

PHYSICAL AND CHEMICAL PROPERTIES OF OLIGOSACCHARIDES
FROM CORN SYRUP

by 1264

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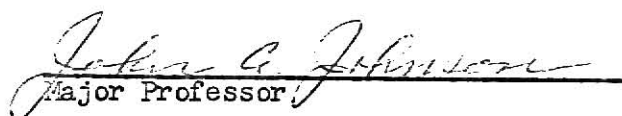
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INTRODUCTION

Corn syrups are produced by acid or acid-enzyme conversion of corn starch. Starch hydrolysis by acid or acid-enzyme is random and the products are glucose and glucose oligosaccharides of various degrees of polymerization. Starch hydrolysates are marketed on the basis of the "dextrose equivalent" (D. E.) of the syrup. "Dextrose equivalent" is a measure of the reducing-sugar content of a product calculated as dextrose and expressed as a percentage of the total dry substance.

The use of corn syrups and sugars has increased in the United States during the past twenty years despite a decline in the over-all consumption of carbohydrates in foods. The ever increasing demand for corn syrups by food and nonfood industries can be attributed to its many unusual properties.

Paper chromatography has become the versatile technique for identifying saccharides. Column chromatography is perhaps the best way of isolating individual oligosaccharides. In recent years, the carbohydrate composition of commercially available corn syrups (Table 1) has become known through the use of paper chromatography.

Considerable knowledge and technical data are available on glucose and maltose, as these are available in the market. However, no detailed information is available on higher oligosaccharides (maltotriose, maltotetraose, maltopentaose and higher chains of the saccharides), due to nonavailability of these products. The only source of these polysaccharides is their isolation in pure form from corn syrups of similar corn starch hydrolysate products. The purpose of this research was to apply chromatography methods to isolation of the various oligosaccharides found in commercial corn syrup and to study certain physical and chemical properties.

Table 1.

Examples of carbohydrate composition of commercially available corn syrups.

Type of Conversion	D. E.	Percent Saccharides						
		Mono-	Di-	Tri-	Tetra-	Penta-	Hexa-	Hepta- Higher
Acid	30	10.4	9.3	8.6	8.2	7.2	6.0	5.2 45.1
Acid	42	18.5	13.9	11.6	9.9	8.4	6.6	5.7 25.4
Acid-enzyme	43	5.5	46.2	12.3	3.2	1.8	1.5	- 29.5*
Acid	54	29.7	17.8	13.2	9.6	7.3	5.3	4.3 12.8
Acid	60	36.2	19.5	13.2	8.7	6.3	4.4	3.2 8.5
Acid-enzyme	63	38.8	28.1	13.7	4.1	4.5	2.6	- 8.2*
Acid-enzyme	71	43.7	36.7	3.7	3.2	0.8	4.3	- 7.6*

* Includes heptasaccharides

Source: Corn Industries Research Foundation, Inc.

REVIEW OF LITERATURE

The manufacture of sweeteners from starch is said to have begun during the reign of Napoleon I. France was at war with England, and cane sugar imports into France had been cut off. Napoleon offered a reward of 100,000 francs to anyone who could discover a way to make sugar in quantity from a native plant. At about the same time, in 1811, a Russian scientist, G. S. C. Kirchoff (19), needing gum arabic in the manufacture of porcelain and unable to get it, tried a starch substitute. Mixing starch and water and subjecting the mixture to the action of sulfuric acid for too long a time for this purpose, he obtained, instead, of a gummy product, a sweetish syrup. The scientist of France, because of the shortage of sugar caused by an embargo placed on its importation by Napoleon, recognized the importance of the discovery, and the manufacture of sugar from starch quickly began. Until 1940, acid-converted corn syrups and dextrose were manufactured essentially by the Kirchoff procedure. In 1940, starch was converted by a dual process involving both acid and enzyme hydrolysis (Dale and Langlois, (7)).

Native corn or wheat starch contains two fractions, amylose and amylopectin. The amylose is a straight chain structure of alpha 1 \rightarrow 4 glucosidic linkages, whereas the amylopectin is chains of alpha 1 \rightarrow 4 glucosidic linkages with branches occurring at the sixth position on approximately every twenty-fifth glucose unit. Only two linkages, therefore, the alpha 1 \rightarrow 4 and the alpha 1 \rightarrow 6, are involved in the hydrolysis of starch.

Enzymic hydrolysis of starch is of great commercial importance. Beta amylases are highly specific for a chain of 1 \rightarrow 4 linked α -D-glucopyranose units. Attack occurs exclusively from the "non-reducing" end group and

attacks the next to last link with the formation of α -maltose. Beta-amylase action has been used to determine the peripheral chain length of amylopectin. Beta-amylase activity is stopped by the presence of α 1-6-D glucopyranose unit.

Amylases hydrolyze the glycosidic linkages in polysaccharides and are grouped with the hydrolases. They are more completely classified by being assigned to sub-group of glycosidases (or carbohydrases) and sub-sub group of polysaccharidases. These enzymes are named as alpha-amylase and beta-amylase and show definite variations in certain properties, such as pH and temperature optima, thermostability and resistance to inactivation by acidity. They are pure, crystallizable proteins that do not require the presence of co-enzymes for their activity. Alpha-amylase, however, shows a higher rate of activity in the presence of calcium. By their over-all action on polysaccharides components of starch, amylases decrease in viscosity which indicates cleavage of the glucose chains, appearance of reducing groups and formation of maltose and dextrans in the form of oligosaccharides of varying chain length.

The action of alpha-amylases from different sources show a substantial similarity. For example, with salivary amylase, the products of action on amylose are oligosaccharides of DP 2, 3 and 4 together with high molecular weight dextrans (Pazur, French and Knapp, (24)). No glucose or oligosaccharides in the range of DP 5-12 are produced. With bacterial amylase, French (8) found the principal early low molecular weight products are of DP 2, 3, 6 and 7 with relatively little DP 4 or 5 produced. French (8) treated isolated branched oligosaccharides of DP 4-7 with a fungal amyloglucosidase. It converted the DP 5, 6 and 7 oligosaccharides to glucose.

Pazur and Ando (23) and Pazur and Kleppe (25) have prepared highly purified amyloglucosidase and have reported detailed studies on the mode of action of these enzymes. The amyloglucosidase hydrolyzes the oligosaccharides

by a multichain mechanism beginning at the nonreducing end of the molecule and proceeds at a faster rate if the terminal unit is linked by an α -D-(1 \rightarrow 4) linkage rather than an α -D-(1 \rightarrow 6) linkage to the remainder of the oligosaccharide molecule. The α -D-(1 \rightarrow 4) linkage in maltose is hydrolyzed at about 15 times the rate for the α -D-(1 \rightarrow 3) linkage in nigerose and about 28 times the rate for the α -D-(1 \rightarrow 6) linkage in isomaltose. Thus, amyloglucosidase converts starch, amylose, amylopectin, amyloextrin and a wide variety of glucosyl oligosaccharides having α -D-(1 \rightarrow 4), α -D-(1 \rightarrow 6), α -D-(1 \rightarrow 3) linkages to glucose in essentially quantitative yields.

Beta-amylase is produced by higher plants, particularly cereals and sweet potatoes. It is a saccharifying enzyme, producing maltose as the only sugar from the nonreducing ends of starch chains when acting on the branched fraction of starch. The action of beta-amylase ceases when it reaches an α (1 \rightarrow 6) linkage, leaving so-called "beta limit dextrins."

Hollo, Laszlo and Toth (13) fractionated products formed during hydrolysis of maize-amylose by α - and β -amylase in a column of layers of Sephadex G-10, G-15 and G-25. Hydrolysis by α -amylase contain products of a high polymerization degree. The distribution provides evidence for "apparent" single chain mechanism in the hydrolysis of natural amylose. Among hydrolysis products, small amounts of glucose were present. During hydrolysis by α -amylase, up to 50% the hydrolysis takes place in three steps. The first step is the formation of intermediate products (with DP 4 to 10) from amyloses of a higher polymerization degree. The second step involves formation of products with DP of 4, 5 and 6, with production of a few molecules having a DP of 3, 2 and 1. The third and final step involves production of low DP products.

Animal and plant tissues and micro-organisms produce amylase enzymes. For commercial application, microbial enzymes have become important sources. Microbial amylases can be produced in unlimited quantities. Commercial enzymes are produced from molds, bacteria and yeasts. These amylase enzymes from animal, plant and microbial origins rapidly hydrolyze starch as follows.

Starch Alpha-amylase Dextrins + Maltose

Starch Beta-amylase Maltose + Dextrins

Starch or Dextrins Amyloglucosidase Glucose

The terms "liquefying" and "saccharifying" amylases are general classifications denoting the types of enzyme action. Alpha-amylases hydrolyze alpha-(1→4) linkages in the starch molecules in a random manner, therefore, liquefying starch rapidly. A considerable amount of low molecular weight dextrins is formed by the low saccharifying or dextrinizing types of alpha-amylases.

Oligosaccharides of Corn Syrups - Column Chromatography

Chromatography revolutionized the study of the structure of carbohydrate polymers. Paper chromatography has become the superior means of identifying saccharides, while column chromatography is the superior means of isolating them.

Tswett (37) was the first to use column chromatography for separation of pigments and colorless substances from natural material. Tswett's method seemed to be forgotten until Kuhn and Lederer (20) separated the carotenes and xanthophyll pigments on a column of aluminum oxide and calcium carbonate. At this time only the classical methods were used for separation of oligosaccharides from syrups. This involved the fractional distillation of the methyl (Hurd and Cantor, (16)) or propenyl derivatives (Hurd and Liggett,

(17)). Column chromatography was applied to resolution of sugar mixtures by Tiselius (30).

Since that time, chromatographic methods have made important contributions to the problems of sugar chemistry. Both partition and adsorption chromatography have been applied. In adsorption chromatography, a polar adsorbent such as clay in water and a non-polar adsorbent such as carbon in water have been used. Quantitative analysis have been successful for monosaccharides and lower molecular weight oligosaccharides.

The progress in using column chromatography for resolution of oligosaccharides started when Tiselius (30) introduced carbon as an adsorbent for the chromatography of colorless substances. Substances to be separated were forced upward through a carbon column contained in a metal tube. Atmospheric pressure and constant temperature were employed. Separation of the substances was followed by measurement of changes in the refractive index of the effluents at the top of the column. Tiselius (29) and Williams, Hagdahl and Tiselius (14) have applied this method to the separation of mono- and disaccharides. This was extended to the separation of raffinose using various carbons (Tiselius, (31)). Tiselius (32) improved the separation of the sugars allowing the developing solvents to pass down through Norite P-3 and thus reducing the intermixing of the zones. Three general analytical procedures have improved this method. They are identified by the mode of development used, namely, frontal, elution and displacement analysis.

Charcoal has been used industrially for the purification of sugars for many years. Hyashi (18) reported that charcoal, stirred in an aqueous acetone-acetic acid solution of glucose and sucrose, completely adsorbed the sucrose but left the glucose in solution. Tiselius (30) proposed that

charcoal might be employed in chromatographic fashion for the separation of glucose and lactose. Later, he employed charcoal and 0.5% aqueous phenol solution to effect the separation of glucose from sucrose, and sucrose from maltose by the displacement development technique in which the components are added to the column and are washed in succession into the effluent without an interval wherein only pure developing solvent is obtained. Tiselius and Hahn (33) demonstrated that a mixture of glucose, sucrose, raffinose and stachyose could be separated, using 0.5% aqueous ephedrin as the displacing agent. The method of Tiselius has been used by Claesson (3) to resolve a mixture of glucose, sucrose and raffinose with 4% aqueous phenol; and by Montgomery, Weakley and Hilbert (22) to isolate 6- α -D-glucopyranosyl β -D-glucose from an enzymic hydrolysate of starch.

Whistler and Durso (40) suggested a modification of the Tiselius technique whereby a mixture of mono-, di- and trisaccharides could be resolved by chromatographic adsorption on charcoal with displacement by water, 5% ethanol and 15% ethanol in succession. The course of the desorption of sugars was affected by the degree of dilution of the sugar mixture or by the presence of salts such as sodium chloride, sodium bicarbonate and sodium acetate in various concentrations.

Whistler and Hickson (41) isolated a tetrasaccharide from corn syrup by column chromatography on charcoal and cellulose. They characterized the sugar as maltotetraose by oxidations with hypiodite and periodate as well as by hydrolysis with β -amylase. Hydrogenation to maltotetraitol followed by acetylation yielded a crystalline pentadeca-O-acetyl derivative. Following the same technique, Whistler and Duffy (39) isolated a pentasaccharide and characterized as maltopentaose by periodate and hypiodite oxidations and by

hydrolysis with β -amylase. Whistler and Moy (42) obtained chromatographically pure maltohexaose by initial separation through carbon columns and final purification through cellulose column. Whelan (43) used carbon column chromatography to separate glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose from incomplete acid hydrolysate of potato amylose. Separation was made with water, 7.5%, 15%, 20%, 23%, 25% and 28% ethyl alcohol, respectively. The saccharides were contaminated with iron, which he eliminated by washing the packed column with 0.2 M-citrate buffer (pH 7.0). He packed his column by placing pads of glass wool and cotton wool in the bottom and adding a 50-50 mixture of charcoal-celite as a thick slurry in water. He used vacuum to speed the elution from the column. Eluates were tested by measuring the optical rotation of each fraction.

Montgomery and Weakly (21) determined the carbohydrate composition of hydrol, using a carbon column packed with equal weights of two types of carbon (Darco G-60 and Pittsburgh S.G.).

Barker, Bourne and Fleetwood (1), using charcoal column, isolated maltohexaose from acid hydrolysis products of amylose. The eluates were filtered, concentrated, freeze dried and then analyzed by paper chromatography. The purity of each saccharide was: maltotriose, 96%; maltotetraose, 95%; maltopentaose, 98%; and maltohexaose, 95%.

Thoma, Wright and French (35) isolated oligo- and megalosaccharides of DP 10-18 using cellulose column chromatography. Thoma and French (34) used the maltodextrin saccharides up to DP 18 which were isolated by chromatography on cellulose columns to examine the effect of chain length on the starch-iodine complex.

Physical and chemical properties of the oligosaccharides from corn syrup have not been studied in detail because of lack of a suitable technique to isolate the saccharides in pure form.

Thompson and Wolfrom (36) established the structure of maltotriose from corn syrup to be 4-~~α~~-maltopyranosyl-D-glucose. French and others (9) determined the specific rotation of maltoheptaose to be $[\alpha]_D + 176^\circ$.

Whelan et al. (43) determined the molecular weight of the saccharides (DP 2 through 7) using the colorimetric reagent of Somogyi (28). Whistler (38) reported the specific rotation of maltotetraose, maltopentaose and maltohexaose to be $[\alpha]_D^{25} + 176.4^\circ$, $[\alpha]_D^{25} + 179.4^\circ$ and $[\alpha]_D^{25} + 182^\circ$, respectively.

Bird and Hopkins (2) reported the specific rotation for maltotriose and a hexasaccharide in water. The $[\alpha]_D$ were found to be $[\alpha]_D + 158 \pm 1$ and $[\alpha]_D + 177 \pm 1$ for maltotriose and hexaose, respectively.

Sato, Ito and Ono (27) isolated isomaltotriose and determined it to have a specific optical rotation of $[\alpha]_D^{21} + 136.1$ in water.

Hoover, Nelson, Milner and Wei (14,15), realizing the major bottleneck to the development of knowledge pertaining to physical and chemical properties of these oligosaccharides, scaled up a charcoal column to pilot plant size (8' x 6") and separated 10 to 50 g. quantities of the sugars up to a degree of polymerization of 10 from acid converted 42 D. E. corn syrup. The saccharides thus obtained were evaluated for their properties of density, refractive index, viscosity, optical rotation and reducing power. Solubility tested in water indicated that dextrose formed a clear water-white solution. Maltotriose and maltotetraose formed clear light-yellow solution. Solutions of maltohexaose

contained a small flocculent precipitate at higher concentrations. Maltoheptaose formed a turbid off-white solution at high concentration which cleared upon dilution. Solutions of maltoheptaose were clear but opalescent even at the lowest concentration. Malto-octaose formed a very slightly turbid yellow solution at high concentration which cleared when diluted. Maltononaose made a clear water-white solution. Maltodicaose solutions were slightly yellow with slight turbidity.

The bulk density of all the oligosaccharides except maltononaose was very low. These low bulk densities were a result of the procedure used to dry the sample, i.e., freeze drying. Specific rotation of maltotetraose was found to be lower than reported by other workers. This may have been due to impurity of chloride ion in their maltotetraose sample. The reducing power of malto-pentaose reported by them was unusually high. They concluded that density, refractive index, viscosity, optical rotation and reducing power were related to the molecular weight of sugars.

Commerford, VanDuzee and Scallet (4) separated large quantities of glucose polymers up to a DP of 11 from starch hydrolysates by paper chromatography. Commerford and Scallet (5) found constant stoichiometry for the members of the homologous series higher than maltotriose, when the results were calculated in terms of equivalents of ferricyanide reagent consumed per equivalent of reducing sugar, G_1 to G_3 had progressively increasing molecular reducing power. They also reported that variation of sugar concentration during the determination had little effect on the reducing power when sodium carbonate was the alkali used but was important when barium or calcium hydroxide was used.

Salem and Johnson (26) separated the oligosaccharides of corn syrup on carbon-celite columns using stepwise increase in ethanol concentrations. They isolated trisaccharide, tetrasaccharide, pentasaccharide, hexasaccharide, heptasaccharide and dextrans using 7.5%, 15.0%, 20.0%, 23.0%, 30.0% and 70.0% ethanol concentration respectively. They used ferricyanide test to follow the progress of separation. They also reported, as observed by previous workers, a trace contamination with a higher oligosaccharide in the elution of tetra-penta-hexasaccharide. Further, the resolution of dextrin was not complete because of the strong adsorbability of dextrans on carbon-celite columns.

Salem and Johnson (26) from the paper chromatogram study of R_G value and chain length relationships, concluded that the oligosaccharides isolated from corn syrup appeared to be a homologous series of maltodextrin. The optical rotation of these oligosaccharides observed by them was in agreement with the same reported by Whistler (38). Their studies on the action of beta- amylase on these oligosaccharides indicated the presence of only the 1-4 type of linkage in these sugars.

The properties of the oligosaccharides, including optical rotation, R_F values of amylose hydrolysate and beta-amylolysis suggested that the oligosaccharides were a homologous series of amylose structures.

Salem and Johnson (26) also studied the effect of the addition of individual oligosaccharides on bread quality. They used two levels, 0.5 and 1.0 percent and observed slight decrease in crust color, break and shred, symmetry, grain and texture with higher oligosaccharides and also noted that addition of oligosaccharides enhanced rather than retarded bread crumb staling.

Commerford and Scallet (6) reported the values for the dextrose equivalent of oligosaccharides (DP 1 through DP 6). Compared with theoretical values, they found that the actual dextrose equivalent values were about 10% higher than expected. Haytko, Burns and Weill (11) separated the malto-oligosaccharides on thin layer plates with n-butyl alcohol:pyridine:water solvent mixtures at 26⁰ on Kieselguhr G.

The foregoing review and the present status of knowledge reveals that considerable data (physical and chemical properties) are available on glucose and maltose. However, knowledge on higher oligosaccharides is still lacking. The only rich source of these oligosaccharides is to isolate them from corn syrup or starch hydrolysate and study their physical and chemical properties. These oligosaccharides may have potential importance for many industrial applications if the physical and chemical properties were known.

MATERIALS AND METHODS

Preparation and Use of Carbon Columns

A chromatographic column, 6" x 12', made of pyrex glass was filled with a mixture of activated carbon (G-60) and celite 545 (60:40). The column was packed with carbon-celite in the form of a slurry. A second column was charged with 50:50 mixture of granular (20:30 mesh) and powder carbon (G-60).

The columns were washed with 1:1 hydrochloric acid solution to assure the removal of basic ash which might otherwise cause isomerization of the sugar applied later. The acid was removed from the column by washing with distilled water. The column was never allowed to dry but covered with a layer of liquid at all times.

The column was charged with a dilute solution of 452 gms. of corn syrup having 42 D. E. (Corn Products Refining Company, Argo, Illinois). Elution of oligosaccharides was followed in sequence by water, 5.0%, 7.5%, 10%, 12.5%, 15%, 17.5%, 20.0%, 22.5% and 25.0% ethanol. The surface of the column was covered with four disks of filter paper to keep the surface even when solvent was added. To speed the elution rate, initial trials were made with vacuum from the bottom of the column. However, this caused short channels and disturbed uniformity of carbon in the column. Another trial was made by continuous flow of solvent from a 35 gallon plastic tank raised to a greater height than the top of the column. This resulted in increasing the elution rate from 0.5 gallon per day to about 1.5 gallons per day without creating any channels or disturbing the carbon pack. Fractions of eluate were collected in one gallon plastic jars, arranged in a row of six. The rate of elution ranged from one-two gallons per day.

Glucose and maltose were eluted by water and 5% ethanol. Eluates containing glucose and maltose were tested as follows.

Analysis of eluant

To detect the presence of carbohydrate in the eluate, a 1.0 ml. aliquot was mixed with 1.0 ml. of 5% orcinol and 5.0 ml. of concentrated sulfuric acid. After placing tube in boiling water for 10 minutes, a red to brown color was obtained with as little as 5 mg. of carbohydrate. The intensity was measured at 490 m, using a Bausch and Lomb spectrophotometer Spectronic 20.

The purity of eluate was tested for sugar by a paper chromatography using Whatman No. 4 filter paper. The development solvent was propanol-ethyl acetate-water (6:1:3). The chromatograms were developed by spraying with silver nitrate, sodium hydroxide and sodium thiosulfate solution.

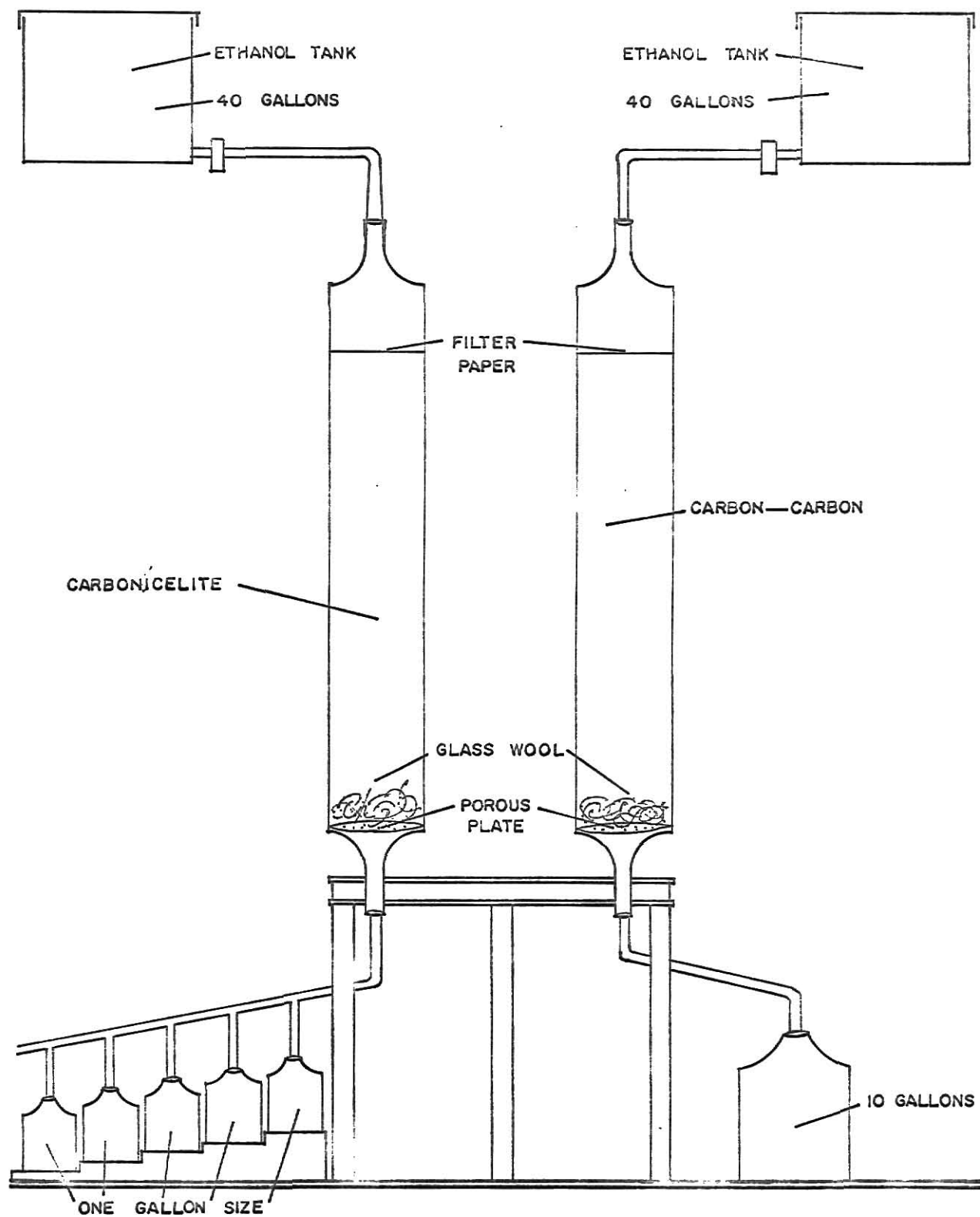


Fig. 1. Design and construction of carbon-celite and carbon-carbon columns.

Methodology for study of properties of oligosaccharides

The isolated oligosaccharides were studied for: (1) hygroscopicity, (2) reducing power, (3) solubility.

Reducing power

The ferricyanide number of a carbohydrate (the number of ml. of 0.1000 N ferricyanide reacting with one g. of substance) was used in determining the reducing power of starches, dextrans and syrups. The Schoch and Jensen (12) procedure for determining the ferricyanide number was applied to glucose and to the series of glucose polymers prepared by carbon column chromatography.

Procedure

Reagents were prepared as cited in Appendix. Alkaline ferricyanide (25 ml.) was transferred to a reaction flask containing about 0.05 milli-equivalent of oligosaccharide dissolved in 25 ml. of distilled water. The flask was covered with a vented cap and the solution heated to boiling for exactly 15 minutes. The reaction was quenched by cooling in tap water. Acid-zinc sulfate solution (60 ml.) was added to complex with the ferricyanide and to acidify the reaction mixture. Potassium iodide solution (20 ml.) was added to reduce the excess ferricyanide and excess titrated with the standard thiosulfate, using the starch as indicator. A blank of 25 ml. of water was also used.

Calculation

The ferricyanide number was obtained from the expression:

$$\frac{(\text{ml. for blank} - \text{ml. for sample}) \times (\text{normality of thiosulfate})}{(\text{sample weight in g.}) \times (0.1)}$$

To determine the equivalents of ferricyanide consumed per equivalent of oligosaccharide, the following formulae is used:

$$\frac{(\text{Ferricyanide number}) \times (0.1) \times (\text{Polymer mol. weight})}{1000}$$

Hygroscopicity

Certain products, because of strong residual valence force, exhibit hygroscopicity, i.e., an ability to absorb large quantities of water through hydrogen bonding. It is not known but it is assumed that various oligosaccharides differ in this property as a function of chain length.

To measure hygroscopicity of oligosaccharides, changes in weight with time at various relative humidities were measured. The pure oligosaccharides from G₁ to G₆ were placed in aluminium dishes kept in dessicators of known humidity. These were weighed periodically and their increase in weight was measured.

Environment of desired humidity was obtained in a desiccator with controlled constant humidity obtained with sulfuric acid solutions, described in Handbook of Chemistry and Physics (10).

Solubility

Water soluble and insoluble fractions of isolated oligosaccharides were separated, dried and tested for solubility and presence of carbohydrates.

RESULTS AND DISCUSSION

The oligosaccharides present in corn syrup were separated on carbon-celite columns. Maltose was eluted with 5% ethyl alcohol. Flow rate was one to two gallons per day and the concentration of sugar in the eluate ranged from a few micrograms to about 2.0 mg./ml.

Collection, concentration and freeze drying of eluate. Isolation of maltotriose

Maltotriose was eluted with 7.5% ethyl alcohol. Maltotriose in the eluate, in the beginning, was mixed with maltose. The mixed fractions of

eluate were discarded and subsequent eluates proved to be pure maltotriose. Pure fractions were collected in gallon jars and regularly tested for their purity by paper chromatography. The flow rate was one to two gallons per day. A total of 64 gallons of eluate containing maltotriose was collected. The eluate was concentrated to about one gallon in a dairy plant, skim milk evaporator operating at a vacuum of about 26 inches of Hg and temperature about 130⁰ F. The concentrated eluate was acidic and salty, indicating the presence of salts and chloride ions as impurities. These impurities were removed by passing the eluate through an ion exchange resin columns (anion and cation exchange resins). Approximately 10 gms. of pure maltotriose was obtained after freeze drying the concentrated purified eluate.

Isolation of maltotetraose

Elution of maltotetraose was achieved with 10.0% and 12.5% ethyl alcohol. Initial fractions showed maltotriose coming out with maltotetraose, but later fractions showed pure maltotetraose. Routine testing of eluate by paper chromatography assured the purity of the collected fraction. The flow rate was 1.5 to 2.0 gallons per day and the maltotetraose saccharide present in eluate ranged from 20 to 225 microgram per milliliter. Seventy-five gallons of eluate containing maltotetraose was concentrated to 1.5 gallons. The concentrate was slightly turbid (milky) with a pH of 4.0. The concentrated eluate contained approximately 1.5% of maltotetraose and was found to be pure by paper chromatography. The concentrate was freeze dried and yielded five to seven gms. of maltotetraose.

Isolation of maltopentaose

Maltopentaose was obtained by increasing the ethanol concentration to 15 to 17.5%. Initial eluates were contaminated with lower homologues but

subsequently pure fractions were collected and about 70 gallons containing maltopentaose was concentrated to about one gallon. About 10.0 gms. of maltopentaose was obtained after freeze drying the concentrated eluate.

Isolation of maltohexaose

Maltohexaose was obtained by elution with 20 and 22.5% ethanol. A relatively large quantity of maltohexaose was eluted as a mixture with maltopentaose, but the last fractions of eluate contained, in about 25 gallons, pure maltohexaose. The concentration of maltohexaose in the eluate ranged from 40 to 126 microgram per milliliter. About 5.0 gms. of maltohexaose was obtained after concentration and freeze drying.

Isolation of oligosaccharides from Liquidex

A second carbon column was filled with granular carbon and charged with 450 gms. of Liquidex, which is a low D. E. starch hydrolysate.

Elution techniques as described above were used to isolate the polysaccharides. Approximately two to three gms. of pure maltose was prepared. The reason for the low quantity was the low content of glucose and maltose in Liquidex. Similarly, about 5.0 gms. of maltotriose was obtained in pure form.

This column had somewhat faster flow rate than the first column. The eluate collected per day was about three gallons. However, saccharide content was only in microgram quantities and, therefore, there was no appreciable reduction in time.

The isolated oligosaccharides were studied for (1) reducing power (2) hygroscopicity and (3) solubility.

Reducing power, a property of analytical interest, was studied by the Schoch and Jensen procedure by determining the ferricyanide number of glucose

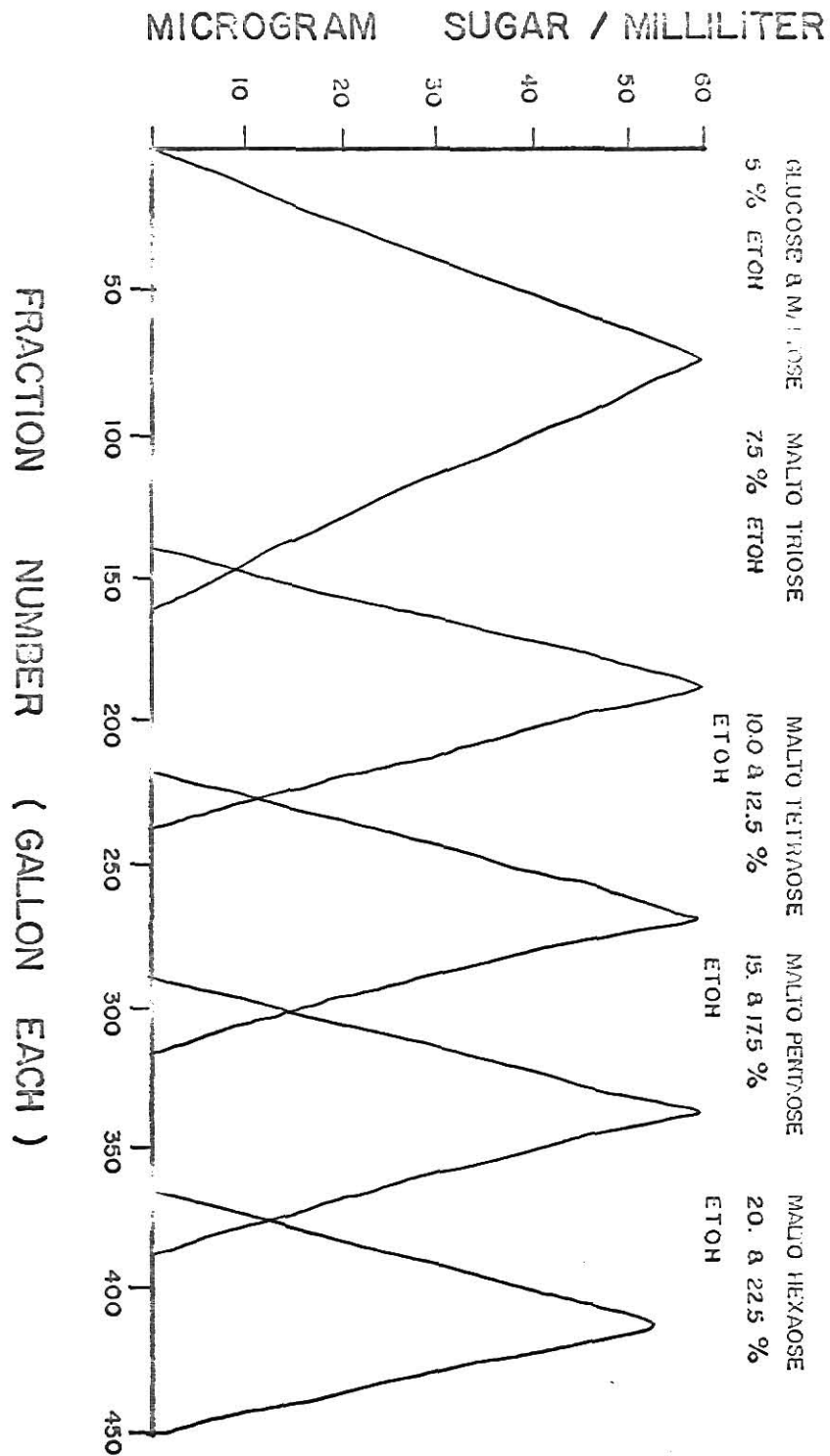


Fig. 2. Elution pattern of corn syrup saccharides from carbon celite column.

and glucose polymers. The data obtained in these experiments are summarized in Table 2 in comparison with those reported by previous workers.

Table 2.
Ferricyanide number of glucose polymers.

	Commerford and Scallet (5)	Present Investigation
(1) Glucose	305	331
(2) Maltose	243	253
(3) Maltotriose	176	139
(4) Maltotetraose	147	73
(5) Maltopentaose	123	47.50
(6) Maltohexaose	105.5	44.50

The ferricyanide numbers obtained for glucose and maltose compare favorably with figures given in the literature. The ferricyanide numbers regularly decreased as the molecular size of the oligosaccharides increased. By the procedure used, the replication of measurements was excellent. The results indicate that ferricyanide number of glucose polymers decreases as the chain length increase from G_1 to G_6 .

The reducing power of the oligosaccharides reported by different workers does not show close relationship. This may be due to presence of impurities in the isolated oligosaccharides. Hoover (14) reported the presence of chloride ions in his preparation of maltotetraose. He also reported that the value for maltopentaose was unusually high but no explanation was given for this.

Table 3 gives the D. E. values found for each saccharide by Hoover (14, 15), Commerford and Scallet (6) and present investigation.

Table 3.

Dextrose equivalents (D. E.)

Saccharide	Hoover et al. (14,15) (Ferricyanide method)	Commerford (6) (Lane & Eynon)	Present Investigation (Ferricyanide method)	Theoretical values
(1) Dextrose	100.08	100	100	100
(2) Maltose	71.95	58.0	74.0	52.60
(3) Maltotriose	61.63	39.50	44.0	35.70
(4) Maltotetraose	52.27	29.80	23.0	27.00
(5) Maltopentaose	53.97	24.20	15.0	21.70
(6) Maltohexaose	41.14	20.80	14.10	18.20

The dextrose equivalent concept assumes that the reducing sugars in corn syrup behave like dextrose. Theoretically, the D. E. of maltose should be 52.60 (the molecular weight of dextrose divided by the molecular weight of maltose times 100). Actually, values ranging from 54 to 74 have been reported. Whether this deviation is due to differences in the reaction conditions or to inherent differences in the stoichiometry of the reaction has not been established. The homologous series of D-glucose polymers higher than maltose normally found in corn syrup is an interesting set of compounds for studying reactions of this type. Whether the observed differences can be correlated exactly with specific reactions is open to speculation. However,

there are many areas in which knowledge of the D. E.'s of the individual oligosaccharides is of immediate value.

Studies on reducing power of oligosaccharides by Hoover et al. (14,15), Commerford and Scallet (5) and our findings indicate that reducing power of oligosaccharides regularly decreased as the molecular size of the oligosaccharides increased. However, the values reported by each worker do not agree with the theoretical values indicating that the isolated oligosaccharides carried some impurities observed by Hoover. This perhaps causes deviation of the values from the theoretical. It is therefore necessary to apply some purification and refining technique to these isolated oligosaccharides before their physical and chemical properties are established.

Hygroscopicity

The oligosaccharides prepared from corn syrup are hygroscopic i.e., they possess the ability to absorb large quantities of water through hydrogen bonding. Hygroscopicity tended to increase as the chain length increased from G_1 to G_6 but the relationship was not linear.

Studies on hygroscopicity of glucose polymers (G_1 to G_6) at a relative humidity of 18.80% and temperature 75°F, indicated that hygroscopicity increased in order of glucose, maltotetraose, maltose, maltotriose, maltopentaose, maltohexaose. These data as shown indicate that glucose has minimum hygroscopicity and maltotetraose (G_4) has lower hygroscopicity when compared with maltose, maltotriose, maltopentaose and maltohexaose.

At a relative humidity of 37.10% and temperature 75°F, hygroscopicity of oligosaccharides increased as chain length increased in order: glucose, maltose, maltotetraose, maltotriose, maltohexaose and maltopentaose. The results indicate that glucose possesses the least hygroscopicity, maltotriose has

higher hygroscopicity than maltotetraose and maltopentaose has higher hygroscopicity than maltohexaose, at a relative humidity of 37.10% and temperature 75°F.

Studies at a relative humidity of 58.30% and temperature 75°F indicated that maltotriose is more hygroscopic than maltotetraose and maltopentaose is more hygroscopic than maltohexaose.

At a relative humidity of 70.0% and temperature 75°F, maltopentaose is more hygroscopic than maltohexaose; other saccharides showed increased in hygroscopicity as the chain length increased.

Hygroscopicity studies of glucose polymers (G_1 to G_6) at a relative humidity of 60% and temperature 75°F showed that maltotetraose had the highest hygroscopicity at this particular relative humidity and was followed in decreasing order as the chain length decreased: maltohexaose, maltotriose, maltopentaose, maltose and glucose.

It is therefore concluded that hygroscopicity of glucose polymers depend on relative humidity and temperature of the environment.

Solubility

Water soluble and insoluble fractions of isolated oligosaccharides were separated, dried and tested for solubility and presence of carbohydrates. The tests indicated that the soluble fraction contained most of the polymer and the insoluble residue which was non-carbohydrate in nature may have been leached from the column or contamination may have arisen from processing of the eluate at some stage during evaporation and/or freeze drying. The insoluble residue was colloidal in nature and had very high adsorption power for the polymers.

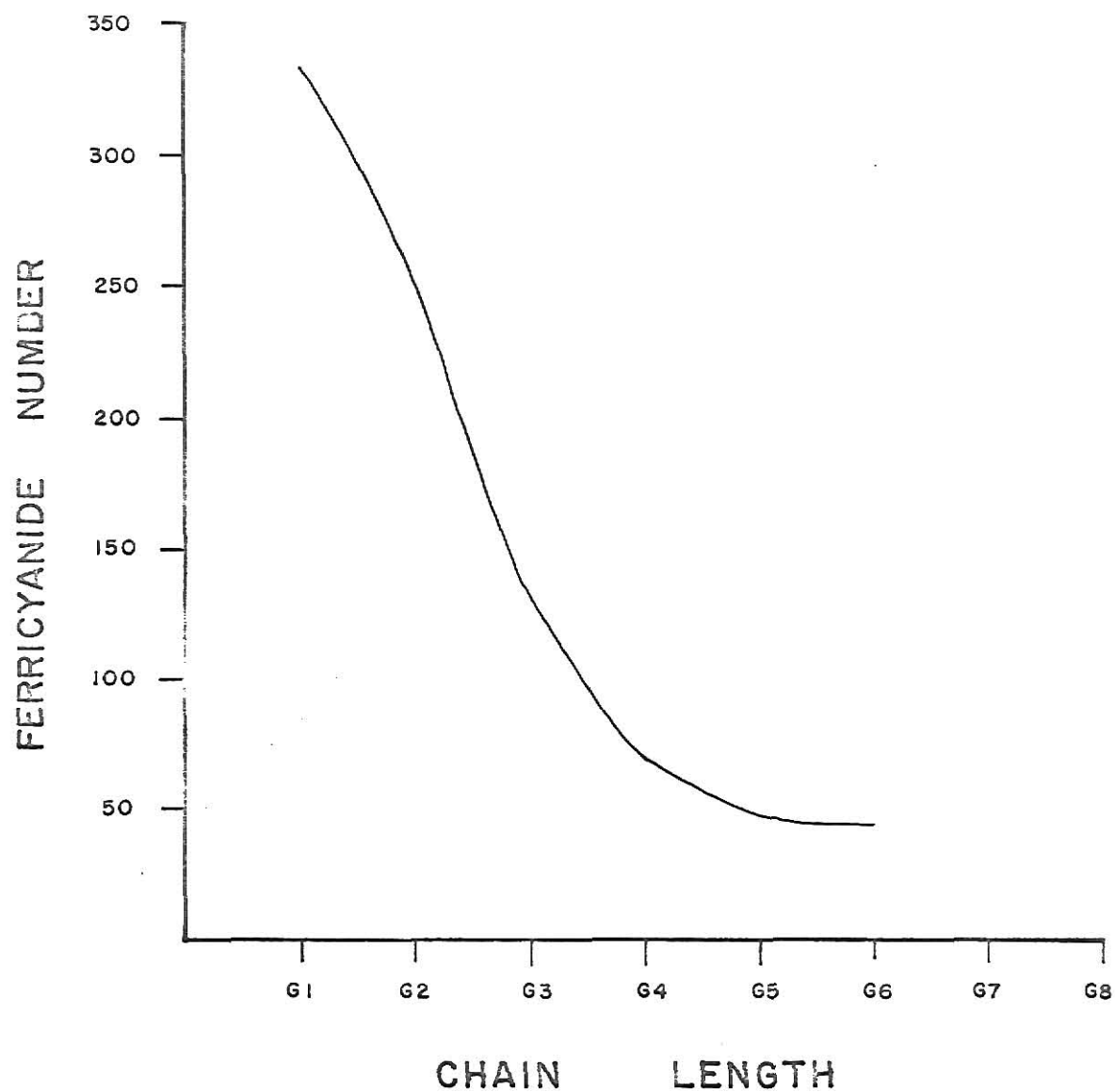


Fig. 3. Ferricyanide numbers of glucose polymers ranging from G_1 to G_6 .

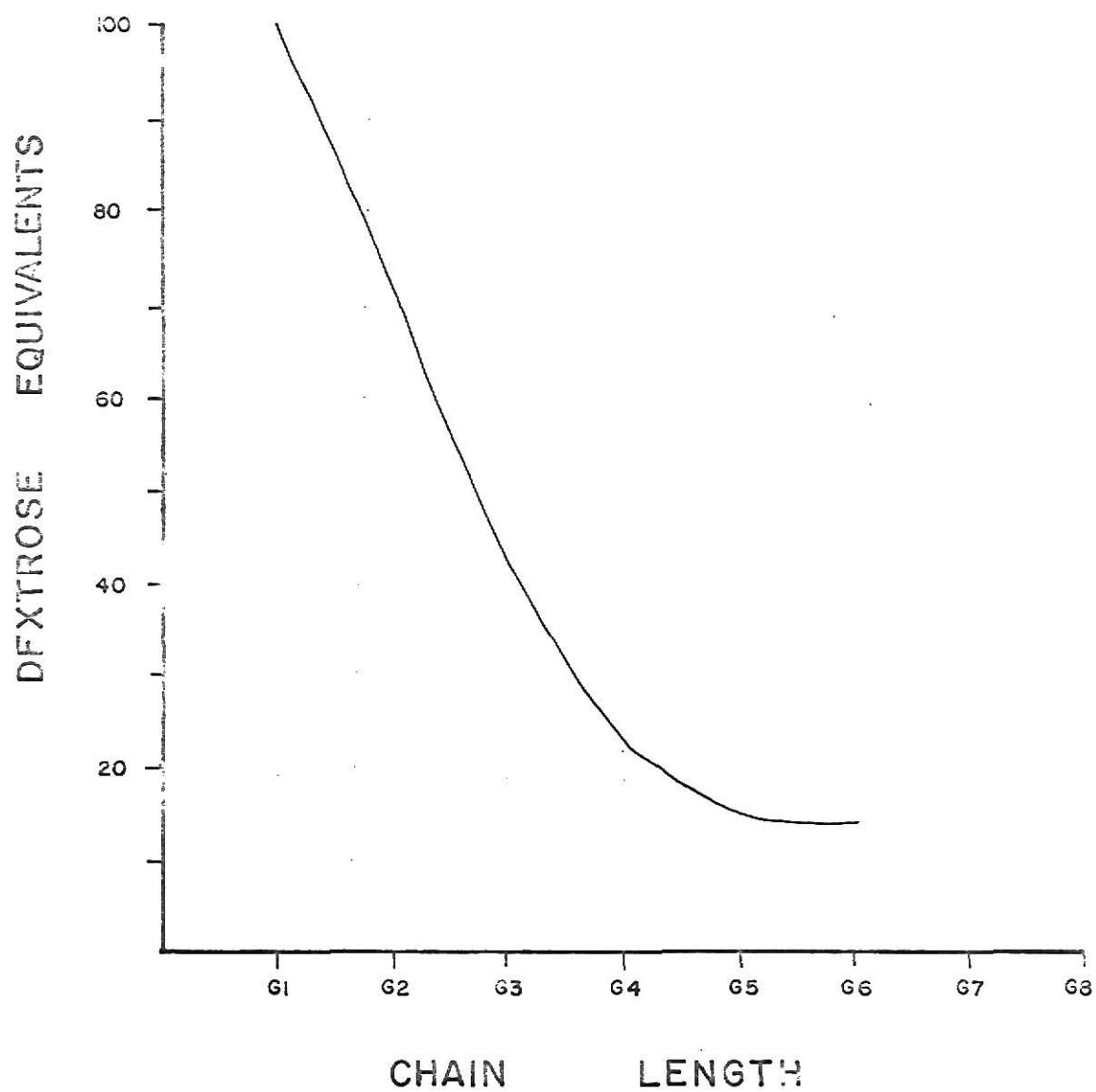


Fig. 4. Dextrose equivalents of glucose polymers ranging from G_1 to G_6 .

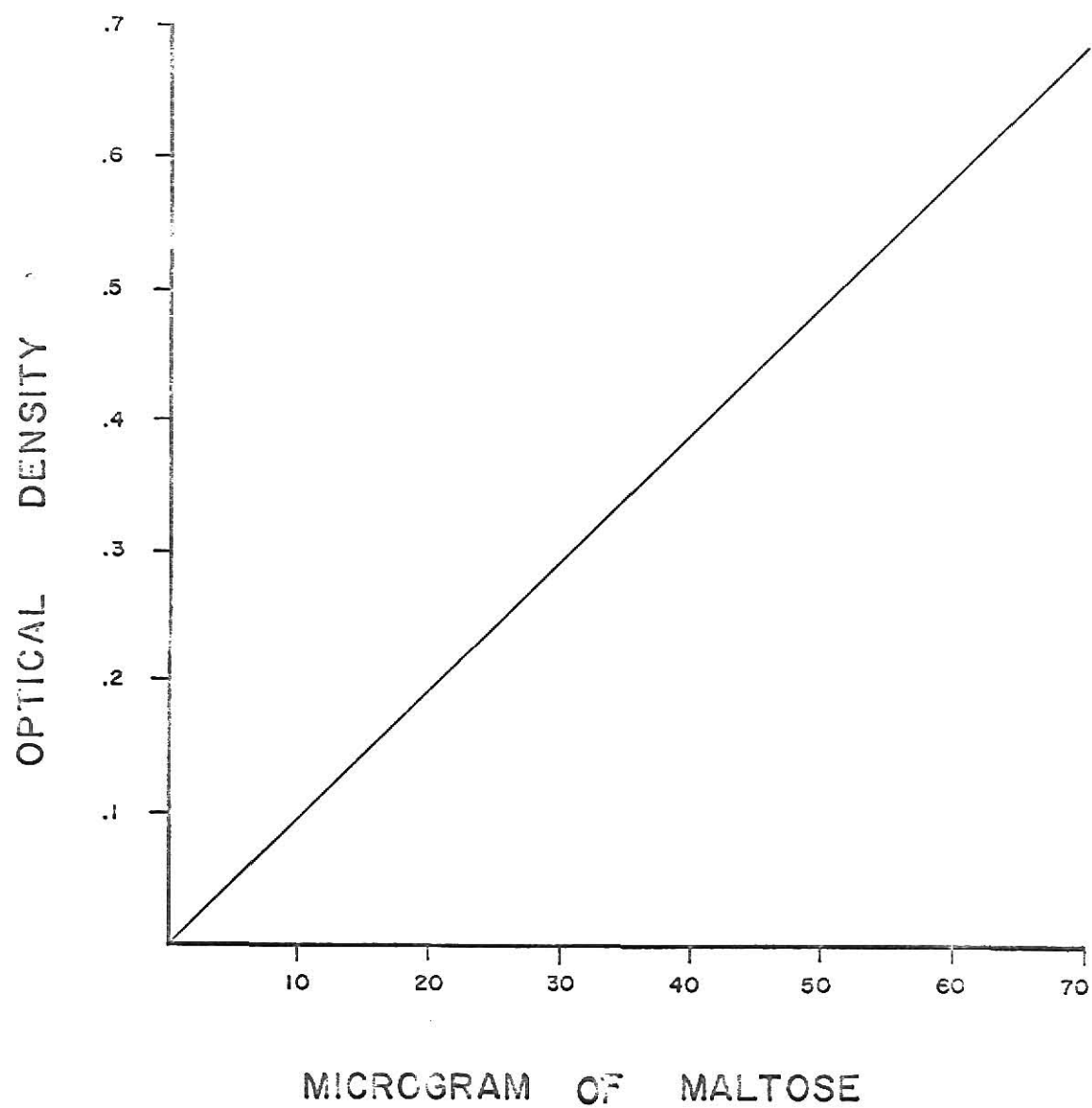


Fig. 5. Standard curve for maltose.

Table 4.

Hygroscopicity of glucose polymers at relative humidity 80.00 and temperature 75° F.

Time in minutes	% Moisture					
	Glucose	Maltose	Maltotriose	Maltotetraose	Maltopentaose	Maltohexaose
15	0.25	4.02	9.61	15.02	10.22	10.76
30	0.25	4.08	9.72	15.11	10.26	10.80
60	0.25	4.20	10.13	15.48	10.50	11.10
120	0.25	4.41	11.13	16.46	11.06	11.77
240	0.16	4.41	12.05	17.63	11.86	12.62
360	0.16	4.32	12.28	17.72	12.18	12.88
24 hours	0.16	4.32	14.19	22.43	14.43	14.77

Table 5.

Hygroscopicity of glucose polymers at relative humidity 70.00 and temperature 75° F.

Time in minutes	% Moisture					
	Glucose	Maltose	Maltotriose	Maltotetraose	Maltopentaose	Maltohexaose
15	0.14	2.28	4.54	4.94	7.21	6.35
30	0.14	2.44	4.79	5.36	7.52	6.61
60	0.14	2.76	5.17	5.92	7.91	7.01
180	0.14	3.47	5.84	7.01	8.70	7.77
240	0.15	3.72	6.01	7.39	8.93	8.00
300	0.12	3.90	6.11	7.88	9.12	8.42

Table 6.

Hygroscopicity of glucose polymers at relative humidity 58.30 and temperature 75° F.

Time in minutes	% Moisture					
	Glucose	Maltose	Maltotriose	Maltotetraose	Maltopentaose	Maltohexaose
15	0.13	1.68	3.03	2.54	4.93	4.40
30	0.14	1.80	3.25	2.79	5.21	4.66
60	0.14	1.92	3.54	3.12	5.77	4.91
90	0.14	1.95	3.71	3.34	6.89	5.61
120	0.14	1.98	3.81	3.50	6.33	5.32
180	0.13	2.00	3.86	3.63	6.49	5.33
240	0.14	2.02	3.94	3.73	6.83	5.50
300	0.13	2.01	3.96	3.77	6.73	5.55
360	0.13	2.01	3.98	3.82	6.85	5.61
30 hours	0.14	2.04	4.19	4.47	6.85	5.96

Table 7.

Hygroscopicity of glucose polymers at relative humidity 37.10 and temperature 75° F.

Time in minutes	% Moisture					
	Glucose	Maltose	Maltotriose	Maltotetraose	Maltopentaose	Maltohexaose
15	0.11	1.08	1.62	1.11	2.20	2.45
45	0.06	1.02	1.59	1.08	2.44	2.75
90	0.06	1.11	1.75	1.25	2.80	2.97
150	0.12	1.26	2.08	1.53	3.27	3.26
180	0.12	1.29	2.14	1.58	3.36	3.30
240	0.13	1.32	2.18	1.58	3.53	3.32
300	0.15	1.42	2.40	1.83	3.92	3.70
360	0.15	1.47	2.48	1.95	4.24	3.85
420	0.14	1.53	2.63	2.05	4.57	4.02
24 hours	0.16	1.47	2.57	2.09	4.49	3.81

Table 8.

Hygroscopicity of glucose polymers at relative humidity 18.80% and temperature 75° F.

Time in minutes	% Moisture					
	Glucose	Maltose	Maltotriose	Maltotetraose	Maltopentaose	Maltohexaose
15	0.06	0.54	0.82	0.37	0.92	1.52
30	0.03	0.64	0.99	0.42	1.12	1.86
45	0.04	0.73	1.05	0.54	1.32	2.11
60	0.04	0.78	1.16	0.60	1.48	2.23
90	0.06	0.81	1.17	0.68	1.68	2.25
120	0.06	0.82	1.18	0.75	1.76	2.25
180	0.07	0.85	1.20	0.82	1.88	2.21
240	0.07	0.85	1.20	0.83	1.88	2.11

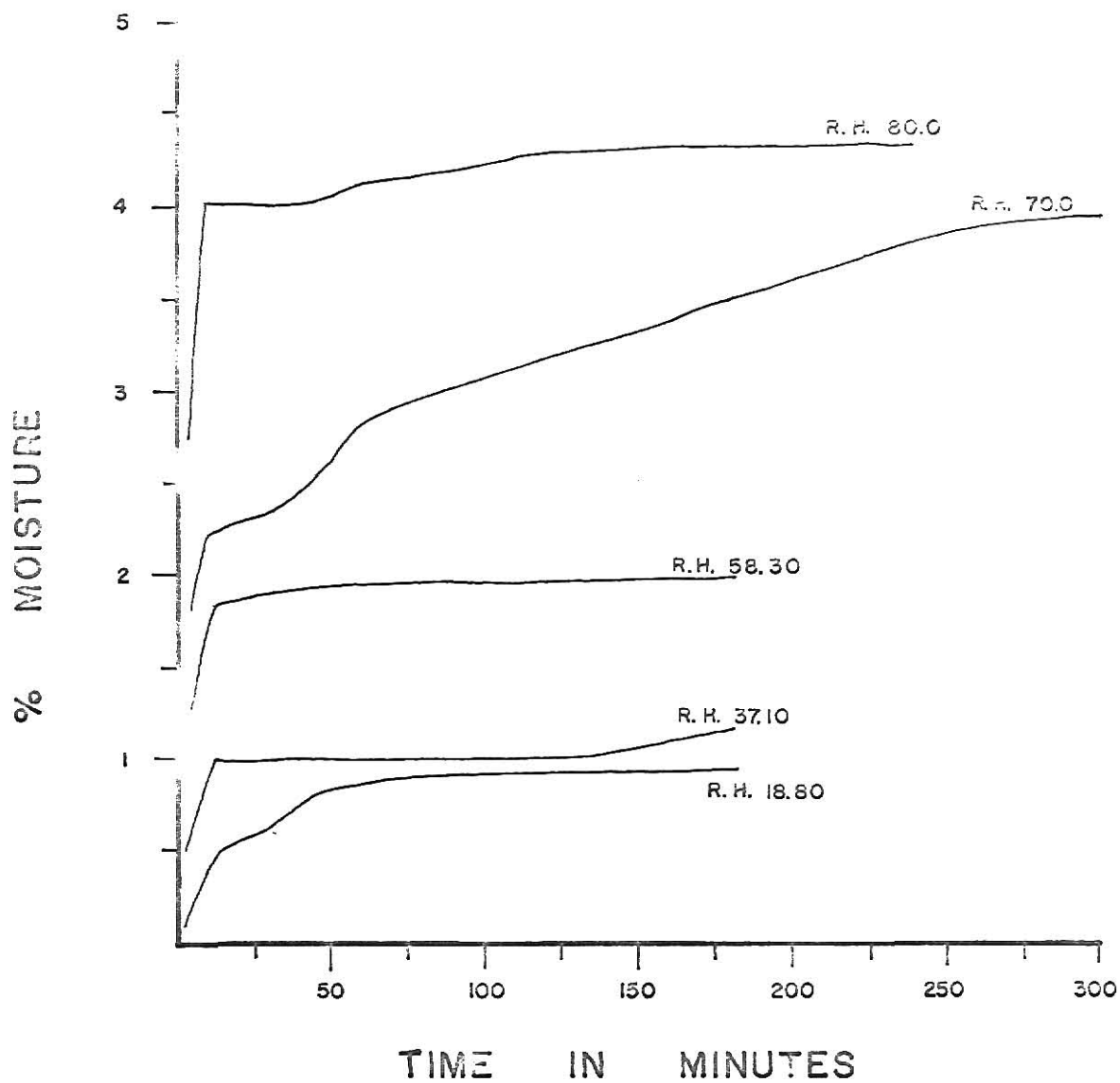


Fig. 6. Hygroscopicity of maltose at various relative humidities, temperature 75°F.

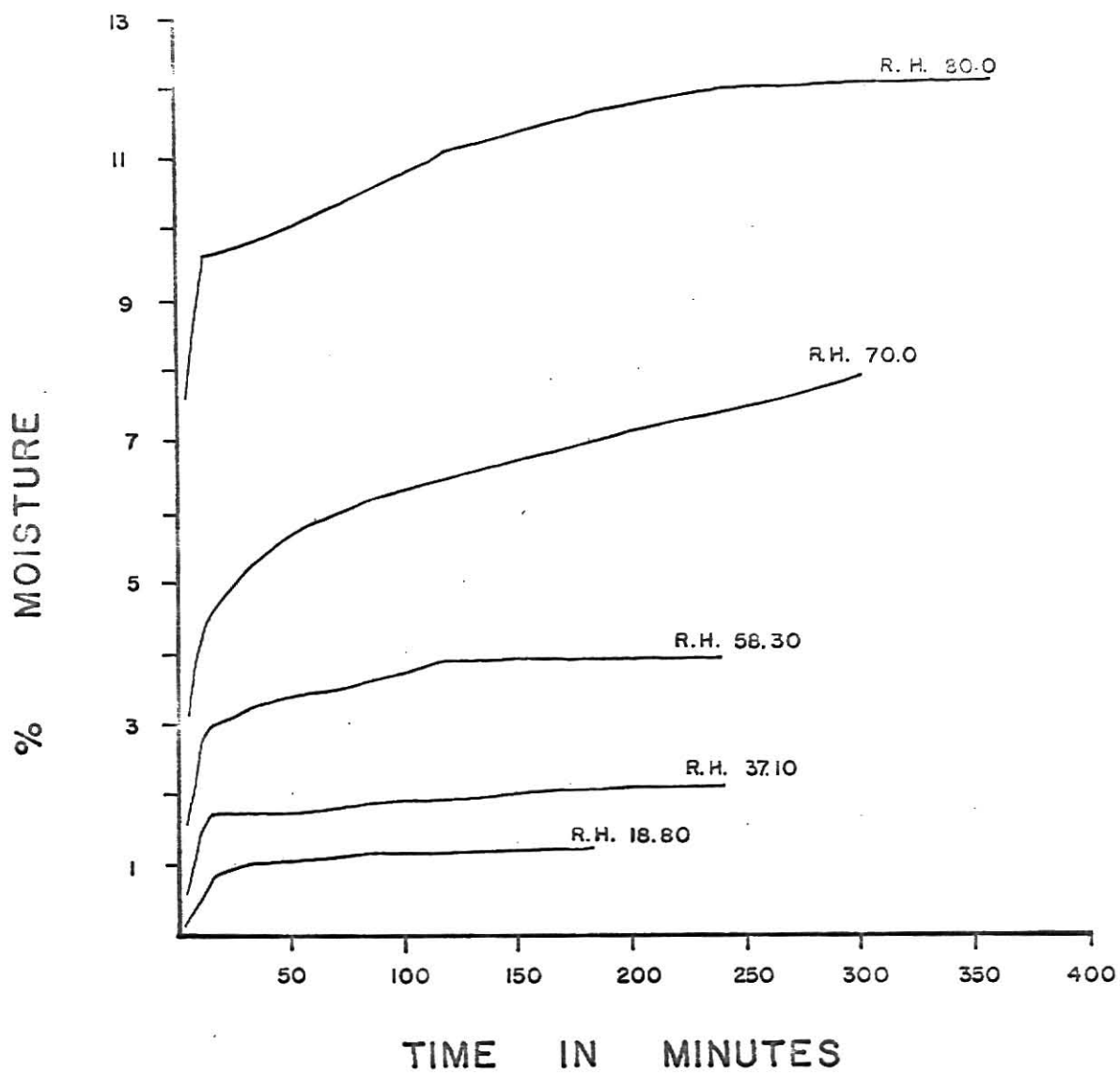


Fig. 7. Hygroscopicity of maltotriose at various relative humidities and temperature 75°F.

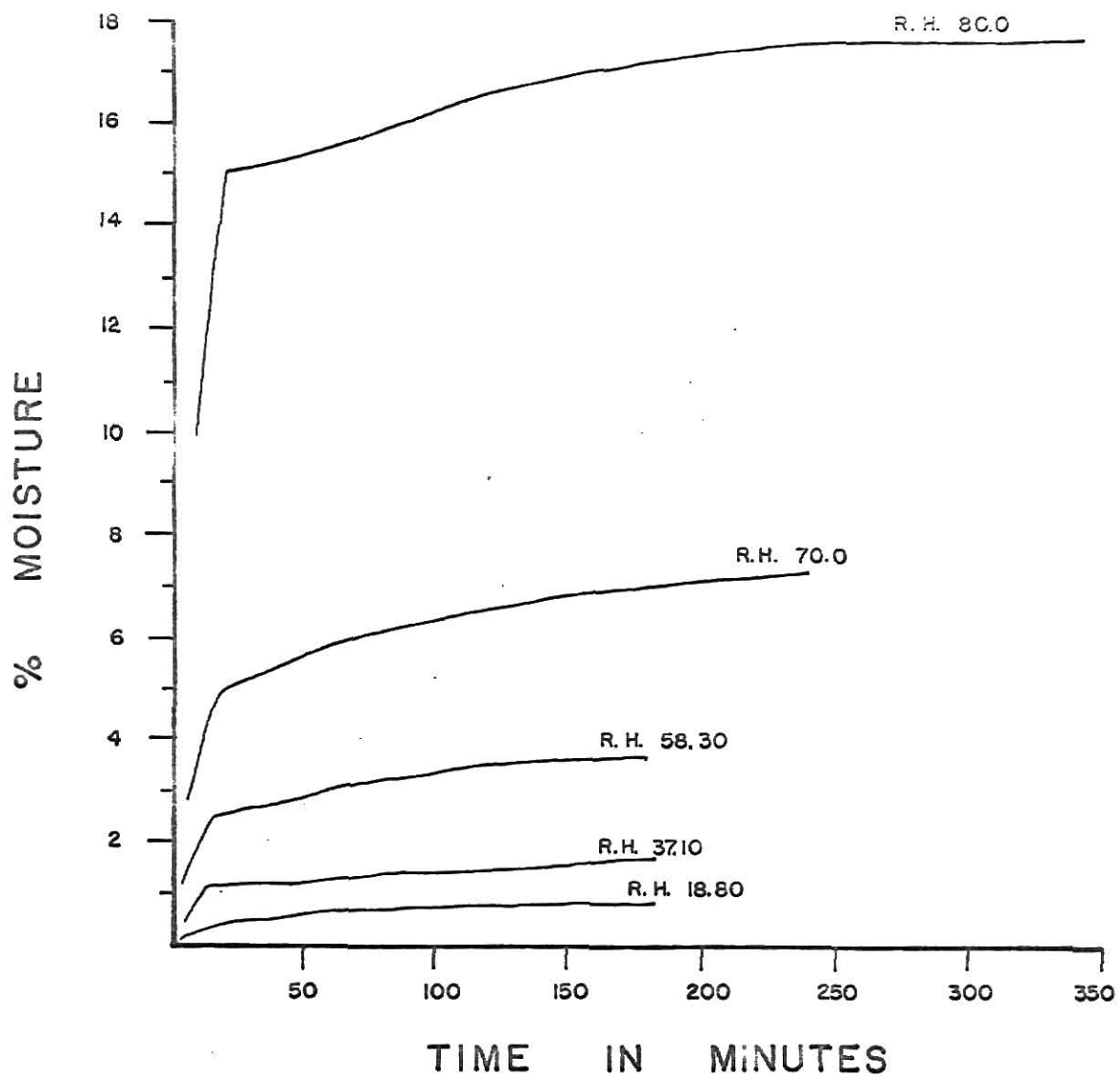


Fig. 8. Hygroscopicity of maltotetraose at various relative humidities and temperature 75°F.

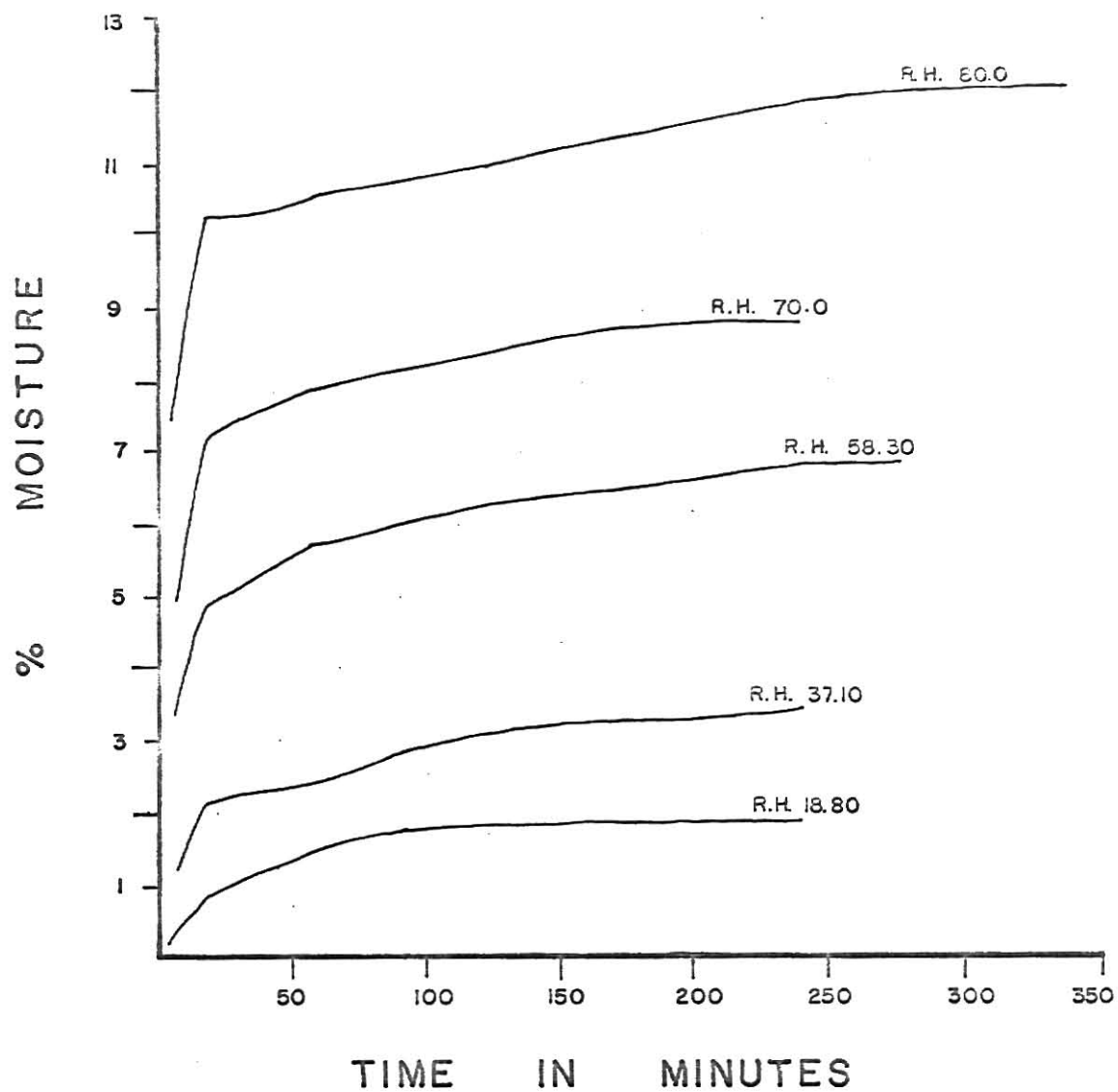


Fig. 9. Hygroscopicity of maltopentaose at various relative humidities and temperature 75°F.

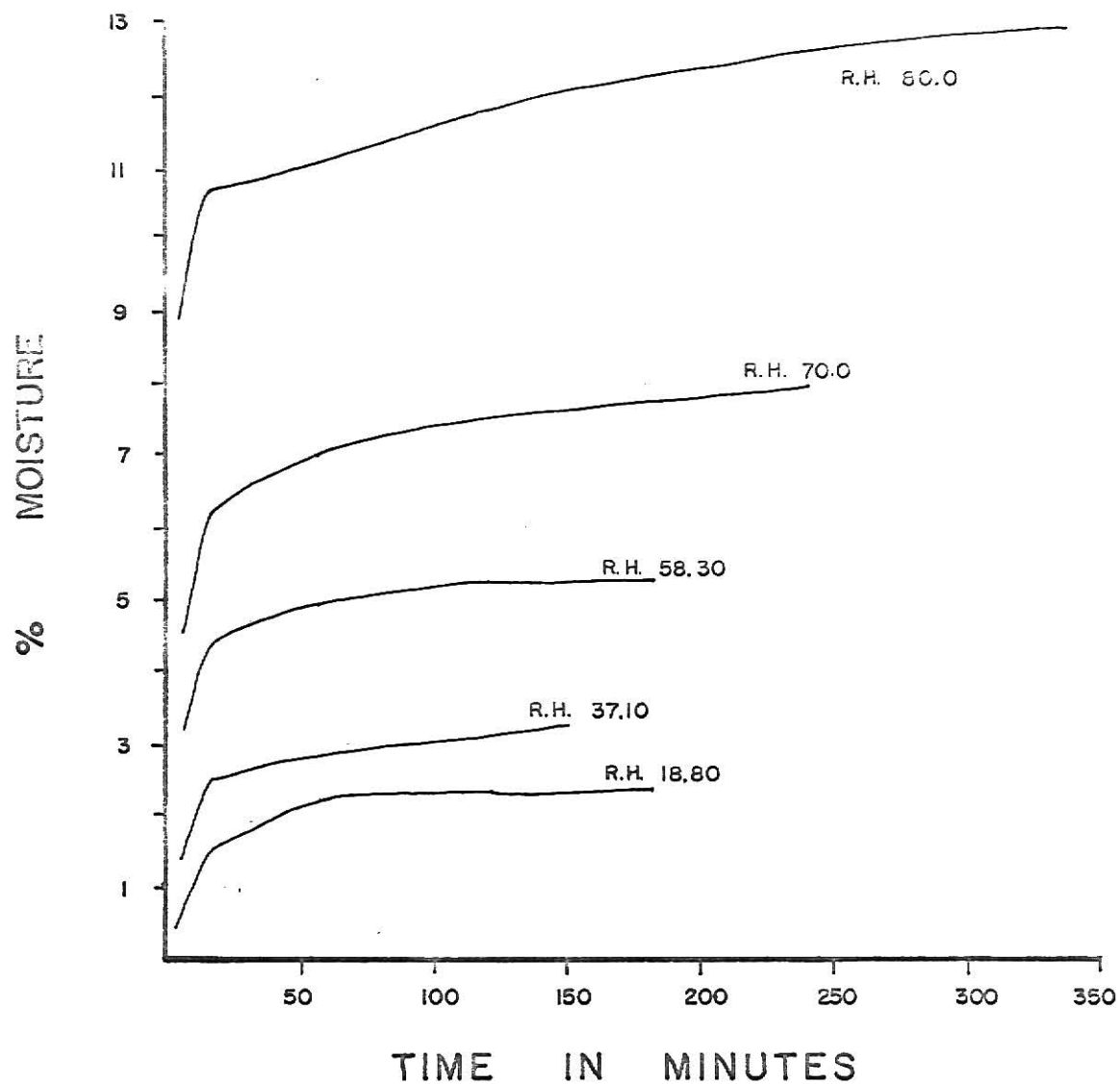


Fig. 10. Hygroscopicity of maltohexaose at various relative humidities and temperature 75°F.

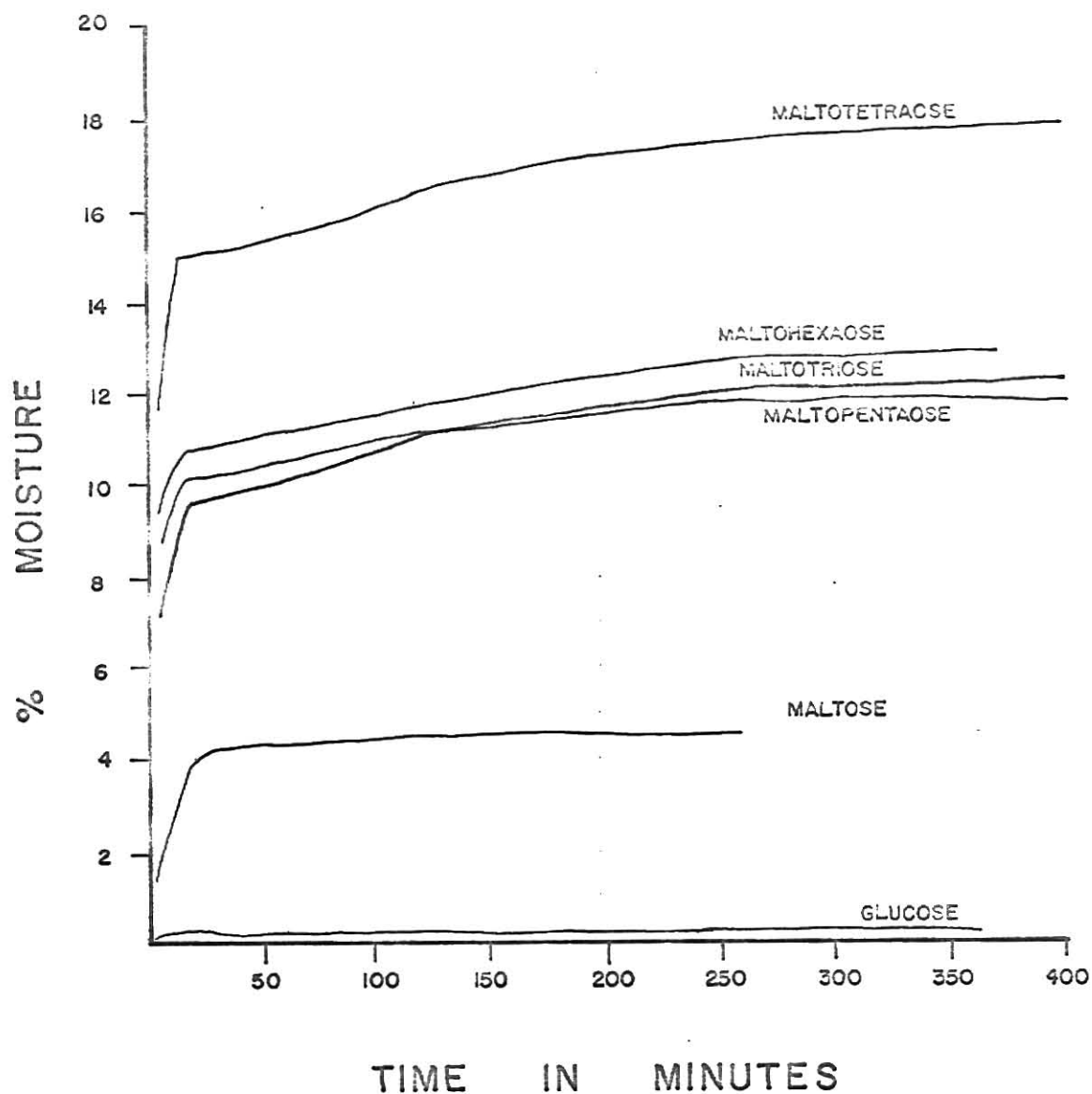


Fig. 11. Hygroscopicity of glucose polymers (G_1 to G_6) at relative humidity 80.0% and temperature 75°F.

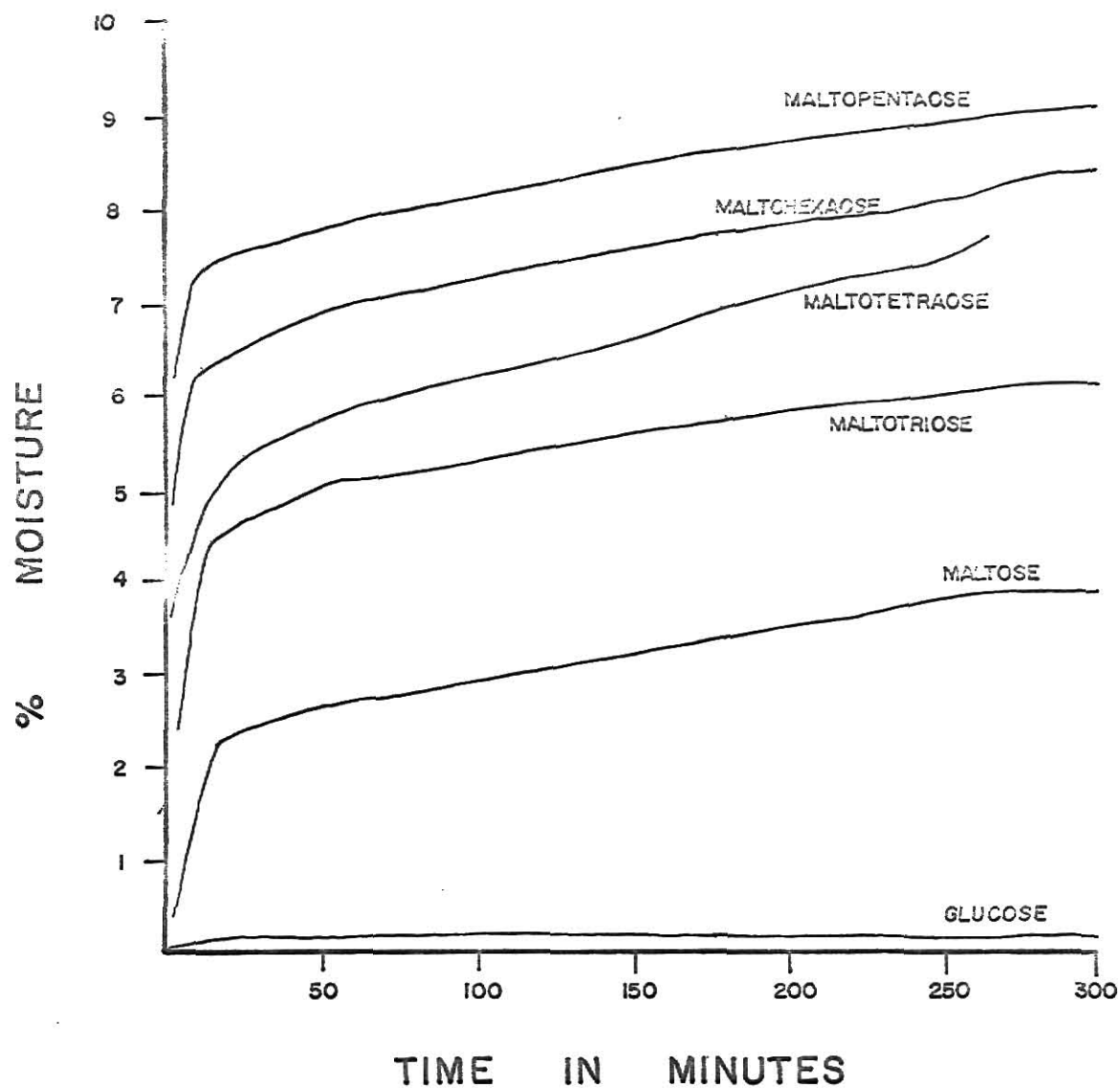


Fig. 12. Hygroscopicity of glucose polymers (G_1 to G_6) at relative humidity 70.0% and temperature 75°F.

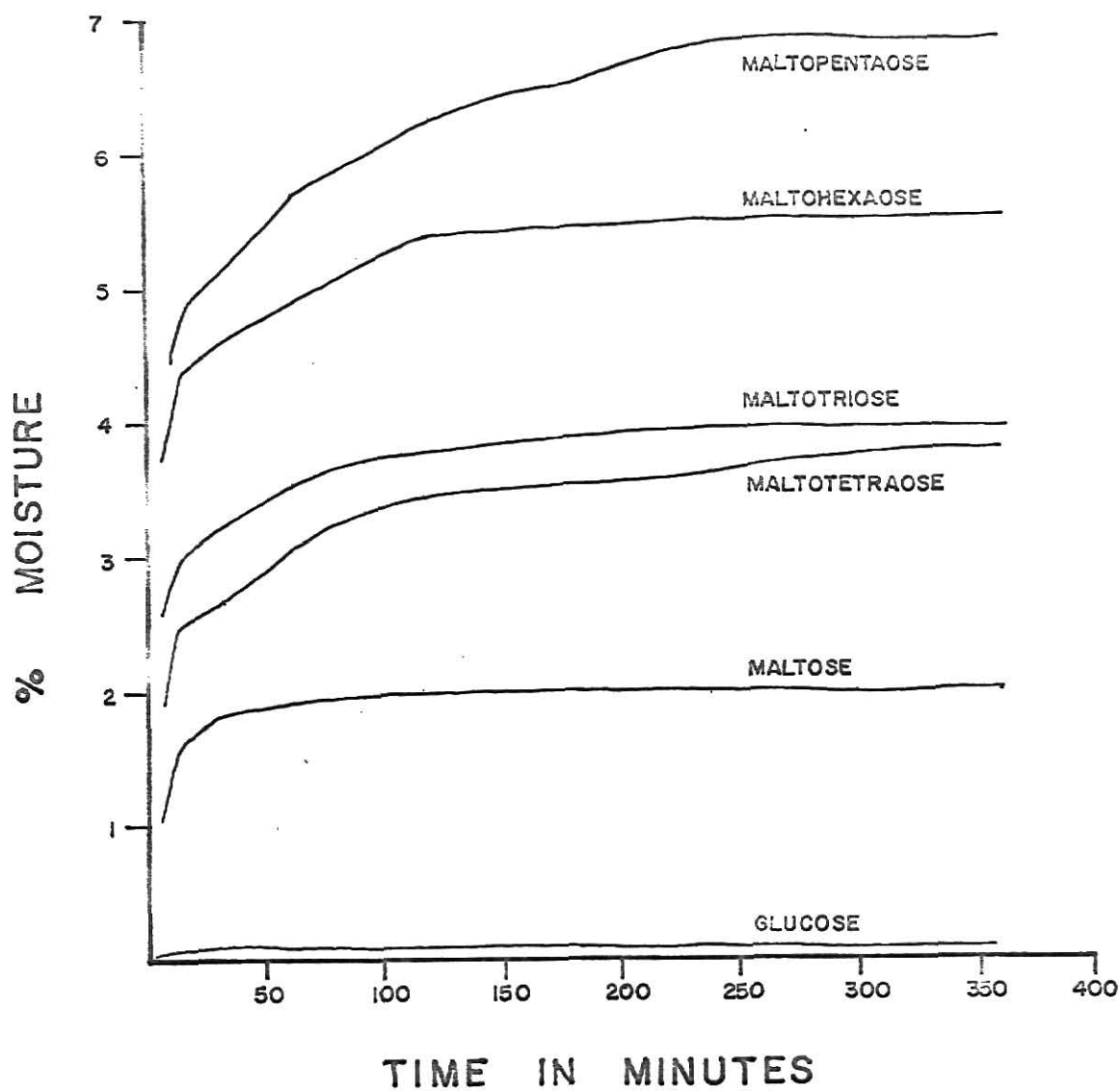


Fig. 13. Hygroscopicity of glucose polymers (G_1 to G_6) at relative humidity 58.30% and temperature 75°F.¹

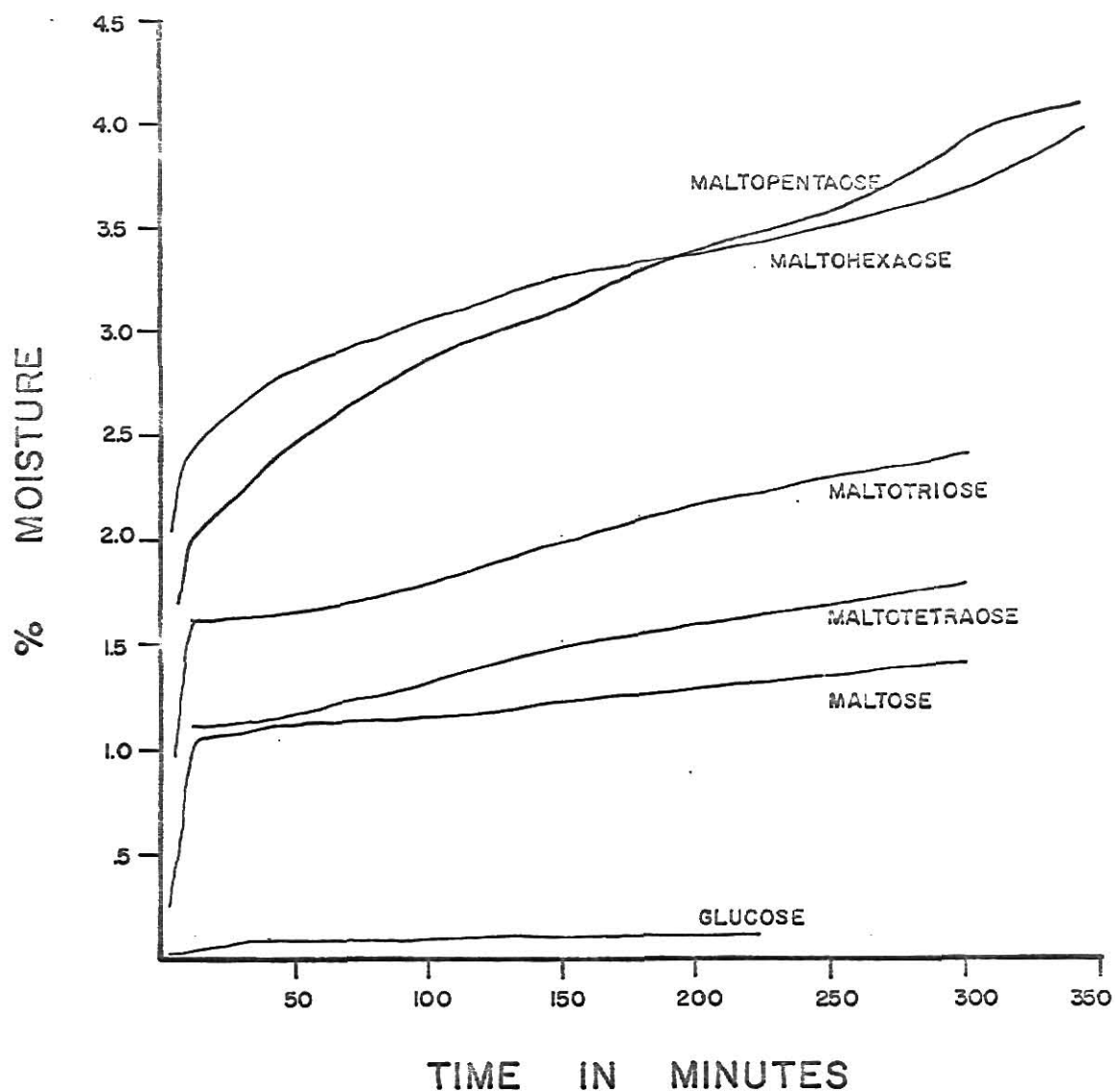


Fig. 14. Hygroscopicity of glucose polymers (G_1 to G_6) at relative humidity 37.10% and temperature 75°F.^1

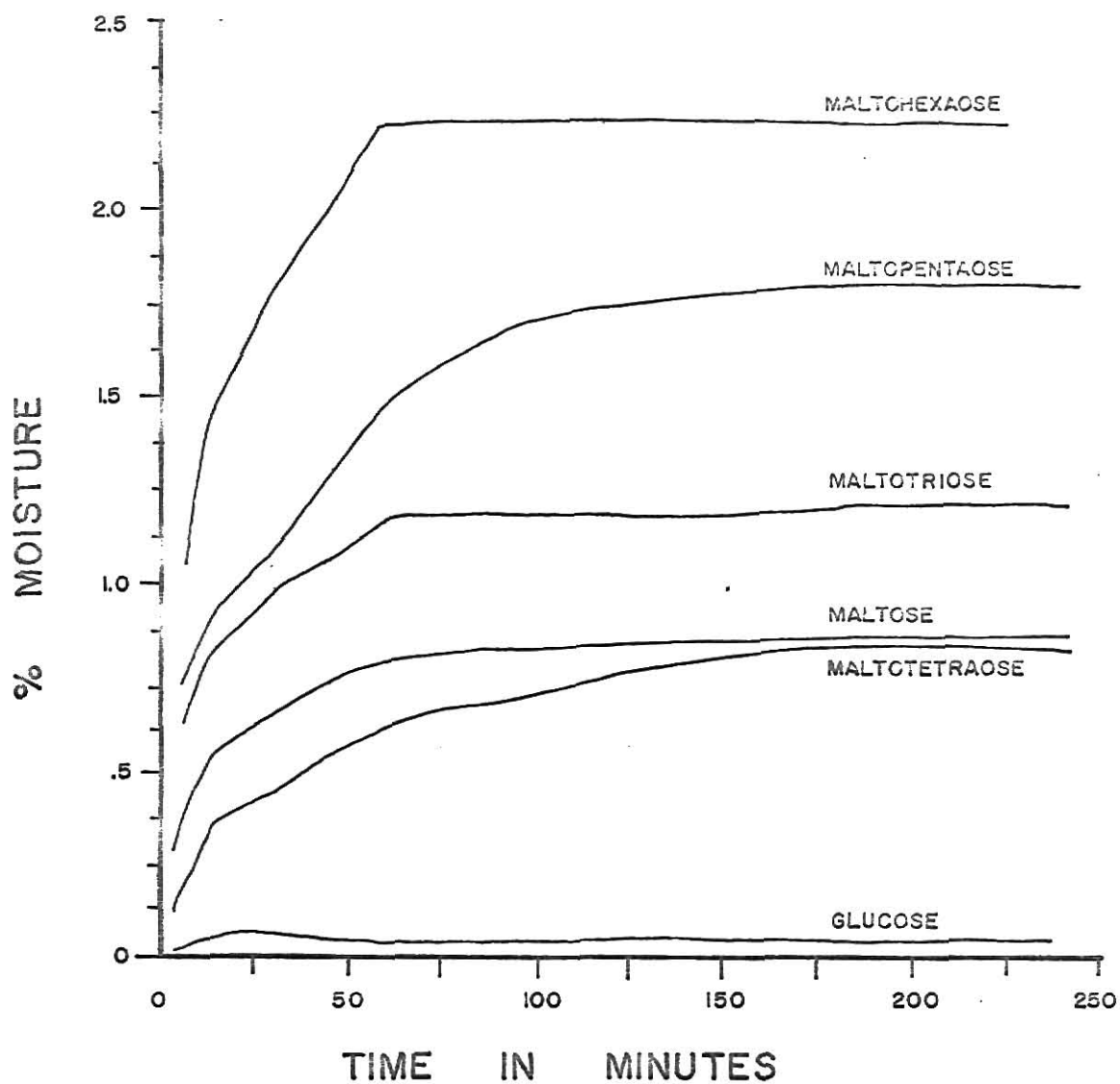


Fig. 15. Hygroscopicity of glucose polymers (G_1 to G_6) at relative humidity 18.80% and temperature 75°F.^{1-6}

SUMMARY AND CONCLUSIONS

The chemical and physical properties of the malto-oligosaccharides are unknown, yet it is recognized that corn hydrolysates made up of mixtures of different oligosaccharides have various properties which must depend on the chemical and physical properties of the individual components. It was the object of this investigation to isolate pure oligosaccharides from a mixture and to establish certain of their chemical and physical properties.

A modification of the Tiselius technique was used to resolve corn syrup saccharides by chromatographic adsorption on charcoal and displacement by water followed by stepwise increase in ethanol concentration. The course of the desorption process was followed by testing the carbohydrates in the eluate by a chemical method (phenol-sulfuric acid method). Presence of sugar in the eluate was tested by a paper chromatographic method using Whatman No. 4 filter paper. The development solvent was propanol-ethyl acetate-water (6:1:3). The chromatograms were developed by spraying with silver nitrate, sodium hydroxide and sodium thiosulfate solution.

The eluate fractions containing one particular oligosaccharide were combined and concentrated in a vacuum evaporator. The concentrate was freeze dried.

The isolated oligosaccharides were studied for: (1) reducing power (2) hygroscopicity.

Reducing power and hygroscopicity of glucose polymers were found to be related to the molecular weight of the sugars. Since physical and chemical properties of oligosaccharides are related to chain length, it can be concluded that the oligosaccharides isolated from corn syrup are members of homologous series of maltodextrin.

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APPENDIX

Reagents

1. Potassium ferricyanide solution:

Dissolve 16.5 g. of potassium ferricyanide and 22 g. of sodium carbonate in water and make up to one liter (0.2 M carbonate). Age the solution for three days and store in brown bottles away from sunlight.

2. Zinc sulfate-potassium chloride-acetic acid solution:

Dissolve 20 g. of zinc sulfate heptahydrate, 70 g. of potassium chloride and 200 ml. of glacial acetic acid in water and make up to one liter.

3. Potassium iodine solution:

Dissolve 200 g. of potassium iodine in water and make up to one liter.

4. Sodium thiosulfate solution, 0.05 N:

Dissolve 12.4 g. of sodium thiosulfate pentahydrate in boiled water and make up to one liter. Standardize the thiosulfate against reagent grade potassium dichromate. 3.2 g. of borax may be added per liter of solution to prevent spoilage.

5. Starch indicator:

Pour slowly 5 g. of soluble starch slurried in 20 ml. of water into a stirred boiling salt solution containing 150 g. of sodium chloride in 480 ml. of water. The cooled starch suspension should remain stable for several months.

All sugars (G_1 to G_6) were dried in vacuum oven for 24 hours at 70°C under a vacuum of 27 in. of mercury.

PHYSICAL AND CHEMICAL PROPERTIES OF OLIGOSACCHARIDES
FROM CORN SYRUP

by

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Baroda, India, 1959

AN ABSTRACT OF A MASTER'S THESIS

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requirements for the degree

MASTER OF SCIENCE

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Manhattan, Kansas

1969

The present investigation was undertaken to separate and identify the oligosaccharides present in corn syrup and to study their physical and chemical properties.

Two commercial corn hydrolysates, one with 43 D.E. and other of very lower D.E. have been used as a source of oligosaccharides. One chromatographic column 6" x 12', made of pyrex glass was filled with activated carbon (G-60) and celite -5H5 (60:40). The column was packed with carbon-celite in the form of a slurry. A second column was charged with 50:50 mixture of granular and powder carbon (G-60). Each column was charged with 450 gm. of hydrolysate and separation of oligosaccharides was followed with stepwise increase in concentration of ethyl alcohol. Presence of sugar in eluate was estimated by a phenol-sulfuric acid method. Purity of eluate was tested by a paper chromatography. Chromatographically pure maltotetraose, maltopentaose and maltohexaose were obtained after concentration and freeze drying the eluate. Paper chromatographic separation identified these oligosaccharides as members of a homologous series of the malto-oligosaccharides.

The isolated oligosaccharides were studied for (1) reducing power, (2) hygroscopicity and (3) solubility.

Reducing power was studied by the Schoch and Jensen procedure for determining the ferricyanide number of glucose and glucose polymers. It was observed that reducing power decreased as the chain length increases from G_1 to G_6 .

Certain products, because of strong residual valence forces, exhibit hygroscopicity, i.e., an ability to absorb large quantities of water through hydrogen bonding. To measure hygroscopicity in the oligosaccharides, changes in weight under various relative humidities as a function of time were measured.

Studies on hygroscopicity indicate that hygroscopicity of glucose polymers depended on relative humidity and temperature.

Water soluble and insoluble fractions of isolated oligosaccharides were separated, freeze dried and tested for solubility and presence of carbohydrate. The tests indicated that glucose polymers (G_1 to G_6) are water soluble and the insoluble residue which was non-carbohydrate in nature was leached from column or from processing of eluate at some stage during evaporation and/or freeze drying. The insoluble residue was colloidal in nature and had very high adsorption power for the oligosaccharide.