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Carbohydrate Polymers 39 (1999) 181–186

Carbohydrate Polymers

Properties of cassava starch–dicarboxylic acid complexes

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Received 22 April 1998; received in revised form 10 November 1998; accepted 26 November 1998

Abstract

Cassava (*Manihot esculenta* Crantz) starch prepared from fresh roots of M4 cultivar was complexed with oxalic, malonic and succinic acids. The products were examined for their physicochemical, structural and enzyme digestibility properties. The results were compared to that of untreated starch sample. The properties looked into were swelling, solubility, intrinsic viscosity, amylose (blue value), free reducing sugars, pH and carboxyl content. Enzyme digestibility pattern was examined with respect to α- and β-amylases. Crystallinity of the sample was checked by means of X-ray diffractometry, and their IR spectra were taken and compared. Pronounced increase in aqueous and DMSO solubility was noticed in the case of starch–oxalic acid and starch–malonic acid complexes. These samples, also showed relatively higher carboxyl content and their pH turned to acidic range. pH value was lowest (2.4) in the case of starch–oxalic acid complex. Amylose content was also found to be the lowest in these samples. Among the three starch–acid complexes, starch–succinic acid complex showed relatively less change in these properties. α-amylase digestibility pattern of both untreated and experimental samples revealed highest resistance to enzyme digestibility by starch–succinic acid complex. However, this resistance could not be observed with respect to β-amylase. Both X-ray diffraction pattern and IR spectra of all the starch samples were generally similar. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Starch; Cassava; Dicarboxylic acid complex

1. Introduction

Chemical modification of starches is usually accomplished by reactions such as oxidation, esterification, hydrolysis and dextrinization. Much information is available on the properties of starches chemically modified by various methods (Craig et al., 1968; Hebeish and Khalil, 1988; Wurzburg, 1986). Besides the types of modification cited earlier, there are also studies on complexation of starch with organic acids (Maier and Bauer, 1972). Cassava starch is a major starch source and can be isolated in a pure form with least contamination of non starchy constituents. The starch has a unique granular property, that exhibits considerably high single stage swelling and peak viscosity, but the swollen granules lack ability to retain the swollen structure and hence collapses instantaneously. The earlier phenomenon reflects the weak nature of the inter and intra molecular hydrogen bonding of the starch granules. In this respect, cassava starch considerably differs from starches isolated from sources such as wheat, maize, potato, etc. The present study was carried out to examine the impact of modification of starch by complexing with three dicarboxylic acids via oxalic, malonic and succinic acids with respect to some of the essential physicochemical and enzyme digestibility properties.

2. Experimental

Cassava starch was prepared in the laboratory from fresh roots of M4 cultivar. Wet starch thus obtained was dried to moisture level of 8%–10% at ≈ 70°C for ≈ 10 h in a hot air-drier.

2.1. Preparation of starch–carboxylic acid complexes

Starch–carboxylic acid complexes of oxalic, malonic and succinic acids were prepared in aqueous medium, without addition of 0.1N hydrochloric acid, but essentially based on the method reported by Tomasik et al. (1995). The reaction products were dried under vacuum and stored in a desiccator pending analysis and characterization studies. For further references, starch–acid complexes are represented by SMA, SMA and SSA for oxalic, malonic and succininc acids, respectively.

2.2. Analysis

Solubility of starch and its carboxylic acid complexes in water was determined as follows: 100 mg sample was

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PII: S0144-8617(98)00164-7

Table 1
Properties[a] of modified cassava starch and its carboxyl complexes (BD – Below detectable)

Sample	Solubility in water at 30°C (%)	Solubility in DMSO at 30°C (%)	pH of the aqueous solution	Free reducing sugars (%)	Swelling volume (ml)	Solubility at 100°C (%)	Intrinsic viscosity (η)	Amylose blue value	Carboxyl content (%)
Original starch	2.38	4.34	6.73	0.077	37.5	20.3	2.44	0.01316	—
SSA	2.82	1.51	4.60	0.025	22.0	35.1	1.30	0.01304	2.0
SMA	4.01	8.25	4.71	0.123	4.25	83.8	0.0663	0.01240	3.0
SOA	6.68	23.40	2.41	1.523	BD	100	0.269	0.01000	4.0

[a] Values reported are the mean of three determinations.

dispersed in 30 ml of distilled water and the solution was stirred for 3 h by keeping on a magnetic stirrer at ambient condition. The solution was filtered and the total carbohydrates in the filtrate was quantified using Phenol–sulfuric acid reagent (Igura et al., 1997). In a similar manner, solubility in dimethyl sulfoxide (DMSO) was found by dispersing 1 g sample in 50 ml of 80% DMSO.

Free reducing sugar content in the samples was determined by the procedure mentioned before, but by estimating the sugars using dinitrosalicylic acid reagent (Bruner, 1964).

Apparent amylose (as blue-value), swelling volume and solubility, intrinsic viscosity and carboxyl content were examined adopting reported methods (Sowbhagya and Bhattacharya, 1971; Schoch, 1964; Meyers and Smith, 1964; Wurzburg, 1964).

2.3. Enzyme susceptibility

Enzyme digestibility of the samples with respect to α-and β-amylases was studied by the method already reported (Raja et al., 1994) after following modification.

2.3.1. α-amylase susceptibility

50 mg sample was gelatinised in 50 ml Citrate–phosphate buffer of pH 5.6 at 65°C–70°C and cooling to ambient conditions. 1.0 ml of the buffer solution was transferred to test tubes and preincubated to 70°C by keeping in a constant temperature water bath. Equal volume of (1.0 ml) the diluted α-amylase prepared by dissolving 25 mg enzyme of 222.5 units (Sigma USA) in 25 ml citrate-buffer and diluting the solution by 1 : 9 using the same buffer, was added to each of the tubes. Samples, in triplicate, were incubated at the temperature mentioned earlier. After successive intervals of 0, 15, 30 and 45 min, each set of samples was removed from the water bath and the reaction was terminated by adding 2.0 ml of DNS reagent and the reducing sugar was estimated (Bruner, 1964) using dextrose as a standard.

2.3.2. β-amylase susceptibility

β-amylase digestibility of the samples was determined using diluted enzyme of 35 units activity (Sigma USA). The enzyme solution was prepared by dissolving 10 mg sample in 25 ml acetate buffer of pH 4.8 and diluting by 1 : 9 with the buffer. The enzyme reaction was carried out in triplicate at 28°C for different incubation intervals as in the case of α-amylase, and sugar content was measured and quantified (Bruner, 1964) against maltose as standard.

2.4. X-ray diffraction

X-ray diffraction pattern of starch and its acid complexes was examined on a Philip's X-ray diffractometer using CuKα radiation.

2.5. IR spectra

IR spectra of the samples were taken on a Perkin Elmer IR spectrometer using pellets of the samples on KBr matrix.

3. Results and discussion

Results of the present study reflecting the changes occurring in some of the essential properties of cassava starch are presented in Table 1.

A comparative study of the solubility (%) of the samples conducted in water and in 80% DMSO at 30°C showed highest value for SOA. In the case of SSA, solubility in both these solvents remained close to that of untreated sample. Solubility of SMA was intermediate between that of SOA and SSA. Solubility in water at elevated temperature also followed the earlier pattern. There was a progressive decline in the carboxyl content in the order of SOA $>$ SMA $>$ SSA. Correspondingly, aqueous solution of SOA indicated the highest acidity evinced by a low pH of 2.4. Aqueous solution of both SMA and SSA had a very close range of pH 4.6–4.7, while unmodified starch solution was practically neutral (pH 6.7). As oxalic and malonic acids have higher dissociation ability (Weast, 1977) and higher solubility in water (Lange's Hand book of Chemistry, 1967), it is likely that the former two acids might have ionised and penetrated the starch matrix. On the contrary, succininc acid having lower dissociation constant and higher hydrophobicity, reaction with starch inside the helix is less likely. This presumption is supported from observation that SOA and SMA had relatively higher water soluble free reducing sugars than both SSA as well as control starch sample, when determined at an ambient temperature of 30° C.

Clarke and Gillespie (1932) have already mentioned that carboxylic acids can hydrolyse starch at a higher rate than even mineral acids. In addition to the earlier observations, both SOA and SMA were found to have lower swelling as well as lower amylose content. Tomasik et al. (1995) have reported the results of complexation of corn starch with simple dicarboxylic acids including the ones employed in the present study. A comparison of Brabender viscogram of untreated and complexed corn starch samples, in the earlier study showed no characteristic swelling pattern for SMA and SOA complexes. Moreover, viscograph pattern of SSA and unmodified starch appeared generally similar, although peak viscosity of the untreated starch sample (350 BU) was marginally higher than that of SSA (310 BU). This indirectly suggests that the swelling capacity was not considerably affected as a result of complexation with succinic acid. In the present study, the swelling volume of unmodified cassava starch and SSA were rather closer than that of SMA and SOA. In the case of SOA, swelling volume was below detectable level. The reduction observed in the swelling volume, amylose content, higher

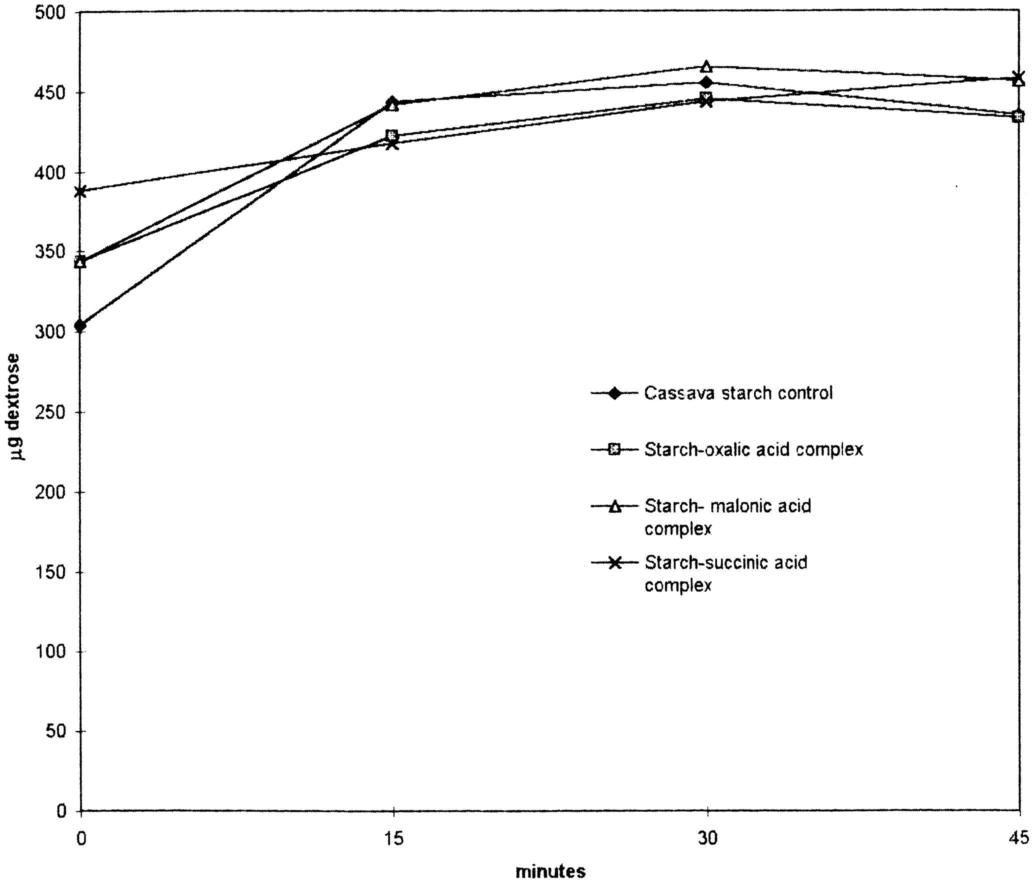

Fig. 1. α-amylase digestibility of cassava starch–carboxylic acid complexes.

amount of free reducing sugars and acidity could be as a result of partial cleavage of intermolecular hydrogen bonding inside the amylose helix, as well as because of partial hydrolytic cleavage occurring in the amylose resulting from concentration of H + ions released from –COOH groups.

The pattern of α- and β-amylase digestibility presented in Figs. 1 and 2 reveal distinct pattern of enzyme action. Among all the samples, SSA showed highest resistance to enzyme action, especially during the initial stage, i.e. 0–15 min, when calculated in terms of µg dextrose released into the substrate per min. In the case of unmodified starch, the earlier value was 9.2 while for SSA, SMA and SOA the values were 1.9, 6.5 and 5.2, respectively. During the second (15–30 min) and third phase (30–45 min), all the samples showed considerable decline, presumably because of product inhibition. Relatively higher resistance to enzyme action by SSA could be visualised as resulting from the presence of undissociated or partially dissociated succinic acid getting attached to amorphous region by means of weak hydrogen bonding or hydrophobic interactions by means of $-CH_2-CH_2-$ groups. The exact mechanisms remained obscure as the study warrants a detailed structural investigation using NMR and X-ray crystallography.

β-amylase digestibility pattern was found to be different in the case of all the samples and showed relatively higher enzyme susceptibility during the initial period (0–15 m) of the reaction. As β-amylase attacks the glucosidic bond from the non reducing end which is open, it is possible that the reaction proceeds more favourably than in the case of α-amylase. The fact that X-ray pattern of the samples were strikingly similar indirectly suggests that complexation could have taken on the amorphous region, and the amylopectin component which is essentially responsible for starch crystallinity (Banks and Greenwood, 1975) has not undergone any visible change. Any possibility of strong organic bonding and ester formation between starch and carboxylic acid is ruled out from a similar type of IR spectra obtained. It is, however to be ascertained whether the modifications in the properties observed as a result of complexation with dicarboxylic acids is by virtue of the intrinsic nature of cassava starch granules comprised of less cohesive granules or it is also applicable to samples of starch isolated from others such as wheat, potato and maize.

4. Conclusion

The present study highlights the major changes occurring

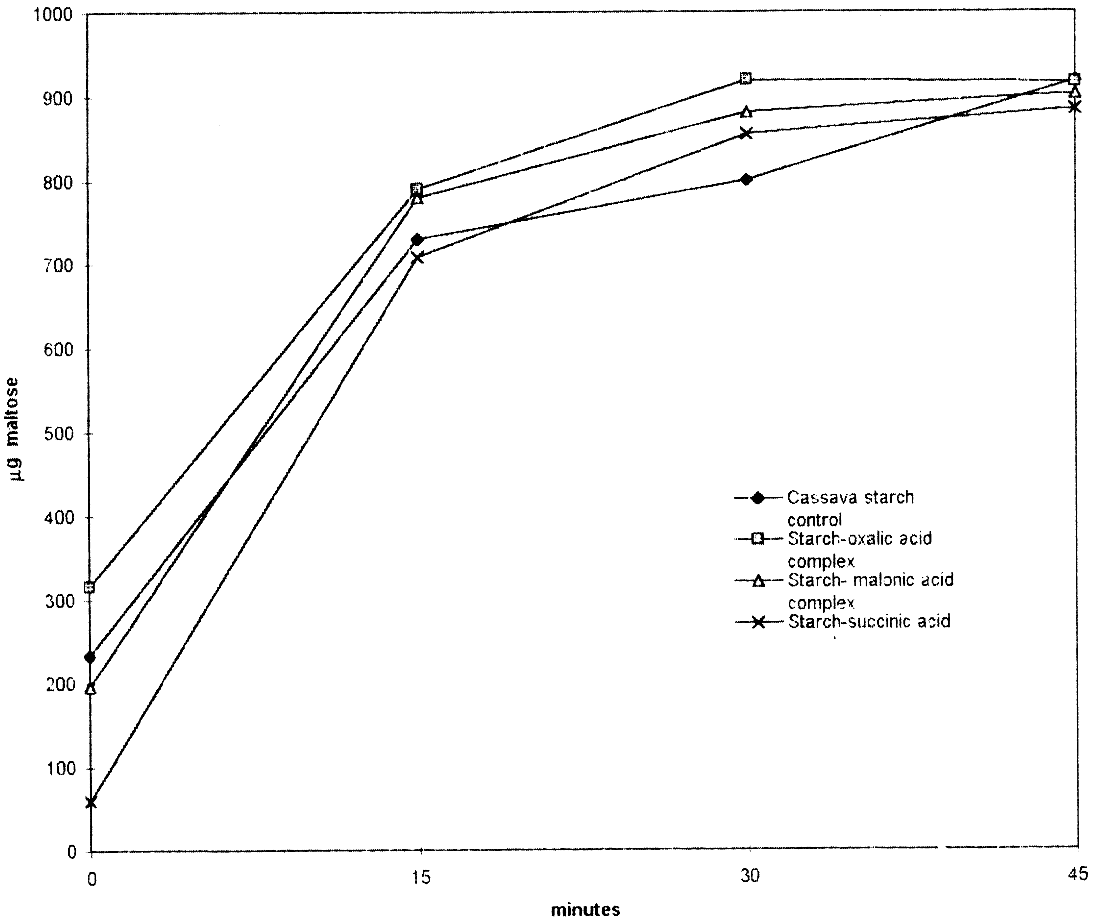

Fig. 2. β-amylase digestibility of cassava starch–carboxylic acid complexes.

in the physicochemical and enzyme digestibility pattern of cassava starch as a result of complexation with oxalic, malonic and succinic acids. The results suggest the possibility of oxalic and malonic acids entering amylose helix and disrupting hydrogen bonding as well as partially hydrolysing starch. Complexation of succinic acid with starch though takes place in the amorphous region, physicochemical properties are not affected as much as in the case of oxalic and malonic acid complexes. However, resistance to α-amylase action was observed to a greater extent in the former than in the latter two complexes. It also could be concluded that amylopectin component is not considerably affected during complexation as reflected from the similar X-ray diffraction pattern observed for all the four starch samples.

Acknowledgements

Authors are grateful to the Director, Regional Research Laboratory for all his encouragement and support. We also gratefully acknowledge the technical help extended by Miss S. Sindhu, Graduate trainee during this study.

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