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Modified properties of lintnerized cassava and maize starches

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Cassava and maize starches were subjected to lintnerization under identical experimental conditions using 2·0 M hydrochloric acid. The filtrate and residual starch samples were separated at 5-day intervals up to 35 days. There were considerable changes in amylose content, viscosity, and cold water solubility values. Despite the α- and β-amylase susceptibility patterns being quite distinct, there were less perceptible changes between cassava and maize. X-ray diffractograms of lintnerized samples showed greater crystalline peaks than native samples.

INTRODUCTION

The intrinsic properties of starches are influenced by their plant source, mainly due to changes in structural framework at both molecular and granular levels. It is now widely accepted that many of the characteristics of a given type of starch could be modified by appropriate modifications at the molecular and granular levels of this polymer.

Studies on the effects of treating potato starch with mineral acids were pioneered by Kirchoff and later pursued by researchers such as Lintner, Nageli, Bellmas, and Duryea (Wurzberg, 1986). The effects of lintnerization on the structural aspects of depolymerized dextrins from potato starch were examined in greater detail by Robin *et al.* (1974). Maningat and Juliano (1979) studied the extent of surface corrosion occurring in rice starch granules prepared from various cultivars. The higher susceptibility of rice starch, other than cereal and potato starches, to lintnerization has also been reported by the above authors.

In the present study, the effects of acid treatment closely similar to lintnerization on material and structural properties of cassava and maize starch, including enzyme susceptibility and X-ray diffraction have been looked into.

EXPERIMENTAL

Starch

Cassava starch was prepared in the laboratory from fresh tubers of M-4 cultivar (Raja *et al.*, 1987). The processed dry starch had a moisture content of 12·0%. Maize starch was supplied by M/s. Maize Products, Ahmedabad (India). The sample had a moisture content of 13·2%.

Amylose

Potato starch amylose was procured from the Centre for Biochemicals (CSIR), Delhi (India).

Enzyme

α-Amylase (E.C.3.2.1.1) and β-amylase (E.C.3.2.1.2) were purchased from Sigma (USA). α-Amylase had an activity of 222·5 units while β-amylase had an activity of 27 units.

All common chemicals, viz. hydrochloric acid, sodium hydroxide, iodine, potassium iodide, etc., were of AR/GR grade.

Preparation of lintnerized starches

Accurately weighed samples of cassava and maize starch (2·2–2·5 g in duplicate) were placed in 250 ml conical flasks and uniformly dispersed in 200 ml of 2·0 M hydrochloric acid. The flasks were stoppered and stored at 29–30°C. One set of flasks from each for cassava and maize was removed at regular intervals of 5 days up to a period of 35 days. The contents of the flask were filtered through a Büchner funnel under suction and the residue was air-dried. Both filtrate and residue were used for analysis. A zero-hour sample was prepared as above, but the suspension was filtered immediately giving only minimum contact time.

Analysis

Dissolved carbohydrates (reducing sugars)

A measured aliquot in the range of 1·0–2·0 ml from the filtrate was used for estimation by dinitro salicylic acid (DNS) as per the procedure of Bruner (1964) using D-dextrose as standard.

Amylose content

Amylose content was estimated by the method described by Sowbhagya and Bhattacharya (1971) based on starch–iodine complex formation and measuring the absorbance at 600 nm. Potato starch amylose was used as standard.

Solubility

The solubility of acid-treated starch samples in water at 29–30°C was determined as follows: accurately weighed samples in the range of 200–250 mg were dispersed in a measured volume (100 ml) of water. The solution was stirred for 2·0 h on a magnetic stirrer, centrifuged, and 25 ml of the supernatant was transferred to a preweighed evaporating dish. The dish was dried in an air oven at 100°C for 16–18 h. The amount of dry residue was determined gravimetrically. Experiments were repeated in triplicate and the mean value calculated.

Relative and intrinsic viscosity

The viscosity of native and acid-treated samples in 1·0 M NaOH suspension was determined at 30°C using an Ostwald Viscometer as per the procedure of Myers and Smith (1964).

α-Amylase susceptibility

Accurately weighed starch samples (~0·01 g) were dispersed in 1·0 ml of citrate–phosphate buffer pH 5·8. An equal volume of α-amylase was added and samples were incubated at 70°C in a constant temperature water bath for intervals of 10, 20 and 30 min. After the respective reaction times, 2·0 ml DNS reagent was added to inactivate the enzyme and the colour was developed by keeping in a boiling water bath for precisely 5 min. The absorbance at 540 nm was measured and the amount of reducing sugar was calculated from a D-dextrose standard graph.

Table 1. Relative and intrinsic viscosity of native and lintnerized cassava and maize starches solution (2 M NaOH)

Name of the sample	Relative viscosity n_r	Intrinsic viscosity n_i
Cassava starch		
Native	3·3162	0·2398
Lintnerized sample (5th day)	1·2837	0·0050
Lintnerized sample (10th day)	1·0405	0·00078
Lintnerized sample (15th day)	1·0397	0·00076
Maize starch		
Native	2·8724	0·0211
Lintnerized sample (5th day)	1·3720	0·0063
Lintnerized sample (10th day)	1·19180	0·0035
Lintnerized sample (15th day)	1·0108	0·0002

β-Amylase

β-Amylase activity of acid treated starch samples was studied in acetate buffer pH 4·8 at 35°C for incubation periods of 15, 30, 45 and 60 min. The reducing sugars were estimated by DNS using maltose as a standard.

X-ray diffraction

X-ray diffraction patterns of native and acid-treated starch samples were studied on a Philips X-ray diffractometer using CuK_α radiation.

RESULTS AND DISCUSSION

A progressive fall in amylose content was found in the acid-treated starch samples as against an increase in the dissolved carbohydrates in the filtrate (Figs 1 and 2). With respect to cassava and maize starches, the rate of the changes appeared to be faster in the former indicating

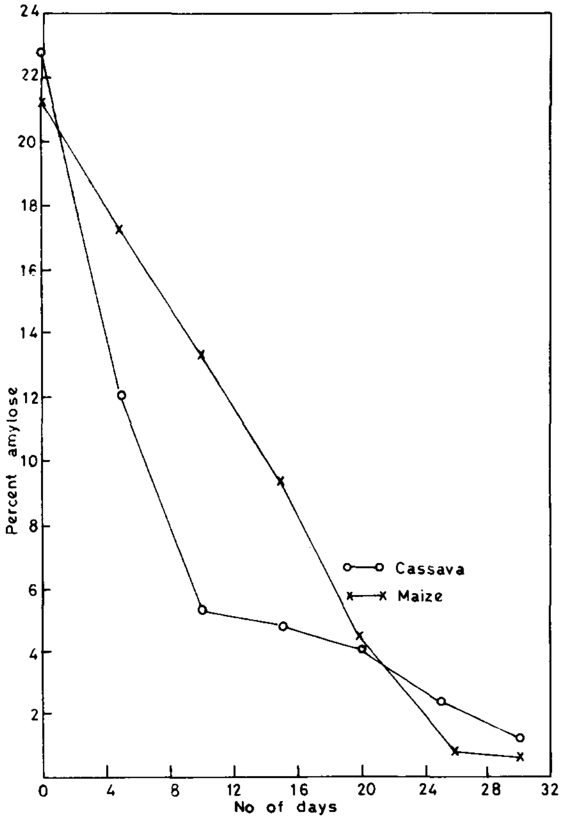

Fig. 1. Amylose content in acid treated starches.

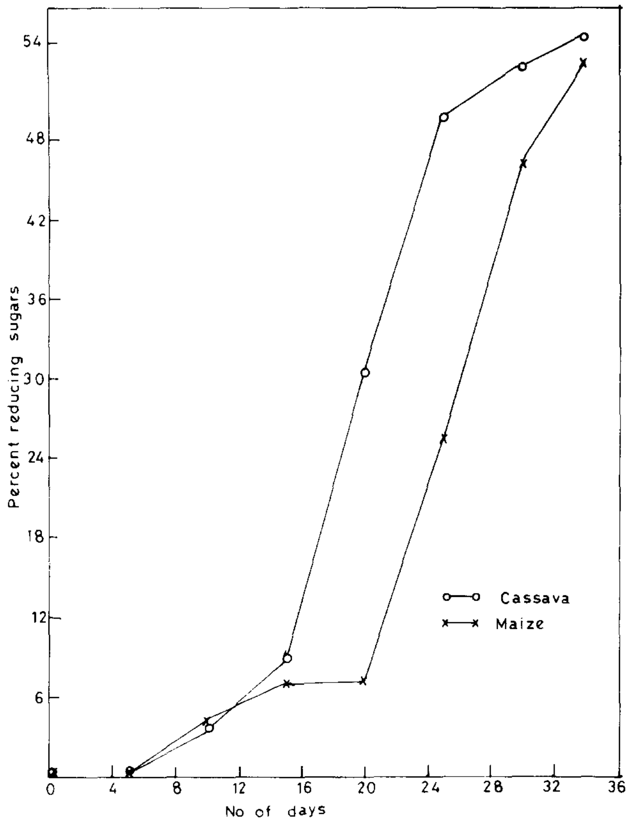

Fig. 2. Dissolved reducing sugars in acid treated starches.

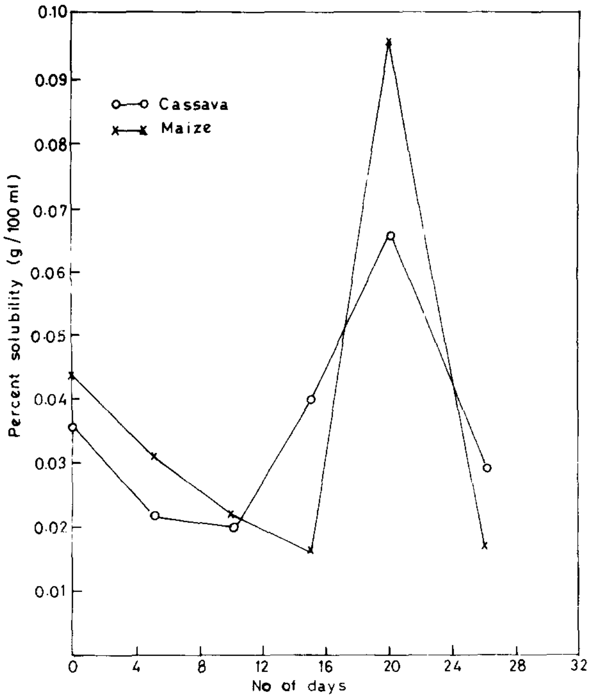

Fig. 3. Cold water solubility of acid treated starches.

higher susceptibility to acid attack. In both starches the rate of amylose leaching and dissolution of carbohydrates progressed incrementally up to 20 days of storage. Subsequently the rate of change showed a tendency to decline. Studies by Robin *et al.* (1974) on potato starch have shown that solubilization of carbohydrate during lintnerization (acid treatment) occurs in two stages, the first stage being relatively higher. Additionally, the above phenomenon was attributed to the hydrolysis of the amorphous part of the starch occurring during the initial stage, followed by hydrolysis of the crystalline region. Wolfrom *et al.* (1963) mentions the preferential action of acid on a 1-4 linkage, i.e. linear portion compared to 1-6, i.e. branching point, linkages. As there is enough evidence which establishes that it is amylopectin that builds up the crystalline region of starch (Eliasson *et al.*, 1987) it should be concluded that earlier action of acid treatment at room temperature is targeted towards linear sub-unit amylose or long branched chains. The progressive fall in the viscosity (Table 1) and starch iodine blue value measured (600 nm), for residual acid-treated starch samples supports the above pattern of acid attack. The higher initial action of acid on cassava starch must be due to relatively higher water absorptivity of this starch (Hellman *et al.*, 1952).

The cold water solubility of acid-treated starch samples showed a three stage pattern (Fig. 3). The highest solubility of cassava starch was noticed between 9 and 10 days of acid treatment, and between 14 and 24 days in maize starch. Subsequent prolongation of storage brought only marginal changes in cold water solubility.

The pattern of α- and β-amylase action on acid treated starches showed a clearly distinct pattern (Figs 4a, 4b, 5a, 5b). The peak α-amylase susceptibility was seen in those starch samples treated for 9–14 days, this was followed by a sharp decline. The above pattern of α-amylase activity remained similar in all three reaction incubation periods (10, 20 and 30 min). Since α-amylase acts randomly on 1-4 glycosidic linkages and its action ceases at the point adjacent to 1-6, it is possible that the initial stage of acid action liberates long chain dextrins which subsequently undergo progressive degradation, accumulating short chain branched dextrins and oligosaccharides of lower DP — detailed studies carried out with potato starch (Robin *et al.*, 1974) have observed that acid hydrolysis of starch initially transfers the intact polymer to long chain branched dextrins of DP 25 and linear fragments of DP 15. The initial higher α-amylase action observed in the present study suggests a similar mode of degradation in the case of cassava and maize starches. A progressive increase in the short chain branched carbohydrate moieties explains the gradual fall in α-amylase action. The β-amylase action was lower in magnitude than α-amylase independent of reaction time (15, 30, 45 and 60 min). Unlike β-amylase, α-amylase action showed a steady state stage prior to a fall in enzyme activity. Kainuma and French (1971) proposed that the formation of single glucose unit stubs offer resistance to β-amylolysis. However, only further

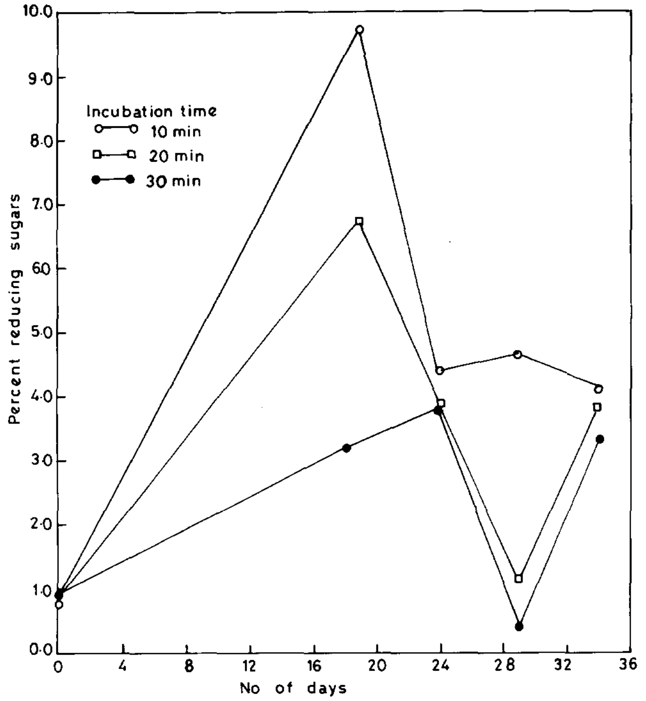

Fig. 4. (a) α-Amylase susceptibility of acid treated cassava starch.

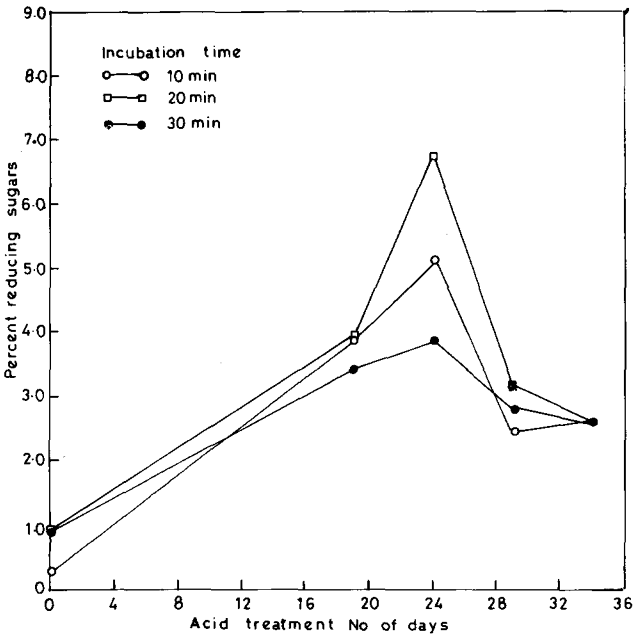

Fig. 4. (b) α-Amylase susceptibility of acid treated maize

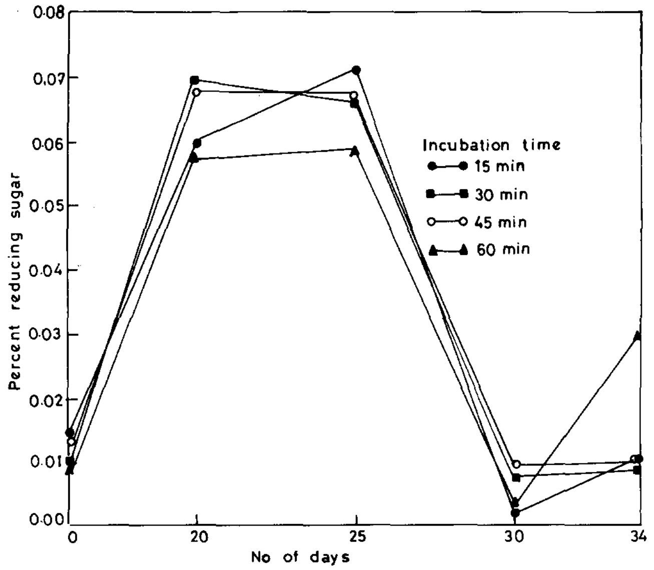

Fig. 5. (a) β-Amylase activity of acid treated cassava starch.

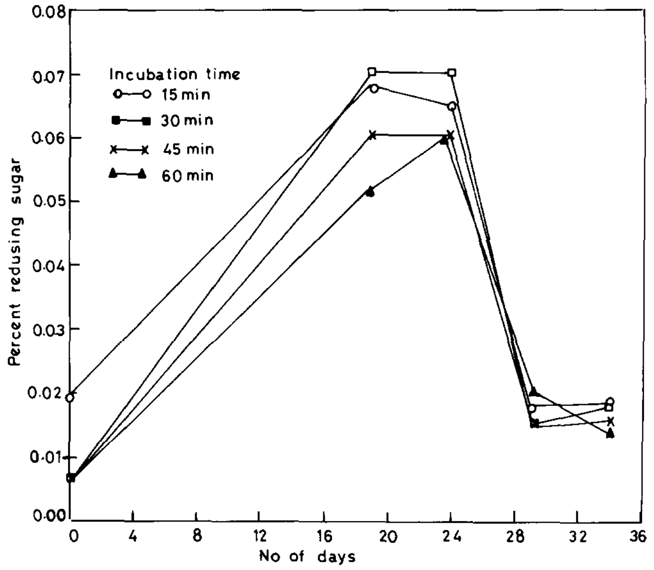

Fig. 5. (b) β-Amylase activity of acid treated maize starch.

detailed structural studies will confirm the above presumption.

X-ray diffraction data showed higher crystallinity in the acid-treated samples. Comparison of the samples treated for 5 and 15 days with their respective native starch (Figs 6 and 7, Table 2) indicated increased crystallinity especially in the 15-day treated samples. Values of the *d* spacing and I/I_0 suggested that the changes are more prominent in cassava starch than maize indicating higher susceptibility of the former to lintnerization (acid treatment) at room temperature.

CONCLUSION

Studies on the effect of lintnerization of cassava and maize starches revealed perceptible changes in starch characteristics. These included amylose content, viscosity, α- and β-amylase susceptibility. The fall in amylose content viscosity, and progressive accumulation of soluble carbohydrates (reducing sugars) predict that the initial actions of acid could be centred on the amorphous region. X-ray diffraction patterns of acid treated starches revealed increased crystallinity.

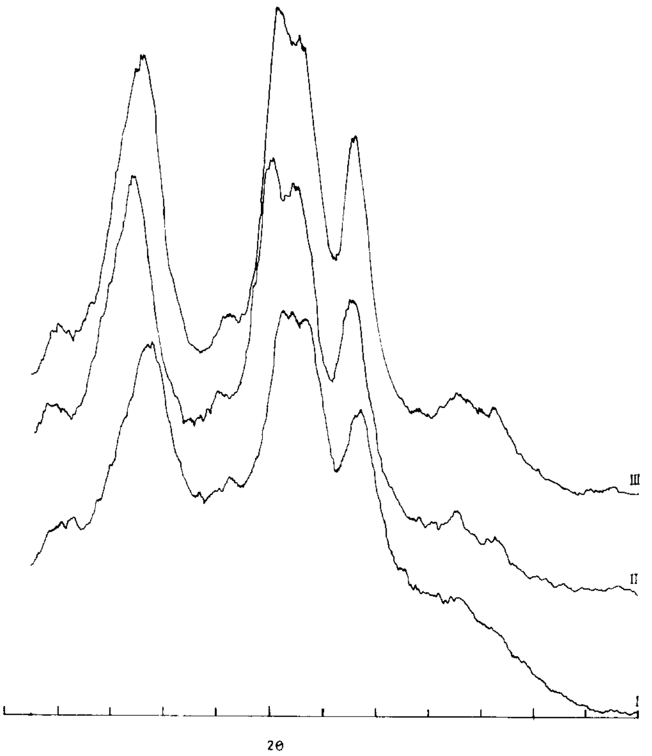

Fig. 6. X-ray diffraction of native and acid treated cassava starch, I: Native, II: Acid treated (5 days), III: Acid treated (15 days).

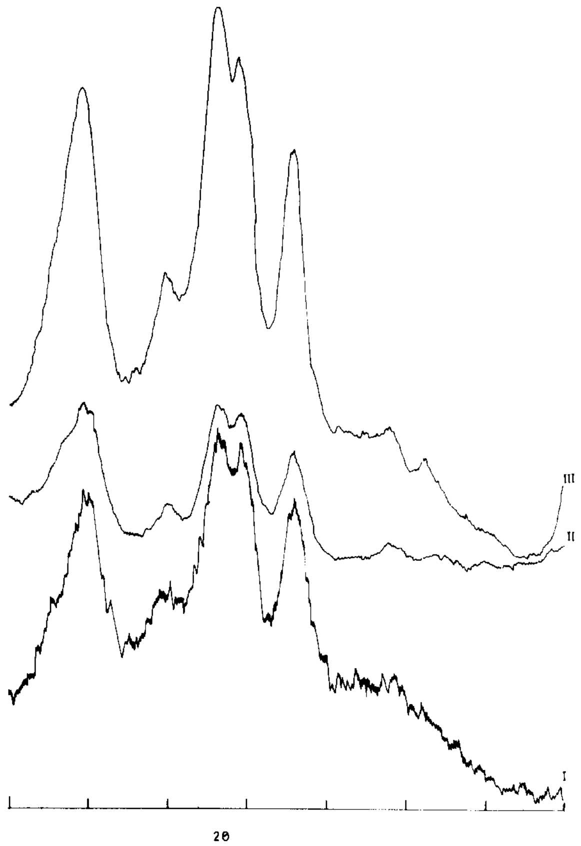

Fig. 7. X-ray diffraction of native and acid treated maize starch, I: Native, II: Acid treated (5 days), III: Acid treated (15 days).

Table 2. X-ray diffraction data of native and acid-treated starches

Sample	Peak Nos/(a) *d* value; (b) angle; (c) $I/I_{\max}$								
	1	2	3	4	5	6	7	8	9
Native cassava starch									
	(a) 7·7796	(a) 5·7940	(a) 5·1365	(a) 4·8258	(a) 3·8233	(a) 2·9039	—	—	—
	(b) 22·365	(b) 15·280	(b) 17·250	(b) 18·370	(b) 23·245	(b) 30·765			
	(c) 14·22	(c) 71·28	(c) 98·37	(c) 95·14	(c) 98·37	(c) 28·39			
Acid-treated samples									
I (5 days)	(a) 7·7186	(a) 5·8243	(a) 5·2498	(a) 4·8678	(a) 3·8497	(a) 3·3658	—	—	—
	(b) 11·455	(b) 15·200	(b) 15·200	(b) 18·210	(b) 23·085	(b) 26·460			
	(c) 7·58	(c) 56·79	(c) 74·36	(c) 90·11	(c) 100·00	(c) 17·06			
II (15 days)	(a) 15·4788	(a) 8·6527	(a) 7·6620	(a) 5·8053	(a) 5·1483	(a) 4·8625	(a) 3·8645	(a) 3·3343	(a) 2·9394
	(b) 5·705	(b) 10·215	(b) 11·540	(b) 15·250	(b) 17·210	(b) 18·230	(b) 22·995	(b) 26·715	(b) 30·385
	(c) 1·40	(c) 9·97	(c) 9·97	(c) 66·55	(c) 96·09	(c) 98·69	(c) 98·69	(c) 23·07	(c) 25·00
Native maize starch									
	(a) 7·8824	(a) 6·4238	(a) 5·9198	(a) 5·2094	(a) 4·7627	(a) 4·4383	(a) 3·8968	(a) 2·3547	—
	(b) 11·225	(b) 13·785	(b) 14·965	(b) 17·020	(b) 18·630	(b) 20·005	(b) 22·820	(b) 38·220	
	(c) 13·11	(c) 28·57	(c) 58·87	(c) 91·57	(c) 64·28	(c) 32·37	(c) 100·00	(c) 40·73	
Acid-treated samples									
I (5 days)	(a) 7·7967	(a) 5·8742	(a) 5·2653	(a) 4·9487	(a) 4·4558	(a) 3·8921	—	—	—
	(b) 11·340	(b) 15·070	(b) 16·825	(b) 17·910	(b) 19·910	(b) 22·830			
	(c) 10·43	(c) 56·25	(c) 76·56	(c) 59·85	(c) 30·48	(c) 100·00			
II (15 days)	(a) 7·8345	(a) 5·8742	(a) 5·2637	(a) 4·9038	(a) 4·4360	(a) 8770	(a) 3·3534	(a) 2·9361	
	(b) 11·285	(b) 15·070	(b) 16·830	(b) 18·075	(b) 20·000	(b) 22·920	(b) 26·560	(b) 30·420	
	(c) 8·67	(c) 57·87	(c) 72·72	(c) 101·23	(c) 38·39	(c) 101·23	(c) 12·66	(c) 22·98	

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