ELSEVIER

Carbohydrate Polymers 41 (2000) 191–195

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Acid degradation of starch. The effect of acid and starch type

V. Singh, S.Z. Ali*

Department of Grain Science and Technology, Central Food Technological Research Institute, Mysore 570013, India

Received 18 June 1997; accepted 16 September 1997

Abstract

Starch from cereals (wheat, maize and finger millet) pulses (chick pea and green gram), tuber (potato) and root (tapioca) was modified with different acids (0.5 N, 1.5 h, 50°C). Molecular weight (number average, $\bar{M}_n$) of these starches decreased after modification, H_3PO_4 causing the least and HCl and HNO_3 the highest reduction. Gel permeation chromatography of native starches using Sepharose CL 4B gave mainly two fractions. Fraction I (Fr. I), a higher molecular weight component eluting in the void volume and Fraction II (Fr. II), a lower molecular weight component that entered the gel and eluted at higher elution volumes.

After acid modification, the carbohydrate content of Fr. II increased while that of Fr. I decreased. The magnitude of the effect for different acids followed the same pattern as was the case for molecular weight. Very high increase in the total carbohydrate content in Fr. II was seen in cereal starches followed by pulses, root and least by tuber. The λ_{max} values of the peak of Fr. I increased in cereal and millet starches after modification by 9 to 14 nm, but either remained the same or decreased to some extent in other starches. The peak of Fr. II of modified starches had similar K_{av} to that of the respective native starch suggesting that the degraded portion had a molecular size similar to that of Fr. II of the native starch. However, the λ_{max} of the peak of Fr. II decreased after modification indicating that degraded portion of Fr. I which entered the gel and eluted with the peak of Fr. II was branched.

Keywords: Starch; Gel permeation chromatography; Acid modification

1. Introduction

Acid modified, thin boiling starch is normally prepared by treatment with hydrochloric acid and is used extensively in food, textile and paper industries (Radley, 1976; Wurzburg, 1986). Our earlier report described comparative study of different starches with respect to changes in some of the starch properties as a result of acid modification using 0.5 N HCl at 50°C (Singh & Ali, 1987). Effect of different acids (HCl, HNO_3, H_2SO_4 and H_3PO_4) under similar conditions of treatment on molecular weight, alkali fluidity number, iodine binding capacity and intrinsic viscosity of various starches has also been studied (Singh, 1995). The present article describes the degradative changes in the molecular profile of various starches as followed by gel permeation chromatography.

2. Materials and methods

2.1. Materials

Maize and tapioca starches were obtained from commercial sources. Potato starch was a gift from Professor W. Kempf of the Federal Research Centre for Cereal and Potato Processing, Detmold, Germany. Wheat (*Astivum durum*) was obtained from a local market and finger millet (Indaf-9 cultivar) from Agricultural Research Station, Mandya, Karnataka, India. All chemicals used were of analytical grade.

2.2. Methods

2.2.1. Isolation of starches

Wheat, finger millet, chick-pea and green gram starches were prepared as reported earlier (Singh, 1995).

2.2.2. Acid modification

A 33% (d.b.) starch slurry was acid modified as described by Ali and Kempf (1986) using 0.5 N acid at 50°C for 1.5 h. The slurry was stirred frequently during the treatment period, neutralised with NaOH at the end and washed with water repeatedly until the filtrate was free from respective anions.

2.2.3. Gel permeation chromatography

About 40–50 mg starch in a graduated test tube was dispersed in 2.5 ml of 0.5 M KOH, mixed well, and heated

* Corresponding author.

0144-8617/00/$ - see front matter
PII: S0144-8617(99)00086-7

Table 1
Molecular weight (Number average, $\bar{M}_n$ of native and modified starches)

Starch	Number average molecular weight, ($\bar{M}_n$ (KD))				
	Native	HCl	HNO_3	H_2SO_4	H_3PO_4
Wheat	236	85	84	140	202
Finger millet	97	56	60	73	91
Maize	120	71	69	104	120
Chick pea	177	69	69	112	158
Green gram	277	76	76	185	244
Potato	335	81	81	151	303
Tapioca	220	87	87	158	208

in boiling water bath for about 15 min in a nitrogen atmosphere. The solution was cooled, neutralised with HCl using phenophthalein as indicator and made up to 10 ml. It was centrifuged at 10 000 rpm for about 20 min, the supernatant collected and carbohydrate content determined. An aliquot containing 10 mg (d.b.) carbohydrate was fractionated by ascending chromatography on a Sepharose CL 4B gel (Pharmacia Fine Chemicals, Sweden) column (1.6 × 70 cm) operating at 30 ml/h flow rate using degassed, double glass-distilled water containing 0.02% sodium azide as eluent. Three ml fractions were collected, from which 0.5 ml aliquot was used for determination of carbohydrate content by phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956), against glucose standard. To the remaining 2.5 ml portion, 0.2 ml of 2% iodine solution was added and absorption maxima of iodine–polysaccharide complex were recorded by scanning using a Beckman Spectrophotometer model DU6. Recovery of the sample loaded on to the column was also calculated.

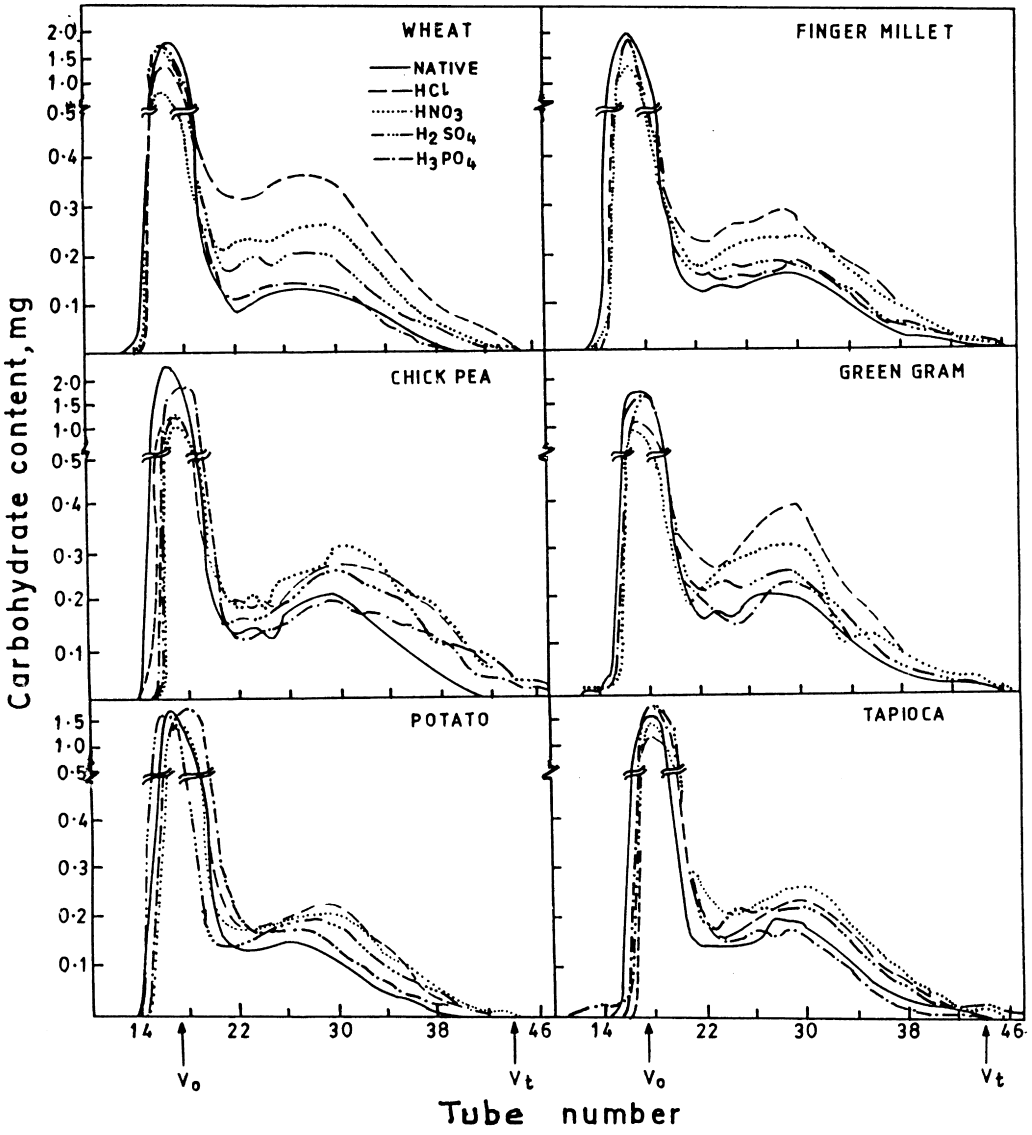

Fig. 1. Elution pattern of native and different acid modified (0.5 N, 50°C, 1.5 h) starches on Sepharose CL 4B column, V_o = Void volume; V_t = total volume.

Table 2
Carbohydrate content (% of total recovered carbohydrate) in Fr. I and Fr. II in native and various modified starches

Starch	Fraction I	Fraction II	% increase[a]
Wheat, native	78	22	
HCl modified	48	52	136
HNO_3 modified	46	54	145
H_2SO_4 modified	66	34	56
H_3PO_4 modified	79	21	− 5
Maize, native	76	24	
HCl modified	45	55	129
HNO_3 modified	55	45	88
H_2SO_4 modified	71	29	21
H_3PO_4 modified	78	22	− 8
Finger millet, native	76	24	
HCl modified	50	50	108
HNO_3 modified	53	47	96
H_2SO_4 modified	65	36	50
H_3PO_4 modified	70	30	25
Chick pea, native	71	29	
HCl modified	44	56	93
HNO_3 modified	41	59	103
H_2SO_4 modified	43	57	97
H_3PO_4 modified	74	26	− 10
Green gram, native	67	33	
HCl modified	42	58	76
HNO_3 modified	46	54	64
H_2SO_4 modified	60	40	21
H_3PO_4 modified	65	35	6
Tapioca, native	68	32	
HCl modified	56	44	38
HNO_3 modified	55	45	41
H_2SO_4 modified	64	36	13
H_3PO_4 modified	72	28	− 12
Potato, native	71	29	
HCl modified	62	38	31
HNO_3 modified	63	37	28
H_2SO_4 modified	66	34	17
H_3PO_4 modified	76	24	− 13

[a] % increase in Fr. II in comparison to Fr. II of native.

2.2.4. Molecular weight

The number average molecular weight ($\bar{M}_n$) of native and modified starches was determined using a slight modification of the method of Cĕh, Stropnik and Leskovar, (1976), (Ali & Kempf, 1986).

3. Results and discussion

Table 1 shows the molecular weight ($\bar{M}_n$) of various starches before and after modification with different acids. The maximum degradation, was obtained with HCl and HNO_3 and the least with H_3PO_4. Sulphuric acid, though a strong acid, promoted less degradation at equal normalities, compared with HCl and HNO_3.

The starches were separated into two main fractions on a Sepharose CL 4B column, one eluting at the void volume (Fr. I, a high molecular weight polymer) and another (Fr. II) that entered the gel and eluted at higher elution volumes. It has been reported that Fr. I represents amylopectin (branched polymer), while Fr. II the amylose (linear polymer) component of starch (Chinnaswamy & Bhattacharya, 1986; Radhika Reddy, Ali & Bhattacharya, 1993). The recovery of the total carbohydrate from the column was 75 to 80% which although not considered good, could not be improved further. Upon acid modification, the resultant starches still showed the two main fractions, however there were qualitative and quantitative changes as discussed below.

A few representative chromatograms are shown in Fig. 1. It may be noted that different acids have produced degradative responses to different extents. The patterns also indicates in general, that Fr. I gets partially degraded and some of it enters the gel and gets eluted in the second fraction. The rise in the second fraction due to the degradation of Fr. I was highest in the case of HCl and HNO_3 treatment and least with H_3PO_4. H_2SO_4 had an intermediate effect. This is in agreement with the trend observed for changes in the number average molecular weight of the starches (Table 1).

Table 2 shows the distribution of carbohydrate content, in Fr. I and Fr. II before and after acid modification. It could be seen that the proportion of high-molecular weight component in native starches was higher in cereals (75 to 78%) as compared to other starches (67 to 71%). Upon modification with different acids, as expected, the proportion of carbohydrate content of Fr. II increased due to hydrolysis, altering thereby the relative proportion of both fractions. It could also be noted that the percentage increase in Fr. II was high due to the treatment with HCl and HNO_3 and much lower with H_2SO_4. H_3PO_4 on the other hand caused a decrease in Fr. I only in the case of finger millet and green gram, but caused an increase in Fr. I of the rest of the starches. This anomaly is at present difficult to explain. The degradative effect of H_3PO_4, however, in these starches is apparent from the data on $\bar{M}_n$ presented in Table 1 which shows a reduction of about 12%.

The increase in the total carbohydrate content in Fr. II on acid treatment was greatest for cereals followed by pulses, tapioca and lowest for potato. This may reflect the differences in the granular organisation and molecular structure of different starches. The greater susceptibility of cereal starches to acid attack may suggest that they contain more D(1 → 6) linkages, as it has been suggested that in the granular state the amorphous portion gets attacked preferentially. These regions are known to contain more D(1 → 6) branch points than the crystalline regions. (Wurzburg, 1986). The increase in carbohydrate content of Fr. II due to acid modification in the case of potato starch was not so dramatic as compared to cereal starches. Although potato starch has a very high molecular weight and there appears to be a substantial reduction in $\bar{M}_n$ after acid modification (Singh & Ali, 1987), there does not seem to be a corresponding increase in the Fr. II in the present data. The amylose of potato starch has the highest molecular weight among the various starches (O'Dell, 1979) and therefore there is

Table 3
Absorption maxima of iodine complex of elute during fractionation of native and modified starches (figures in parentheses indicate the tube number wherever different from the one indicated in the tube number column; a dash in place of value indicates not determined)

Starch	Tube no.	Native λ_{max}	HCl λ_{max}	HNO_3 λ_{max}	H_2SO_4 λ_{max}	H_3PO_4 λ_{max}
Wheat	15	572	581	581	578	575
	17	555	558(16)	561(16)	566(16)	567
	24	633	585	587	588	629(25)
	29	648(28)	636(31)	622	617	639
	39	590	575(41)	597(36)	588	617
Finger millet	15	564	578	575	575	560
	17	561	570(16)	564	560	563
	24	609	572	570	590	593
	27	640	–	–	–	–
	31	645	620	620(32)	626	620(30)
	35	620	612(36)	606(37)	615	620
Maize	16	557(14)	566	563	563	566(17)
	19	560(17)	561(18)	555	563	567
	23	603	558	560	573(22)	591(22)
	27	640	–	–	–	–
	35	603	620(33)	608(36)	618	623(34)
Chick pea	15	570	569	569	570	
	18	573(17)	582	570	576	
	23	620	567	576	–	–
	26	645	615(27)	600(28)	619(29)	
	29	639	627(33)	619(35)	619	
Green gram	16	557(17)	554	555	558	557
	19	579(18)	562	570	586	587
	23	630	597(22)	629(22)	616(21)	629(21)
	28	643	–	–	–	–
	31	643	635	629	624	624
	35	620(33)	611	615	615	613
Potato	15	559	560	557	555	557
	17	558	560	561	554	560
	21	593	561	566	581	594
	24	650	566(22)	563(22)	606(23)	620(26)
	30	650	623	618	624(34)	630(31)
Tapioca	16	546	542	545	551	552
	17	542(18)	547	546	543	546
	20	563	555	548	548	560
	23	607	561	554	603	594(22)
	28	643(27)	594(26)	598	627	635

possibility that it may elute along with the void volume fraction itself, which might explain the comparatively low Fr. II carbohydrate content of native potato starch. It may also be related to a relative resistance of potato amylopectin towards acid hydrolysis.

It is not known whether granule size relates to the extent of degradation of the starch, although the present data on degradative susceptibility may point towards such a possibility. Potato starch has the biggest granule size, cereals the smallest with pulses falling in between (Moss, 1976). The rise in the carbohydrate content of Fr. II on gel permeation chromatography after modification in the present case has shown an inverse relation to the granule size of starches in general. It may be of interest to mention here that it has been observed that the bigger granules gelatinize first and the smallest in the last (Leach, 1965; Bhattacharya, 1979).

Table 3 shows the λ_{max} values for different aliquots of Fr. I and II in native and modified starches. The iodine complex is formed by inclusion of iodine into the helix of the linear portion of amylose or amylopectin, and hence gives an indication of linearity of the molecule. Generally, the Fr. I was completely eluted by tube numbers 21 or 22. The λ_{max} values shown before this tube therefore are mainly for the branched component of the starch. It could be seen that the λ_{max} values of tube numbers 15–17, which contain the peak of Fr. I increased by 9–14 nm in the case of cereal and millet starches after modification. The increase was more for HCl and HNO_3 modified starches. In the case of other starches either it remained constant or showed a negligible decrease. This would imply, perhaps that in cereal and millet starches more of the degraded portions were branched, which are released (and enter into gel as Fr. II) thereby removing the steric hindrances caused by their branching, and allowing the remaining freed/exposed chains of the Fr. I to interact with the iodine. Thus the λ_{max} of Fr. I showed an increase. Correspondingly, therefore, there are changes in the λ_{max}

Table 4
K_{av} of peak of Fr. II of native and acid modified starches from various sources fractionated on Sepharose CL 4B gel

Starch	K_{av} of Fr. II peak				
		Modified using			
	Native	HCl	HNO_3	H_2SO_4	H_3PO_4
Wheat	0.44	0.43	0.45	0.41	0.41
Finger millet	0.47	0.43	0.45	0.40	0.48
Maize	0.46	0.47	0.46	0.46	0.46
Chick pea	0.54	0.55	0.54	0.51	0.54
Green gram	0.39	0.43	0.43	0.40	0.42
Potato	0.31	0.41	0.44	0.41	0.33
Tapioca	0.41	0.44	0.44	0.41	0.41

value of Fr. II. It could be seen from Fig. 1 that the peak of Fr. II elutes at tube numbers 28–30 in all the native starches, (except in the case of potato and tapioca, in which the peaks eluted at tube numbers 26–27). These peak tubes show λ_{max} in the range of 640 to 650 nm indicating the predominance of linear chain amylose molecules. Banks, Greenwood and Khan (1971) have reported a λ_{max} of 642 nm for amylose of 1500 DP while enzymatically synthesized amyloses of lower molecular weight (DP) had a λ_{max} of 496 nm to 610 nm for chain lengths (DP) of 22 to 140, respectively. It could be seen from the present data (Table 3) that the peak of Fr. II in native starches had λ_{max} with highest value of 650 nm for potato and lowest of 640 nm for maize and finger millet indicating that the DP of these amyloses was about 1500 or more.

Upon modification, there was a rise in the area of Fr. II, corroborating the results of Table 2, that the proportion of Fr. II increased. One can note however that the peak of Fr. II in all the modified starches coincided with the peak of Fr. II of native starches, i.e. it also occurred in aliquots eluting in tube numbers 28–30 and having K_{av} close to that for the peak of Fr. II of respective native starches (Table 4). It therefore suggests that the hydrolysis of the native granular starches by the acids, under the conditions tested occurs in an orderly manner and the degraded products have more or less similar molecular weights. Interestingly, the λ_{max} of these tubes was much less (9 to 45 nm) than that of the respective native starches. This is understandable, as it has been already pointed out that the degraded portions of Fr. I are branched, and an increase of their proportion in Fr. II peak containing linear molecules would reduce the λ_{max} of the aliquot in the respective tube.

References

Ali, S. Z., & Kempf, W. (1986). On the degradation of potato starch during acid modification and hypochlorite oxidation. *Starch/Staerke, 38*, 83–86.

Banks, W., Greenwood, C. T., & Khan, K. M. (1971). The interaction of linear, amylose oligomers with iodine. *Carbohydrate Research, 17*, 25–33.

Bhattacharya, K. R., (1979). Starch and its derivatives: their properties and food uses. *Proceedings of the Symposium on the Status and Prospects of the Confectionery Industry in India*. AFST, CFTRI, 25–26 May 1979, Mysore (1980), pp. 42–55.

Cěh, M., Stropnik, C., & Leskovar, S. (1976). Potentiometric determination of molecular weights of starches. *Starch/Staerke, 28*, 51–54.

Chinnaswamy, R., & Bhattacharya, K. R. (1986). Characterstics of gel-chromatographic fractions of starch in relation to rice and expanded rice-product qualities. *Starch/Staerke, 2*, 51–57.

Dubois, M. K., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*, 350–356.

Leach, H. W. (1965). Gelatinization of starch. In R. L. Whistler & E. F. Paschall (Eds.), *Starch: chemistry and technology*, 1. (pp. 289–306). New York: Academic Press.

Moss, G. E. (1976). The Microscopy of starch. In J. A. Radley (Ed.), *Examination and analysis of starch and its products*, (pp. 22–27). London: Applied Science Publishers.

O'Dell, J. (1979). The use of modified starch in the food industry. In J. M. V. Blanshard & J. R. Mitchell (Eds.), *Polysaccharides in food* (pp. 171–181). London: Butterworths.

Radhika Reddy, K. R., Ali, S. Z., & Bhattacharya, K. R. (1993). The fine structure of rice-starch amylopectin and its relation to the texture of cooked rice. *Carbohydrate Polymers, 22*, 267–275.

Radley, J. A. (1976). *Industrial uses of starch and its derivatives*, London: Applied Science publishers.

Singh, S., & Ali, S. Z. (1987). Comparative acid modification of various starches. *Starch/Starke, 39*, 402–405.

Singh, S., (1995). Properties of starches modified by different acids. In *Studies on modified starches*. Annexure III, Thesis submitted to the University of Mysore, India.

Wurzburg, O. B. (1986). Converted starches. In O. B. Wurzburg (Ed.), *Modified starches: properties and uses*, (pp. 17–40). Boca Raton, Florida: CRC Press.