

FACTORS INFLUENCING ALKALI SOLUBILITY

by

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## INTRODUCTION

Starch is the chief reserve carbohydrate of plants. It is therefore one of the most widely distributed substances in the vegetable kingdom. However, even though starch is so widely distributed, it still remains the subject of much research. The basic composition of the starch molecule is known, but the structure and spatial configuration of the starch granule have long been the subject of much controversy among chemists. According to prevailing theories, starch usually contains two carbohydrate substances, both polymers of glucose but differing in properties and structure. The minor component, called amylose, is a linear polymer with the repeating units connected by 1,4- $\alpha$ -glucosidic bonds, while the major fraction, called amylopectin, has the regularities of the fundamental starch chain interrupted by varied degrees of branching through the 1,6- $\alpha$ -glucosidic linkage. Therefore, the starch granule may be considered to be an association of starch molecules of different size, shape, and degree of branching. It is believed that the intermolecular association is made possible through forces operating between two polar groups sharing a hydrogen atom. Such bonds of low and variable energy content are called hydrogen bonds.

Recent studies (5) in this laboratory have shed new

light on the granular structure of starch and placed added emphasis on the role of the hydrogen bond. More particularly, a quantitative inverse relationship between the rate of gelatinization and alkali lability has been proven. In the explanation of this phenomenon, Barham and Campbell (5) have proposed the use of the concept of a free radical mechanism activated through granular strain.

The present work has been undertaken to further the knowledge concerning the interpretation of alkali lability and its relation to granular strain.

The simplified alkali lability procedure by Echoch and Jensen (15) was to be investigated with consideration given to the effect of time of digestion, various alkali metal hydroxides, and several other factors. Furthermore, it was desired to find some means of relieving internal strain within the granule and thereby causing a decrease in alkali consumption by the starch granule.

#### REVIEW OF LITERATURE

A technique which has proved valuable in routine and commercial examination of starch was published by Taylor and Salzmann (17), and modified later by Taylor, Fletcher and Adams (18). Since the publication of this procedure, the interpretations of the significance of alkali lability measurements have been as numerous as the investigators who

have used the method.

Taylor and Salzmann pointed out that by allowing hot aqueous potassium hydroxide to act on a sample of starch or amylose, then neutralizing and determining the reducing matter iodometrically, it was possible to magnify small differences among the samples which were not significant when based on the initial reducing value alone.

When a starch or amylose was modified, apparently only a part of the original polysaccharide was changed, the un-attacked portion remaining stable to alkali. Native starch which, in Taylor's opinion, had no history of hydrolytic attack from aqueous acid, enzymes, etc. was unaffected by repeated treatments with hot aqueous alkali, and the amyloid material after such treatments was still like the original as indicated by such qualitative means as specific rotation, blue color with acidulated iodine-iodide test solutions, and quantitative production of glucose.

It was also shown that aqueous acid, certain heat treatments, and grinding in the presence of moisture caused an increase in the alkali labile portion of the starch material. However, when a starch sample was treated by acid and heat to form a yellow dextrin, it was found that a decrease in alkali labile material ensued.

As a result of studies of granular disintegration of cornstarch, Taylor and Keresztesy (19) concluded that the alkali labile value was apparently a sensitive measure of the

change in available reducing groups since, presumably, it was at the aldehyde end group that the attack by alkali began on the amylose chain. These authors suggested two definite ways in which available aldehyde groups may be produced from organized starch granules. If amylose consists of a chain of n glucopyranose rings linked through the usual glucosidic oxygen bridge, there will be one free aldehyde group on the end of every chain. The ether linkages and hydroxyl groups located along the chain would tend to form hydrogen bonds with those of other chains, thus producing bundles of linear fragments and segments of amylose dovetailed together to shield part of the aldehyde groups.

Using this hypothesis, the alkali labile value, which reflects the reducing value, would be increased in one of two ways. One means would be the dissociation of coordinately-linked chains to uncover vulnerable existing terminal aldehyde groups; the other would be the hydrolytic scission of the glucosidic linkings giving shorter chains and consequently new aldehyde groups.

Kerr (13) studied the intrinsic viscosities, conversion limits, alkali labilities, and percentages of linear material in starch fractions. His conclusion was that alkali labile numbers vary inversely with the chain length of an unbranched, 1,4- $\alpha$ -glucosidically linked glucopyranose polymer and with the complexity of structure, explaining that alkaline degradation involved in the test starts from the terminal aldose group, proceeds by enediol splitting, not by

direct hydrolytic scission, and that the time of reaction is limited in the test.

Caesar and Cushing (8) discussed the structure of the starch molecule at great length and included unpublished work by the late Dr. Taylor, author of the first alkali lability investigations. Stress was placed on the importance of the covalent coordinate valence linkage (the hydrogen bond) causing the primary valence chains to associate into bundles or micelles.

In a subsequent publication, Caesar (9) stated that the interpretation of data for lability to alkali was difficult and uncertain. However, he viewed alkali lability as being associated with chain degradation, state of aggregation of micellar complexes, and chemical modifications, but that its chemistry was still obscure. He found that white dextrans gave a maximum iodine absorption. Further, during the preparation of Canary type dextrans, during which heat plays an important role, the curve for alkali lability passes through a definite peak and then declines. This phenomenon was also noted in the conversions of British gums but to a lesser extent.

Some evidence was offered that the mechanism of heat conversion includes dissociation of primary valence chains through the breaking of hydrogen bonds, dehydration to form inner ether linkages, and possible oxidation effects, especially at high temperatures. Taylor stated in a private

communication to Caesar (9) that "alkali-stable" substances such as inner anhydrides of the type of levoglucosan are probably formed from chain fragments through progressive loss of hydroxyl groups, followed by consequent dissociation of chains. The increased solubility in water is promoted by formation of a multiplicity of linkages, such as



Inner anhydride formation was substantiated by the fact that torrefaction dextrans of the highly converted, yellow type, acetylate to a very small extent, indicating few, if any, hydroxyl groups. However, Brimhall (7) found it difficult to interpret the change as a conversion of end groups to levoglucosan residues, since the reducing values continued to increase with the decline of the alkali lability.

In 1940 Schoch and Jensen (15) published a modified alkali lability procedure which has replaced the complicated and tedious iodometric technique by Taylor that gave results which could not be duplicated by independent operators. These authors stressed the glucosidic hydrolysis of the glucopyranose chain as a source of increased aldehydic properties and further suggested that enolization of the free terminal aldehyde resulted in decomposition of the starch into simple acidic substances. They defined the alkali number, which measures the rate of alkali consumption by starch, as the



cubic centimeters of 0.1 N sodium hydroxide consumed by one gram of starch during digestion in alkali for one hour at 100°C. The authors conclude their publication by stating that alkali lability, however determined, should not be construed as a quantitative evaluation of aldehyde content but the method did afford a simple empirical index of hydrolysis.

During the course of several years, numerous experiments conducted in this starch laboratory have placed a new interpretation on the meaning of alkali lability. Barham and Wagoner (1) found that the trend of alkali lability during the cure of sweet potatoes was opposite to that of the rate of gelatinization, which paralleled the increase in granule diameter. Also, the trend in alkali lability varied directly with the quantity of material removable by solvent extraction. It appeared from this study that increased alkali lability was associated with a reduced rate of availability of water for swelling. Data obtained by Schrenk, Andrews and King (16) supported this view in as much as solvent extraction had no effect on the magnitude of the heat of wetting of a starch, but it markedly decreased the rate at which the wetting occurred.

In the work involving the evaluation of sorghum starches, Barham, et al. (2) found that solvent extraction of the naturally occurring adsorbed substances very often changed to a marked degree the viscosity record of a starch, the alkali number, granular diameters, density, and moisture. This

indicated that solvent extraction caused a structural change in the granule. It was also noted that alkali lability closely paralleled the empirical ratio  $W_2/W_1$  obtained from viscosity records. Two maxima are developed in the viscosity records, one in the heating period ( $W_1$ ) and the other in the cooling period ( $W_2$ ). The maximum which appears during the heating period closely corresponds to the temperature at which the granules lose their anisotropy (the upper limit of the gelatinization temperature). The maximum of the cooling period appears when the temperature has reached some value within the range, 22° to 30°C. In as much as the two viscosity maxima are dependent on granule permeability, it again emphasized the relation of alkali lability to rate of availability of water for swelling.

Alkali lability gained added significance as the result of work carried out by Barham and Campbell (5) in this laboratory. This work involved the progressive adsorption and desorption of palmitic acid on solvent extracted starch (Blackhull kafir) samples. Selected points on the adsorption and desorption isotherms were chosen and the starch samples corresponding to these points were individually investigated with regard to the properties influencing starch behavior. Cycles representing changes in density and specific surface of the granule which accompanied the process were completely closed, while the adsorption-desorption process as

well as other properties of the treated starch indicated a reasonably close approach to reversibility.

It was found that an irregular increase in the alkali number resulted from the progressive adsorption and desorption of palmitic acid with a maximum increase in excess of six alkali units above the solvent extracted sample. However, the complete cycle approached very nearly a reversible process. This reversible procedure precluded the possibility of explaining alkali lability on the basis of hydrolysis or increased availability of terminal aldehyde end groups, since the adsorbed palmitic acid would tend to shield the end groups rather than make them more available for alkaline attack.

Furthermore, this work showed a quantitative inverse relationship between the rate of gelatinization and alkali lability. In other words, the more inhibited the starch to water, the greater its vulnerability to alkali.

Because of the inadequacy of the concepts of alkali lability as proposed by other workers, Barham and Campbell (5) proposed the use of the concept of a free radical mechanism activated through granular strain. This proposal resulted from work on "active" glucose carried out by Levene (14) and Clifton and Ort (11).

As Barham and Campbell stated:

If it can be admitted that an occasional glucose molecule in a glucose solution can be activated through strain into a free radical, it is even more

probable that similar free radicals could be formed in greater numbers from the glucose residues of the rigid, metastable organization of the starch granule or in a starch substance where organization still persists.

Further evidence of the free radical character of the starch granule was given by the work of Thomson (20) in which benzene was chlorinated in the presence of a black starch induction product.

## METHODS

### Preparation of Starch

Continuous study of starch chemistry has emphasized the necessity of care in the preparation of starch samples to prevent inhibitions such as retrogradation and damage to the starch through steeping or bacterial action.

A study of milling procedures extending over a period of several years gave a procedure of wet milling which yielded starches of high quality from sorghum grain. This procedure, described below, was used to obtain the Pink kafir starch employed in this work.

The Pink kafir grain was first dehulled in the presence of water by means of a hydraulic mill. The grain was then steeped, in 500 gram portions, for a period of two hours at 52°C. using a solution of water and chloroform as the steeping medium. At the end of the steeping period, the grain

was removed from the steep water, cooled and then ground for 10 minutes in the hydraulic mill. The ground grain was then screened through a 200-mesh sieve and washed with water to separate the starch from the fibrous residue. At this point, the starch and water suspension was passed through a colloid mill to facilitate removal of protein material adhering to the starch granules. The starch slurry was allowed to settle for two hours, the clear liquid decanted from the settled starch, and then tabled for a period sufficient to remove the starch from the water solution. Two additional tablings were carried out to insure a clean starch. Air drying of the starch was followed by grinding to pass through a 60-mesh sieve.

An 1800 gram sample of the starch was stored in a glass container for use in the alkali lability studies.

#### Starch Samples

In addition to the large Pink kafir starch sample discussed previously, several other starch samples, previously prepared in this laboratory, were investigated.

The samples, Original C-8, Extracted C-8, and Palmitic Acid Adsorbed C-8, were prepared by Campbell according to the procedure described in her thesis (10). The Palmitic Acid Adsorbed C-8 sample was prepared by refluxing the extracted starch in a five per cent solution of palmitic acid

and 85 per cent methanol. The peripheral fatty acid was then removed by Soxhlet extraction using carbon tetrachloride. This resulted in a sample which contained approximately 0.73 per cent fat adsorbed in the granule. These Blackhull kafir starch samples were selected as representative of the native starch, the defatted granule resulting from removal of adsorbed materials, and granules under strain resulting from adsorption of palmitic acid on the starch granule.

The hydrated cornstarch samples were prepared by Barham and Lanning (4) during 1946 in a study of granular behavior resulting from hydration at temperatures below the gelatinization temperature. Each sample was prepared by suspending 550 grams of starch in 1500 cc. of water. With continuous stirring, the temperature was raised one degree centigrade per hour from 61°C. to the temperature of the run as indicated in Table 6. After the maximum temperature was attained, the starch sample was held at this temperature for 17 additional hours. The starch was then dried and ground to pass through a 60-mesh sieve.

#### Alkali Lability

The method of Schoch and Jensen (15) was used to determine the alkali labile values reported in this work. The alkali labile value, called the alkali number, expresses the

rate of alkali consumption by the starch in terms of the cubic centimeters of 0.1 N sodium hydroxide consumed by one gram of starch during digestion in alkali for one hour at 100°C.

The Schoch method is given in detail in order to present a clear picture for later discussion of the effect of the several variables as reflected by the alkali consumption.

In Schoch's procedure, the starch sample is pulverized to pass through a 60-mesh sieve and a moisture determination is made to allow calculation of the alkali number on a dry basis. The digestion bottle is an 8-ounce, narrow-mouth, Pyrex nursing bottle, which is fitted with a regular one-hole, No. 0 rubber stopper to allow the exit of steam. Powdered starch in the amount of 500 mg. is introduced into the digestion bottle and suspended in 10 cc. distilled water with gentle swirling. To this suspension is added exactly 25.00 cc. of 0.4 N sodium hydroxide during which time agitation is continued to prevent lumping of the starch during gelatinization. Sixty five cubic centimeters of hot distilled water is added, the bottle capped, and then placed in a vigorously boiling water bath. The bottle is heated in the bath for exactly 60 minutes, then placed in cold water and 60 cc. cold distilled water is added to the digested sample. One cubic centimeter of thymol blue indicator is added and the excess alkali is titrated to the yellow end-point with standard 0.2 N sulfuric acid. The titer value of the alkali is determined by neutralizing 25.00 cc. of 0.4 N sodium hydroxide with the

standard acid, thus balancing out indicator errors.

The alkali number is calculated in accordance with the following equation:

$$\text{Alkali Number} = \frac{(\text{cc. acid to titrate blank} - \text{cc. acid to titrate sample}) \times \text{normality of acid} \times 10}{\text{weight of sample on dry basis}}$$

This value represents the alkali consumption during one hour of digestion at 100°C. when 0.1 N sodium hydroxide is used. This term, alkali number, is reserved for Schoch's special conditions of reaction. When such variables as the time of digestion, temperature, or alkaline pretreatment of the starch are changed, the term "alkali consumption" is used to express the rate of reaction. The calculation of the alkali consumption value is in accord with the above equation.

Experience in the determination of alkali lability in this laboratory has shown that four simple modifications, when incorporated into the Schoch procedure, aided materially in gaining better reproducibility of results. These modifications, which are given below, were incorporated in the procedure used during this investigation.

First, the Pyrex bottles, in which the digestions were carried out, became etched and cloudy when in continuous use. In order to keep the bottles clear and also to maintain the consumption of alkali by the bottle surface at a fairly constant amount, the bottles were cleaned after each experiment by heating a one per cent solution of hydrochloric acid in the bottles for one hour in the boiling water bath. The



bottles were then rinsed with distilled water and dried.

A second change required that all distilled water, whether hot or cold, be boiled to free it of dissolved gases.

The third improvement in the method required the starch to be suspended in 20 cc. of water instead of the prescribed 10 cc. This facilitated better suspension of the starch sample during agitation and addition of the alkali. This change in the volume of water used to suspend the starch necessitated a reduction in the hot water added to 55 cc. in order to maintain the total volume at a constant 100 cc.

As a final improvement, the water bath used for the digestion of samples was enclosed except for a few holes for steam outlets. This aided materially in maintaining a constant temperature around the Pyrex bottles.

#### RESULTS AND DISCUSSION

An extensive survey of the starch literature since 1933 revealed the continuous use of alkali lability determinations as a means of starch evaluation. Numerous investigators used the procedures in studying granular behavior and, invariably, the significance of the alkali lability data was explained in terms of the terminal aldehyde end group content of the starch, or as an evaluation of hydrolysis which had taken place within the granule. However, Barham and Campbell (5) have placed a new interpretation on the significance of

alkali lability data. They showed that alkali lability was quantitatively and inversely related to the rate of gelatinization. In other words, the more inhibited the starch to water, the greater its vulnerability to alkali. Furthermore, they proposed the use of Levene's "active" glucose concept (14) to account for the change in alkali number in the absence of degradation. Active glucose possesses the properties of a free radical resulting from strain in the configuration of the glucose molecule, and it is even more probable that similar free radicals could be formed in greater numbers from the glucose residues of the rigid, metastable organization of the starch granule.

The starch granule may be considered to be an association of the starch molecules (amylose and amylopectin) of different size, shape, and degree of branching. Furthermore, it is believed that the intermolecular association is made possible through forces operating between two polar groups sharing a hydrogen atom. Such bonds of low and variable energy content are called hydrogen bonds.

Experimental evidence strongly indicates that these polymers of anhydro- $\alpha$ -d-glucopyranose, if unencumbered, will relax into a helical form corresponding to the lowest energy state, each turn of which possesses six glucose units. However, the relaxation to this helical form will be obstructed by branching and intermolecular association. According to the prevailing opinion, the linear molecules and segments of

the granule are extended. This, if true, would mean that the relaxed helix would not exist in the granule. Nor should the granule possess properties which are presumably characteristic of the relaxed helix, such as the formation of the stoichiometric, blue iodine complex. Still, most starches do form a blue iodine complex, this in spite of the extended form of molecules and fragments in the granule. This and other facts suggest that the dominating structure in the granules of most starches is one in which a number of extended molecules or branches are so associated that it possesses properties similar in some important respects to those of the relaxed helix. This structure might be regarded as a multiple helix (6, 8).

Cassava and glutinous starches possess very similar pasting characteristics, yet the former gives a blue (violet) color with iodine while the latter are colored brown by iodine. Both have high hydration rates and low alkali numbers. Tapioca starch pastes, however, possess greater "body" than glutinous starches. This comparison suggests that the greatest randomness of arrangement of molecules occurs in the granules of glutinous starches, less in those of tapioca starch, and still less in other non-glutinous starches.

A limiting granule structure would be one having a minimum randomness and one stoichiometric with respect to hydrogen bonds. This condition would seldom be reached. Among natural starches, the closest approach to this con-

dition is by cereal starches, with cornstarch having the greatest degree of organization. Solvent extraction and ageing would cause still further progress toward complete organization.

The statement that the molecules of the granule are extended (and therefore possess energies in excess of those in the lowest energy state - the relaxed helix) is equivalent to saying that the molecules are under strain or are distorted. The distortion may be increased through the adsorption of various substances within the granule. Furthermore, the existence of the hydrogen bond as such does not of necessity mean that strain exists within the glucose units of the granule. If it is supposed that the hydrogen bonds holding the multiple helices together (those of the primary alcohol groups) are destroyed, or, if they exist, they are without strain, then the multiple helix could change to its most stable form. The granule would then be without strain. It is still possible, however, that strain would again be produced with swelling even though the granule had been without strain. When the strainless granules are used in a reaction under conditions which allow a maximum adsorption of reactants, it may be that, at incipient reaction, the disorganization will be the most uniform and thus result in a maximum randomness of the glucose units and of reactivity.

Although the existence of hydrogen bonds in the granule

is not evidence for strain, they are instrumental in its retention. Perhaps much of the strain is the result of the tendency of the multiple spiral to contract and the opposing forces holding the radially arranged multiple spirals together. If the concept of the multiple spiral has merit, the aggregate hydrogen bonding energy of the groups in the "seams" may well exceed that of the intra-spiral bonds, even though the energy per bond is less. Thus, the primary alcohol hydrogen bonds may be so weakened that they can be ruptured at relatively low temperatures. It is these bonds which may be broken during low temperature hydration. This may explain why a reactive starch results by heating at the incipient gelatinization temperature after low temperature hydration.

The present work was planned with the desire to gain additional understanding of the significance of alkali lability and the factors which influence the behavior of the starch granule toward hot aqueous alkali. In as much as the Schoch method of alkali lability determination (15) had been universally adopted by research and commercial investigators because of its simplicity, it was investigated in this laboratory. The results reported herein are an average of at least two determinations and in all cases the deviation from the average is well within the limits of reproducibility reported by Schoch.

In the original work by Taylor and Salzmann (17),

aqueous potassium hydroxide had been used as the alkaline digestion medium. They pointed out that the production of reducing substances passed through a maximum after a 30 minute digestion period at 100°C. when 0.1 N potassium hydroxide was used. When Taylor, Fletcher, and Adams (18) later modified the original alkali lability method, 0.1 N sodium hydroxide was designated the digestion medium and a digestion time of one hour at 100°C. was specified.

Schoch and Jensen (15), in reporting the simplified alkali lability procedure, used the same digestion time, temperature, alkaline solution as reported by Taylor, Fletcher, and Adams. However, since Schoch had not shown the effect of various alkali metal hydroxides or varied times of digestion, it was deemed advisable to consider these variables and their effect on the alkali consumption of the starch sample.

The behavior of starch in reactions has indicated that the energies of hydrogen bonds cover the whole "spectrum" within limits. Furthermore, the stability of the hydrogen bonding pattern in the starch granule has been evidenced by the near reversibility of the palmitic acid adsorption-desorption isotherms (5). Moreover, the interstices within the granule network would not be expected to be uniform and if not, would materially affect diffusion rates of reactants. Therefore, the granule in its initial condition probably is not uniformly available to a given ion or equally available

to different ions.

As the metal ion diffuses into the granule, it might conceivably add to points of unsaturation (reaction 1). Moreover, the addition compound could be such that it could be titrated with acid. It would then be expected that the added, but still titratable, alkali would undergo further change (reaction 2), depending upon the temperature and upon the strain. At a sufficiently low temperature, the diffusion process could dominate to the extent that the granule would be completely saturated (to the extent allowed by steric hindrance) before reaction 2 became appreciable. More particularly, if a temperature should exist at which reaction 2 did not occur (and, therefore, no change in the granule lattice), the granule could be completely "saturated" with any particular ion, in which case, the alkali consumed in digestion would be a maximum. If the temperature should be such that reaction 2 is appreciable, the latter might be followed by structural adjustments resulting in decreased granule strain and a decreased alkali consumption upon digestion. Furthermore, other than a sufficiently low temperature, another condition might be necessary if reaction 2 is to be avoided. It might be necessary to employ such alkali concentrations that local concentrations in the granule could not become excessively high and cause premature swelling.

In the investigation of the effect of the time of digestion upon the alkali consumption, natural Pink kafir

starch was treated according to the modified Schoch procedure except that the time of digestion was varied. At the same time, a comparison was made of the consumption of lithium, sodium, and potassium hydroxides (Table 1).

Table 1. The rates of consumption of different alkalis by starch (Schoch's procedure).

Time of digestion (min.)	Lithium hydroxide	Sodium hydroxide	Potassium hydroxide
0	0.44	0.16	0.07
10	1.22	0.91	0.66
20	1.95	1.59	1.59
30	3.02	2.86	2.60
45	4.31	4.69	3.82
60	6.36	6.01	5.37
120	14.86	15.06	15.30
240	30.68	32.61	33.30
360	40.37	44.05	42.67
480	47.26	51.13	48.67

This work definitely established the fact that the alkali number was a numerical value expressing the rate of reaction of the starch and alkali (Fig. 1). It was also evident that the reaction failed to produce a maximum in alkali consumption during an eight hour period of digestion. This fact was contrary to the data published by Taylor and Salzmann (17) and indicated that the substances measured iodometrically by Taylor were not the substances measured by the Schoch method. Furthermore, in order to achieve reproducible results, the time of digestion, as specified by Schoch, must be followed to the minute.



It has long been known that the reactivity of the alkali metals increases with increasing atomic weight. Thus, potassium is more reactive than sodium, which in turn, is more reactive than lithium. In addition, the ionic radius of the alkali metal increases with increasing atomic weight.

The data (Table 1), which are graphically illustrated in Fig. 1, gave evidence for the two proposed reactions between the metal ion and the starch molecule. That is, as the metal ion diffuses into the granule, it might add to points of unsaturation (reaction 1) and later undergo further change (reaction 2), depending upon the temperature and upon the strain.

When Schoch's procedure was applied to Pink kafir starch with lithium, sodium and potassium ions, these ions initially were not equally available to the granules. Therefore, early in the digestion, the alkali consumption seemed to depend upon the ionic radii or upon the diffusion rates. Since the temperature was high ( $100^{\circ}\text{C}.$ ), the rate of reaction 1 was probably slower than reaction 2 and thus gave rise to continuous structural adjustments. With time (2 hours), structural changes may have occurred to such an extent that the diffusion rates may have become zero order. In such a case, the relative amounts of the ions consumed would follow the order of ionic activities. Still later (4 and  $2/3$  hours), the residual lattice may once again become more restricted. It is possible that the extent of restriction was so sharp

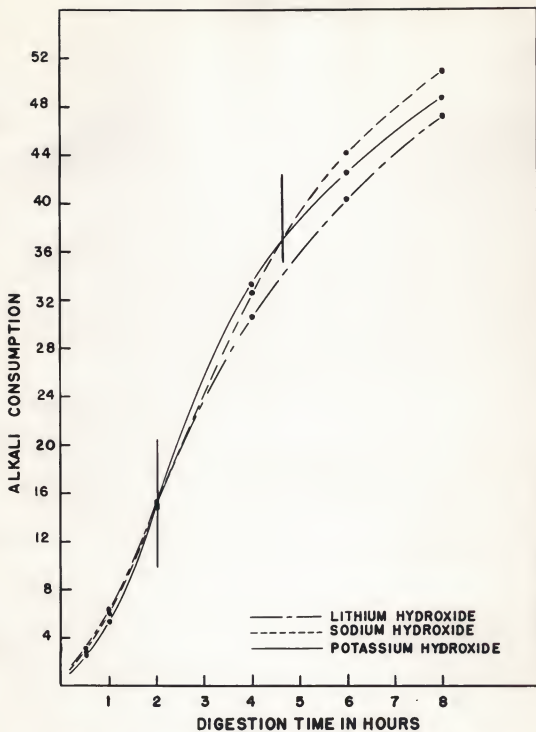


Fig. 1. The consumption of different alkalis by starch (Schoch's procedure)

that the rates of consumption of sodium and lithium ions depended upon their reactivities, while that of the potassium ion again depended upon diffusion.

In as much as the alkali consumption was not entirely dependent upon the rate of diffusion or upon the reactivity of the ion, this work gave further evidence for the proposal that small but measurable structural adjustments could take place within the granule during alkaline attack and thus revealed the stability of the hydrogen bond toward alkali.

During the investigation of alkali lability, it was learned that a 0.1 N sodium hydroxide solution, when added to starch, produced very little gelatinization and that increasing concentrations above 0.1 N produced increased swelling. However, preliminary results indicated that tolerance to sodium hydroxide could be built up in the starch granules by slow step-wise increases in the concentration of the alkali. This permitted a study of the effects of time allowed for diffusion and of temperature on the rate of swelling.

The data of Table 3 show the effect on the alkali consumption of adding alkali step-wise at a temperature of 4°C. as compared with samples held at room temperature. The regular alkali numbers of the samples are included for the purpose of comparison.

The 0.5 gram samples of the starch were suspended in 20 cc. water and then brought to the temperature indicated.

The addition of 0.4 N sodium hydroxide was then made in the amounts indicated at 24 hour intervals (Table 2). The required 25.00 cc. of alkali was thus added in five daily intervals and then the starch was allowed to remain in contact with the 0.22 N solution for an additional 24 hours. At the end of 144 hours, all samples were allowed to warm to room temperature, then 55 cc. hot distilled water added and digested for one hour at 100°C.

Table 2. The step-wise additions of 0.4 N sodium hydroxide.

Hours	: Alkali addition	: Resulting normality
24	3 cc.	0.05 N
48	3 cc.	0.09 N
72	6 cc.	0.15 N
96	8 cc.	0.20 N
120	5 cc.	0.22 N*

\* This normality is equivalent to the solution normality as specified by Schoch.

Table 3. Effect of the step-wise addition of alkali on its consumption by starch at two different temperatures.

Starch	: Stored : : at : : 4°C. :	$\Delta_1^*$	: Stored : : at : : 25°C. :	$\Delta_2^*$	: Regular : : alkali : : number
Original Pink kafir	6.69	+0.13	6.82	+0.81	6.01
Original C-8	11.04	-0.23	10.81	+0.98	9.83
Extracted C-8	11.39	-0.95	10.44	+0.05	10.39
Palmitic Acid Adsorbed C-8	13.27	-1.36	11.91	-0.45	12.34

\*  $\Delta_1$  is the difference in the alkali consumption value of the sample stored at 25°C. and the sample stored at 4°C.

\*  $\Delta_2$  is the difference in the alkali consumption value of the sample stored at 25°C. and the regular alkali number.

The data in Table 3 show the effects of time allowed for diffusion and of temperature on the rate of swelling. Four starches were selected for this work which represented extremes in internal strain. The two original starches are under less strain than the Extracted C-8 sample or the Palmitic Acid Adsorbed C-8 sample. This is substantiated by the increased alkali consumption as determined by the Schoch method. The rates of diffusion, to most regions of the granule, seemed to be high in all cases. At 4°C., swelling was so slow that it permitted "saturation" of the less accessible regions to occur with a minimum of swelling, and therefore

produced a maximum alkali consumption. At 25°C., the rates of swelling were sufficiently slow to allow structural adjustments to occur with a subsequent decrease in strain. However, under the conditions of the Schoch procedure, the addition of all the sodium hydroxide at one time forced the granule to swell before complete penetration had taken place. When high concentrations of alkali are used, it might be assumed that locally high concentrations of the ions are instrumental in causing swelling before the diffusion of ions is complete. Therefore, the structural adjustments took place in the granule without the metal ions being available and, consequently, a greater decrease in alkali consumption resulted than was noted in the 25°C. treatment.

The highly distorted Palmitic Acid Adsorbed C-8 sample would be expected to show more structural adjustment on swelling at 25°C. than would the Original C-8 starch which is under considerably less strain. This was substantiated by the fact that the former decreased 1.36 alkali units, whereas the latter decreased only 0.23 alkali units as shown in the column,  $\Delta_1$ . Furthermore, the Original C-8 starch, which is under less strain, would be expected to show a greater increase in alkali consumption as expressed by  $\Delta_2$  than would the highly distorted samples. This would be due to the fact that diffusion could take place more readily in the original starch and thus allow the less accessible regions of the granule to become saturated before swelling.

This is substantiated by the data shown in column  $\Delta_2$ . It also appeared, in the case of the Pink kafir sample, that the slow swelling, which occurred at 25°C., allowed an opening of the less accessible regions which were not available in the case of the sample stored at 4°C. and thereby caused an increase in the alkali consumption as shown by  $\Delta_1$ .

In an effort to determine the consumption of alkali during diffusion, consideration was given to the swollen granules. The starch samples were pasted in 20 cc. water for 30 minutes, then allowed to cool to room temperature in three hours. The required 25.00 cc. of 0.4 N sodium hydroxide was then added and the volume brought to 100 cc. These samples were allowed to stand at room temperature for varied periods of time. At the end of the specified period of time, two of the samples were then treated with 55 cc. of hot distilled water and digested one hour to determine the hot alkali consumption. Two other samples were titrated, without digestion, using the standard acid to obtain the cold alkali consumption. The amount of alkali consumed was calculated in the usual manner.

When the data in Table 4 were expressed graphically, Fig. 2, it became evident that the trend of alkali consumption for both the digested and cold samples followed the same rate of increase in nearly all cases. When the alkali consumption value of the cold sample was subtracted from the value of the digested sample, the curve (III) indicated a nearly

constant alkali consumption value.

Table 4. The alkali consumption of starch pastes. The effect of time of standing in contact with alkali before digestion.

Time of standing (hrs.)	Original C-8		Extracted C-8		Palmitic Acid Adsorbed C-8	
	Digest	Cold	Digest	Cold	Digest	Cold
1	11.22	0.02	11.49	0.02	12.60	0.16
24	11.24	0.16	11.76	0.05	12.56	0.18
48	11.15	0.19	12.55	0.53	13.45	0.75
72	11.47	0.37	12.95	0.55	13.50	0.75
96	11.70	1.01	13.12	1.00	13.42	0.78
120	12.15	0.81	13.19	1.05	13.83	1.10

In as much as the corrected alkali consumption curve remained nearly constant, it was evident that internal strain was not decreased in the pasted granule as a result of the reactions taking place during diffusion. It indicated that diffusion was slow and that there were less accessible regions even in the swollen granule. This and other facts gave indication that a true evaluation of internal strain would be realized by a very slow process of diffusion of the alkali into the unswollen granule so that all points of strain would be saturated with metal ions before the digestion was carried out. This would then result in a maximum alkali consumption value as obtained by a specified digestion time and temperature.

This work also gave evidence that the strain in the swollen granule may exceed that of the natural granule.



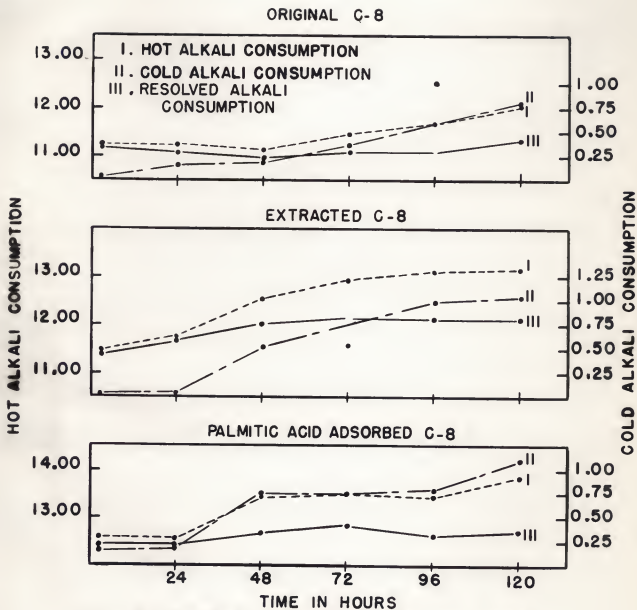


Fig. 2. The alkali consumption of starch pastes. .

When a comparison was made of the alkali consumption values of the three C-8 samples as determined by the regular Schoch procedure (Table 3), it was evident that the strain in the swollen granule exceeded that of the natural granule since there was an increase in alkali consumption.

In carrying out preliminary alkali lability studies in this laboratory, little attention was given to the number of samples placed in the boiling water bath during the one hour period of digestion except that the titration procedure limited the number of samples to one every 10 minutes. However, since the evidence revealed the importance of diffusion and temperature, these effects were given further consideration.

The Schoch method required addition of hot distilled water to the alkali swollen starch samples before placing them in the water bath. These swollen starch samples resulted from the addition of the required 25.00 cc. of 0.4 N sodium hydroxide. When the starch was treated in this manner, complete diffusion of the metal ions into the granule was prevented by swelling due to localized high concentrations of metal ions and by the effect of the hot distilled water. Therefore, the structural adjustments took place in the granule without the metal ions being available and consequently a decrease in alkali consumption resulted (Table 3). Therefore, this method was compared with the procedure of adding cool distilled water (kept at room temperature) to the alkali swollen starch samples, which were then digested for one hour

at 100°C. Furthermore, these two procedures were carried out so that six samples were digested during the hour as compared with the digestion of one sample per hour (Table 5).

Table 5. The influence of the temperature of the dilution water and the number of samples simultaneously digested on alkali consumption.

Starch	Alkali consumption			
	Hot water*		Cool water*	
	6/hour	1/hour	6/hour	1/hour
Pink kafir	6.01	6.33	6.14	6.74
Original C-8	9.83	10.24	10.31	11.02
Extracted C-8	10.39	11.11	10.97	11.66
Palmitic Acid				
Adsorbed C-8	12.34	13.51	13.27	13.61

\* Hot water: 55 cc. hot distilled water added to alkali swollen starch before digestion.

\* Cool water: 55 cc. cool distilled water added to alkali swollen starch before digestion.

In all cases, the placing of more than one sample in the bath during the hour of digestion lowered the temperature of the bath sufficiently to affect the rate of reaction. Therefore, to attain reproducible data, the number of samples digested per hour should be fixed by the operator and followed throughout.

This work also brought out the fact that when the cool starch sample was placed in the boiling water bath, the slower

temperature increase was sufficient to allow a greater extent of diffusion and a consequent increase in the amount of alkali consumed. Furthermore, when a comparison was made of the cool water samples digested six per hour and the samples which had alkali added step-wise at 4°C. (Table 3), it was again apparent that the original starch had a more loosely woven lattice which allowed diffusion to take place more readily than did the highly distorted samples.

During the year of 1943, Barham and Hagoner (3) had materially increased the rate of gelatinization of sweet potato starch by low temperature hydration. The procedure for hydrating at low temperatures consisted in uniformly increasing the temperature of a 10 per cent starch and water suspension from 20° to 65°C. during a two hour period. The suspension was then held at 65°C. for varied periods of time ranging from 1 to 96 hours. In all cases, the rate of gelatinization was increased.

In 1946, Barham and Lanning (4) continued the low temperature hydration work using commercial cornstarch. The procedure used by them is discussed under the heading, "Starch Samples", page 11. Again, the viscosity records showed an increased rate of availability of the starch for water.

Since the recent work of Barham and Campbell (5) revealed the quantitative inverse relationship between the rate of

gelatinization and alkali lability, investigations were made on five hydrated samples prepared by Barham and Lanning. Complete data are presented in Table 6. The column, per cent granules stained, represented the per cent of granules either injured or swollen. This was determined by a special stain (12) consisting of equal parts of a 0.1 per cent aqueous solution of congoecorinthe and a 0.25 per cent aqueous solution of benzopurpurine, which was mixed just before placing on the starch granules. The color of the injured or swollen granule was pink, whereas the unchanged granule retained the white color. The per cent of granules showing crosses was determined by observation of the granules under polarized light. The natural granule showed a cross due to structural orientation; however, as the granule swelled, the cross disappeared, thus giving a measure of swelling. The alkali number was determined by the usual Schoch method.

The natural cornstarch is presented in the table for purposes of comparison. The temperature given in the column, temperature of hydration, was the lower limit of gelatinization as determined from viscosity data.

Table 6. Low temperature hydration of starch.

Sample number	Temperature of hydration	Per cent granules stained	Per cent granules showing crosses	Swelling	Alkali number
XVIII	63	5	97	none	11.73
XVI	64	5	95	none	11.72
XX	65	15	90	slight	11.45
XIX	66	50	70	moderate	11.43
XVII	67	65	50	high	11.25
XXI	68	75	45	high	11.15
Natural starch	68 <sup>†</sup>	1	99	none	11.86

<sup>†</sup> The lower limit of gelatinization as determined by viscosity data.

The purpose of low temperature hydration was an attempt to cause a granular rearrangement to take place which would decrease internal strain. Since it is thought that much of the strain is the result of the tendency of the multiple spiral to contract and the opposing forces holding the radially arranged multiple spirals together, the primary alcohol hydrogen bonds may be so weakened that they can be ruptured at relatively low temperatures. It is these bonds which may be broken during low temperature hydration. The fact that water was able to break the primary alcohol hydrogen bonds was shown in the chlorination of starch carried out in this laboratory (20). When the relaxed granules are used in a reaction under the conditions which allow a maximum adsorption of reactants, it may be that, at incipient reaction,

the disorganization would be the most uniform and result in a maximum randomness of the glucose units and therefore cause a decrease in the alkali consumption which would be the consequence of decreased internal strain.

The data in Table 6 indicated a limited amount of granular relaxation. Furthermore, it was noted that the alkali number did not decrease appreciably until part of the granules began to swell. This would seem to indicate that granular adjustment can take place after a many-fold increase in diameter of the granule and that adjustment is facilitated by higher temperatures. It appeared that maximum granular relaxation could be achieved by slowly raising the temperature of a starch suspension to a temperature slightly below the lower limit of gelatinization and then holding it at this temperature a sufficient time to allow the rupture of the primary alcohol hydrogen bonds; then again raising the temperature slightly above the gelatinization temperature and holding it for a period sufficient to break the secondary alcohol hydrogen bonds located in the seams of the multiple spirals.

## SUMMARY

1. The alkali number, as determined by the Schoch method, expressed the rate of reaction between starch and alkali in terms of the cubic centimeters of 0.1 N sodium hydroxide consumed by one gram of starch during digestion in alkali for one hour at 100°C. This was further substantiated by studies concerning the rates of consumption of different alkalis.

2. Alkali consumption was influenced by the rate of diffusion of the alkali into the starch granule and by the reactivity of the alkali metal hydroxide.

3. Sodium hydroxide was consumed during the process of diffusion into the starch granule and resulted in a decrease in internal strain.

4. Two reactions were proposed to account for the alkali consumption by starch. First, when the metal ion diffuses into the granule, it adds to points of unsaturation (reaction 1). Later the added alkali may undergo further change (reaction 2) depending on temperature and strain.

5. The strain in the swollen granule may exceed that of the natural granule.

6. Low temperature hydration of starch materially increased the rate of gelatinization and consequently decreased the alkali number. This was further evidence for the quantitative inverse relation between the rate of gelatinization and



alkali lability.

7. Experience has indicated that the consumption of alkali by starch is due to a labile reducing structure caused by strain. Such a structure may arise from the rupture of the lactal oxygen bridge of the glucose unit. An unsaturated structure of a free radical character would result. Because of the variability of strain operating a point of unsaturation, each structure would have its own equilibrium constant.

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