

STARCH HYDROLYSIS BY HEATING IN HERMETICALLY
SEALED SYSTEMS AT NEUTRAL PH

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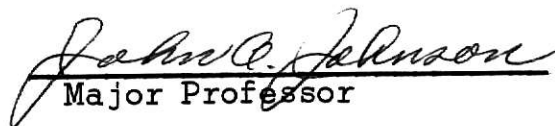
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INTRODUCTION

Starch is one of the most abundant plant materials in the world. It has been in the diet of man and his ancestors since the beginning of time. Because of its availability, much research has been conducted on starch. This research has dealt in great part with the physical and chemical changes that take place when starch is heated. This includes heating dry, acid solutions, and aqueous suspensions. Most of the studies have been concerned with heat treatments below 100°, especially when these studies dealt with the heating of aqueous suspensions or acid solutions. Temperatures in this range cause starch to undergo several physical changes. The most important change is the irreversible swelling of the starch granule. This hydration and swelling is called gelatinization. Gelatinization primarily affects the film and paste characteristics of the starch.

A second area of study has dealt with the hydrolysis of starch. Upon hydrolysis, the polysaccharide starch yields glucose and higher oligosaccharides due to incomplete hydrolysis, as well as dehydration and decomposition products of glucose. In this area of study, there has been a great deal of interest in the amount of hydrolysis, the reactions produced under various

conditions and the products formed. While much is known about these products after acid or enzyme hydrolysis, very little work has been done on trying to hydrolyze starch in a neutral, aqueous system. This investigation was undertaken to determine if starch could be hydrolyzed in a neutral starting solution, and if so, what were the necessary conditions, what products could be formed and what starch concentration could be used in this hydrolysis.

REVIEW OF LITERATURE

Structure of Starch

Naturally occurring starch is found in many different sizes and shapes. The characteristics of different plants are such, however, that starch can be identified microscopically. Under a microscope, the starch granules are minute structures built up of molecules arranged in layers usually concentrically. The size of these granules vary from less than 1 micron to over 150 microns in diameter (1). Wheat starch has been found to vary between 2 u and 35 u in size (2). Sponsler (3) reported that the starch granule has a regular and fairly uniform arrangement of atoms. He also reported (4) that the starch granule is built up of units arranged in concentric layers and that the structure was neither strictly amorphous or crystalline. Meyer (5) reports that the starch granules consists of intermixed linear and branched starch molecules arranged in a radial fashion. When these molecules are branched or when linear molecules are parallel to each other, hydrogen bonding takes place. These forces pull the chain together in associated crystalline bundles or micelles. It is conceivable that the long chain starch molecules pass through many micellar areas and that those chains on the outer edges of

the granule participate in several separate micelles. The bundles tend to hold the granule together and allow it to swell, but prevent dispersion and solution of the individual molecules. While the micelle bundles are tightly packed, the spaces between them are more disorderly and less densely packed. These characteristics render starch its elastic characteristic.

When starch is being developed naturally, it shows a layered or shell structure (6). Within this granule is a spot, considered by some, to be the organic center or nucleus of the starch granule (7). As the starch granule is developed, stresses develop causing cracks in the granule. When these granules are observed under polarized light, the granules light up except for an interference pattern. This pattern intersects at the hilum causing a "Maltese Cross" configuration. Reichert (8) has shown many photographs of different starch granules taken in both ordinary and polarized light showing this configuration.

As starch granules grow, the peripheral layers have a greater swelling power and this exerts a pulling force on the internal layers closer to the hilum which can cause cracks in the granule. This is especially true if the swelling capacity is low (9). As these cracks appear within the structure, they make the granule more available to internal as well as external digestion. When starch is suspended in water, these fractures provide a path for it to enter the internal portion of the starch granule.

This water is then available to dissolve the amylose leaving amylopectin as an insoluble residue. This technique has been used quite successfully for the separation of starch granule components. It is within these faults also that starch molecules become partially hydrated and are more subject to amylolysis (10).

Several workers (11, 12, 13) have shown that starch consists of two discrete polymer fractions, amylose and amylopectin. It was found (14) that these two compounds vary greatly with the variety and environmental conditions under which the plant was grown. This fact led to many disagreements over the existence of amylopectin in starch. Later studies (15, 16) have shown that actually amylopectin is the major component in most cereal starches. This polymer ranges from around 65% to as high as 100% in waxy rice, corn and maize. Legumes were shown to have a narrower range of amylopectin than cereals. This was reported to range from 30% - 66% in peas (17).

Amylopectin, the branched component of starch, is primarily responsible for the organization and crystallinity of the granule according to Meyer and Bernfield (18) and is thought to be fairly well distributed throughout the entire granule. The results of Baker and co-workers (21) indicate that this branching is through 1 - 6 glucosidic linkages. The molecular weight of amylopectin has been reported to range from 50,000 to 1,000,000. These values were obtained by osmotic pressure changes and may be artificially

low due to the difficulty of obtaining complete dissolution of the swollen starch granule without some molecular degradation. Further work by Kerr and co-workers (19) indicates that the molecular weight range is significantly lower. They made osmotic pressure measurements of amylopectin acetates dissolve in chloroform. These data showed molecular weights in the range of 160,000 to 230,000. These data showed reasonably good agreement with research done by Potter and Hassid (20).

Amylose is the linear fraction of the starch granule. This has been shown to be a linear unbranched chain of glucopyranoside units, joined by 1 - 4 glucoside lineages (22). Data summarized by Greenwood (22) shows that the molecular weight of amylose ranges from 50,000 to 200,000. Of the various starches, cereal starches (corn and wheat) generally show the lowest molecular weights while tuber and root starches (potato and tapioca) show the highest.

The chemical and physical properties of amylose and amylopectin vary greatly. Amylose is, at best, only slightly soluble in cold water. Kerr (24) and co-workers studied the effect of extracting starch using hot water. They found that as the temperature increased, the solubility of amylose also increased. Foster (23) reported that the best way to dissolve amylose in a neutral solution was to place it in aqueous alkali and then neutralize. After neutralization and upon standing, the amylose will then

ultimately precipitate out. This phenomenon is called retrogradation. He further stated that while aqueous solutions of amylose must be regarded as metastable, amylopectin will form relatively stable solutions in water.

Amylose was found to stain bright blue with iodine while amylopectin gave a purple color. This starch-iodine complex formation was the basis for the work by Bates et al. (15). He measured the iodine affinity potentiometrically. When iodine was added to a solution containing amylose, the iodine affinity, as measured by E.m.f. increase, remained constant as long as amylose was available to form the complex. When the amylose complex formation was complete, there was a continual rise in the E.m.f. value. When iodine was added to an amylopectin solution, there was a continual rise in E.m.f. instead of an initial stable period. When this rise was plotted, the curve obtained closely matched one obtained by adding iodine to water and measuring the E.m.f. increase. Both of these curves approximated the one obtained from the amylose sample after all available amylose had complexed with the iodine.

Effect of Heat on Starch

There have been much research involving the heating of starch. Research has been conducted on the heating of dry starch, starch in an aqueous solution, and in acids, alkalies and salts.

Air-dried starches, which retain small amounts of water, show characteristic X-ray patterns indicating a high degree of crystalline order in the granule (6). As air-dried starch is subjected to successively higher temperatures, various changes take place. Katz (25) reported that as long as the temperature remained below 120°C . there was no observable difference in the X-ray pattern upon rehydration. However, above this temperature the moisture uptake on rehydration is less, and the X-ray pattern indicates a loss of crystallinity. Around 210°C . a completely amorphous X-ray pattern appears, caramelization occurs and there is extensive conversion to volatile products. Wheat starch was found to lose its crystallinity at a temperature of 220°C . It was carefully pointed out by the researchers that the starch was extremely dry and all moisture possible had been removed from the air in which the samples were heated. Sjostrom and Brimhall (26, 27) in separate papers reported that granular starches heated to $180 - 200^{\circ}\text{C}$. retained their typical appearance under a microscope and were strongly birefringent in aqueous glucerol. These did not, however, show normal swelling properties; instead, the granules underwent a dissolution by sloughing off in concentric layers. It was also noted by Katz (25) that the cold water solubility of the heated starches increased markedly after being heated to 180°C . in dry heat. Temperature below 140°C . appeared to have little effect upon the solubility. Bryce and Greenwood

(28) studied the effects of heating dry starch to temperatures between 200 and 400°C. These studies indicate that major amounts of formaldehyde, formic acid, acetic acid and 2-furaldehyde were produced. Their studies indicated that there was little difference in the products, whether this was conducted in air or oxygen.

In contrast to studies on the effect of heat on dry starch, most of the studies on starch in aqueous solutions have been at temperatures below 100°C. These studies have included such variables as the percent water, varieties of starch, length of heating temperature and environmental effects within the varieties. The techniques used included loss of birefringence, changes in swelling patterns and increased sorption, increase in viscosity and change in optical transparency.

In the natural state starch exhibits a limited capacity to absorb cold water and swell, and at room temperature it will establish an equilibrium with moisture in the surrounding atmosphere. If this starch is again dried, the swelling which occurs is reversible and no visual changes in the starch granule structure are noted. When aqueous solutions of starch are heated, the micellar network within the granule is weakened by disrupting the hydrogen bonding. This permits further hydration and finally irreversible granule swelling called gelatinization. After this swelling there is a pronounced increase in the viscosity of the suspension (29). Gelatinization has been defined by Labanov and

Lovacheva (30) as a process of breaking down the natural structure of the starch granule, the degree depending on the relationship between the amount of starch and water, temperature, and length of heating.

French (31) states that the breakdown of the starch granule takes place in three distinct phases. The first is when water is slowly and reversibly taken up. During this phase limited swelling occurs and the viscosity of the suspension does not change noticeably. The granule retains its characteristic appearance and birefringence. Around 65°C. and within a small temperature range, characteristic of the variety of starch, the granule suddenly swells increasing many times in size, takes up a great deal of water and loses its birefringence. During this stage there is also a marked increase in the viscosity of the suspension. As additional heat is added, the third phase of swelling develops. Here the granules become almost formless sacs, and the more soluble part of the starch granule is leached out.

Kerr (31) discussed in detail several methods for measuring the gelatinization temperature of starch. Several different criteria have been used to detect the gelatinization temperature--loss of birefringence, increase of optical transmittancy, a rise in viscosity. Of these, the loss of birefringence has been shown to be very sensitive, accurate, and reproducible. The Kofler microscope hot stage technique provides a rapid and simple method

for detecting the loss of birefringence (36). Sullivan and Johnson (32) reported a close relationship between loss of birefringence and susceptibility of starch to beta-amylolysis. This method was found to be useful in measuring starch gelatinization in complex systems where measurement of loss of birefringence was difficult.

Leach, McCowen and Schoch (2) investigated several different varieties of starch to determine their swelling patterns. They found that each starch showed characteristic swelling patterns. They reported that the major factor controlling the swelling behavior of the starch is the strength and character of the micellar network within the granule. On the molecular level many factors were shown to influence the degrees of association. This included not only the size, shape, composition and distribution of the micellar areas but the ratio of amylose to amylopectin. These also included characteristics of each fraction in terms of molecular weight and molecular weight distribution. The degree of branching and the length of the outer branches in the amylopectin was shown to have an influence on the degree of association. Naturally occurring noncarbohydrate impurities were also shown to be an important factor. Using the water sorptive and swelling capacities, Sair and Fetzer (33) showed that normal starch varieties could be divided into three groups (cereal, pith, or root and tuber) based on their degree of association.

Probably the most important physical property of starch is the change in viscosity produced upon gelatinization. This change occurs during the heating of an aqueous starch solution and continues during cooking if the gelatinization temperature is reached. As the starch suspension is heated, the granules gelatinize and swell freely until they assimilate virtually all the available water. In addition the bonding forces within the granule are less. As a result of these two factors the granule becomes more susceptible to thermal and mechanical breakdown (34). During the initial swelling, prior to reaching gelatinization temperature, the starch granule becomes partially hydrated. The intricate micellar network tends to separate, and the amylose fraction dissolves. The granule continues to swell until the entire volume is occupied. There was associative bonding of the dissolved amylose and starch granule as well as crowding of the swollen granules. Therefore, Kite, et al. (37) felt that the apparent viscosity change was somewhat superficial since the change was due not only to the properties of the swollen granules themselves but also due to the interaction of the starch solubles and the swollen granule. Katz (36) has further suggested that as some granules are swelling, others are simultaneously disintegrating under continued heating and stirring. The maximum viscosity is reached when the increase in structural viscosity caused by swollen starch is counterbalanced by the decrease in viscosity

resulting from disintegration and solubilization of the starch.

As starch granules are gelatinized, the aqueous suspension loses its initial opacity and becomes progressively more transparent. Kite (37) and co-workers have recently shown a quantitative relationship between gelatinization and light transmittance. At the same time they found that a visual comparison of paste clarity showed differences with respect to uniformity of hydration. Beckord and Sandstedt (38) measured the light transmittance as a starch suspension was heated. They found that if during the heating process, the starch suspension was held at a constant temperature for ten minutes, a significant increase in the optical transmittance occurred. They felt that this was due to either that starch gelatinization was not instantaneous and occurred over time or that granule disintegration was taking place.

Hydrolysis of Starch

Starch is one of the major sources of commercial sugars, and hydrolysis of starch to produce many commercial products has become a major world industry. This hydrolysis may be either complete or partial and is usually done by acid, enzyme, or a combination of both.

The discovery in 1811 by Kirchoff (39), a Russian chemist, that when starch and acid were heated together a crude sugar syrup was formed, became the basis for this industry. The fact

that this reaction was one of hydrolysis and the sugar formed was identical to grape sugar was determined a short time later by de Saussure (40).

Kerr (41) states that early attempts to study the mechanism of this reaction were complicated by three factors: (1) failure to recognize and appreciate the importance of secondary reactions; (2) lack of suitable analytical methods to study the reactions; (3) and a lack of knowledge concerning the chemical nature of the substrate, starch. He concludes that these factors make much of the early work on starch chemistry of little value to anyone other than historians.

Recent research by Cowie and Greenwood (42) has extended investigations of starch hydrolysis, and they reported further information concerning the structure of starch granules. They studied the effect of 0.2 M hydrochloric acid on potato starch heated to 45°C. It was observed that under these conditions there was no swelling of the granules and the birefringence properties were unchanged. This was interpreted by them to mean that acid treatment preferentially affected the amorphous rather than the crystalline region of the granule. Their study also indicated that the amylopectin was degraded at a considerably faster rate than the amylose. Since the hydrolysis rate of amylopectin must be at a minimum, they interpreted the data to mean that the alpha 1 - 6 glucosidic linkages are more acid resistant than alpha 1 - 4

linkages (68). They concluded that the amylopectin was preferentially degraded and therefore far more accessible to attack than the amylose and that this must be associated with the non-crystalline region of the granule. On the basis of these facts they reported that acid modification takes place in two steps. The first is a rapid attack in the outer non-crystalline regions followed by a slower attack on the crystalline areas.

In a later study by Arbuckle and Greenwood (43) on wheat starch under similar conditions the amylose and amylopectin had a similar relationship in rate of degradation. This rate was considerably slower than that of potato starch, however, and was interpreted to mean that the wheat starch had a more compact structure.

The relative degree of acid resistance by alpha 1 - 6 and alpha 1 - 4 glucosidic linkages was discussed in a paper by Swanson and Cori (68). They studied the comparative rates between amylose (1 - 4) and dextran (1 - 6) and found that the alpha 1 - 4 linkages ruptured more readily. This was shown to be true whether the starch molecules were linear or branched. They concluded that the alpha 1 - 6 glucosidic linkage was more acid resistant than the alpha 1 - 4 linkage.

Many theoretical models have been proposed for the random hydrolysis of polysaccharides. Frudenberg and Kuhn (44) assumed that the hydrolysis is a monomolecular reaction involving the

scission of alpha 1 - 4 glucosidic bonds. They applied a statistical treatment to predict values for glucose, maltose, and higher homologues at any given point during the early stages of hydrolysis. From this study they determined the rate at which any degradation product of any given length is formed and the maximum yield of this product which may be obtained during hydrolysis of a polysaccharide chain. According to Freudenberg and Kuhn (44) the maximum yield or maximum weight fraction ($W[n]_{\max}$) of a polymer during random degradation can be calculated. The following equation was derived:

$$W(n)_{\max} = \frac{NZn_{\max}}{N} = N \left(\frac{2}{n+1} \right)^2 \left(\frac{n-1}{n+1} \right)^{(n-1)}$$

Where Zn is the number of chains of length n , n is the number of units per chain and N is the total number of units. When Canton and Moyer (45) studied the accuracy of this in relation to several sugar determinations, they found that there was little agreement between the calculated results and actual values found for all sugars except maltose. They proposed that the terminal dextrose unit is more easily hydrolysed, and this would account for the higher amount of dextrose found. Simha (69) proposed that early products of hydrolysis (mono and oligosaccharides) could be formed by cleavage near a chain end. He stated that for random depolymerization the rate of formation of each product should be proportional to the number of chain ends from which the

particular product could be formed. The rate of formation would be maximum when the number of chain ends were at a maximum. Therefore, in the early stages the rate of formation of glucose, maltose, and maltotriose would be identical due to the fact that the number of cleavage points which would form each product would be identical.

As stated earlier the basic end product of acid hydrolysis is glucose. In addition, depending upon the conditions, there are also small amounts of gentiobiose, derivatives of furfural, levulinic acid and other degradation products formed by heating carbohydrates at high temperatures. It appears that after glucose is formed, further degradation takes place in highly acid system and at high temperatures to form furfural type compounds. The formation of 5-hydroxymethylfurfural is the principal product of this dehydration (46). Mizuguchi and co-workers (47) reported that small quantities of amino acids or proteins also promote the formation of 5-hydroxymethylfurfural as a result of the Browning Reaction.

The most recent commercial development in starch hydrolysis has been the discovery of practical method for the enzymatic conversion of starch to glucose or intermediate conversion products. Enzymes capable of catalyzing hydrolysis reactions are widely distributed in nature. These are found in digestive secretions of animals and within the cells of most animals, plants,

and microorganisms. These enzymes can be divided in groups according to their action patterns: alpha amylase, beta amylases, and glucoamylases.

Alpha amylases are found in virtually every type of living cell. They effect a rapid fragmentation of the whole starch molecule by cleaving the alpha 1 - 4 linkages thus bringing about a slow, but eventually extensive, conversion of starch to dextrins.

Beta amylases are found primarily in plant material. These promote a rapid hydrolysis of the outer chain of starch molecules to maltose. Beta amylase, however, can not hydrolyze the alpha 1 - 6 linkage, thus leaving a high molecular weight dextrin.

Glucoamylases are found in animal tissue and are produced by species of fungi. These enzymes have been found to hydrolyze amylopectin, amylose, and malto-oligosaccharides completely to D-glucose by breaking the alpha 1 - 4 linkage. Enzymes of this group affect hydrolysis of starch by a single chain mechanism in which glucose units are removed from the non-reducing end of the chain (48).

The enzyme hydrolysis of starch and oligosaccharides involve the addition of water to a D-glucosidic bond. In these reactions the catalysis occurs on the surface of the enzyme. Initially, some of the functional groups at the active sites of the enzyme interact with the functional groups of the substrate (starch) to form an enzyme-substrate complex. In this complex

the first carbon attracts electrons from hydroxyl groups. This strained structure breaks and finally a water molecule is added to the carbonium ion formed immediately. Mayer and Lerner (49) state that the experimental verification of this mechanism is very difficult to achieve.

Separation of Starch Hydrolysis Products

The use of chromatography has been an indispensable tool in the analysis of starch and separation of starch hydrolysis products. The methods used include paper, column, thin layer and gas.

Paper chromatography has been used by a number of researchers to study starch hydrolysis products. Salem and Johnson (50) used a system of butanol, pyridine and water in a 6:4:3 (v/v) ratio. Commerford and co-workers (51) used a solvent system composed of n-propanol, ethyl acetate and water, 14:2:7 (v/v) ratio for the separation of macro quantities of oligosaccharides. Shaw (52) used n-propanol, ethyl acetate and water, 6:3:1 (v/v) to study the purity of oligosaccharides separated from corn syrup on a carbon column.

Column chromatography has been used both in quantitative analytical and preparative work (53). Carbon columns have been used a great deal because of their large holding capacity. Hoover, et al. (54) used a carbon column to separate the oligosaccharides

found in 42-DE corn syrup. The columns were prepared from a mixture of two charcoals purified with concentrated hydrochloric acid and rinsed until pH 3.5 was reached. The oligosaccharides were eluted from the column by employing a gradient of ethyl alcohol, the concentration going from 0 - 30%. Paper chromatography of the eluted samples indicated homogeneous products were obtained. Sephadex G-75 was used by Nordin (55) to separate starch dextrans. He found good resolution according to molecular size.

Columns prepared from cellulose powders have proven to be very reliable in this work also. Neukom and co-workers (56) used DEAE advantageously for the fractionation of acidic as well as neutral polysaccharides. Acidic polysaccharides were absorbed readily on the DEAE at a pH near 6 and were eluted depending upon their number of acidic groups. Also, DEAE has been used successfully in the separation of impurities from polysaccharides. The impurities were absorbed very strongly and remain on the DEAE cellulose column while the polysaccharides were easily eluted (57).

Gas chromatography was used by Bryce and Greenwood (28) in their study of the high temperature heating of dry starch, as reported earlier. The main volatile products included carbon dioxide, water, furaldehyde and 5-(Hydroxymethyl)-2-furaldehyde.

Microwave Heating

The fact that high frequency electrical fields might affect biological systems has been known since 1893. A great deal of work has been done over the last few years to determine how this fact could be utilized in the processing of foods. One of the important discoveries was the fact that radio-frequencies could be used to rapidly increase the heat content of water. Unlike thermal heating where the air is first heated and then transferred to the surface of the material, microwaves were found to pass through matter producing a volume heat effect.

The first type of microwave process device to be developed was the batch type oven. Radiation was emitted from a power tube into the heating chamber. Here, it bounced back and forth, being absorbed in the food product. This caused the molecules present to rapidly align themselves with the electromagnetic field produced. Since this field changed quite rapidly, heat of friction increased at a rapid rate. The product was heated throughout at the same time since these waves penetrated the entire volume of food. Also, it has been reported that the effect of the microwaves on food is that of heat (58).

Microwave heating has been used to good advantage in the area of "in-package sterilization." When a material is placed in a suitable container that will withstand the necessary pressures, sterilization can be accomplished in a very short time. The

advantage of using microwaves for this purpose involves its rapid heat penetration. This results in shorter time periods necessary to get the material to a desired temperature. Glass containers especially designed to withstand high pressures serve these needs well. Since much of the time presently required for sterilization is involved in heat penetration to the center of the container, adaptation to microwave equipment could increase the efficiency of sterilization techniques appreciably (59).

MATERIALS AND METHODS

Starch Preparations

This investigation was conducted on hydrolysis of wheat starch which was prepared by extracting and purifying the starch in the laboratory at Kansas State University. The wheat starch was extracted from Hard Red Winter wheat flour by the method used by Wolf (60) with certain modifications. A dough was prepared using 70% water (100% flour basis) and allowed to rest one hour. The dough was broken up and placed in a mixer with a hook beater. It was slurried in 4 - 5 volumes of water per volume of dough on a weight basis for 20 minutes at 115 R.P.M. After mixing, the resulting slurry was filtered through filter cloth to separate the large gluten particles from the starch. The resulting starch slurry was allowed to settle, and most of the free water was decanted off. The starch and remaining water was slurried and centrifuged at 1500 R.P.M. for 20 minutes. By centrifuging, bran particles, gluten masses, and contaminated starch granules had separated from the prime starch. These were removed by carefully scraping them away from the starch with a metal spatula. There was a well defined, sharp boundary between the contaminants and the prime starch which was easily detected visually by the color

change and texture difference. The prime starch was reslurried in a minimum amount of water and recentrifuged. This process was repeated until all visual evidence indicated the bran, gluten and other contaminants had been completely removed. When this process had been completed, prime starch was left containing no visual contaminants. The starch was dried at $42 \pm 2^{\circ}$ C. for 16 hours in a forced air oven.

The starch was defatted by a modification of the method of Schoch (61). The dried starch was placed in a boiling flask with 85% methanol. A ratio of 3:1 (v/w) was used. A condensor was connected, and the mixture refluxed for two hours. After refluxing the starch was filtered and rinsed with hot 85% methanol. This procedure was repeated three times. After the final hot alcohol rinse, the starch was thoroughly washed with distilled water and filtered. This washing was repeated three times. The starch was then dried as before and stored at 40° C.

After the purification samples of the dried starch were submitted for analytical analysis. The purified and dried starches had 0.15% nitrogen (Kjeldahl) and 0.1% fat (ether extract).

Hydrolysis Tubes and Source of Energy for Heating

The hydrolysis tubes were heavy glass cylinders made at Kansas State University. These were made from pyrex glass having an inside diameter of 1.25 inches. The wall thickness was 5/32

inches and length approximately 11 inches. These tubes held approximately 125 ml. of starch slurry. In each test a total of 100 grams was added to the reaction vessel leaving about 1.5 inches of head space.

Two sources of heat energy were investigated for this work. A radiant heat convection air oven was first tried. Using this oven, it was found that it required one hour for the starch slurries to reach 180° - 200°C . It was not known what effect this extended heating had on the hydrolysis, and other energy sources were investigated to reduce this time. Microwave energy was selected as the second source.

The microwave energy was provided by a bench model Amana microwave oven. This oven operated at 2450 megacycles and provided 160 kilowatts of power. The internal cavity measured 15 x 15 x 10 inches. The tubes were held 1.5 inches above the base plate on glass supports to allow for more concentration of the microwaves in the starch slurry.

DEAE Cellulose Column

At the concentrations of starch finally selected for this work, colored side products formed at such a rate that paper chromatography separation of the sugars formed was extremely difficult. The separations were not distinct, and dark paths were left as the sugars migrated down the paper. A method to remove

these contaminants was necessary to increase the accuracy of our sugar determinations.

Charcoal columns were first used in an attempt to remove the contaminants. This did not provide the degree of clarification desired, and it was almost impossible to elute all the sugars with water. Due to undesirable structural changes which occur in sugars at extremely basic conditions, strong anion columns were eliminated. Strong cation column would not remove the contaminants. A DEAE cellulose column, a weakly basic substitution material, was investigated and successfully used to remove impurities from polysaccharide solutions. A column 4 cm in diameter and 25 cm in height was prepared by a modification of the method reported by Neukom and Kuendig (57). Twenty grams of DEAE was weighed into a large glass container and covered with 6 inches of distilled water. The mixture was slurried, and after twenty minutes settling the water and suspended fines were removed by suction. This process was repeated four times. The water was then removed by vacuum filtration and was continued until the DEAE took on a dry appearance. The procedure described by (57) was followed. This included suspension of the DEAE in 0.5 N hydrochloric acid, washing with water, resuspension in 0.5 N sodium hydroxide solution, washing with water and, finally, suspension in 0.1 N sodium hydroxide. The slurry was then added to the chromatography column and washed with water under slight pressure. This washing was

continued until the effluent was below pH 8.5. The cellulose DEAE used was obtained from the National Biochemicals Company.

Carbohydrate Analysis and Separation

Total carbohydrates were determined by the Phenol-Sulfuric method (62) and reducing sugar concentrations by the Folin Wu method (63).

A solvent system using n propanol-ethyl acetate-water 6:1:3 (v/v) was used in combination with Whatmann No. 1 and No. 4 paper. Both papers gave good resolution with the No. 1 paper taking 24 hours for the sugars to separate completely. The chromo-colors were developed in silver nitrate. The silver nitrate was prepared by adding 0.1 ml. of saturated nitrate solution to 20 ml. acetone. Drops of water were added until the silver nitrate dissolved. The papers were dipped in the silver nitrate solution and allowed to dry. They were then dipped in a 0.4% sodium hydroxide in methanol solution. When the spots had developed, they were fixed in a 5% sodium thiosulfate solution. The papers were then washed with water and dried (64).

Glucose determinations were made using Glucose Oxidase and O-dianisidine (65). This test makes use of a coupled enzyme reaction where glucose in the presence of water, oxygen, and glucose oxidase produces hydrogen peroxide and gluconic acid. The hydrogen peroxide then reacted with the o-dianisidine to give the

oxidized form. The color change was measured spectrophotometrically.

Commercial preparations of Glucose Oxidase (Glucostat Special) and o-dianisidine (Chromogen) were obtained from Worthington Biochemical Company. These were dissolved in 50 ml. distilled water just prior to each determination. Two ml. of this reagent were added to 4 ml. of unknown and a standard glucose sample. These were allowed to react exactly 10 minutes at which time the color was stabilized with 3 drops 4N HCl. The color development was read at 420 mu. The unknown and standards had been preserved with thymol and test conducted established that the addition of thymol did not affect the results.

Analysis of Contaminants and Acidity

Furfural and Hydroxymethylfurfural concentrations were determined by the method of Linko (66). This method made it possible to determine quantitatively 2-furaldehyde (furfural) and 5 (Hydroxymethyl)-2-furaldehyde in the presence of each other.

Furfural stock solution was prepared by dissolving 500 ug liquid furfural in a liter of distilled water. This stock solution was diluted to obtain standards containing 50, 40, 30, 20, and 10 u grams furfural. Five mls. of each dilution was then extracted with 25 ml. of benzene to remove the furfural from the water. This extraction was carried out 5 times using 5 ml. of

benzene each time. The samples were extracted in 50 ml. erlenmeyer flasks by shaking 2 minutes. The water and benzene layers were separated with a separatory funnel. The benzene fractions were combined and brought to 25 ml. These benzene extracts were the samples used to prepare the standard curve for furfural and hydroxymethylfurfural determination. The actual determinations for furfural and hydroxymethylfurfural were conducted as in the method cited (66) with one minor addition. In determining furfural with m-PhDA, after cooling for one minute, the samples were centrifuged at 15,000 R.P.M. for 5 - 7 minutes. This gave a better separation of the two layers and facilitated in extracting the sample from the test tube. The test for furfural and hydroxymethylfurfural in the presence of each other were determined as reported in the literature (66).

Titratable acidity was determined on 10 ml. aliquots of the sample diluted to 50 ml. with deionized water. They were titrated with 0.1 N NaOH to pH 7.

Temperature Determination

The use of microwave radiation for a heat source introduced a problem of determining the temperature inside the sealed glass tubes used in the reaction. Metal could not be placed in the microwave field since it might reflect the radiowaves away from the desired heating areas; or, if the metal absorbed the wave

length emitted, excessive heating would have resulted. This ruled out the use of a potentiometer or mercury thermometer. Alcohol thermometers could not be used since they would also absorb the radiowaves. There are a number of materials, waxes in general, that have well defined melting points. These materials can be obtained suspended in an inert, volatile, non-flammable liquid. Once applied to a surface they dry to a thin film. Upon heating, these materials will melt at specified temperatures. By placing sample tubes which had been streaked with these materials it was found that they were not affected by the microwave radiation. Each sample tube was marked with different indicators ranging from 176.5°C . to 239.5°C . These gave estimations of the temperatures reached during the heating. The temperature indicators were obtained from Tempil Division, Big Three Industrial Gas and Equipment Company, New York.

RESULTS AND DISCUSSION

Research by Calentine (67) suggested that starch suspension could be hydrolysed in neutral water solutions. This research indicated that at temperatures around 180°C . in hermetically sealed containers hydrolysis of starch to sugar would take place.

Calentine's (67) research was carried out in 1 percent starch solutions heated in stainless steel tubes. The tubes were sealed with stainless steel caps containing a lead gasket and were heated to temperatures between $150^{\circ} - 200^{\circ}\text{C}$. for one hour. At temperatures below 170°C . he reported that almost no hydrolysis took place and the optimum temperature found was 180°C . The method used to heat the tubes was to place them in an air oven set at the desired temperature and allow the solution to reach that temperature. Once reached, the samples were left for one hour and then removed for cooling. The total time necessary from first insertion in the oven to removal was approximately two hours. Data on the resulting solutions indicated that the starch was being hydrolysed into glucose, other reducing sugars and unknown products. Based on this research (67) it appeared possible to hydrolyse starch in a neutral starting solution.

The original design of this investigation was to determine

the true parameters under which hydrolysis in a neutral water solution took place. This was first concerned with the starch concentrations which might be used. Calentine (67) reported using only a concentration of 1% starch. There was need for information on the effect of metal ion contamination on the process. The elimination of any possible metal ion contamination was accomplished by using glass tubes. These had the added advantage over metal tubes of allowing visual observation of physical changes in the sample. Tubes containing one percent starch were heated to 180°C. for one hour and cooled. During this heating visual observation indicated that once the critical temperature (180°C.) was reached, physical changes took place within 3 - 4 minutes. The most prominent change was that the starch passed from a gelatinized paste to a clear viscous liquid quickly. Paper chromatography of the resulting liquid indicated that the starch had been hydrolysed to reducing sugars. A second observation revealed that the liquid went from one with a clear-yellowish to brown to dark black color. A question then arose concerning the possibility of eliminating the hour necessary to bring the temperature of the suspension to the desired level.

Since microwave radiation was known to rapidly increase the temperature of water (59) and since Goldblith (58) reported that microwave radiation had no effect on foods except one of heating, this was tried. When a one percent starch solution was

heated in a microwave oven, it was observed that after seven minutes the sample had undergone similar visual changes as in the air oven when heated to 180°C . and held for one hour. Paper chromatography of the syrup again gave typical hydrolysis products.

With the elimination of possible metal ion contamination and having available an energy source that would rapidly raise the heat content of the water, work was started to develop the parameters under which the hydrolysis would take place and all further work was conducted using the methods described.

Starch Concentration

Since one percent starch slurries had been tried previously, two and five percent sample were heated and visual examination indicated these had hydrolysed. To determine the upper limit slurries of 50 and 75 percent starch were heated. The 50 percent concentration was a thick slurry and hydrolysed uniformly to give a thick, viscous, syrupy material. The 75 percent concentration was extremely difficult to work with since not all of the starch granules were uniformly wet. The sample would not form a slurry; hydrolysis was erratic; and the dry particles became burnt and charred. Since the 50 percent concentration gave uniform hydrolysis based on visual appearance of the liquid produced, it was selected as an upper concentration. Starch concentrations of 10 and 25 percent were selected as intermediates.

Effect of Time in Microwave Field on Temperature Rise

By the use of temperature sensitive materials, it was possible to estimate the temperatures reached by the starch slurry after being subjected to microwave energy for various times. The results indicated that the starch slurries could be brought up to temperatures between $177^{\circ}\text{C}.$ and $218^{\circ}\text{C}.$ in 4.75 - 6.25 minutes. Table 1 shows the relationship between time heated, temperatures, and pressures obtained in the starch slurries. This compared to approximately one hour necessary to reach these temperatures in the air oven.

Table 1. Time held in microwave compared to temperature and pressure reached.

Time Heated (Minutes)	Estimated Temp. °C	Pressure*
10% Concentration		
5.00	198/190	270
5.25	204/198	230
5.50	204/198	230
5.75	212/204	266
6.00	218/212	313
25% Concentration		
5.25	191/184	171
5.50	198/190	201
5.75	198/190	201
6.25	204/198	230
50% Concentration		
4.50	184/177	146
4.75	191/184	171
5.00	191/184	171

*The pressure was determined by the use of steam tables and reported in pounds per square inch.

Effect of Concentration and Time Heated on Sugar Production

The effect of time heated and temperature obtained and increase in total carbohydrates, reducing sugars and glucose for the three concentrations, is summarized in Table 2 and Figures 1 - 3. These show that as the temperature increased the total reducing sugars and glucose followed a very similar increase in concentration. Figure 4 shows the distribution of the oligosaccharides separated by paper chromatography. Samples hydrolysed at the maximum temperature are shown.

Table 2. Effect of concentration and heating time on sugar production.

Time Heated Minutes	Estimated Temp. °C	Total Carbohydrates mg/ml	Reducing Sugars mg/ml	Glucose mg/ml
10% Concentration				
5.00	198/190	96.5	6.9	1.4
5.25	204/198	109.0	13.0	3.4
5.50	204/198	116.0	13.4	4.2
5.75	212/204	166.0	32.5	20.5
6.00	218/204	125.0	56.5	38.0
25% Concentration				
5.25	191/184	282.0	27.0	7.0
5.50	198/190	297.0	77.0	12.0
5.75	198/190	311.0	95.0	58.0
6.25	204/198	311.0	195.0	131.0
50% Concentration				
4.50	184/177	598.0	57.0	12.0
4.75	191/184	635.0	190.0	39.0
5.00	191/184	668.0	368.0	250.0

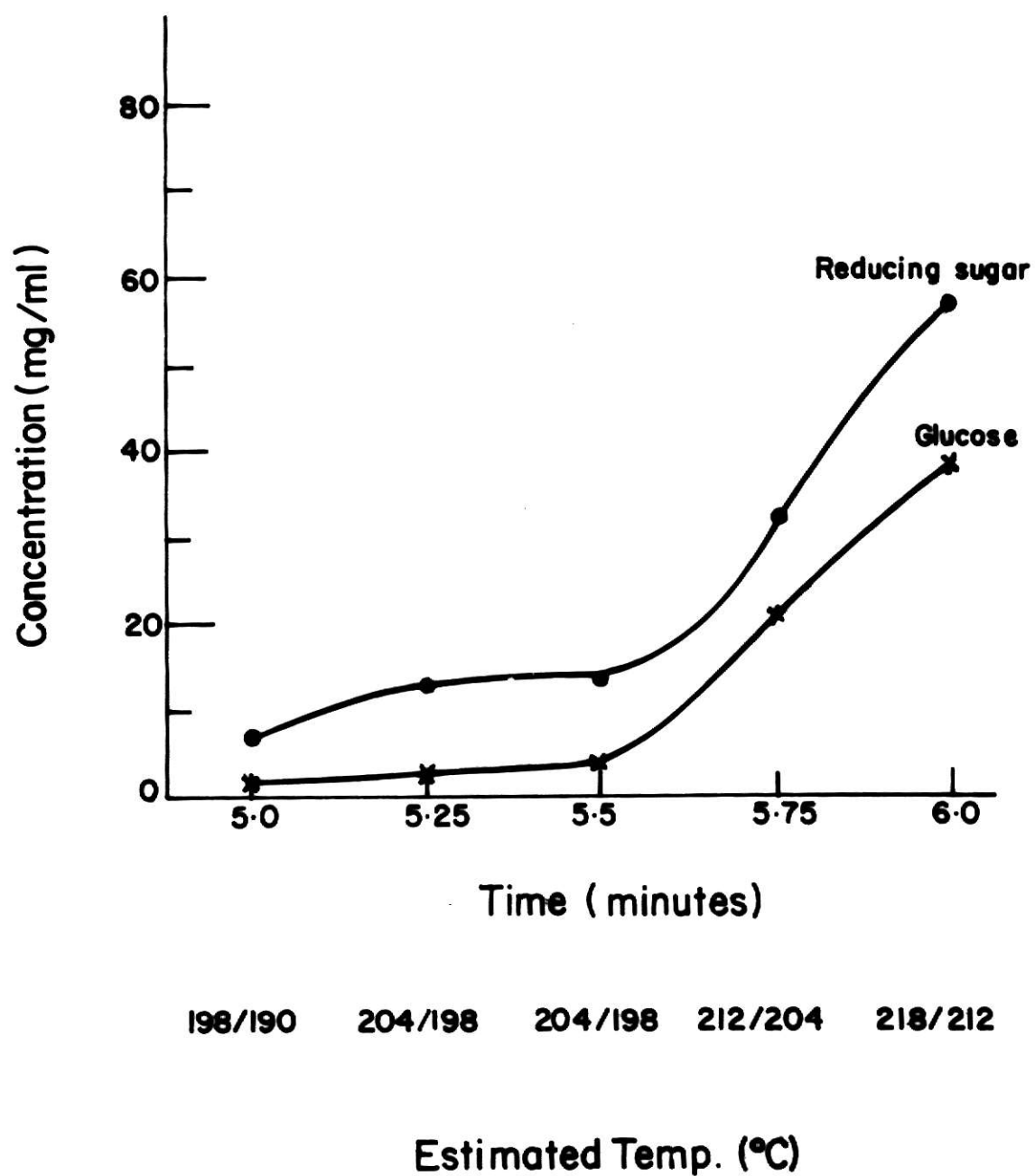


Figure 1. 10% Starch. Increase in the concentration of hydrolysis products.

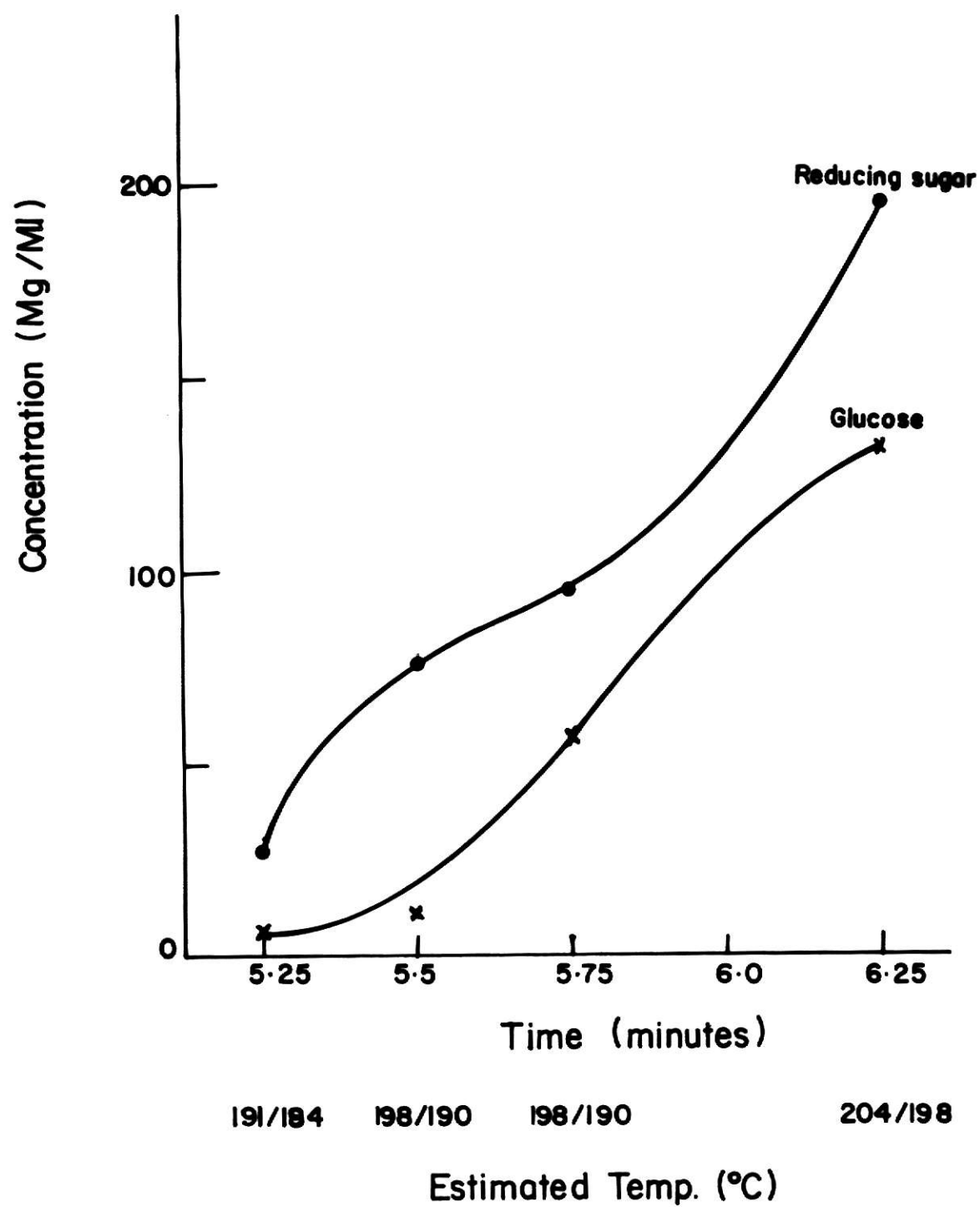


Figure 2. 25% Starch. Increase in the concentration of hydrolysis products.

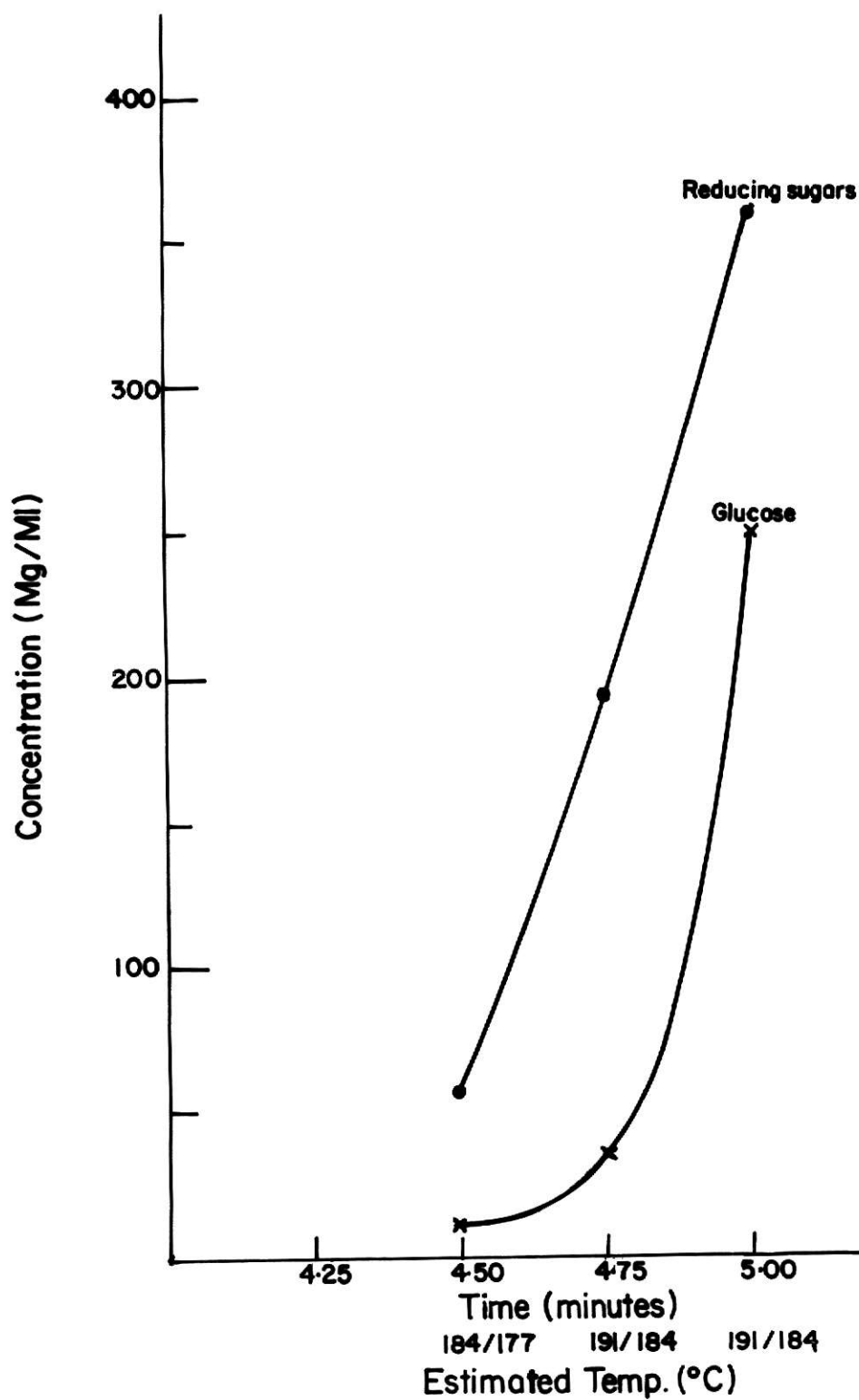


Figure 3. 50% Starch. Increase in the concentration of hydrolysis products.

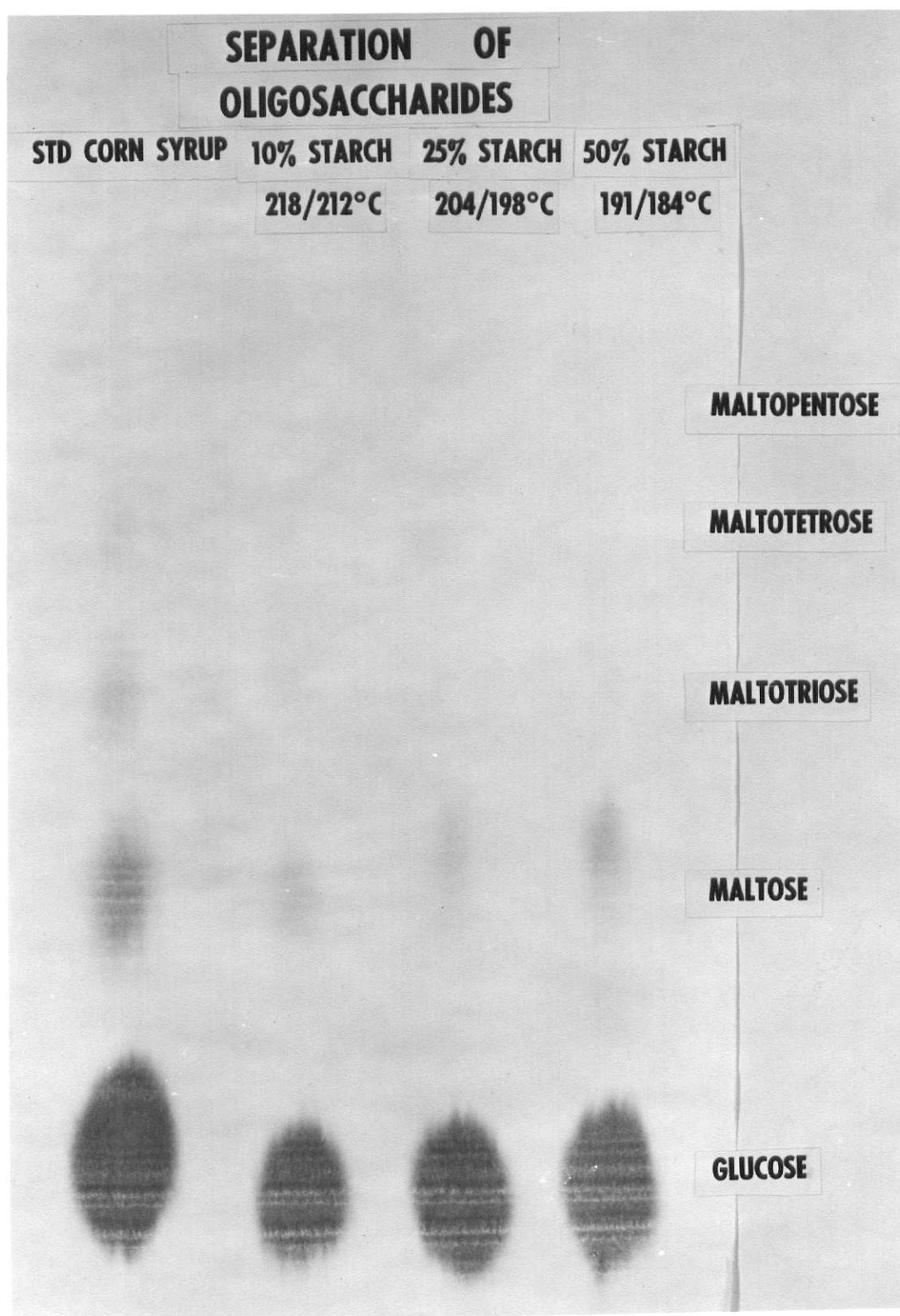


Figure 4. Separation of oligosaccharides by paper chromatography.

Effect of Concentration and Time Heated on Total Acid Production

As the starch slurries were heated the pH and titratable acidities were found to change in a similar pattern.

These changes would support the theory that as the temperature and pressure increase, acids are produced. These acids appear to react with the starch as would be observed under normal acid hydrolysis. Table 3 and Figures 5 - 7 show these changes.

Table 3. Effect of concentration and time heated on total acid production.

Time Heated Minutes	Estimated Temp. °C	pH	Titratable acidity expressed as ml. 0.1N NaOH to pH7
10% Concentration			
5.00	189/190	4.55	.12
5.25	204/198	4.00	.16
5.50	204/198	3.75	.22
5.75	212/204	3.50	.44
6.00	218/204	3.15	.71
25% Concentration			
5.25	191/184	3.65	.38
5.50	198/190	3.40	.71
5.75	198/190	3.10	.88
6.25	204/198	2.95	3.20
50% Concentration			
4.50	184/177	3.65	1.00
4.75	191/184	3.20	2.20
5.00	191/184	2.70	5.50

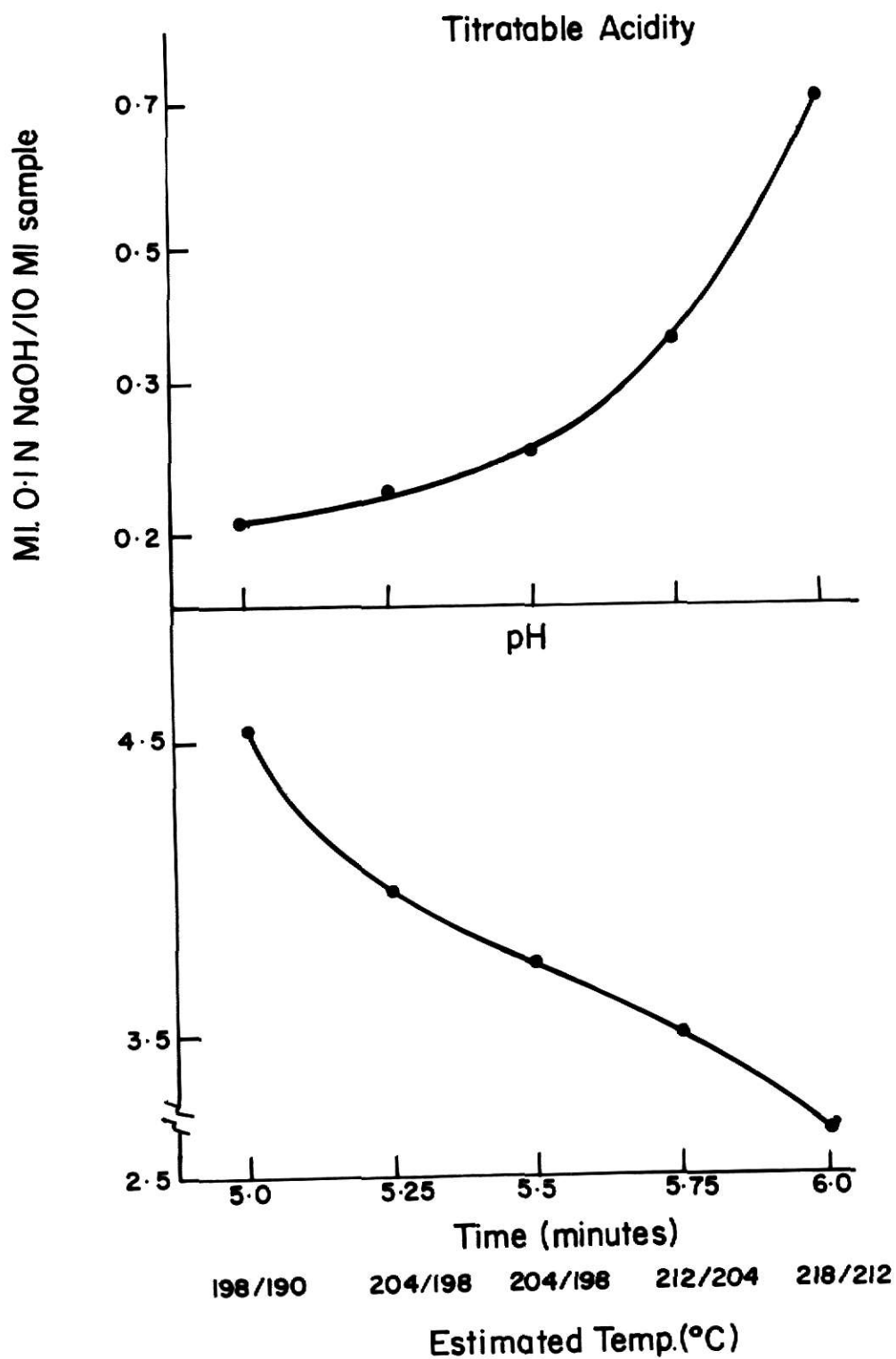


Figure 5. 10% Starch. Effect of concentration and time heated on pH and titratable acidity.

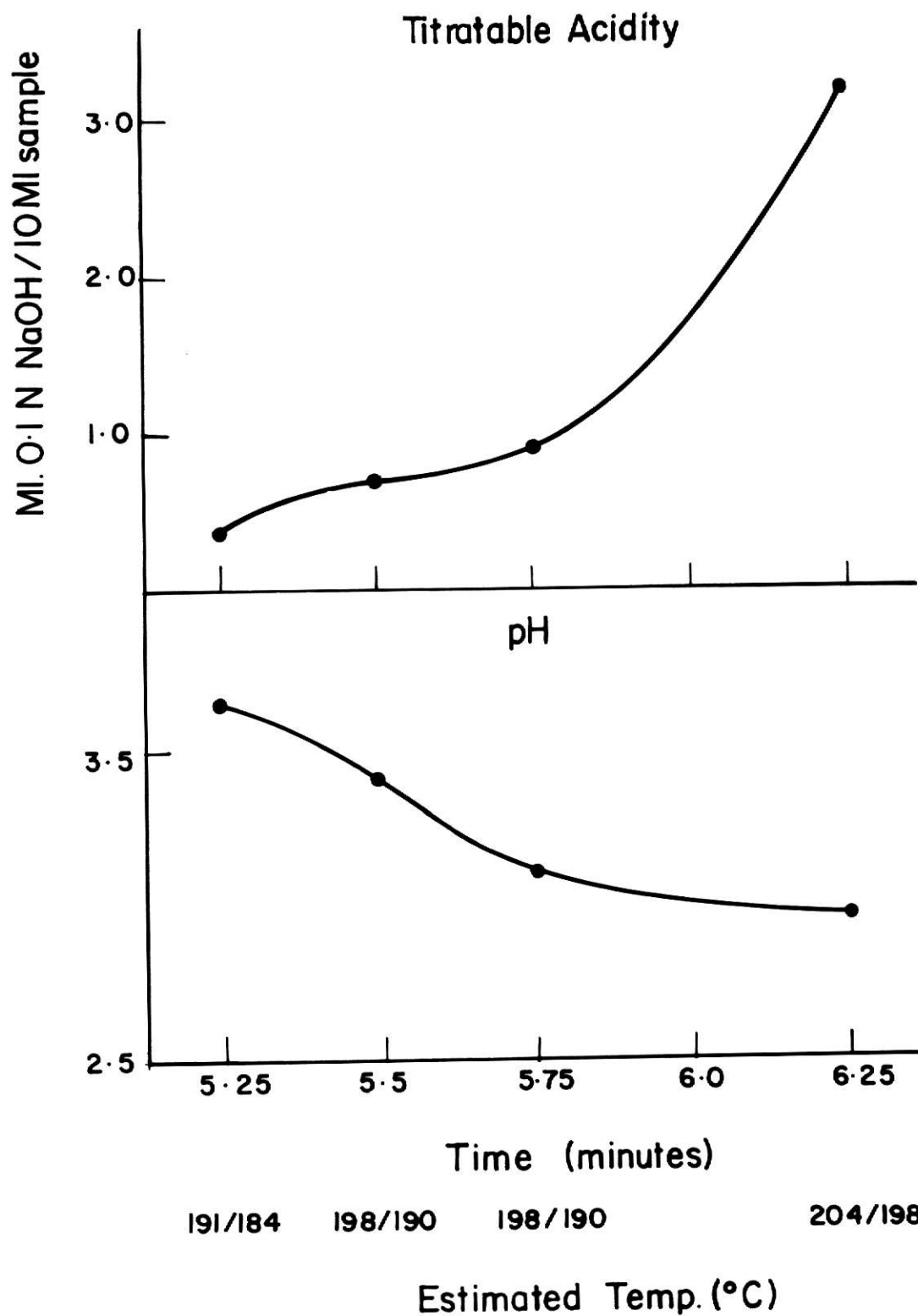


Figure 6. 25% Starch. Effect of concentration and time heated on pH and titratable acidity.

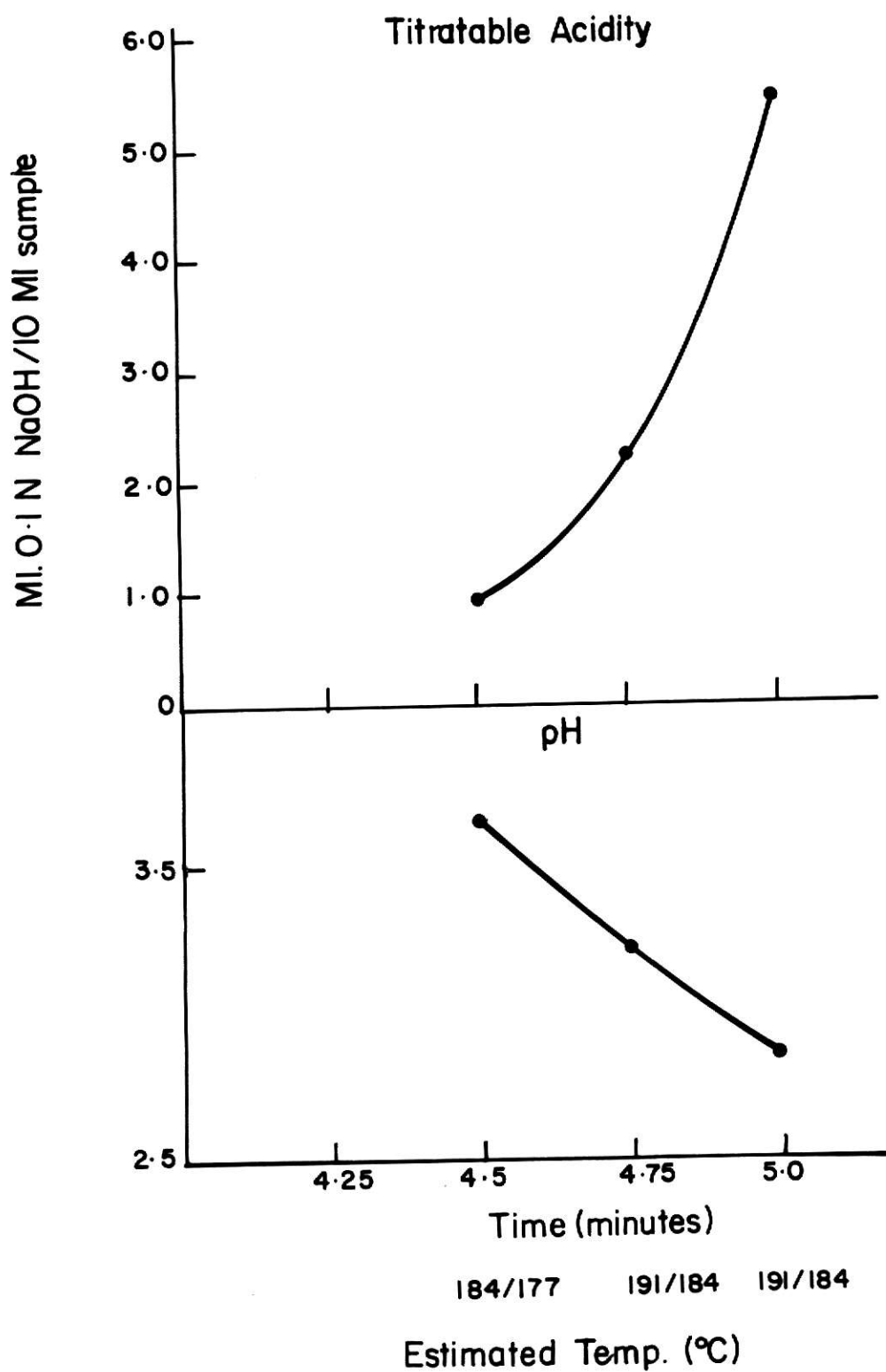


Figure 7. 50% Starch. Effect of concentration and time heated on pH and titratable acidity.

Effect of Concentration and Time Heated on Furaldehyde
and 5-(Hydroxymethyl)-2-Furaldehyde Produced

Since the starch slurries were hydrolyzed in glass tubes, it was possible to observe the color changes during heating. The samples were observed to go from an opaque starch suspension to a clear gel and then to a clear viscous liquid. As heat was continued and the temperature raised beyond the optimum, the color changed to a yellow and finally dark brown. Upon opening these tubes they had a strong caramelization odor with an underlaying note of furfural. It was also noted that these color changes took place after the microwave energy was turned off. It was impossible to cool the hydrolysed starch immediately upon the removal from the heat and some glucose degradation continued to take place.

Research by Kerr (46) showed that 5-(Hydroxymethyl)2-furaldehyde could be produced from the heating of glucose in acid conditions. This would account for the marked increases in this product after continued heating of the samples. The increase in this would also contribute to the darkening of the hydrolysed sample. Table 4 and Figures 8 - 10 show the increase in Furaldehyde and 5-(Hydroxymethyl)-2-furaldehyde as related to starch concentration, time heated and temperature obtained. Table 5 shows the relationship of the concentrations of furfural and hydroxymethylfurfural to glucose.

Table 4. Effect of Concentration and time heated on Furaldehyde and 5-(Hydroxymethyl)-2-Furaldehyde produced.

Time Heated Minutes	Estimated Temp. °C	Furfural ug/ml	Hydroxymethylfurfural ug/ml
10% Concentration			
5.00	189/190	N. D.	7.0
5.25	204/198	N. D.	12.2
5.50	204/198	N. D.	15.2
5.75	212/204	10.0	29.2
6.00	218/204	15.0	80.6
25% Concentration			
5.25	191/184	N. D.	28.0
5.50	198/190	10.0	53.0
5.75	198/190	15.0	104.0
6.25	204/198	105.0	261.0
50% Concentration			
4.50	184/177	15.0	38.0
4.75	191/184	35.0	147.0
5.00	191/184	242.0	698.0

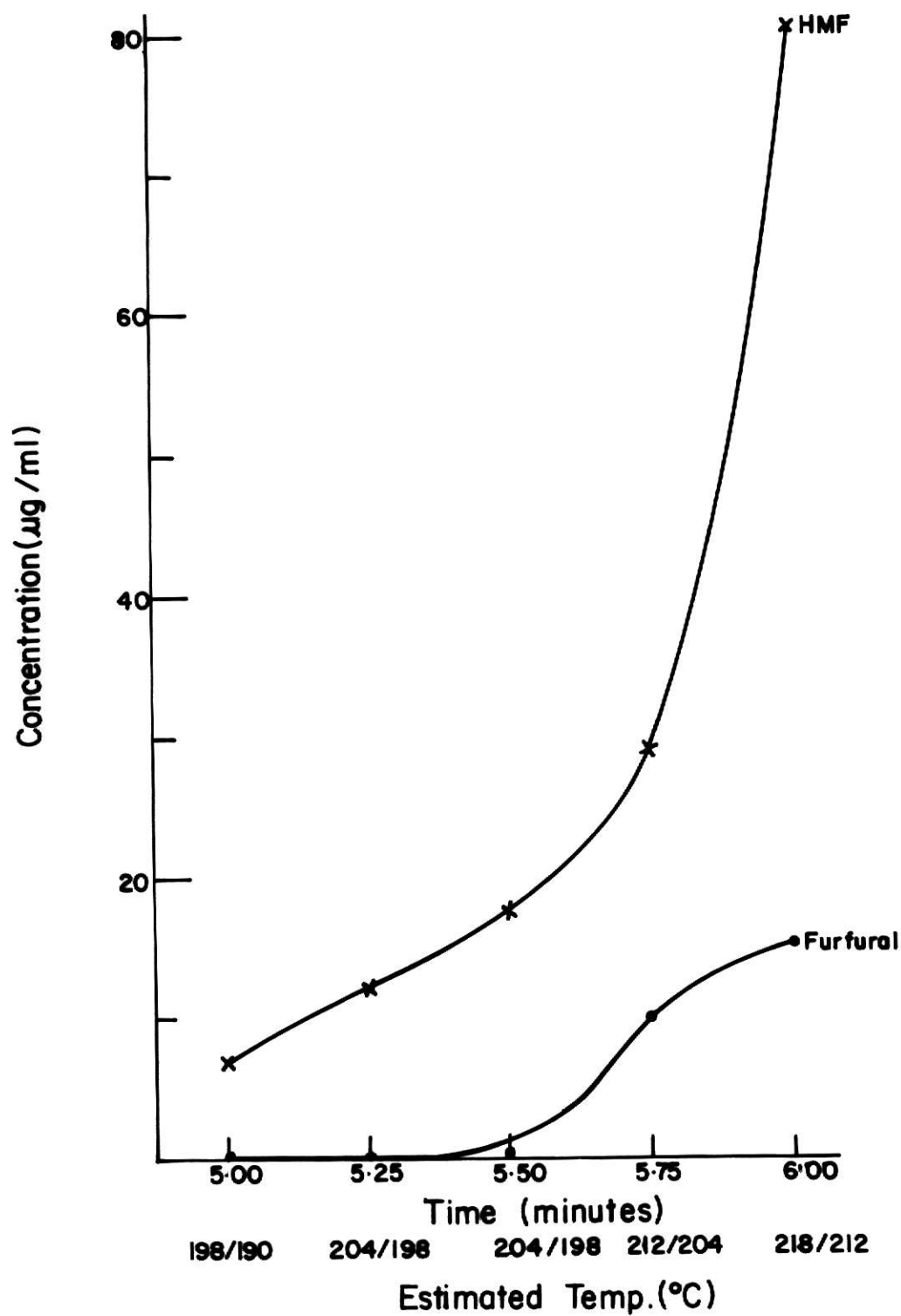


Figure 8. 10% Starch. Effect of temperature and time heated on furaldehyde and 5-(Hydroxymethyl)-2-furaldehyde produced.

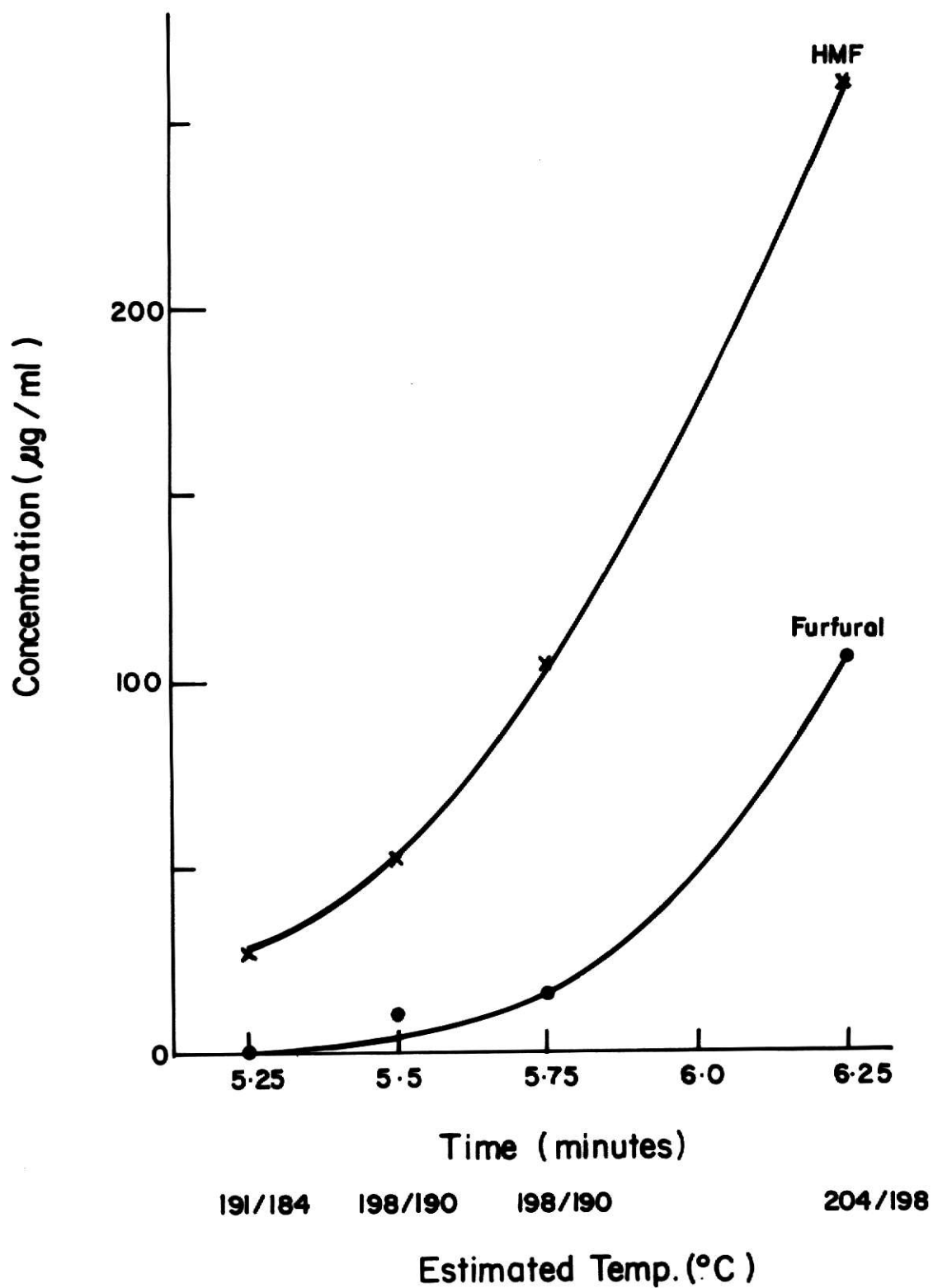


Figure 9. 25% Starch. Effect of temperature and time heated on furaldehyde and 5-(Hydroxymethyl)-2-furaldehyde produced.

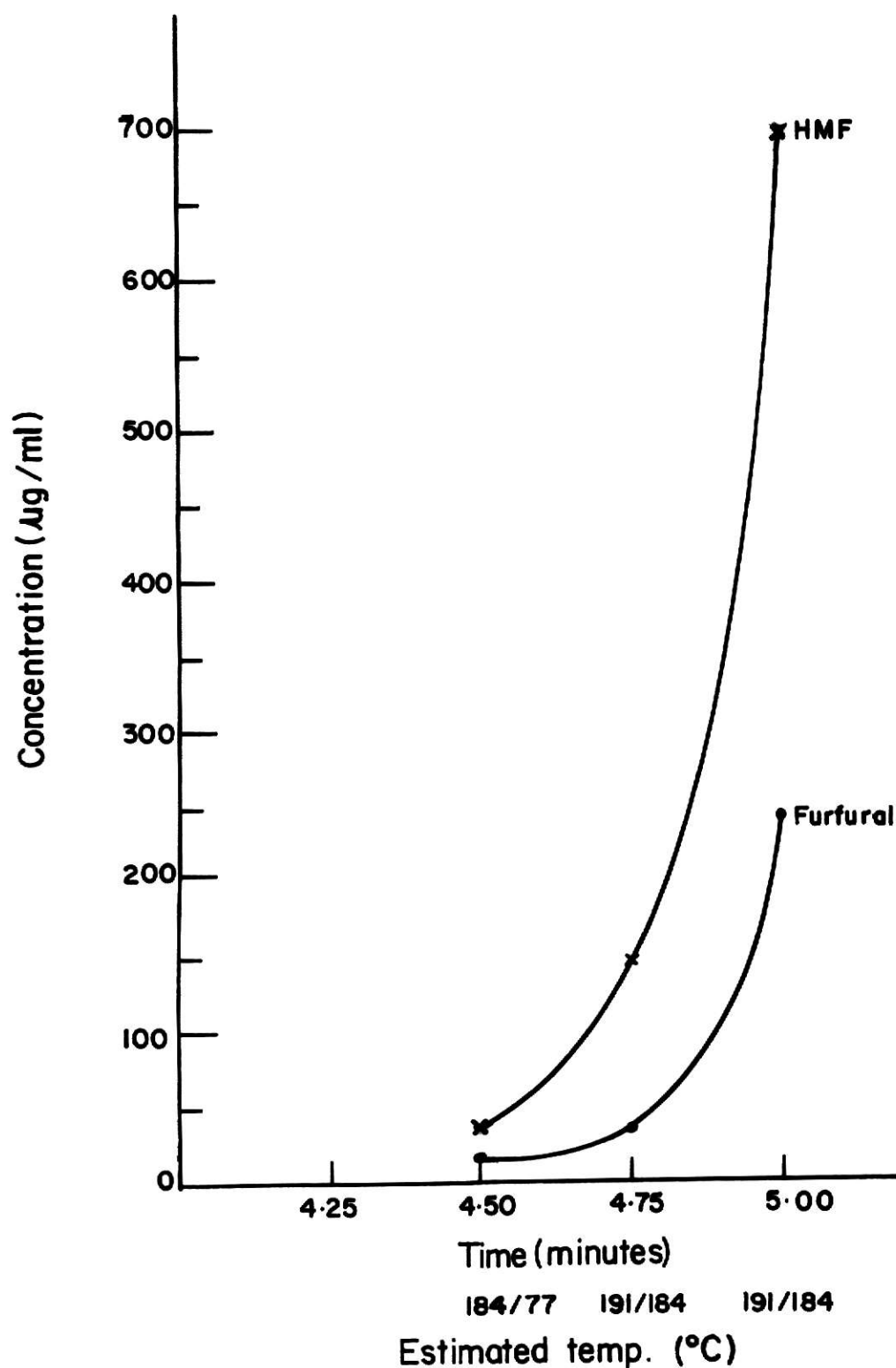


Figure 10. 50% Starch. Effect of temperature and time heated on furaldehyde and 5-(Hydroxymethyl)-2-furaldehyde produced.

Table 5. Relationship of Furfural and Hydroxymethylfurfural concentration to glucose concentration.

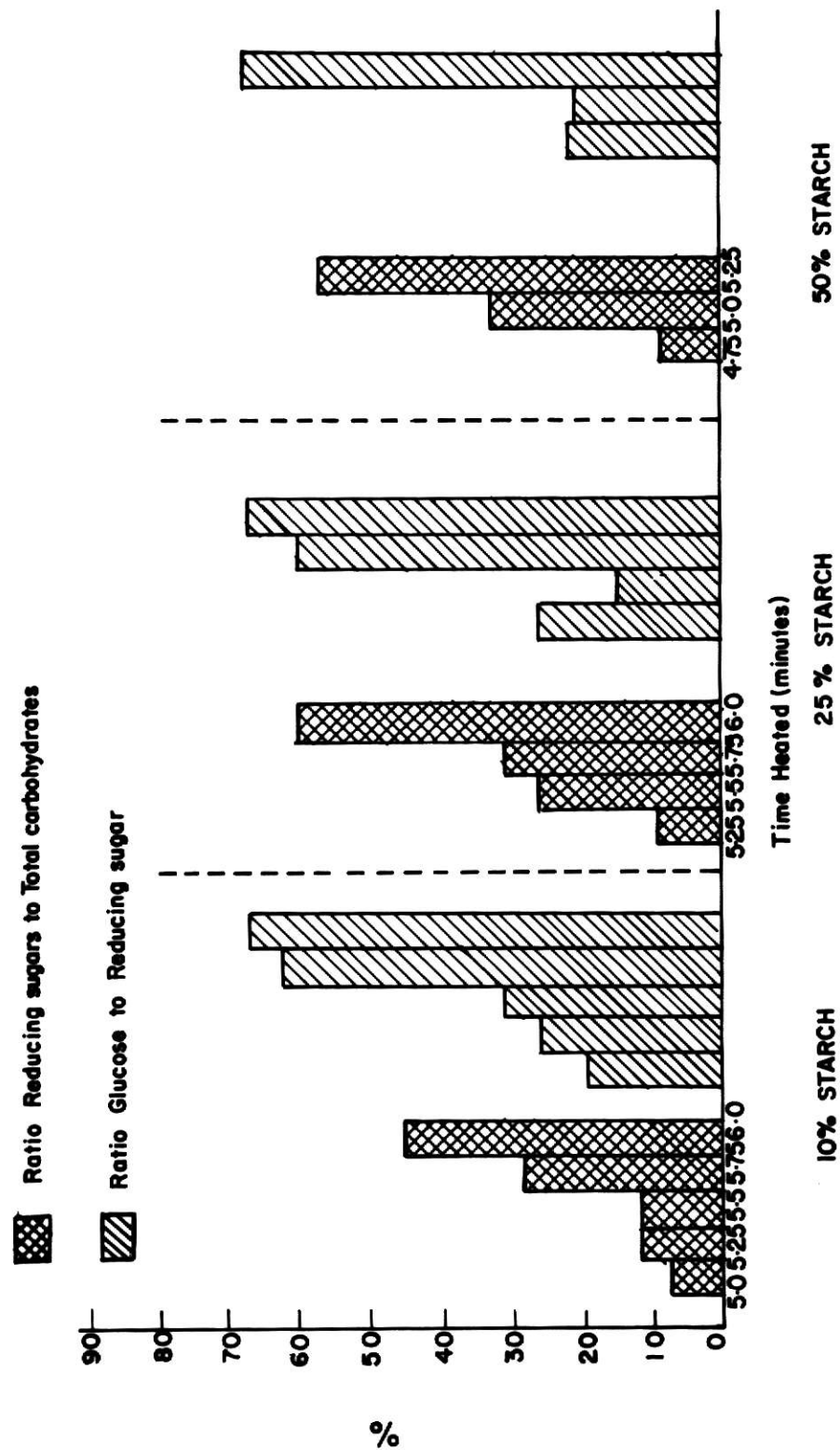
Time Heated (Minutes)	Estimated Temp. °C	Furfural and Hydroxymethylfurfural/ Glucose (percent)
10% Concentration		
5.00	189/190	0.50%
5.25	204/198	0.36%
5.50	204/198	0.36%
5.75	212/204	0.19%
6.00	218/204	0.25%
25% Concentration		
5.25	191/184	0.40%
5.50	198/190	0.52%
5.75	198/190	0.21%
6.25	204/198	0.28%
50% Concentration		
4.50	184/177	0.35%
4.75	191/184	0.47%
5.00	191/184	0.37%

Comparison of Total Carbohydrates/
Reducing Sugars/Glucose

Since widely different starch concentrations were used in this study, the only real comparison between the samples which would show the degree of hydrolysis would be a comparison of ratios between the different components. The mg./ml. of reducing sugars was compared to the total amount of carbohydrates in the sample. The mg./ml. of glucose were then compared to the amount of reducing sugars present. These ratios indicate that at the maximum temperature reached the percent glucose produced to total reducing sugars available was constant. They also indicate that optimum hydrolysis as determined by the ratio of total reducing sugars to total carbohydrates is probably between 25 and 50 percent starch concentration. Table 6 and Figure 11 show these comparisons.

Table 6. Relationship of reducing sugars to total carbohydrates and glucose to reducing sugars.

Time Heated Minutes	Estimated Temp. °C	Reducing Sugar/ Total Carbohy- drate	Glucose/ Reducing Sugars
10% Concentration			
5.00	189/190	7.2	19.7
5.25	204/198	11.9	26.1
5.50	204/198	11.6	31.3
5.75	212/204	28.2	62.9
6.00	218/204	45.2	67.3
25% Concentration			
5.25	191/184	9.6	26.1
5.50	198/190	25.9	----
5.75	198/190	30.7	60.6
6.25	204/198	60.3	66.8
50% Concentration			
4.50	184/177	8.8	21.9
4.75	191/184	32.8	20.7
5.00	191/184	57.8	68.0



Reducing sugars / Total carbohydrates-Glucose / Reducing sugars.

Figure 11. Relationship of reducing sugars to total carbohydrates and glucose to reducing sugars.

SUMMARY AND CONCLUSIONS

The effect of heating neutral starch slurries in temperatures around 180°C . in sealed glass reaction vessels caused typical hydrolysis reactions to take place. Acids are produced and the starch chains are hydrolysed to glucose, other sugars, and non-sugar compounds. Microwave energy can be used to rapidly bring starch slurries to the necessary temperature to effect this hydrolysis.

Concentrations as high as 75 percent starch can be hydrolysed in this manner. At concentrations above 50 percent, there is not enough water present to effect a slurry, and erratic results are obtained.

The resulting hydrolyses products are similar to those formed during acid hydrolysis. Paper chromatography and analytical test indicate that a high Dextrose Equivalent syrup is produced.

The heating of starch slurries cause caramelization and other browning reactions to occur. After glucose formation there is a further dehydration of glucose to form 5-(Hydroxymethyl)-2-Furaldehyde. If this dehydration could be stopped immediately upon reaching a specified temperature and pressure it would appear

that much of the concentration of furfural and Hydroxymethyl furfural would be reduced.

The results obtained indicate that starch can be hydrolysed in a neutral (water) starting solution. The critical conditions for this reaction to progress are temperatures above 170°C . and the pressures associated with these temperatures. This heat and pressure appears to promote the formation of acids which accelerate the hydrolysis. The reaction of hydrolysing starch to glucose can be completed very quickly when an energy source that will rapidly bring the slurry to a temperature of 180°C . is used.

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STARCH HYDROLYSIS BY HEATING IN HERMETICALLY
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by

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A study was conducted to determine if starch could be hydrolysed without the use of added acid or enzyme.

Starch slurries were prepared containing 10, 25, and 50% concentrations. These slurries were heated by microwave radiation for times ranging from 4.5 - 6.25 minutes. The resulting syrups were analysed for total carbohydrates, reducing sugars and glucose. The results of these tests indicated a direct relationship between concentration, time heated, and sugar produced.

Visual observation of the samples showed a definite darkening upon heating. Tests were made for Furfural and Hydroxymethylfurfural, and these tests showed an increase in both of these products with time and temperature increase.

It was concluded that starch could be hydrolysed in a neutral solution under conditions of certain heat and pressure. This hydrolysis is similar to that of acid hydrolysis with the acids being formed in the hydrolysis vessel.