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Role of Hydrogen Ion Concentration in Retrogradation of Starch Sols

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Introduction

It has been reported[1,2] that slightly acidic and slightly basic sols of potato starch retrograde to a greater extent than do neutral sols. On the other hand, Fouard[3,4] found that the greater the hydrogen ion concentration, the faster the rate of retrogradation. More recent evidence has not clarified the situation. Paschall and Foster[5] stated that when the pH was varied from 4 to 9, the maximum rate of retrogradation for potato amylose occurred at pH values of 6 and 7, the minimum between 4 and 5.5, and intermediate rates at 8 and 9. Further studies by Holló and co-workers[6] established that the maximum rate of retrogradation of potato amylose is at pH 7 (that of wheat amylose is at pH 5) and that hydrogen ion concentrations higher and lower than these values are inhibitory.

The present study reinvestigates the role of hydrogen ion concentration in starch retrogradation. Changes in cold water solubility, enzymatic digestibility, and crystallinity (by x-ray determination) have been determined in cornstarch sols at a series of pH values, imposed before and after gelatinization.

Experimental

Preparation of 2% cornstarch sols has been described elsewhere.[7] In a first series of experiments (I), the pH was adjusted, before gelatinization, by the addition of hydrochloric acid or ammonium hydroxide. (Sodium and potassium hydroxides will lower the gelatinization temperature markedly, as will sulfuric and phosphoric acids.[8,9]) The pH values ranged from 2 to 10, in unit intervals. Certain questions, arising from the results of series I, suggested a second series of experiments (II), in which the pH was adjusted after gelatinization. The pH values of series II were limited to 1.3, 1.8, 2.2, 4.8, and 10. All sols were stored at 1°C.

In series I, samples were taken immediately after gelatinization and following 2, 5, 9, and 28 days of storage. In series II, samples were taken immediately upon pH adjustment and after 7, 27, 53, and 216 hr. of storage—in order to study the initial rapid phase of retrogradation more closely.

Analytical Methods

Crystallinity Determination

Because retrogradation has been interpreted as crystallization,[10–12] the crystallinity of the starch sol was examined by means of an x-ray diffraction technique.[7]

Enzymatic Digestibility

Invariably, retrogradation has been related with decreased enzymatic susceptibility of aged starch pastes.[1,2,13,14] Here, duplicate aliquots of the starch sol were diluted 1:11 with 0.025 molar phosphate buffer to attain a pH value of 7.0. To 6 ml. of this dilution was added 1 ml. of a solution, containing 0.50 mg. α-amylase (Nutritional Biochemicals Co.) and 2.5 mg. calcium chloride dihydrate. The solutions were mixed, and 1 ml. aliquots were removed immediately and after 2 hr. at 26°C. Each aliquot was placed in 10 ml. of ferricyanide solution[15] at pH 10.4, and the mixture was shaken vigorously to inactivate the enzyme. Maltose was determined by the method of Schales and Schales,[15] following a standard curve for maltose monohydrate.

Cold Water Solubility: Acid Hydrolysis

The diluted starch sol, as described above, was centrifuged at approximately 12,000 g. for 5 min. Two milliliters of the supernatant were hydrolyzed with 0.2 ml. of hydrochloric acid (sp. gr. 1.19) at 100°C. for 90 min. Reducing power, in terms of glucose concentration, was evaluated by the method of Schales and Schales[15] following a standard curve for glucose.

Cold Water Solubility: Iodine Sorption

One milliliter aliquots of the supernatant from centrifugation were placed in 10 ml. of dilute Lugol solution (10 mg. iodine and 20 mg. potassium iodide in 100 ml. distilled water). Optical density of the starch-iodine complex was read with a

spectrophotometer at 590 mμ. (Although the peak maxima varied from 570–610 mμ, in all cases the optical density at 590 mμ was at least 97% of the peak maximum.) The relationship of optical density to starch content has been documented by Nielsen and Gleason.[16] Hence changes in optical density indicate changes in the cold water solubility of the starch molecules. Because measurement of iodine sorption was the most sensitive and rapid method for determining the extent of retrogradation, this measurement alone was used for series II (see also Loewus and Briggs[17]).

Results

The x-ray indices of retrogradation for series I sols are plotted against pH values in Figure 1. The maximum index occurs at pH 7. Note, however, that the indices between pH 4 and 7 are quite close in value, so that the true peak probably is somewhere within this range.

Enzymatic digestibility during storage of sols of series I is shown in Table I. The greatest decrease in digestibility occurs about the pH values of 5 and 6, with that at 5 greatest. Cold water soluble starch, as measured by acid hydrolysis, is least at pH 5 and slightly greater at pH 6. Similarly, the lowest optical density is that at pH 5 (Fig. 2).

In series II (Fig. 3), the lowest values for optical density of the starch-iodine complex are those for pH 1.3, 1.8, and 2.2. Thus, retrogradation is occurring most rapidly at the lowest pH values. (There is little difference in retrogradation among these pH values, so that all are represented by the curve for pH 2.2.) The results for series I and II have been duplicated in two sets of experiments.

TABLE I
Enzymatic Digestibility (γ maltose/milliliter) in Sols of Series I during Storage at 1°C.

pH	Days in storage				
	0	2	5	9	28
2	524	535	679[a]	535	695[a]
3	550	602	534	523	500
4	544	577	502	460	400
5	557	575	485	410	347
6	540	565	510	431	354
7	606	502	485	430	390
8	590	600	555	476	391
9	572	804[a]	534	498	425
10	578	660[a]	540	504	455

[a] Values exceptionally high (>650), possibly due to contamination.

Because Figures 2 and 3 represent logarithmic plots of optical density versus time, it is of further interest that, except for a short initial portion, the curves are virtually rectilinear. First-order rate constants, calculated from the slopes of these lines, are given in Table II together with final optical densities. For each sample, the rate constant, the final optical density, and the steepness of the initial portion of each curve seem to be related: thus, in series I, the highest rate constant is that at pH 5 (3.35×10^{-3} hr.$^{-1}$); after 28 days the greatest amount of retrogradation has occurred at this pH value; and the sample at pH 5 has the steepest initial portion of the logarithmic curve.

In series I, the value of the rate constant de-

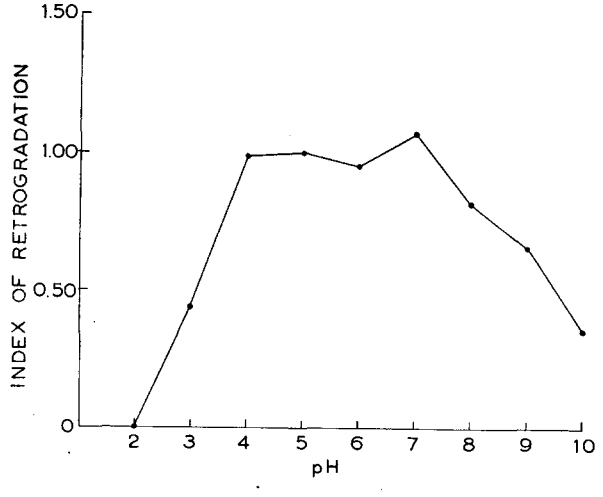

Fig. 1. Index of retrogradation (measured by ratio of peak heights in x-ray diffraction pattern) as function of pH in series I sols (pH adjusted before gelatinization).

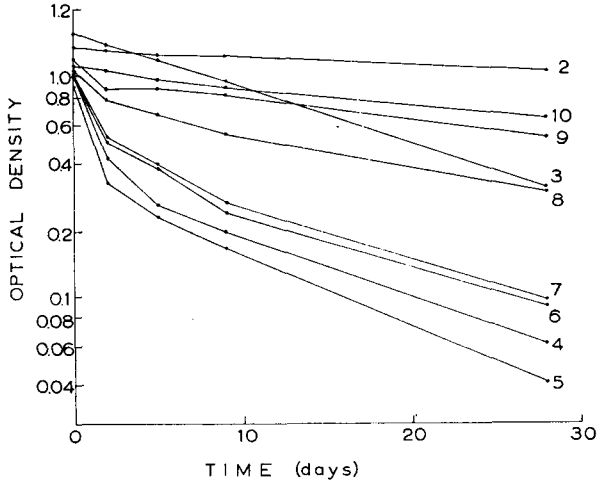

Fig. 2. Logarithm of optical density of starch-iodine complex as a function of time in series I sols (pH adjusted before gelatinization); pH values indicated at end of respective curves.

TABLE II
First-Order Rate Constants (k) and Final Optical Density (O.D.) for Cornstarch Sols of Series I and II

pH	$k \times 10^3$, hr.$^{-1}$	O.D. 590 mμ[a]
	Series I	
2	0.43	1.000
3	2.34	0.310
4	2.68	0.060
5	3.35	0.040
6	2.59	0.090
7	2.59	0.090
8	1.56	0.300
9	0.89	0.520
10	0.81	0.620
	Series II	
1.3–2.2	4.44	0.190
4.8	4.09	0.245
10.0	3.14	0.420

[a] For series I, 28 days; for Series II, 216 hr.

creases at pH values higher and lower than 5. However, in series II, the highest rates occur at the lowest pH values, and the rate becomes lower as the hydrogen ion concentration decreases. Also, the effect of pH is greater in series I. For example, in that series the rate constant at pH 10 is one-fourth that at pH 5, whereas in series II, it is three-fourths that at pH 5 (4.8). In series I, the rate constant at pH 2 is one-eighth that at pH 5, in series II it is 1.1 times that at pH 5 (4.8).

Discussion

The difference in results between series I and series II indicates the complexity of the role of hydrogen ion concentration in retrogradation. In series I, starch which is put into solution at low pH undergoes glycosidic bond scission and thereby experiences a reduction in molecule length. The decrease in molecule size is related to a corresponding decrease in the extent of retrogradation.[18,19]

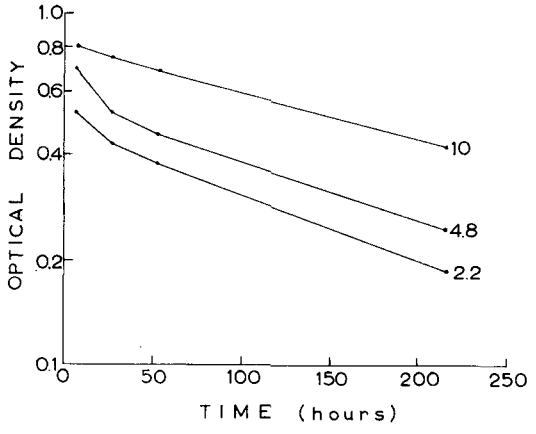

Fig. 3. Logarithm of optical density of starch-iodine complex as a function of time in series II sols (pH adjusted after gelatinization); pH values indicated at ends of respective curves.

At high pH values in series I, extensive hydrogen bond rupture must occur when the starch is heated. The extent of bond breakage is evidently a direct function of the pH value, which also is a significant factor in the rate of retrogradation (compare Fig. 2 with Fig. 3). Perhaps another impediment to retrogradation at high pH is the weakly acidic character of starch. This effect must be slight and may be evident only in the results with series II sols. Thamsen[20] determined for glucose an acid dissociation constant of 1.2×10^{-13} at 0°C. If the starch molecule acquired a negatively charged surface at high pH by the dissociation of protons, the charged layers would give rise to an electrostatic repulsion between adjacent molecules. Hence, in series II, in which little hydrolysis occurs at low pH at 1°C., there are essentially no charged layers, and the molecules can aggregate more readily if they are of suitable length.

It is noteworthy that the retarded rate of retrogradation at low pH in series I agrees with the findings of Holló et al.[6] and Paschall and Foster.[5] The opposed results of Fouard[3,4] and Maquenne,[1,2] i.e., enhanced rate of retrogradation at low pH, is confirmed in the results of series II. Hence the contradictory reports of the literature may be the consequence of the method of adjustment of hydrogen ion concentration.

If retrogradation be regarded as crystallization, gel formation is a first step in the retrogradative process.[21] It is of interest, then, that the most rigid gels of potato starch are those pasted at pH 5 or 6, rather than at higher or lower pH values.[22]

Loewus and Briggs[17] have presented data for starch to the effect that in retrogradation (*1*) the time required to reach any stage is a constant fraction of the time required to reach any later stage, independently of concentration, and that (*2*) the time required to reach any given stage varies inversely as the square of the original amylose concentration.

The first statement implies first-order kinetics, but the second implies third-order kinetics. Results of the present study indicate obedience to first-order kinetics after a very short, initial stage.

Information on entropy changes during retrogradation is not available. Most recent evidence[23,24] indicates that the starch molecule is a random coil in solution. In order for the highly

restricted, linear form of B-starch[25] to be assumed, there must be a loss in configurational freedom, i.e., a negative entropy change. Thus, the datum that partially hydrolyzed starch retrogrades more slowly is explicable in terms of the above model. Because the configurational freedom of many molecules of low molecular weight is greater than that of few molecules of high molecular weight, the amount of negative change in the entropy in the first case must be greater than in the second.

A question of interest is the possible analogy between starch retrogradation and protein denaturation. Although, like starch retrogradation, protein denaturation is usually indicated as a first-order reaction,[26] the latter is accompanied by a large *positive* entropy change.[27]

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Synopsis

Cornstarch sols were prepared with pH adjusted to various values both before and after gelatinization. Retrogradation was ascertained by measurement of crystallinity (x-ray), enzymatic digestibility, and cold water solubility. When the starch was gelatinized after pH adjustment, maximum retrogradation was at a pH value of about 5, with diminishing retrogradation at higher and lower values. When the starch was gelatinized before pH adjustment, maximal retrogradation occurred at pH values 1–2, with diminishing retrogradation at higher values. After an initial period of rapid retrogradation, retrogradation at all pH values studied follows first-order kinetics. The data are explained in terms of molecular size, role of pH in hydrogen bond rupture, the weakly acidic character of starch, and changes in the configurational entropy of starch molecules.

Résumé

Des sols d'amidon de blé ont été préparés avec ajustement de pH à diverses valeurs tant avant qu'après la gélatinisation. La rétrogradation a été étudiée par mesure de cristallinité (rayons-X), par digestibilité enzymatique et solubilité à froid dans l'eau. Lorsque l'amidon a été gélatinisé après ajustement du pH, le maximum de rétrogradation se trouve à un pH voisin de 5. Lorsque l'amidon a été gélatinisé avant l'adjustement du pH, le maximum de rétrogradation se situe à un pH de 1 à 2. Après une période initiale de rétrogradation rapide, celle-ci suit une cinétique de premier ordre à toutes les valeurs de pH étudiées. Les données sont interprêtées sur la base de dimensions moléculaires, du rôle du pH dans la rupture du lien hydrogène, du caractère légèrement acide de l'amidon et des variations d'entropie configurationnelle des molécules d'amidon.

Zusammenfassung

Es wurden Maisstärkesole mit pH-Einstellung auf verschiedene Werte sowohl vor als auch nach der Gelatinierung hergestellt. Retrogradierung wurde durch Messung der Kristallinität (Röntgenstrahlen), enzymatischen Verdaulichkeit und Löslichkeit in kaltem Wasser ermittelt. Bei Gelatinierung der Stärke nach der pH-Einstellung trat maximale Retrogradierung bei einem pH-Wert von etwa 5 auf; bei höheren und niedrigeren pH-Werten war die Retrogradierung geringer. Bei Gelatinierung der Stärke vor der pH-Einstellung trat maximale Retrogradierung bei pH-Werten von 1–2 auf; bei höheren Werten war die Retrogradierung geringer. Nach einer Anfangsperiode mit rascher Retrogradierung, folgt die Retrogradierung bei allen untersuchten pH-Werten einer Kinetik erster Ordnung. Eine Erklärung der Ergebnisse wird auf Grundlage der Molekülgrösse, der Rolle des pH bei der Spaltung von Wasserstoffbindungen, des schwach sauren Charakters der Stärke und der Änderung der Konfigurationsentropie der Stärkemoleküle gegeben.

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