Starch Hydrolysis Under High Temperatures and Pressures[1]

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ABSTRACT

Starch was hydrolyzed in a closed system using elevated temperatures and pressures and water at neutral pH. Gas-liquid chromatography revealed the following alcohols: methanol, ethanol, isopropanol, propanol, isobutanol, and butanol. Total alcohol by volume varied from 1.54% at a hydrolysis temperature of 120°C. to 2.77% at a hydrolysis temperature of 180°C. Carbonyl compounds formed were: methanal, ethanal, propanal, butanal, pentanal, and hexanal; 2-propanone, 2-butanone, 2-pentanone, and 2-hexanone; 2-methylbutanal, 2-methylpentanal; and diacetyl, 5-hydroxymethylfurfural, and furfural. Total carbonyl compounds – 116 p.p.m. at a hydrolysis temperature of 120°C. and 230 p.p.m. at 135°C. – increased to 69,000 p.p.m. at 155°C., and to 94,000 p.p.m. at 180°C. At the hydrolysis temperature of 180°C., the p.p.m. value approximated 10% of the reaction mixture. Total acidity increased and pH decreased as hydrolysis temperatures increased. The free fatty acids found were straight- and branched-chain, having chain lengths between C_1 and C_6. Identified were formic, acetic, propionic, butyric, isobutyric, valeric, isovaleric, caproic, and isocaproic acids. As the hydrolysis temperature increased, the C_5 and C_6 fatty acids decreased, but formic and acetic acids increased. Small amounts of glucose and glucose polymers were formed by starch hydrolysis at temperatures of 120° and 135°C. and pressures of 32 and 80 p.s.i.g., respectively. Extensive starch hydrolysis to sugars occurred at a temperature of 155°C. and a pressure of 140 p.s.i.g. At 180°C. and 165 p.s.i.g., maximum glucose was produced, and small amounts of mannose, xylose, and arabinose were detected. Total sugar ranged from 11.2 mg. per 100 ml. at 120°C. to 265 mg. per 100 ml. at 180°C. Those values corresponded to 0.37 and 8.83% sugar production, respectively, based on starch. Glucose comprised from 3.1 to 41.5% of the total sugar.

Acid hydrolysis of starch to produce sugars has been a commercial method since 1814, when the first plant to produce glucose syrup was built in France (1). Starch is hydrolyzed by acid to produce D-glucose. In addition to D-glucose, other products are formed, such as oligosaccharides, and products of decomposition and dehydration of D-glucose, such as 5-hydroxymethylfurfural, levulinic acid, formic acid, and colored products (2).

Today, enzymes are used commercially to increase the efficiency of glucose production from starch. In practice, starch is partially hydrolyzed with acid and the hydrolysis is completed by enzymes. Use of enzymes to complete hydrolysis retards formation of reversion and decomposition products (3).

A long list of carbohydrate decomposition products formed by caramelization or via the Maillard reaction has been compiled by Hodge (4). Carbonyl compounds have been isolated from fresh bread, from preferments, and from oven gases during bread-baking. During fermentation, starch is partially converted to glucose which, in turn, is responsible for forming carbonyl compounds during the baking process (5,6).

Hunter et al. (7), Johnson et al. (8), and Wiseblatt (9) have found C_1 to C_6 fatty

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acids in fresh bread, bread dough, and preferments. The breakdown of starch (the major component of those systems) to glucose through the action of acid, enzymes, or heat, followed by breakdown to aldehydes and oxidation, would produce acids with chain lengths of from C_1 to C_6.

Recent reports (10,11) indicate the possibility of starch hydrolysis in a closed system using water at neutral pH. Calentine (10) studied the effect of heat treatment on 1% starch suspensions sealed in stainless-steel tubes and heated 1 hr. between 150° and 250°C. He found 180°C. the optimum temperature for hydrolysis. Glucose and other oligosaccharides were identified in the reaction mixture. Solution color darkened and the pH decreased as temperature increased. In addition to sugars, furfural and 5-hydroxymethylfurfural were identified.

Pappas (11) heated neutral starch slurries in thick-walled glass tubes in a microwave oven and investigated hydrolysis of starch suspensions as high as 75% concentration. As had Calentine, Pappas observed decreased pH and the reaction mixture darkening as temperature increased. Pappas (11) concluded that a high dextrose-equivalent syrup could thus be produced successfully.

This investigation was undertaken to determine the composition of starch hydrolysates as a function of temperature and pressure. This paper deals with the general composition, as determined by gas chromatography.

MATERIALS AND METHODS

The starch was prepared from a commercial bread flour (protein 12.1%; ash 0.44%) using a procedure Calentine described (10), and defatted by Schoch's method (12). Respective AOAC methods (13) were used for analyses for moisture, protein, and total fat.

Three-percent starch suspensions (3 g. starch and 100 ml. water) were hydrolyzed in sealed, heavy-walled glass tubes which were heat-sealed over an oxygen flame, and heated in an oven at 120°, 135°, 155°, and 180°C. internal temperature for 1 hr. Since approximately 1 hr. was required to reach the respective temperatures, total time in the oven was 2 hr. Internal temperatures of sealed-glass tubes during hydrolysis were measured with a No. 20 Ga. Iron/Constantan Thermocouple, sealed into the glass tube airtight with the aid of an epoxy sealant.

Internal pressure in the tubes during hydrolysis was measured by inserting a stainless-steel tube (1/8 in. o.d.) into the glass tube. The insert was sealed with epoxy sealant. The stainless-steel tube was connected to a pressure meter outside the oven. Internal pressure of the glass tubes was measured at regular intervals during hydrolysis at each temperature.

High pressures developing inside the tubes during hydrolysis prevented their being opened immediately after removal from the oven. All tubes were allowed to cool to room temperature (2.5 hr.) before being opened.

ANALYSIS OF CONSTITUENTS

Carbonyl Compounds

One hundred milliliters of chloroform was added to each hydrolysate. The solution was filtered to eliminate any starch material or undissolved dark-hydrolysis end products. The chloroform layer was removed. An additional 50 ml. of chloroform was added to the hydrolysate to recover any carbonyl compounds

remaining after the first extraction. The combined extracts were evaporated under mild vacuum at 35°C. to a final volume of 10 ml.

Total carbonyl compounds were determined by the method of Lappin and Clark (14). The absorbance of the hydrazones was measured at 480 nm. with a Bausch and Lomb Model 20 spectrophotometer. A standard curve was prepared with butanal as the standard, and results were expressed as p.p.m. relative to the color of that standard. The mixture of carbonyl compounds was separated by gas chromatography by the flash-exchange technique of Stephan and Teszler (15). Resolution and identification were accomplished with a Hewlett-Packard Model 5750 gas chromatograph equipped with a dual-column, hydrogen-flame ionization detector. The columns were 8 ft. long, and of 1/8 in. o.d. The liquid phase was Carbowax 20M (weight % 20) and the solid support was Chromosorb P (AW-HMDS) with a 60/80 mesh. The carrier gas was nitrogen (N) at a flow rate of 15 ml. per min. The column temperature was 100°C.; the detector, 250°C.; and the injection port, 230°C.

To identify the carbonyl compounds, hydrazones of known carbonyl compounds were prepared and their retention times measured and compared with the retention times of compounds in the unknown mixture. Additionally, they were compared with retention times reported by El Dash (16) and Maga (17), who separated carbonyl compounds under similar conditions.

Free Fatty Acids

After hydrolysis of the 3% starch suspensions at 120°, 135°, 155°, and 180°C., the hydrolysates were centrifuged 10 min. at 10,000 r.p.m. to eliminate any starch material or undissolved dark-hydrolysis end products. The supernatant was extracted with 100 ml. of a petroleum ether-diethyl ether mixture (60:40) and titrated with 1N NaOH to the phenolphthalein end point (to convert the free fatty acids to corresponding sodium salts). The water-soluble salts were extracted with two portions of 2 ml. of distilled water. The extracts containing the sodium salts were transferred to small vials, evaporated to dryness, and stored in a refrigerator until analyzed.

Resolution and identification of the acids were accomplished on a Beckman GC 5 gas chromatograph equipped with a dual-column, hydrogen-flame ionization detector. The column (8 ft. long; 1/8 in. o.d.) was packed with 5% LAC IR 296 (glycol-adipate polymer) on firebrick (40/50 mesh). The carrier gas was helium at a flow rate of 12 ml. per min. The column temperature was 115°C. isothermal; the detector was 225°C.; and the injection port, 185°C.

The free fatty acids were recovered from their corresponding sodium salts by adding 0.5 ml. of a 7N dichloroacetic acid solution in acetone to the small vials containing the salts. Three microliters of the mixture of free fatty acids was injected into the gas chromatograph.

Fatty acids were identified by measuring relative retention times of pure commercial fatty acids separated under the same gas-chromatographic conditions.

Methyl-Esters of Free Fatty Acids

To confirm the findings reported above and obtained with free fatty acids, methyl-esters of these acids were prepared, separated, and identified by gas chromatography. The ether extract of the fatty acids, along with 100 ml. of 1%

concentrated sulfuric acid in methanol, was placed in a 300-ml. round-bottomed flask and refluxed for 1.5 hr. After cooling, the condenser was rinsed with 50 ml. of distilled water. The total mixture was saturated with sodium chloride and transferred to a separatory funnel to collect the ether layer containing the methyl-esters. This was followed by three additional extractions with 5 ml. of petroleum ether. All ether extracts were combined and evaporated to 10 ml. final volume under refrigeration. Sodium sulfate was added to absorb any residual moisture.

Resolution and identification were accomplished with a Hewlett-Packard Model 5750 gas chromatograph equipped with a dual-column, hydrogen-flame ionization detector. The column (8 ft. long; 1/8 in. o.d.) was packed with 60/80 mesh Chromosorb G AW-DMCS coated with 7.5% HI-EPP 1 BP (diethylene glycol succinate). Because methylation decreased the boiling point of the fatty acids considerably, the column temperature of 65°C. was used to separate the C_2 to C_6 methyl-esters. The C_1 methyl-ester has a boiling point lower than that of the solvent. Injection-port temperature was 95°C. and the flame detector, 160°C. The carrier gas was N with a flow rate of 5.5 ml. per min.

Alcohols

A method of Wiseblatt and Kohn (18) was adopted to separate the alcohols from the reaction mixture. After hydrolysis of the 3% starch suspension at 120°, 135°, 155°, and 180°C., the hydrolysates were centrifuged for 10 min. at 10,000 r.p.m. to eliminate residual starch and undissolved dark-hydrolysis end products. The supernatant was titrated with 1N NaOH using phenolphthalein as the indicator to convert the free fatty acids to their corresponding sodium salt. The hydrolysate mixture was distilled at atmospheric pressure, leaving the sodium salt in the distillation flask. The first 35 ml. of the distillate was collected. Temperature during distillation did not exceed 100°C. The distillate was treated with 5 ml. of a 1% solution of 2,4-dinitrophenylhydrazine to form the 2,4-dinitrophenylhydrazones of the carbonyl compounds in the distillate. The hydrazones were separated by filtration and the filtrate made approximately neutral with 10% NaOH. The neutral filtrate was again distilled at atmospheric pressure, and the first 15 ml. collected. The second distillation eliminated any remaining 2,4-dinitrophenylhydrazones. The 15 ml. collected during distillation was extracted with 15 ml. of dry diethyl ether and dried over anhydrous sodium sulfate. The extract was analyzed by gas-liquid chromatography (GLC) on a Beckman GC-5 gas chromatograph equipped with a dual-column, hydrogen-flame ionization detector. The column was 8 ft. X 1/8 in. o.d. The liquid phase was Carbowax 20M (weight % 20) and the solid support was Chromosorb P (AW-HMDS) with a 60/80 mesh. The carrier gas was helium at a flow rate of 12 ml. per min. The column temperature was 100°C. isothermal; the detector was 225°C. Identification of the alcohols was made by measuring relative retention times of known alcohols under the same gas-chromatographic conditions as those described and by comparing retention times with those measured in the unknown mixture of alcohols.

Total alcohols were determined by the AOAC method 9.012 (13). Total alcohols were separated from the reaction mixture as described above up to and including the second distillation, except that during the second distillation at atmospheric pressure, 25 ml. was collected instead of only 15 ml. as for

gas-chromatographic preparation of the alcohols. Specific gravity of the distillates was determined with 25-ml. calibrated pycnometers (weight of 25 ml. of each of the distillates divided by the weight of an equal volume of water). The percentage of alcohol based on ethanol was obtained from table 43.022 of AOAC methods (13).

Carbohydrates

After heating 3% starch suspensions, the hydrolysates were centrifuged at 10,000 r.p.m. for 10 min. Carbonyl compounds were extracted from the reaction mixture by using 100 ml. of chloroform and free fatty acids, and by using 100 ml. of a 60:40 mixture of petroleum and diethyl ethers. The solutions containing the sugars were further purified by passing them through diethylaminoethyl (DEAE) cellulose columns. Pre-swollen DEAE No. 52 was used (column height, 16 cm.; diameter, 3 cm.). Fractions of 25 ml. were collected and analyzed by the phenol-sulfuric acid method until all sugars were eluted from the columns. Fractions containing sugars were combined and the volume reduced to 15 ml. by a rotary evaporator at 40°C. Glucose was determined enzymatically by the glucose-oxidase method. Total sugar in the purified sugar concentrates was determined by the phenol-sulfuric acid method with glucose as a standard.

The purified sugar concentrates were freeze-dried, then transferred to vials; 2.5 ml. of "Tri-Sil" reagent (Pierce Chemical Co.) was added to form trimethylsilyl (TMS) derivatives; the vials were tightly sealed (to prevent entrance of moisture) and the reaction was allowed to proceed overnight. The sugars were resolved and identified by a Hewlett-Packard Model 5750 gas chromatograph equipped with a dual-column, hydrogen-flame ionization detector. The columns (2 ft. long; 0.25 in. o.d.) were packed with 2% Analabs GP 91 SE 52 and 98% Chromosorb W-AW DMCS (mesh size 80/100). The carrier gas, N, had a flow rate of 80 ml. per min. A linear program (140° to 275°C. with an increase of 4° per min. and no post-injection interval) was used. The injection port temperature was 290°C. and the flame detector, 270°C.

Monosaccharides were separated by a Hewlett-Packard Model 5750 gas chromatograph equipped with a dual-column, hydrogen-flame ionization detector. The column (12 ft. long; 1/8 in. o.d.) was packed with 3% SE-52 as the liquid phase and with Anakrom SE mesh 90/100 as the solid support. The carrier gas, N, had a flow rate of 20 ml. per min. A linear temperature program was followed: 125° to 190°C. with an increase of 6°C. per min. and no post-injection interval. The injection port temperature was 120°C., and the flame detector, 250°C.

Trimethylsilyl derivatives of known mono-, di-, and trisaccharides were prepared and their relative retention times measured under the same gas-chromatographic conditions as those just described. Retention times were compared with those found for sugars in the unknown. For additional information, they were compared with TMS-sugar retention times reported by Brobst and Lott (19) and by Sawardeker and Sloneker (20).

RESULTS AND DISCUSSION

Proximate Analyses

The results of the proximate analyses are summarized in Table I. The laboratory procedure was more efficient than the commercial procedure in removing protein

TABLE I. PROXIMATE ANALYSES OF STARCH SAMPLES

Content	Sample No. 1[a] %, as-is	Sample No. 2[b] %, as-is
Moisture	6.9	10.4
Protein	0.3	0.6
Total fat	none	trace

[a]Prepared from baker's patent flour.

[b]Commercial wheat starch.

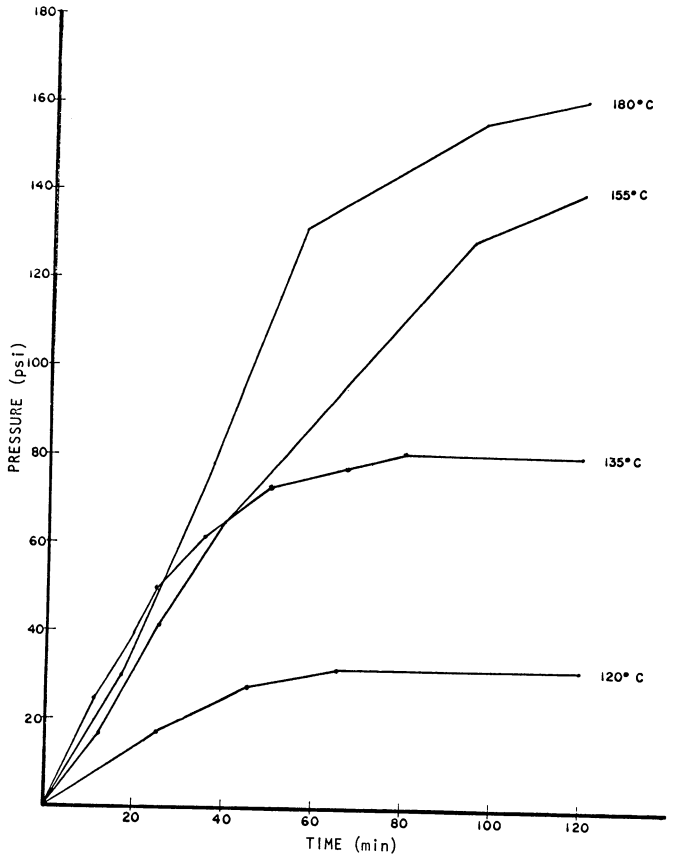

Fig. 1. Relationship between temperatures and internal pressure applied to starch slurries.

and total fat. The small amount of protein, which could not be removed during the preparation of the starch sample, likely affected formation of some of the compounds, which will be discussed later.

Internal pressures of the glass tubes at different temperatures and times of starch hydrolysis are given in Fig. 1. At all four temperatures of starch hydrolysis

(120°, 135°, 155°, and 180°C.), the increase in pressure in the tubes was very rapid in the first 40 to 50 min. At hydrolysis temperatures of 120° and 135°C., maximum pressure was reached within 50 min. At higher hydrolysis temperatures (155° and 180°C.), 90 min. was required to reach the maximum. Maximum pressures varied from 32 p.s.i.g. for hydrolysis at 120°C. to 165 p.s.i.g. for hydrolysis at 180°C.

Change in color was the most obvious of the changes during hydrolysis of starch suspensions. Solution colors varied from milky white, to very light brown, to dark brown or black. Color change was very slight as temperature of hydrolysis increased

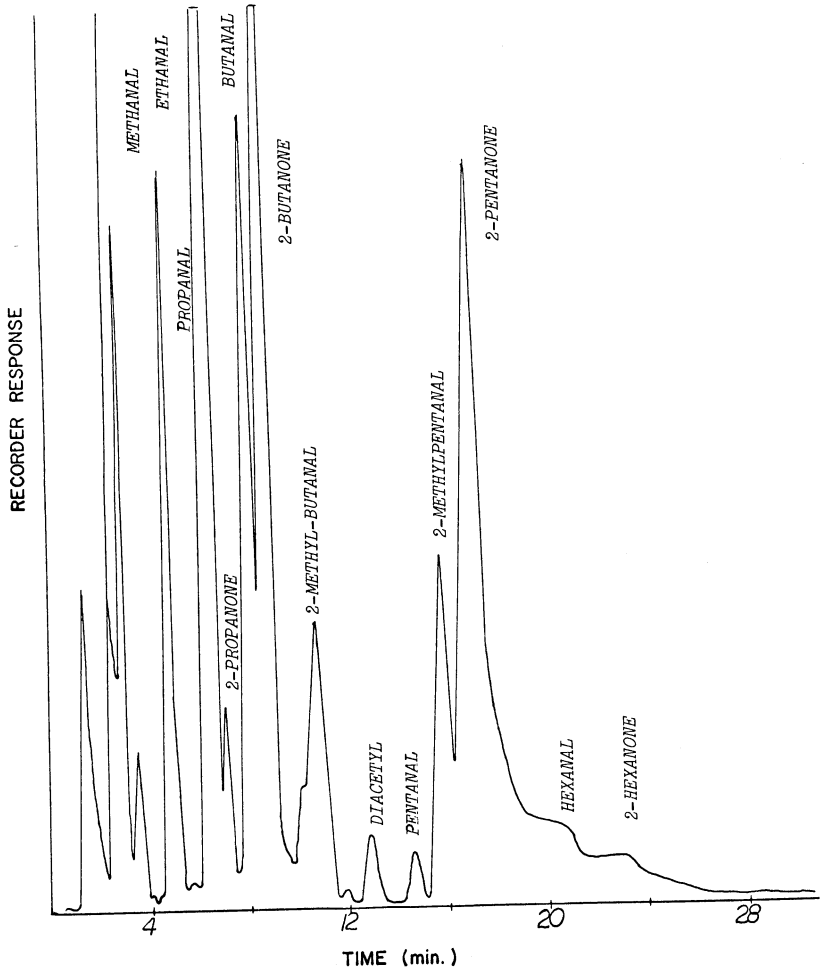

Fig. 2. GLC separation of carbonyl compounds in starch hydrolyzed at 180°C.

from 120° to 135°C. Slight darkening occurred at 155°C.; greatest color change was from 150° to 180°C.

Heat treatment at 120° and 135°C. completely gelatinized the starch, as expected, but did not completely hydrolyze it. Hydrolysis at 155° and 180°C. left no starchy residue but rather a dark residue that increased with hydrolysis temperature.

Carbonyl Composition

Carbonyl compounds separated after starch hydrolysis at 180°C. are illustrated in Figure 2. The C_1 to C_6 straight-chain aldehydes ethanal, propanal, butanal, pentanal, and hexanal were separated and identified by GLC (15). Methanal could not be detected by this (15,16,17), but one of its major decomposition products produced a peak that presumably represented methanal. Also identified were the C_3 to C_6 straight-chain ketones: 2-propanone, 2-butanone, 2-pentanone, and 2-hexanone. Identifying 2-methylbutanal, 3-methylbutanal, 2-methylpentanal, and 3-methylpentanal was not possible because they have the same retention times (16). Carbonyl compounds with chain lengths longer than C_6 were not present. The same 12 carbonyl compounds were present at all hydrolysis temperatures, differing only in concentration. Each invariably appeared at its highest concentration in the samples hydrolyzed at 180°C. The presence of any of the reported compounds was not surprising; all have been reported as degradation products of carbohydrates (4).

Separating furfural and 5-hydroxymethylfurfural from carbonyl-compound mixtures would have been possible under gas-chromatographic conditions used for the straight- and branched-chain carbonyls, but the retention time would have been more than 2 hr. Therefore, the temperature of the gas-chromatographic column was increased to 160°C. and the flow rate of the carrier-gas (N) to 80 ml. per min., which reduced furfural and 5-hydroxymethylfurfural retention time to less than 30 min. These compounds have been reported by many to be degradation products of pentoses and hexoses, respectively (21). Calentine (10) and Pappas (11) also identified them during investigations of starch hydrolysis at neutral pH.

By adding all peak areas at each hydrolysis temperature, it was concluded that total carbonyl compounds increased sharply as the hydrolysis temperature was increased from 120° to 135° and to 155°C. (Fig. 3). Amount of total ketones exceeded that of total aldehydes at each hydrolysis temperature. Although carbonyl compounds in the reaction mixtures were separated and identified by GLC, the colorimetric method of Lappin and Clark (14) was used to obtain a precise value of the total amount of carbonyl compounds. Total carbonyl compounds were expressed in p.p.m. based on the color of the arbitrary standard, butanal. Trends were the same as those reported for total peak areas. At a hydrolysis temperature of 120°C., total carbonyl compounds were 116 p.p.m.; they increased to 230 p.p.m. at a hydrolysis temperature of 135°C., and to 69,000 p.p.m. at 155°C. They leveled off to 94,000 at 180°C. At the hydrolysis temperature of 180°C., the reported value amounted to approximately 10% of the reaction mixture.

pH and Titratable Acidity

The pH of the reaction mixtures was measured each time a hydrolysis was performed. Recorded pH values varied only slightly from day to day; averages,

TABLE II. pH AND TITRATABLE ACIDITY

Temp. of Hydrolysis °C.	Average pH (after hydrolysis)	Titratable Acidity (0.01N NaOH) ml.
120	6.03	3.4
135	4.60	4.3
155	3.75	7.2
180	2.98	12.2

together with values for titratable acidity, determined by titrating 20 ml. of the hydrolysates with 0.01N NaOH to a pH of 7, are reported in Table II. Measured from a neutral starch solution, the pH decreased and the titratable acidity increased as the hydrolysis temperature was elevated.

Free Fatty Acid Composition

The free fatty acids, straight- and branched-chains, had a chain length between C_1 and C_6. Identified were formic, acetic, butyric, isobutyric, valeric, isovaleric,

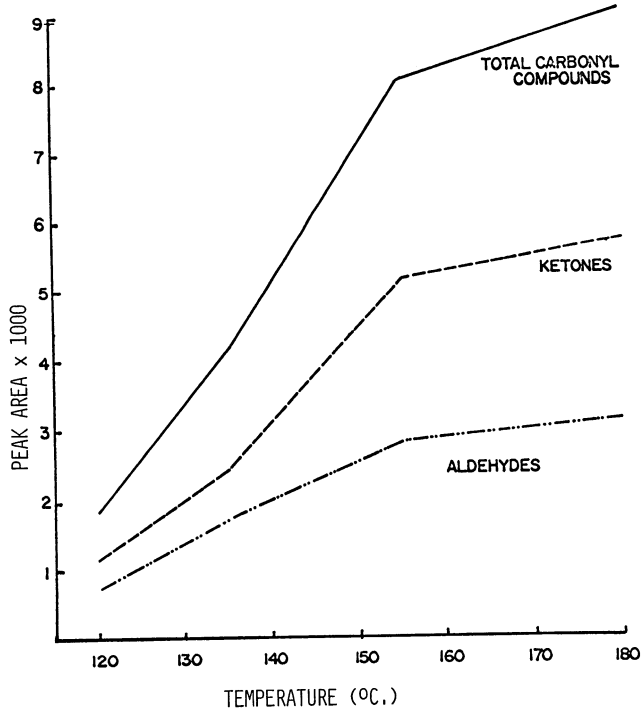

Fig. 3. Formation of carbonyl compounds during starch hydrolysis at different temperatures.

caproic, and isocaproic acids. Fatty acids with chain lengths longer than C_6 were not found. Air oxidation of the corresponding aldehydes was a possible source of these acids.

At hydrolysis temperatures of 120° and 135°C., the C_5 and C_6 fatty acids, valeric, isovaleric, caproci, and isocaproic, predominated in the acid mixture. As the hydrolysis temperature was increased to 155° and to 180°C., amounts of C_5 and C_6 acids decreased; caproic and isocaproic acids were absent at a temperature of 180°C. As the hydrolysis temperature increased, the amounts of the C_1 and C_2 acids increased. Formic acid, more highly ionized than valeric or caproic, had a greater effect on the pH of the solution. Figure 4 illustrates change in acid composition as a function of the hydrolysis temperature.

Preparing methyl-esters of the free fatty acids and separating and identifying them under different gas-chromatographic conditions revealed the same trend as described above. The amounts of the C_5 and C_6 fatty acids decreased with increased hydrolysis temperature, but amounts of formic and acetic acids increased.

Methylated free fatty acids decreased the boiling point considerably. Therefore, formic acid could not be detected, as methyl formate has a boiling point slightly lower than that of the solvent, diethyl ether.

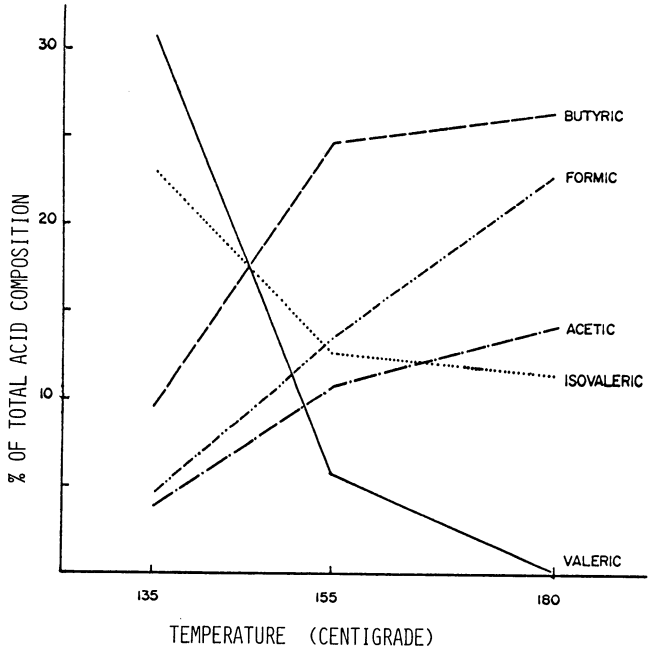

Fig. 4. Effect of temperature on free fatty acid composition of starch hydrolysate.

Alcohol Composition

GLC revealed alcohols in the hydrolysates. Separated and identified were methanol, ethanol, isopropanol, propanol, isobutanol, and butanol. Varying only in concentration, all appeared in the reaction mixtures at each of the four temperatures of hydrolysis. Invariably, most of each of the alcohols was in the sample hydrolyzed at 180°C. The data showed alcohols only in the range from C_1 to C_4, perhaps as a result of the sample-preparation method. Alcohols were distilled from the mixture under atmospheric conditions. During distillation the temperature never exceeded 100°C. The C_5 and C_6 alcohols, which have boiling points near 200°C., therefore, were not recovered. The percentage of alcohol by volume varied from 1.54% at a hydrolysis temperature of 120°C. to 2.77% at a hydrolysis temperature of 180°C.

Carbohydrate Composition

Paper chromatography showed that sugar production occurred as hydrolysis temperature increased. At 120°C. and 38 p.s.i.g., sugar concentration in the sample was too small to be demonstrated by paper chromatography. At 135°C. and 80 p.s.i.g., a few sugar spots, representing polysaccharides with a degree of polymerization (DP) of 4 and higher, were faintly visible. At 155°C. and 140 p.s.i.g., glucose was apparent, as were oligosaccharides and polysaccharides with a DP of 9 or even higher. At 180°C. and 165 p.s.i.g., glucose was by far the major component of the sugar mixture; no other sugars with a DP of more than 5 were evident. Thus, it was concluded that extensive starch degradation to glucose, oligosaccharides, and polysaccharides commenced at a temperature of about 155°C. or slightly lower. Maximum glucose production was found at a hydrolysis temperature of 180°C.

The presence of glucose and polymers of glucose in the hydrolysates was confirmed by GLC. Separations of the trimethylsilyl derivatives of the sugars are presented in Fig. 5. At a hydrolysis temperature of 120°C., the highest possible

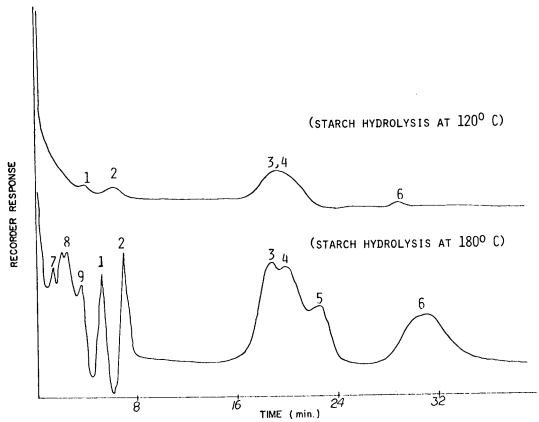

Fig. 5. Gas chromatograph of the trimethylsilyl sugar derivatives. 1) α-glucose, 2) β-glucose, 3) α-maltose, 4) β-maltose, 5) isomaltose, 6) maltotriose, 7) arabinose, 8) xylose, 9) mannose.

TABLE III. EFFECT OF TEMPERATURE ON SUGAR FORMATION DURING STARCH HYDROLYSIS[a]

Temperature of Hydrolysis °C.	pH after Hydrolysis	Total Sugar		Glucose	
		mg./100 ml.	% based on starch	mg./100 ml.	% of total sugar
120	6.03	11.2	0.37	0.35	3.13
135	4.60	123.0	4.10	0.39	3.17
155	3.75	200.0	6.66	5.52	2.76
180	2.98	265.0	8.83	109.95	41.49

[a]3% starch suspensions.

sensitivity for the gas chromatograph was necessary to reveal the presence of α- and β-glucose, maltose, and maltotriose. Trimethylsilyl derivatives with a DP of 4 or higher could not be separated with the method used. At a hydrolysis temperature of 180°C., α- and β-glucose, as well as α- and β-maltose, isomaltose, and maltotriose, were identified. Because wheat starch is approximately 70% or more amylopectin, the presence of isomaltose in the sugar mixture was expected. In the mixture of sugars from the 180°C. hydrolysates, several smaller peaks appeared prior to those of α- and β-glucose. Retention times of known trimethylsilyl derivatives of pentoses and hexoses obtained under the same gas-chromatographic conditions overlapped, making positive identification of any early peaks impossible.

Separating the trimethylsilyl derivative mixtures by using a column especially designed for monosaccharide separation revealed the presence of arabinose, xylose, and mannose in the mixture of sugars from the 180°C. hydrolysate, but only glucose at hydrolysis temperatures of 120°, 135°, and 155°C. Mannose has been reported (10) in a 1% starch suspension heated to 180°C. Pentoses have not been identified previously.

Total sugar ranged from 11.2 mg. per 100 ml. in the 120°C. hydrolysate to 265.0 mg. per 100 ml. in the 180°C. hydrolysate (Table III); the values corresponded to 0.37 and 8.83% sugar based on the original starch. Glucose ranged from 0.35 mg. per 100 ml. in the 120°C. hydrolysate to 109.95 mg. per 100 ml. in the 180°C. hydrolysate; these values corresponded, respectively, to 3.13 and 41.41% of the total sugar.

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