphosphate and hydroxypropylated using propylene oxide. The level and position of phosphorus and hydroxypropyl groups within cross-linked and hydroxypropylated sweet potato starch was investigated by phosphorus and proton nuclear magnetic resonance spectroscopy (^{31}P, ^{1}H NMR). The cross-linking reaction produced monostarch monophosphate and distarch monophosphate in a molar ratio of 1:1.03, indicating that only half of the introduced phosphorus resulted in a possible cross-link. One cross-link per approximately 2900 glucose residues was found. Phosphorylation leading to monostarch monophosphate mainly occurred at O-3 and O-6 (ratio 1:1). It was inferred that the majority of the cross-links formed in distarch monophosphate were between two glucose residues positioned in different starch chains, while a minor part of the cross-links may be formed between two glucose residues within the same starch chain. Hydroxypropylation under alkaline conditions resulted in the formation of intra-molecular phosphorus cross-links, subsequent hydroxypropylation following cross-linking lowered both the level of intra- and inter-molecular cross-linking.

Using ^{1}H NMR the molar substitution of hydroxypropylation was determined to be 0.155–0.165. The hydroxypropylation predominantly occurred at O-2 (61%), and the level of substitution at O-6 (21%) was slightly higher than that at O-3 (17%). In dual modified starch, the preceding cross-linking procedure resulted in a slightly lower level of hydroxypropylation, where the substitution at O-6 decreased more compared to the substitution at O-2 and O-3.

1. Introduction

Starches are often modified to improve their functional properties for specific food applications. Cross-linking and hydroxypropylation are two important chemical modifications commonly used in the starch industry (Goff, 2004). For cross-linking, starch can be treated with a variety of multiple functional reagents, such as phosphorus oxychloride ($POCl_3$), sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), or epichlorohydrin (EPI), forming either ester or ether cross-links between starch through their hydroxyl groups (Singh, Kaur, & McCarthy, 2007). Cross-links between starch chains reinforce the starch granule resulting in a good stability against heating, shear force and acid conditions (Wurzburg, 1986). Hydroxypropylation is one of the methods to make starch more stable towards retrogradation and represents a base-catalysed nucleophilic substitution reaction of propylene oxide and starch. Hydroxypropylation is used to increase paste consistency and clarity, and to improve freeze-thaw and cool-storage stabilities (Tuschhoff, 1986). Cross-linking of starch is often combined with hydroxypropylation (Wurzburg, 1986).

Even a very few cross-links can significantly change the paste and gel properties of starch (Singh et al., 2007). For controlling the modification reactions and hence, to control and optimise the production and use of modified starches, it is necessary to be able to correlate the extent and location of cross-links along the starch backbone with the functionality obtained. Depending on the type

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of starch, phosphorus may be present in the native starch at a low level (0.002–0.089%, w/w, dry basis) and may be divided over different ester forms (Dona et al., 2007; Jane, Kasemsuwan, Chen, & Juliano, 1996). Depending on starch source, phosphorus can even be present at low levels as phosphate or phospholipids. Therefore, the accurate determination of the level and location of phosphorylation within the starch is rather complicated. During the crosslinking reaction using STMP, distarch phosphate cross-links are formed, while also monoesters are formed as monostarch monophosphate (MSMP) without connecting starch molecules (Singh et al., 2007). For this reason, the spectrophotometric method to determine the degree of cross-linking according to the change in phosphorus content before and after cross-linking reaction might not be correct (Kasemsuwan & Jane, 1996).

^{31}P nuclear magnetic resonance (NMR) spectroscopy has been used to analyse the location of phosphorylation in native and modified starches. Phosphorus present in different forms results in different chemical shifts in the NMR spectrum. The results of phosphorus quantified by ^{31}P NMR show that phosphate monoesters were primarily present in potato, mung bean, and tapioca starch (0.0062–0.086%), while phospholipids were mainly present in wheat, maize, and high amylose maize starch (0.0097–0.058%) (Kasemsuwan & Jane, 1996). The phosphorylation of wheat starch prepared with sodium tripolyphosphate under semidry conditions was mainly at the O-6 position of glucose units with concomitant lower extents of phosphorylation at the O-3 and O-2 positions (Lim & Seib, 1993). In phosphorylated cross-linked wheat starch prepared with sodium trimetaphosphate and sodium tripolyphosphate, the molar ratio of distarch monophosphate and monostarch monophosphate formed was around 1:1 (Sang, Prakash, & Seib, 2007).

^{1}H NMR spectroscopy has also been used to determine the substitution of hydroxypropyl groups in hydroxypropylated starch. It has been shown that hydroxypropylation of the glucose unit occurred primarily (67–79%) at the O-2 position for potato, wheat and maize starch (Richardson, Nilsson, Bergquist, Gorton, & Mischnick, 2000; Xu & Seib, 1997). However, previous studies focussed only on the hydroxypropylation of single modified starch and the effect of two subsequent modifications on the achieved level of cross-linking and hydroxypropylation is still unknown.

The aim of this study was to investigate the level of modification and the position of substituents on glucose residues in cross-linked and/or hydroxypropylated sweet potato starches. The starches were hydrolysed with enzymes and the hydrolysates were analysed with ^{31}P and ^{1}H NMR.

2. Materials and methods

2.1. Starch samples

Native sweet potato starch (NT-SPS, SuShu2 species) was isolated as described previously (Chen, Schols, & Voragen, 2003). The modified starches were prepared at the AVEBE facilities and all modifications were conducted in aqueous suspensions of the granular starches (Chen et al., 2003). The cross-linked sweet potato starch (CL-SPS) was obtained by adding 0.002 mol sodium trimetaphosphate per mole glucose residue within the starch. The hydroxypropylated sweet potato starch (HP-SPS) was prepared by addition of 0.2 mol propylene oxide per mole glucose residue. The dual modified starch (cross-linked and hydroxypropylated sweet potato starch, CLHP-SPS) was prepared first with sodium trimetaphosphate, followed by the propylene oxide treatment (Zhao et al., 2012). The glucose/starch content was measured as described previously (Zhao et al., 2012).

Crystallised *Bacillus* sp. α-amylase (Type II-A, A6380, ≥1500 units/mg protein), and Rhizopus sp. amyloglucosidase (A7255, 11,600 units/g) were purchased from Sigma-Aldrich (St. Louis, MO, USA). One unit of α-amylase will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9, 20 °C. One unit of amyloglucosidase will liberate 1.0 mg of glucose from soluble starch in 3 min at pH 4.5, 55 °C. Nicotinamide adenine dinucleotide (NAD) was obtained from Boehringer Mannheim (Germany). Sodium azide, Ethylene diamine tetraacetic acid disodium salt dehydrate were purchased from Merck (Darmstadt, Germany). Deuterium oxide (99.9 atom% D) was from Sigma–Aldrich.

2.2. Sample preparation for ^{31}P NMR analysis

Each starch sample was converted to α, γ-limit dextrins by enzymatic digestion before ^{31}P NMR spectroscopy according to published methods (Sang et al., 2007) with minor modification. Starch (1 g, dry basis) was dispersed in 45 mL of distilled water containing 2.0 mM calcium chloride ($CaCl_2$). Alpha-amylase (200 units) was added to the mixture, followed by gradual heating in a boiling shaking water bath for 15 min to enable gelatinisation with vigorous stirring in 3 min intervals. The suspension was cooled down and the pH was adjusted to 6.5. Then, additionally, 200 units of α-amylase were added and the mixture was incubated at 70 °C for 1 h. The hydrolysate was adjusted to pH 4.5 with 0.5 M hydrochloric acid (HCl) and amyloglucosidase (200 units) was added and the mixture was sequentially incubated at 60 °C for 2 h in a shaking water bath. Next, the mixture was boiled for 15 min to inactivate the enzymes. After cooling to ambient temperature, the mixture was adjusted to pH 7; centrifuged (3000 × *g* for 10 min) and the supernatant obtained was freeze-dried. The recovered soluble hydrolysates accounted at least for 97% weight (dry basis) of original samples and allowed to make high concentrations of starch with relative low viscosity in order to accelerate the NMR measurement and to obtain sharp peak signals.

2.3. Sample preparation for ^{1}H NMR analysis

Each starch (0.1 g, dry basis) was converted to α-limit dextrins by α-amylase treatment before the ^{1}H NMR measurement, using the similar treatment as described above leaving out the amyloglucosidase digestion. The recovered soluble hydrolysates accounted for at least 95% weight (dry basis) of the original samples.

2.4. ^{31}P NMR

The freeze-dried starch hydrolysates were mixed with 1.5 mL distilled water, 0.2 mL nicotinamide adenine dinucleotide solution (5 mg/mL), 0.2 mL ethylene diamine tetraacetic acid, disodium salt dihydrate (EDTA, 0.2 M), 0.05 mL sodium azide (NaN_3, 1 mg/mL), and 0.2 mL deuterium oxide. The solution was adjusted to pH 8 ± 0.1 by adding 0.5 M sodium hydroxide, and then transferred to a 10 mm NMR tube (Wilmad-LabGlass, Vineland, NJ, USA).

The proton decoupled ^{31}P NMR spectra were acquired on a Bruker AVANCE 300WB NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany) equipped with a 10 mm probe operating at a frequency of 121.5 MHz. For each spectrum, 2500–6000 transients were collected with 10 s relaxation delay between pulses at a temperature of 25 °C. The spectra were processed with Bruker TopSpin 1.3 software. Chemical shifts are reported in δ (ppm) based on the external reference signal of 85% phosphoric acid at 0 ppm. The NAD signal (−10.9 ppm) was used as internal reference for the calculation of phosphorus content using integrals of the signals. This calculation of phosphorus content was based on the ratio of peak area of phosphorus in the sample as compared to the peak area of the internal reference phosphorus NAD with known concentration. A small amount of inorganic phosphorus was found in the