SEPARATION OF OLIGOSACCHARIDES FROM CORN SYRUP AND THEIR EFFECT ON BREAD

by

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INTRODUCTION AND REVIEW OF LITERATURE

Corn syrups are commercial sweetening agents used by the baking industry for production of bread and various confections. They provide a readily fermentable source of carbohydrates for the yeast. They may be shipped and stored in bulk cars and tanks and they may be conveniently metered. Under certain circumstances, corn syrup is more economical to use than crystalline sugars for production of bakery products.

Corn syrups are produced by acid and/or enzyme conversion of corn starch. While they consist mainly of glucose, maltose, and water, they usually contain varying amounts of higher oligosaccharides depending on the degree of conversion. Considerable knowledge on the effect of glucose, fructose, maltose, and sucrose on baking is available but there is a dearth of information on the effect of the higher oligosaccharides on baking. The reason for this, perhaps, has been the difficulty associated with isolation and purification of large quantities of the higher oligosaccharides.

Investigations on composition of corn syrup have involved chromatographic methods of separation. These methods have provided only microgram quantities sufficient to establish the identity of the oligosaccharides. For purpose of investigation of the effect of these carbohydrates on baking, gram quantities would be required.

The problem of separating and isolating the various oligosaccharides is complex. They are present in corn syrup in relatively small amounts. They have great similarity of structure and functional groups and therefore differential migration in a chromatograph column can be achieved only by careful selection of carrier and solvents.

The purpose of this research was to apply chromatography methods to the

separation and isolation of the various oligosaccharides found in commercial corn syrup and to study the effect of a homologous series of oligosaccharides on breadmaking.

Column Chromatography of Oligosaccharides

It is evident from the early history of chromatography that Tswett (1903) was the first to use column chromatography for separation of pigment and colorless substances in natural material. Tswett's method seemed to be forgotten until Kuhn and Lederer (1931) separated the carotenes and xanthophylls on a column of aluminum and calcium carbonate. At this time only the classical methods were used for separation of maltose, maltotriose and maltotetraose from syrups. This involved the fractional distillation of the methyl (Hurd and Cantor, 1938) or propanyl derivatives (Hurd and Liggett, 1941). Column chromatography was applied to resolution of sugar mixtures by Tiselius (1940).

Since that time, the study of chromatographic methods has 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; especially for monosaccharides and lower molecular weight oligosaccharides.

The progress in using column chromatography for resolution of oligosaccharides started when Tiselius (1940) introdued 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 (1941) and Williams, Hagdahl and Tiselius (1954) have applied this method to the separation of mono-di-saccharides. The method was extended to the separation of raffinose using various carbons (Tiselius, 1942). Tiselius (1943a and 1943b) improved the separation of the sugars allowing the developer 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.

Tiselius, Claesson and their collaborators (1942) devised a modification of adsorption chromatography which they called "frontal analysis." In this procedure the solution containing the substances is forced continuously through the column. The change in effluent concentration and composition was detected by measuring the refractive index. Hagdahl (1948) attempted to use this method for separation of a mixture of sucrose, glucose, and raffinose but he was only partially successful. He used "elution analysis" successfully for separation of a similar mixture of sugars by changing the ethanol-water ratio in the solvent. The difficulty in this method is due to the fact that many substances are absorbed more strongly from dilute solution and hence the substances "tail" through the column.

Tiselius (1943a) has shown that it is advantageous to develop the chromatogram with a solution of a substance more strongly adsorbed than the substance to be separated which is called "displacement analysis." Tiselius

(1943b) has applied this technique for separation of sacchrose and glucose on a charcoal column by displacement with 0.5% phenol in water. Tiselius and Hahn (1943) used displacement with 0.5% ephedrine in water for separation of degradated products of corn starch with average molecular weight 975. They showed the presence of di-, tri-, tetra-, pent-, and hexa-saccharide components. Losses due to irreversible adsorption were prevented by pretreating the charcoal with a solution of the displacement agent at a tenfold dilution. This treatment increased the differences in the absorbability of the substances being separated. Claesson (1947) obtained a sharper separation of sugars using small carbon columns with 4% phenol in water as a solvent.

Whistler and Durso (1950) separated glucose, maltose, and raffinose in 90% yield on a charcoal column by successive elution with water, 5% ethanol and 15% ethanol, respectively. Desorption was followed polarimetrically. They found that neither adsorption or desorption was affected by dilution or by presence of salt, calcium chloride, sodium bicarbonate or sodium hydroxide. They listed other solvents including methyl alcohol and acetic acid for separation of mono-saccharides and phenol, citric acid and hydrochloric acid for separation of disaccharides. The operation of the flowing chromatogram was facilitated by use of an automatic apparatus for adding the solvent and an automatic fraction collector, (Hickson and Whistler, 1953). This method has found wide application, especially for purification of oligosaccharides. Whistler (1954) has given experimental details for the preparation and operation of these columns.

Roberts and McFarren (1953) have used the Whistler method (1950) for isolation of oligosaccharides formed during lactase hydrolysis of lactose. Several members of malto-oligosaccharides have been isolated from corn syrups.

Malto-tetraose has been isolated using 15% ethanol by Whistler and Hickson (1954), malto-pentaose using 20% ethanol by Whistler and Duffy (1954), malto-hexaose by Whistler and May (1955). Whistler and collaborators have used stepwise increases in concentrations of ethanol on carbon columns followed by purification on cellulose columns. Whelan, Bailey, and Roberts (1953) have separated malto-oligosaccharides as high as maltoheptaose on charcoal-celite column (1:1 by weight) from an acid hydrolyzate of potato amylose. Other workers have used this method for separation of a variety of oligosaccharides. Kuhn, Gauhe and Bear (1953) isolated a nitrogenous tetra-saccharide from human milk. Isomaltose, malto-triose and panose were isolated from the hydrolytic product of amylopectin (Wolfrom et al., 1952, Thompson and Wolfrom, 1951 and 1952). D xylo-biose, -triose, -tetraose, -pentaose, -hexaose, and -heptaose were separated from partial hydrolysis of Xylan, (Whistler and Yu (1952a, 1952b, 1953). An improved procedure for the preparation of stachyose was presented by Laidlow and Wylan (1953). Lactobiose and galactobiose were separated by Wallenfilo (1953). Ball et al. (1956) eluted oligosaccharides from a hemicellulose hydrodyzate using a carbon column with 10% aqueous ethanol. Barker, Bourne and Theander (1955) have found that separation of glucose, maltose, malto-triose, panose, and isomaltotetraose was best achieved using a solvent consisting of boric acid and sodium hydroxide buffer.

Bird and Hopkins (1954) followed the action of alpha-amylases on amylose by using carbon-celite columns for isolation of the enzyme degradation products. Aso and Shibasaki and Arisako (1958) obtained a separation of glucose, maltose and maltotriose using Whistler's method (1950). Barth and Timel (1958) used cocoanut charcoal of 50-200 mesh pretreated with 6 N hydrochloric acid, water,

and ethanol for separation of methylated and uronic monosaccharides. Aqueous ethanol with stepwise elution technique was used in a more recent application of Whistler-Durso method (1950) for following the synthesis of oligosaccharides by Penicellium chrysogenum from lactose was reported by Ballio and Russi (1960). Stefanovic (1961) obtained complete separation of glucose, mannose and raffinose on activated carbon and aluminum oxide (3:4) columns using 1, 5, and 15% ethanol, respectively, as elution solvents. Taylor and Whelan (1962) were able to separate isomaltose, isomaltotriose, isomaltotetraose and isomaltopentaose from neutral or acid charcoal column. Radomski and Smith (1962) used columns of carbon-celite for fractionation of di- and tri-saccharides from an alpha-amylase hydrolyzate of waxy corn starch. Further purification of the maltulose was attained on heavy filter paper.

A technique for better and sharper resolution of sugars called, gradient elution, has been developed by Tiselius (1952). The theoretical aspects of this technique was explained by Alm, Williams, and Tiselius (1952). They pointed out that elution analysis cannot be successfully applied to those substances which have non-linear isotherms because of the spreading of the zones. They showed that the concentration of the eluting solvent must be changed gradually in such a manner that the trailing end of the band comes in contact with a more strongly eluting solvent. This procedure diminished the trailing and caused better separation of the sugars. Alm (1952) applied this technique for separation of oligosaccharides from an acid hydrolyzate of an alpha-schardinger dextrin containing a mixture of mono- to hexa-saccharides and dextrins. He used a gradient development with ethanol in water from zero to 20 percent and a Darco G 60-celite column pretreated with 1% ethanolic stearic acid. He found, in comparison of many activated carbons

as adsorbent, that carboraffin C, Norite F N X special and Darco G 60 gave the best resolution of sugars. Jermyn (1957) used the gradient technique for separation of cello-oligosaccharide using straight chain aliphatic alcohols and acids, aliphatic and cyclic primary and secondary amines as desorbing agents. Aso et al. (1960) used activated carbon-celite columns, displacing glucose by water and higher oligosaccharide by gradient aqueous ethanol solutions. Miller (1960) used micro columns, 1.0 x 5.5 cm., packed with a stearic acid treated mixture of charcoal (Darco G 60) and celite 545 in analysis of malto-dextrin, cello-dextrin, oligosaccharide and mono-saccharides.

Barker, Bourne, and O'Mant (1955) suggested a method for the fractionation of mixtures of oligosaccharides. The method depends on a mild treatment of the oligosaccharides with methanolic hydrochloric acid and subsequent elution from charcoal columns. Those components which form a methyl furanoside can then be separated from others as they are more strongly adsorbed on charcoal. By this method they were able to separate maltose and nigerose, celloboise and laminaribiose and two trisaccharides.

George, Bower, and Wolfrom (1946) have found that sileneEF (calcium acid silicate) is suitable for separation of such groups of sugars as pentoses from hexoses and mono- from oligosaccharides. Dickey and Wolfrom (1949) were able to separate a series of homologous polymers of crystalline alpha-D sugar acetate ranging in degree of polymerization from one to six. They used the acetolysis product of cellulose on silene E F-celite columns and the effluent containing the lower sugar acetates were then fractionated on megnesol. Wolfrom and Dacon (1952) extended the series through alpha-D-celloheptose acetate. Later Wolfrom and co-workers (1956, 1957) used the same technique for separation of

a homologous series of alpha-D-oligosaccharide acetates and beta-D-oligosaccharide acetate from cotton litner.

Carbohydrates such as starch and cellulose powder as a chromatographic adsorbent have been recognized by several investigators for separation of mono-saccharide and their derivites as well as for fractionation of starch. Cellulose columns of Hough, Jones, and Wadman (1949) have also been used for purification of malto-dextrin obtained from carbon columns by Whistler and collaborators (1954, 1955). Recently Thoma, Wright, and French (1959) have extended application of paper chromatography technique to powdered cellulose columns. They were able to isolate an homologous series of oligosaccharides up to D.P. (degree polymerization) of 18 in amounts of 50-100 mg. from linear starch and inulin hydrolyzates. The preparation of megalosaccharides (D.P.> 10) was made possible by the use of relatively high temperature of 53°C., and by successive increases of the water content of the solvent containing water, ethanol and butanol. Lemieux (1956) used partition chromatography for separation of mono-saccharides on celite columns. Thoma, Wright, and French (1959) failed to isolate a homologous series of saccharides by this method.

Oligosaccharide Formation by Amylolysis

The production of malto-oligosaccharides can be attained by acid or enzyme hydrolysis of starch. A certain limited hydrolysis of starch occurs during breadmaking due to the use of alpha-amylase. Pazur and Sandstedt (1954) found that as a result of the action of malt alpha-amylase on starch, a series of sugars of low molecular weight including glucose, maltose, maltotriose, malto-tetraose, and malto-pentaose were formed. This is in contrast to the action pattern of salivary amylase which produces only three reducing

sugars of low molecular weight, maltose, malto-triose and malto-tetraose, (Bird and Hopkins, 1954). The smallest limit dextrin formed by malt alpha-amylase is penose while animal and fungal amylases produce malto-tetraose and Bacilus subtellus alpha amylase produces a minimum size of pentasaccharides (Whelan, 1961).

Johnson and Miller (1948) have found that fungal alpha amylase was slightly more effective than malted barley or wheat in increasing gas production and in yielding improvements in bread designated as malt response. Conn et al. (1950) suggested that the difficulties involved in using bacterial amylases as flour enzyme supplements were due to their high thermostability and their low affinity for molecular weight dextrins. Accordingly, bacterial alpha-amylase were believed to be free to split greater numbers of starch molecules with a corresponding increase in dextrin formation and stickiness of bread crumb. Gas production of water extracts of bread was largest in bread baked with the bacterial enzyme, fungal amylase produced the smallest amounts of fermentable sugars while malted wheat flour alpha-amylase produced less fermentable sugar than bacterial supplements (Miller et al., 1953). Beck et al. (1951) found that the residual maltose in bread crumb increased much less with increasing quantities of fungal enzymes than with comparable quantities of cereal or bacterial alpha-amylases. Unlmann and Seidemann (1958) studied the behavior of amylases on potato and wheat starches by paper chromatography of the sugar formed. At 40°C. fungal amylase produced maltose and malto-triose in large quantities along with traces of malto-tetraose and malto-pentaose. After 20-30 minutes, malto-hexaose and malto-heptaose appeared. Drapon (1962) reported that when starch was acted upon by bacterial alpha-amylases, the formation of glucose and maltose occurred

first. After 10 minutes the largest moieties were malto-heptaose and malto-octaose, both of which disappear concomitant with a rapid formation of malto-triose malto-hexaose and malto-hepataose. Fungal alpha-amylase was found to produce larger amounts of fermentable sugars, than malt alpha-amylase. According to Haydon (1961) fungal amylase produced primarily maltose while the main product of cereal amylases was a hexasaccharide.

Bread Staling

Bread staling is a complex phenomenon on which a great deal of research has already been performed but the mechanism and nature of changes are still not clearly understood. The literature indicates that staling is accompanied by certain changes in bread such as loss of taste and aroma, softness of crust, hardness and dryness of crumb, loss of crumb swelling power and decrease in the amount of soluble starch in the crumb. Excellent reviews of the staling have been reported by Alsberg (1936), Platt (1930), Cathcart (1940), Schoch and French (1947) and Geddes and Bice (1946). An approximate chemical analysis shows little appreciable difference between fresh and stale bread (Cathcart, 1940). Karacsonyi (1928) found that the acidity either remains constant or shows some decrease on bread staling.

The first ideas of bread crumb staling were that it was due entirely to the loss of moisture. However, as early as 1853, Boussingault distinguished between the hardening of bread due to the evaporation of water and crumb firming due to starch retrogradation. He also recognized the reversible nature of crumb firming by heating of bread at 60°C. or higher. Von Bibra (1861) confirmed Boussengault's findings. He showed that bread containing less than 35% moisture could not be refreshened by heating unless moistened

first. Alsberg (1936) stated that it was significant that native starch granules when fully hydrated contain about 30% of water. Horsford (1876) was the first one to suggest that staling of bread was due to changes in the distribution of water between gluten and starch, explaining that gluten was dehydrated during baking while the starch retained most of the water. During aging gluten took up water from starch.

Lindet (1902) suggested that the non-soluble form of starch in aged bread crumb was aggregated starch. This change was called retrogradation of starch and was accompanied by the setting free of moisture to other components. This process was more rapid than the development of crumbliness of the crumb. Katz (1928) explained the delay in crumbliness in terms of the time it takes for water to diffuse from the starch to protein. This was later verified microscopically by Verschaffelt and Van Teutem, (1915). They observed that in fresh bread the starch granules were surrounded with a continuous light gluten matrix. As bread aged, the matrix separated from the starch forming air cells. This occurred with no change in moisture. Katz believed that the moisture transferred from the starch to other components of the crumb as that starch retrogradated. Ostwald (1915) believed that the transfer of moisture was not a matter of retrogradation but rather one of syneresis. Actually, both view points may be compatable since syneresis does not exclude the possibility of retrogradation. Alsberg (1938) believed that separation of moisture during retrogradation was a special case of syneresis.

Katz (1928) did much to establish means of following changes in bread crumb with time of storage. He followed the increase in crumbliness, hardness by feel, decrease in swelling of crumb in water, decrease in amount of soluble

starch that could be extracted from the crumb at 30°C. and changes in the X-ray pattern. He also found that at temperatures lower than 50°C., bread staled and the rate of staling increased as the temperature was lowered reaching a maximum at 3°C. By lowering the temperature below this point the rate was decreased because freezing immobilized the water and starch molecules. Katz (1928) has shown by X-ray studies that changes which take place in bread staling are very similar to those taking place in a starch gel. Later Katz (1930) stated that the process is much more complex and involved a heterogenous equilibrium. In 1937, Katz indicated that in fresh bread the starch existed as a mixture of amorphous and crystalline forms while in stale bread it existed in a totally crystalline form. Hellman et al., (1954) showed by X-ray diffraction pattern that the rate of crystallinity development at different moisture with age differed. However, in all cases, it was completed in 8 days. Reheating did not retain X-ray diffraction of fresh bread completely. Kuhlmann and Golessowa (1936) have shown that the water binding capacity of bread crumb gradually decreases during staling.

Fuller (1938) suggested that during staling, gelatinized starch undergoes a reduction in hydration capacity and becomes less susceptible to alpha-and beta-amylase action. He stated that freezing and addition of sugars altered the former but not the latter. Moderate heating restored the susceptibility of the starch to alpha- and beta-amylase.

Hixon (1943) pointed out that waxy maize starch was unable to retrograde rapidly due to its branched molecular structure and therefore he concluded that bread staling was due to the amylose component of starch. Moznick, Merrit, and Geddes (1946), based on the concept, found that increasing the percentage of waxy maize starch in bread caused the crumb rigidity to decrease,

whereas in substituting wheat starch with waxy maize starch, crumb compressibility increased but upon storage the compressibility of loaves decreased at about the same rate. The results suggested that bread staling was associated in the branched component (amylopectin) of wheat starch. Bechtel (1959) found that bread from synthetic flour was increasingly stale as the proportion of cross linked starch increased. Whereas bread with increasing levels of bacterial alpha-amylose was less firmed. Schoch and French (1947) showed by potentiometric iodine titration that water soluble starch leached from crumb is predominately the branched fraction. Upon aging the solubles declined, suggesting a spontaneous aggregation of branched fraction. Heating restored the high percentage of soluble fraction. The same results were obtained from defated wheat starch. He concluded that staling was inherent in the B fraction. Zobel and Senti (1959) showed by X-ray patterns that bread made with 40% cross bonded starch and a heat-stable enzyme changed less with storage than conventional bread. Changes in the X-ray patterns, however, were not parallel to the changes in crumb firming. Gilles, Geddes and Smith (1961) analyzed the soluble starch in both fresh and stale bread and showed differences in the percentage of water soluble pentosans relating this factor to the retrogradation of amylose.

Jackel, Schoeder and Schultz (1953) showed an increase in soluble crumb polysaccharides on storage being most predominant at 8°. They found also significant increases in the soluble polysaccharides in bread when bacterial alpha-amylase was used. Jackel and co-workers (1952), (1953b) found that soluble starch fraction of bread was not capable of appreciable retrogradation as evaluated by decreased susceptibility to beta-amylase and that the phenomena of crumb starch staling was confined to the insoluble part of

crumb. They found (1953c) that the digestibility of starch by pancreatic amylase decreased during crumb aging while in bread supplemented with alpha-amylase, a high rate of digestibility was maintained over a prolonged storage period.

Bradley and Thompson (1950) found that the rate and extent of changes in crumbliness and compressibility of the intact and decrusted bread were not appreciably different in spite of a loss of moisture from the crumb to the crust in the intact loaves. Later Bechtel, Meisner and Bradley (1953) found that crustless bread maintained its freshness longer than the intact loaves.

Bechtel and Meisner (1954a,b,c,d) have dealt with the staling problem by using flour fractions. If the moisture content of the bread remained constant, even though the flour fractions were varied, staling rate of the crumb was essentially constant. However, if the moisture content of the bread was increased through the effect of the flour fraction such bread remained fresh longer. The amount of tailing fraction affected directly the amount of water absorption. Their results also suggested that staling of bread crumb was most directly related to the starch during the first three days of storage but that gluten had its main effects after 3 days. Prentice, Cuendet and Geddes (1954) used a synthetic flour consisting of gluten, starch, starch tailing and the water solubles of rye flour to reveal the effect of individual fractions on the crumb firmness. They found that increasing the protein content in synthetic flour, maintaining a constant ratio of gluten to water solubles, decreased the average crumb firmness and crumb firming rate. By using soft flour gluten, the average crumb firmness increased but the firming rate was not affected. Starch tailing decreased the average

crumb firmness but not the rate.

Cluskey, Taylor and Senti (1959) compared the rate of firming of gels of flour, starch and gluten. They found flour and starch gels become rigid much faster than gluten. Reheating of flour and starch gels restored their original elasticity but gluten was unaffected indicating that firming of bread crumb can be attributed entirely to the starch fraction.

It has long been known that reheating will freshen bread provided it contains sufficient moisture. Katz (1928) reported that bread stored at 60° to 90°C. in proper humidity control did not stale, but Fuller (1938) reported that even at these temperatures bread may stale at a slow rate. The bacterial growth is a serious problem when bread is stored at such temperatures.

Bailey (1932) found that bread stored three days at -9°C. retains the characteristics of fresh bread after thawing. However Cathcart and Luber (1939b) and Cathcart (1940) found that bread stored at a temperature of -22°C. was somewhat stale as judged by the crumb swelling power test. At a temperature of -35°C., the bread retained its organoleptic freshness for 70 days. The development of an off-flavor was a limiting factor.

The effect of wrapping as a method to retard bread staling has been investigated by Cathcart (1940). He studied the changes in the losses and distribution of moisture in the crust and the crumb of bread and the effect of wrapping in preserving the aroma, flavor, and softness of the bread.

Numerous substances have been reported by many workers as retarders of bread staling. Substances such as volatile and water soluble aldehydes and strongly basic substances show a strong retarding effect. Unfortunately, they are toxic. The search for staleness retarders has consequently concentrated upon naturally occurring food. Stellor and Bailey (1938) found

that bread baked from the high protein flour did not stale as rapidly as did the bread made from the low protein flour. Alsberg (1936) reported that addition of 5 to 15 percent rye flour exerted slight improving effect upon keeping quality. He recommended that not too much yeast be used. Platt and Powers (1940), Alsberg (1936), Hutchinson (1936) and others have found that milk in the formula has only a slight effect on retarding staleness. Harris, Sibbett, and Banasik (1952) found that potato flour reduced the rate of staling as measured by change of hydration. Thomas (1947) found that sugar in amounts of 2 to 4% produced a slight retarding effect on the staling rate. However, Bailey (1932) does not confirm the retarding effect of invert sugar and dextrinized starch on staling of the bread. On the other hand, malt extract was found by him to exert an improving action which was subsequently attributed by Sandstedt and co-workers (1939) to amylase action on the starch, whereas Brook (1927) thought the hygroscopic nature of malt sugar accounted for its moisture retaining effect. Kuhlmann and Balasheva (1938) reported that carbohydrates served as anti-staling agents in the following order of their effectiveness: maltose syrup, glucose syrup, dextrin, beet sugar, maltose, glucose, soluble starch, and potato starch. Barham and Johnson (1951) reported that crumb firmness was affected by sugar concentration, with minimum crumb firmness occurring at 2-4% concentration for all sugars. The rate of firming of bread crumb was not affected. Hester, Brandt, and Personius (1956) reported a decrease in the hydration of starch granules in the presence of sucrose. The rigidity of starch gels decreased and at high concentration of sucrose no gels could be formed. Otterbacher (1961) reported that bread baked with 4-20% dextrose or corn syrups while being firmer initially, remained stable and more palatable for a longer period of time. Johnson (1961)

found 8% sugar or syrup produced the softest bread crumb with the lowest crumb firming rate. He did not find any difference in quality of bread made with sugar or syrup.

Shultz et al. (1951) reported the addition of appropriate amounts of bacterial alpha-amylase to bread baked on commercial scale resulted in retarded crumb firming rate. Volts and Ramstad (1951) reported that addition of polyoxyethylene monostearate or fatty acid monoglyceride had no effect on the enzyme susceptibility of starch. Shortenings have been known to enhance the keeping quality of bread. Carlin (1947) reported that loaves containing 6% shortening will be softer than bread made without shortening after 72 to 96 hours of storage. Edelmann, Cathcart, and Berquist (1950) studied the effect of various methods on the rate of firming of bread crumb. They found 3% lard as ingredient, maximum adsorption, optimum baking time, optimum ratio of baking pan volume to dough weight and use of either polyoxythylene monostearate or glycerol monostearate retarded the rate of crumb firming. Non-fat dry milk solids, sucrose, yeast, and sodium chloride were found to have little effect on crumb firming. Carson, et al. (1950) reported the effect of polyoxyethylene monostearate as anti-staling adjunct in bread. Measuring the staling rate by different methods, crumb firmness gave the most pronounced positive results. They explained that polyoxyethylene monostearate appeared to combine, probably by hydrogen bonding with the alcoholic groups protruding from the starch granules. This addition of a hydrophobic moiety hindered the forces which caused alignment of starch molecules. Ofelt et al. (1958) showed that 0.25-0.3% monoglyceride from lard or cottonseed oil had retarding effect on crumb firming. Diglyceride did not show any retarding effect. Ofelt et al. (1958) reported that

3-stearoyl-D-glucose was as effective as anti-firming agent as polyoxyethylene monostearate, while ascorbyl palmitate and isoascorbyl palmitate were about as effective as monoglycerides. Edelmann and Cathcart (1949) studied the relative effectiveness of 24 different surface action agents on bread staling. Polyoxyethylene monostearate was the most effective on softness, crumb color, grain, and texture. Marnett and Selman (1950) reported a significant increase in maximum paste viscosity produced by polyoxyethylene monostearate in the absence of alpha-amylase. Voltz and Ramstad (1951) showed that the addition of 0.5% polyoxyethylene monostearate or fatty acid monoglyceride did not affect the enzyme susceptibility of starch. Favor and Johnston (1947) did not find any effect of quantities up to 2% of polyoxyethylene stearate on the physical characteristics of bread except softness although it raised the gelatinization temperature of starch. Strandine et al. (1951) reported that monoglycerides reduced the swelling power of wheat starch and the amount of water-solubles starch. Schoch and French (1947) observed the same phenomenon explaining that monoglycerides similar to fatty acids were shown to be absorbed by the amylose factor. Lai, Finney, and Milner (1959) found that bread from wheat irridated with gamma radiation increased crumb firming on storage despite the diastatic changes in the starch fraction.

In another direction many investigators have studied the effect of different dough treatment for decreasing crumb firming. Hutchinson (1936) recommended a slack dough for prolonging freshness. Alsberg (1936) and Kent-Jones (1958) reported the superiority of the sponge over the straight-dough method. On the other hand, Hutchinson has found that short time process gave better keeping quality bread. Alsberg (1936) found that optimum fermentation at 71-79°F. increased the bread's keeping quality. He recommended

slow baking at relatively low temperatures to avoid excessive moisture loss.

Staling is accompanied by a number of measurable physical changes as a measure of the degree of staleness. The methods used most widely for following crumb changes including those which measure compressibility of the crumb, have been described by Bailey (1930), Comb (1944), Noznick and Geddes, (1943) Platt and Powers (1941). All instruments described by the various authors involve subjecting the bread crumb of specified thickness to a weight required for a specified amount of compression. Methods which measure the swelling of crumb have been described by Katz (1913) and modified by Cathcart and Luber (1939), Karasconyl (1929). A viscometric method which measures the absorption changes by means of a determination of the mixing strength of a crumb dough in the farinograph was devised by Fuller (1939). Other methods have been based on the changes in the decrease in the amount of water soluble starch during staling (Katz, 1928), the changes in X-ray diffraction patterns, Katz (1930), the increase in starch crumb opacity as measured by photoelectric cell, Glabau and Goldman (1938), the reduced susceptibility of retrograded starch to amylase attack, and the changes which occur in the crumbliness on staling (Bice and Geddes, 1949). Banasik and Harris (1953) have found a correlation of 0.93 between crumb firmness and sedimentation rate of a 1% crumb suspension.

This investigation was undertaken to separate the oligosaccharides in corn syrup by adsorption chromatographic means and to gain a knowledge of the carbohydrate components of corn syrup. Although the literature did not show any study on the effect of the addition of oligosaccharides on breadmaking, there was some evidence of their indirect effect as products of starch hydrolysis. The object of the present study, therefore, was to

isolate the oligosaccharides and to study their effect on breadmaking and bread staling.

MATERIALS AND METHODS

A commercial corn syrup was used as a source of oligosaccharide. It was obtained from Corn Products Refining Company, Argo, Illinois, under code No. 1122. It had a 42 dextrose equivalent.

The supporting material used for the chromatographic column included: Darco G 60 from Hercules Powder Company, New York, and Celite No. 535, 545, or 560 from Johns Manville Co., Kansas City, Missouri.

The solvents used for both paper and column chromatography included butanol, pyridine, phenol, ethanol, and glacial acetic acid.

The reagents used for qualitative and quantitative analysis of sugars were orcinol, aniline hydrogen phthalate, silver nitrate, sodium hydroxide, acetone, and methanol.

Beta-amylase used for identification of the composition of the dextrins was obtained from the Wallerstein Company, New York.

An automatic fraction collector (Technicon) with 20 test tubes was employed to collect fractions from the carbon celite columns.

A rotary flash evaporator (Precision) with vacuum was used for concentrating the eluted fractions.

A Bloom Gelometer was used to test crumb compressibility.

Preparation and Use of Carbon-Celite Columns

Carbon-celite columns were used to separate the oligosaccharides. Equal parts, by weight, of carbon Darco G 60 and Celite 535 or 545 were initially mixed and washed with distilled water, about three liters of water per 100 gm. of mixture. After vacuum filtration, the mixture was thoroughly dried at 80°C. prior to use.

Glass columns, 60 x 750 mm, were used supported with a pad of glass wool and cotton at the bottom. Two methods for packing the column, dry or slurry method, were attempted. Either method was good when properly handled. In packing a column with such mixture of supporting material differing in density, the mixture was added as a thick slurry in water, 1 inch at a time, and allowed to settle first before light tapping with a plunger. This precaution was necessary for homogenous column and best flow rate.

The column was 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 kept covered with water until used and the liquid level was never allowed to fall below the level of charcoal-celite.

Several experiments were performed to determine the best concentration of corny syrup for the greatest efficiency of the column. Ten, 20, 30, and 50 gm. quantities were diluted to 100 ml. or 100 gm. to 200 ml. Thirty gm./100 ml. was found to give maximum capacity and efficiency and best separation while the higher concentrations caused intermixing of zones.

The concentrated aqueous solution of corn syrup (30 gm./100 ml.) was added carefully to the surface of the celite-carbon column. When the solution was absorbed, the surface was covered with 4 disks of filter paper to keep the

surface even when solvent was added. A liquid head of 5% ethanol was provided from a separatory funnel fixed over the column to provide a head pressure to speed the elution rate. Fractions of 20 ml. were collected by an automatic fraction collector. The fractions were combined in 200 ml. aliquots. At first the column delivered 250 ml. of eluate per hour but the rate gradually decreased to less than 100 ml. per hour.

To detect the presence of the carbohydrate in the eluate, 1.0 ml. aliquots were mixed with 1.0 ml. of 1% orcinol and sulfuric acid 115% (3 parts of concentrated sulfuric acid and 2 parts fuming sulfuric acid containing 30% sulfur trioxide). A red to brown color was obtained with as little as 2 ug. of carbohydrate, (Whistler, 1954). The eluant was changed as indicated by the orcinol test and confirmed for purity by paper chromatography.

The fraction for each level of ethanol elution from each column, excluding those fractions containing more than one spot on the chromatogram were combined and evaporated under reduced pressure at 50°C. to approximately 50 ml. using a rotary flash evaporator or simple direct distillation under vacuum. Celite sometimes dissolved in the developing solution and was obtained as a flocculent suspension in the concentrated effluent. Celite was removed from the concentrated effluent by filteration through a sinistered glass funnel. For further purification it was found advantageous to redissolve the solid residues by warming with small amounts of ethanol and adding water until solution of the sugar was completed. The white, powdery residue of celite, with the exception of the dextrin fraction which contained a small amount of carbon, was removed by filteration.

Further concentration to a syrup was achieved in a small vacuum flask. Evaporation to dryness was carried out in a vacuum desiccator over phosphorus

pentaoxide. The final products are white with a tinge of yellow. It was amorphous and crystallization was not attempted.

Analysis of Eluant

The ferricyanide method was used to quantitatively follow the progress of the fractionations. Five ml. of alkaline ferricyanide reagent (0.1 N) was added to 5 ml. of eluant in a 200 ml. size tube which was immersed in vigorously boiling water for 20 minutes and cooled in flowing tap water. The tube contents was transferred to 125 ml. Erlenmeyer flasks and rinsed with 20 ml. acetic-acid-salt solution. One ml. soluble starch-potassium iodide was added and titration was carried to the end point with 0.1 N sodium thiosulfate.

Examination of the Oligosaccharides

Whatmann No. 4 filter paper was found superior to Whatmann 1 or 3. The solvent butanol-pyridine-water (6:4:3 by volume) was found superior to phenol-water or butanol-acetic acid-water for separation of oligosaccharides from corn syrup. The chromatograms were developed for 16 to 24 hours, at about 25°C. depending on the extent of separation desired. Development for periods of time longer than 24 hours caused loss of dextrose. Shorter periods of time did not permit the separation of oligosaccharides having DP in excess of nine.

One hundred lambda of the fractions and 10 lambda of a 5% corn syrup or a starch hydrolysate used as reference were spotted and spaced 1 1/2 inch on the starting line. To place this quantity of starting material on the paper, alternate spotting and drying to increase the amount of sugar deposited

without increasing the size of the spot was necessary. The sheets of paper used were 6-inches wide and 22 1/2 inches long. Descending technique of Partridge (1949) was used in developing this chromotogram. After developing, the solvents were removed from the paper by drying 15-20 minutes at room temperature.

Two principal reagents were used to detect the oligosaccharides on the paper chromatograms. Silver nitrate was used for general detection of sugars. The chromatograms were dipped into silver nitrate solution (0.1 ml. of saturated, aqueous silver nitrate diluted to 20 ml. with acetone after which silver nitrate was brought back to solution by addition of a few drops of water), dried at room temperature, and then dipped into 0.5 N sodium hydroxide-aqueous methanol. Black spots developed immediately for mono- and di-saccharide and slowly for the oligosaccharide. Chromatograms were dried at room temperature for 5 to 10 minutes, after which they were slowly pulled through Kodak X-ray fixing solution. Chromatograms were finally, thoroughly washed with tap water to remove excess fixing solution. The method yielded relatively stable chromatograms showing black spots against white background (Benson et al., 1952). Aniline hydrogen phthalate was prepared by adding aniline (0.93 gm.) and phthalic acid (1.66 gm.) to water-saturated butanol (100 ml.). The chromatograms were sprayed with this mixture and heated for 5 minutes at 105°C. for monosaccharides, 30 minutes or longer for oligosaccharides to develop the color. This method was sensitive for 1 ug. of sugar showing a stable bright red spot or brown depending on sugar and solvent used (Partridge, 1949).

Beta-amylolysis of dextrin was used to determine the type of linkage of the dextrin. Solutions containing 0.2 gm. of oligosaccharide in 25 ml.

of distilled water were hydrolyzed by 1 mg. of beta-amylase for 72 hours at 37°C. The solutions were covered with toluene to prevent bacterial growth. Ten ul. of the aqueous solution was spotted on Whatmann No. 4 filter paper and chromatographed.

Optical rotation of the isolated oligosaccharides was measured using 1% solution and 1 decimeter tube in the polarimeter.

Baking Tests

The baking experiments were performed on a laboratory scale employing the straight-dough procedure. The basic formula was:

Ingredients	Parts
Flour	100.0
Water	62.0
Yeast	2.5
Shortening	3.0
Salt	2.0
Yeast food	0.5
Dry Milk Solids	1.0
Dextrose	0.5

The oligosaccharides were introduced in two levels, 0.5 gm. and 1.0 gm. in four replicates.

After mixing to optimum consistency, the dough was fermented at 90°C. for 110 minutes. The doughs were punched 50 minutes later, divided, rested for 20 minutes and proofed for 50 minutes. The bread was baked for 25 minutes at 210°C.

The crumb compressibility was measured with a Bloom gelometer. The plunger, 25 mm. in diameter, was depressed in a crumb of 1-inch slice to 4 mm. The weight in grams required to depress the plunger was taken as the compressibility value. The firmer the crumb the greater the weight required to depress the surface. The end slices were not used. The compressibility

value was the average of 4 different compressibility readings. The loaves designated for aging were wrapped, sealed in heavy waxed paper and stored until analyzed. In all series, compressibility tests were made at 0, 24, and 72 hours after baking.

RESULTS AND DISCUSSION

The oligosaccharides present in corn syrup were separated on carbon-celite columns using stepwise increases in ethanol concentrations. The concentration and volume of eluant used for each sugar eluted is presented in Table 1.

Table 1. Stepwise in ethanol concentration of the elution solvent of successive oligosaccharides.

Substance	Conc. of ETOH	Vol. by liter	Fractions combined
	%		
Glucose & maltose	5.0	7.0	—
Trisaccharide	7.5	6.5	39-55
Tetrasaccharide	15.0	5.5	62-78
Pentasaccharide	20.0	5.0	84-101
Hexasaccharide	23.0	4.0	106-118
Heptasaccharide	30.0	4.0	125-140
Dextrins	70.0	2.0	

The dextrins (chain length of glucose unit > 7) were desorbed from the adsorbent with 70% ethanol overnight, filtered free from absorbant on Buchner funnel. The column was washed with ethanol until a nearly negative test of orcinol was obtained. The column could not be reused. Fig. 1 shows the elution diagram for this separation. Oxidation of sugar by ferricyanide

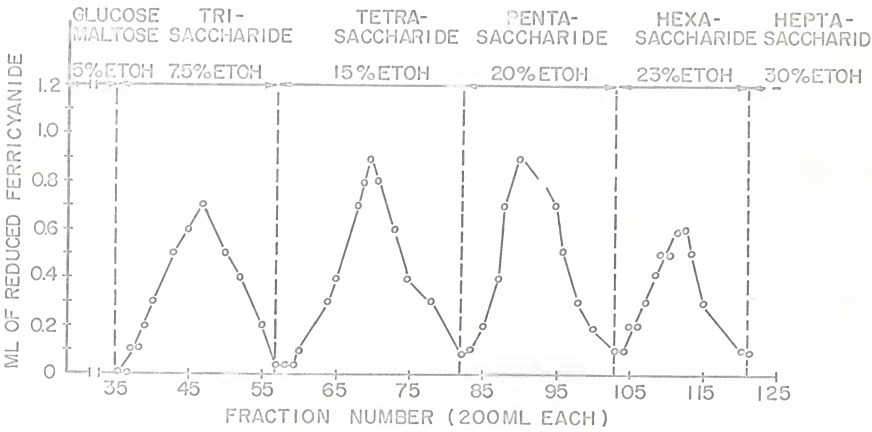

Fig. 1. Elution pattern of corn syrup saccharides from charcoal celite column.

ion in alkaline solution was used to follow the progress of separation. Glucose and maltose were the only sugars to be eluted by 5% ethanol, trisaccharide by 7.5% ethanol and further stepwise increments in the alcohol concentration eluted the higher oligosaccharides up to heptasaccharide. An absolute separation of trisaccharide was achieved but some mutual contamination with a higher oligosaccharide occurred in the elution of tetra-penta-hexasaccharide as evident from the tailing of fractions as illustrated by the schematic representation of stepwise elution analysis in Fig. 2.

The tailing usually takes place in larger columns and in the resolution of higher concentrations. Tailing cannot be avoided even after developing the new technique of gradient elution for separation of oligosaccharides, Tiselius et al. (1952), or after pre-treatment of charcoal columns with 1% ethanolic stearic acid to prevent irreversible elution (Alm, 1952). Whistler and co-workers (1954, 1955) have used two cellulose columns for refractionation of the sugars obtained from carbon columns using different solvents to deplete them from the contaminating sugar.

It was found advantageous to reject the portions of the eluates after inspection of the elution curve and paper chromatogram and to evaporate only those which gave one spot on papergram. Although care was taken in choosing the fractions for evaporation, the chromatogram of the final product showed slight contamination ($< 5\%$) of a higher member of the homologous series. This contamination occurred from accumulation of the small amount of contaminent although these were not evident on papergram before concentration.

The approximate carbohydrate constituents of commercial corn syrup, DE 42, based on the yield of oligosaccharides resolved from the column are shown in Table 2. The yield of these oligosaccharides reported was resolved

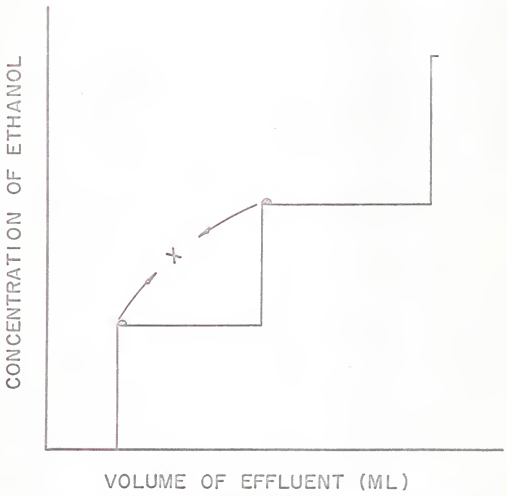

Fig. 2. Schematic representation of stepwise elution analysis in which one substance occurs two times.

Table 2. Carbohydrate composition of commercial corn syrup (DE 42).

Oligosaccharide	Yield	Percent*
	gm.	
Glucose	-	18.5
Maltose	-	13.9
Trisaccharide	1.4	11.6
Tetrasaccharide	1.5	9.0
Pentasaccharide	1.2	8.4
Hexasaccharide	1.0	6.6
Heptasaccharide	0.9	5.7
Dextrin	2.0	25.2

*Source: Technical Advisory Committee, Corn Industries Research Foundation.

from 30 g. of commercial corn syrup.

The resolution of dextrin was not complete because of the strong adsorbability of these dextrins on carbon-celite columns. No attempt was made to quantitatively analyze the sugar constituents of corn syrup in the present work because of the lack of pure sugars to establish the standard curve. Whistler and co-workers (1954 and 1955) reported the analysis of 42 DE corn syrup, showing 7% for malto-tetraose, 8% for malto-pentaose and 6.2% for malto-hexaose. Otterbacher (1961) reported for the same corn syrup 5.9% for glucose, 12.7% for trisaccharide, 13.3% for tetrasaccharide, 1.8% for pentasacchloride, 1.5% for hexasaccharide and 30.5% for higher oligosaccharides.

Properties of Dextrin

Paper Chromatography. Chromatographic separation of the oligosaccharides obtained with descending technique developed by butanol, pyridine and water (6:4:3) revealed a regularity of papergram mobilities of the sugars. Several plots were made obtaining a straight line relationship between the average chain length and some functions which are shown in Table 3.

Table 3. Relative mobilities of oligosaccharide members.

Oligosaccharide	R_G at 25°C.[1]	log R_G	log n	R'M
G_1	1	0	-	-
G_2	0.689	-.156	0.3326	-0.3072
G_3	0.504	-.298	0.0086	0.0070
G_4	0.327	-.486	-0.318	0.3135
G_5	0.220	-.658	-0.553	0.550
G_6	0.142	-.848	-0.796	0.780
G_7	0.095	-1.02	-0.979	0.980
G_8	0.055	-	-1.23	1.234

[1]Mobility with respect to mobility of glucose.

These physical constants were derived from the principles of column chromatography.

Consden, Gerden and Martin (1944) showed that the theory developed for a column chromatography could be applied to partition chromatography. He introduced R_f which is more conveniently measured in paper chromatography than R of column chromatography.

$$R = \frac{A}{A_1 + \alpha A_s}$$

$$R_f = \frac{RA_1}{A} = \frac{A_1}{A_1 + \alpha A_s}$$

where

A = area of cross section of the column,

A_s = area of cross section of non-mobile phase,

A_1 = area of cross section of mobile phase,

α = partition coefficient (gram solute per milliliter of non mobile phase per gram solute per milliliter of mobile phase at equilibrium.

$$\alpha = \frac{A_1}{A_s}\left(\frac{1}{R_f} - 1\right)$$

The relationship between the partition coefficient of a solute and its chemical structure has been presented by

$$\text{Ln}\,\alpha = \frac{\Delta\mu A}{RT}$$

where $\Delta\mu_A$ is equal to the free energy required to transport one mole of solute A from one phase to another phase.

Considering the partition coefficient α_A and α_B of two substances A and B which differ in that B contains a group X in addition to those contained in A.

$$\text{Ln}\,\alpha_A = \frac{\Delta\mu A}{RT} \quad , \quad \ln \alpha_B = \frac{\Delta\mu B}{RT} + \frac{\Delta\mu X}{RT}$$

$$\ln \frac{\alpha B}{\alpha A} = \frac{\Delta\mu X}{RT}$$

Thus, the addition of a group x changes the partition coefficient by a given factor, depending on the nature of groups and on the pair of phases employed, but not on the rest of the molecule.

Since A_1/A_s is assumed to be a constant for a given temperature, α, is directly proportional to $1/R_f)-1$ and

$$\frac{\ln(1/R_{f_B})-1}{\ln(1/R_{f_A})-1} = \frac{\Delta\mu_x}{R_f}$$

calling $R_M = \log_{10}(1/R_f-1)$,

$$\frac{R_{MB}}{R_{MA}} = \frac{\Delta\mu_x}{RT}$$

Jeanes, Wise and Dimler (1951) found on plotting R_M values against the number of similar group of homologous series, a straight line was obtained. They used a single or multiple ascending technique for separation, which enabled them to calculate R_f value by this equation:

$$(1 - R_F)^a = 1 - R_f^a$$

where a is the number of ascents.

In this work which required very many papergrams, a descending technique was choosen for the separation because it provided a fast development and an effective resolution. Hence, the R_f value of the oligasaccharides could not be obtained because of inability to measure the movement of solvent front. Instead of R_f values, R_G values were substituted in their equation excluding glucose, plotting R_M values versus chain lengths. A straight line was obtained as shown in Fig. 3.

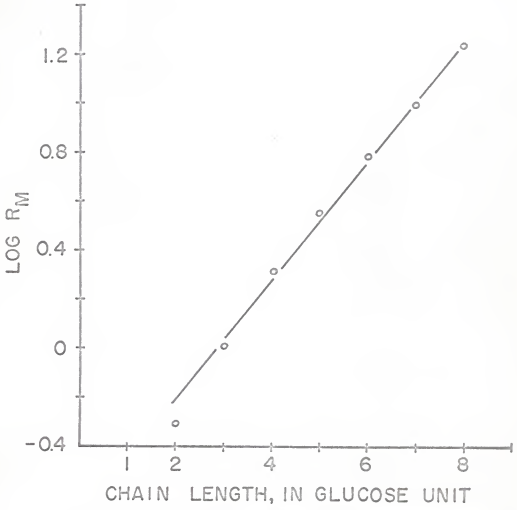

Fig. 3. Relationship between R_M function and average chain length of the oligosaccharides.

Another way of presenting the quadratic relationship between the average glucose unit of the oligosaccharides and relative mobilities was obtained by transformation of the value R_G to the corresponding logharithmic values. A straight line relationship is shown in Fig. 4.

In a study of a series of oligosaccharides, French and Wild (1953) deduced a partition fraction α', from the above equations.

$$\alpha' = \frac{R_f}{1-R_f} = \frac{1}{\alpha} \cdot \frac{A_1}{A_s}$$

From this equation, it appeared that, as the R_f values became small α' approached the R_f value. By plotting log α' against the oligosaccharides chain length in a homologous series, a straight line was obtained.

The same equation was used to obtain α' by replacing R_G values for R_f values for the corresponding sugar. Fig. 5 shows a straight line relationship between log α' and average chain length.

It is noteworthy that glucose does not confirm to the relationships expressed by French (1953) and Jeanes and co-workers (1951). It would be expected that maltose would also deviate from a straight line. Glucose, therefore, should not be regarded as standard for comparison of maltodextrins. This stresses an important fact sometimes overlooked, namely, that in such a polymeric series of sugar, the lowest normal number should not be monosaccharide but rather a disaccharide which contains a glycosidic linkage. The same behavior was observed for optical rotation.

From the paper chromatogram study of R_G values and chain length relationships, it was concluded that the oligosaccharides isolated from corn syrup appeared to be a homologous series of maltodextrin.

Optical Rotation. The optical rotation of the sugars reported are the

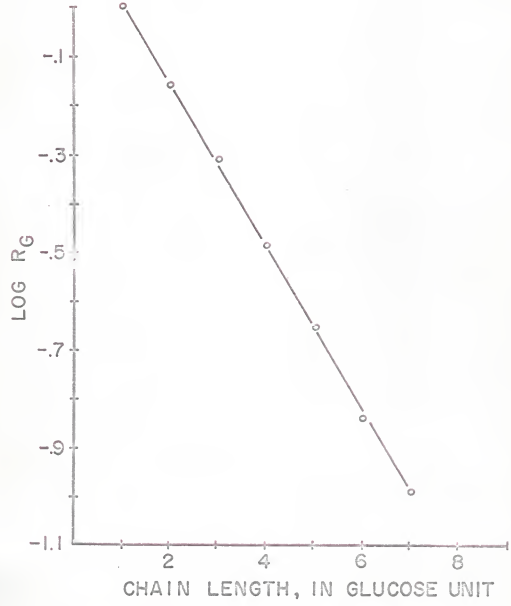

Fig. 4. Relationship between log R_G and average chain length of the oligosaccharides.

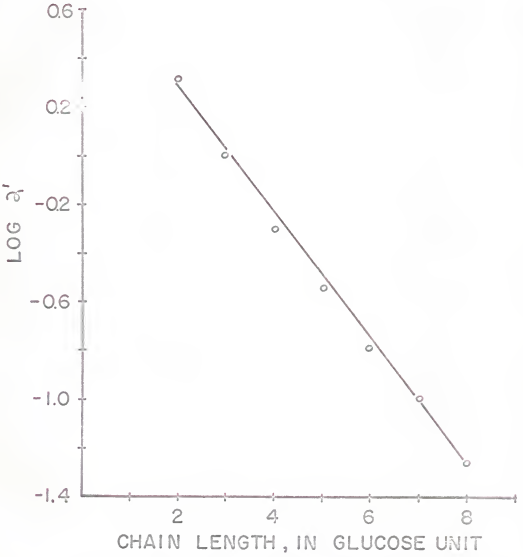

Fig. 5. Relationship between partition coefficient, α', and average chain length of the oligosaccharides.

average of 5 readings. They are presented in Table 4.

Table 4. Optical rotation of oligosaccharides separated from corn syrup.

Oligosaccharide	$[\alpha]^{D}_{25}$	Literature values for malto-dextrin Whelan(1953)	Whistler(1954)	Molecular weight	Molecular rotation
G_1	51	52	—	180	918
G_2	135	136.0	—	342	46170
G_3	155	160	—	504	78120
G_4	162	177.0	165	666	107892
G_5	175	180.3	178	828	144900
G_6	181	184.7	182	990	179190
G_7	186	186.4	—	1152	214272
Dextrin	192	201*	—		

*Optical rotation of starch.

The Freudenberg and Blomqvist's (1935) relationship was used in plotting of Fig. 5 in which A/n is plotted against $\frac{n-1}{n}$; where A is the molecular rotation and n the number of glucose residues per molecule. A linear relationship existed between the values for all oligosaccharides but this relationship does not hold for the monomer, glucose. Whistler and Chen Chue Tu (1952) have reported the isolation of a series of xylodextrins by a similar method. It is noteworthy that from a plot of the specific rotation of these xyldextrins using the coordinates of Fig. 6, a linear curve was also obtained except for the monomer, xylose.

Action of Beta-amylase. Further evidence about the type of linkage in these oligosaccharides was obtained from a study of their behavior towards beta-amylase. The enzyme was allowed to act on 0.2 gm. of all members of

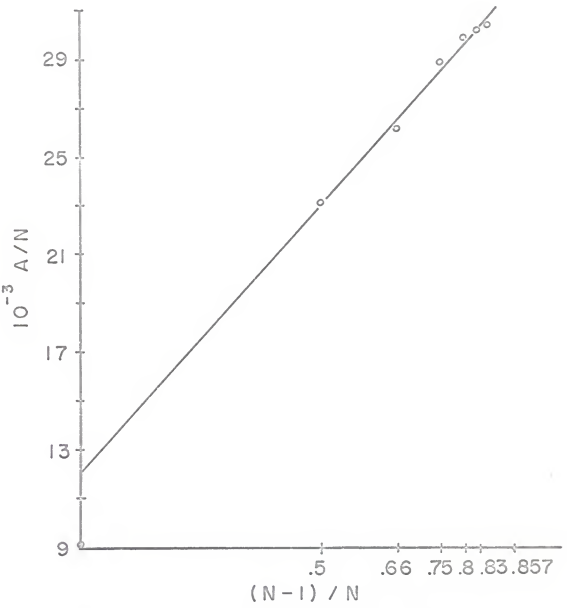

Fig. 6. Application of Freudenberg and Blomqvist.

oligosaccharides isolated. The results by qualitative paper chromatography showed no hydrolysis for trisaccharides, but there was hydrolysis of the other four dextrins forming maltose from tetrasaccharide and hexasaccharides but maltose and malto-triose for pentasaccharide and heptasaccharide. These results indicate only the presence of the 1-4 type of linkage in these sugars.

From the properties of the oligosaccharides, including optical rotation, R_f values of amylose hydrolyzate and beta-amylolysis, it can be concluded that the oligosaccharides are a homologous series of amylose structures.

Effect of Corn Syrup Oligosaccharides on Breadmaking

Seven members of malto-oligosaccharides from glucose to malto-heptaoses were added at two levels, 0.5 and 1.0 percent, to the bread formula. The data showing the effect of these oligosaccharides on crumb color, crust color, volume, symmetry, break and shred, texture and grain are given in Table 5.

The loaf volume decreased on the addition of these oligosaccharides to bread. The correlation coefficient between the loaf volume and average chain length of the oligosaccharides was 0.76 for both levels. However, the rate of decrease of loaf volume was greater with the addition of the higher concentration, as shown in Fig. 7. The regression coefficient was -17.9 for the higher concentration compared to -11.8 for the low concentration. This phenomena was observed also for glucose, sucrose, and fructose by Barham and Johnson, (1951). The higher oligosaccharides had a deleterious effect on the texture regardless of the concentration used. However, the data suggested that the addition of glucose and maltose improved the grain and texture. Glucose and maltose improved the crust color but higher oligosaccharides did not improve the color because they do not actively participate in the browning reaction.

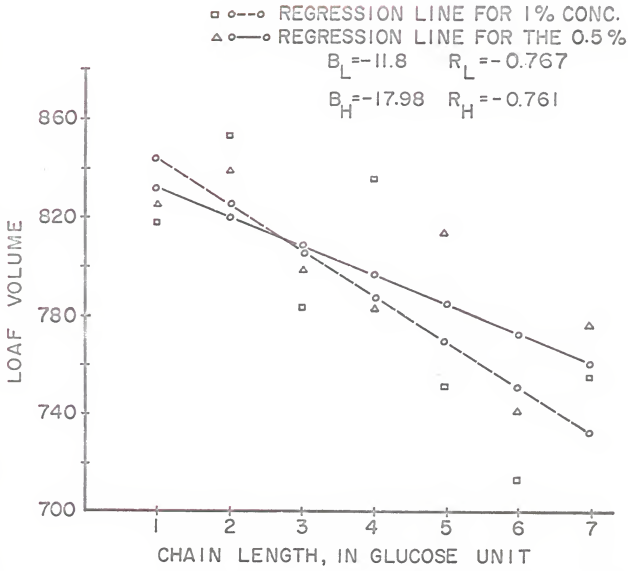

Fig. 7. Correlation between loaf volume and average chain length.

Table 5. Effect of various oligosaccharides on baking characteristics.

Oligosaccharide	Conc.	Loaf Volume c.c.	Quality scores							
			Vol.	Crust color	Symmetry	Break & shred	Texture	Grain	Crumb color	Total
Maximum score			20	10	10	10	20	20	10	100
Control	-	838	16	6	9	9	18	17	10	85
G_1	0.5	825	16	6	8	7	18	16	10	87
	1.0	818	16	7	8	8	17	16	10	82
G_2	0.5	835	16	8	8	8	17	16	10	83
	1.0	853	17	9	9	9	17	16	10	87
G_3	0.5	798	15	6	8	6	17	15	10	75
	1.0	783	15	6	7	6	16	15	10	75
G_4	0.5	783	15	6	7	6	17	15	10	76
	1.0	835	16	6	7	6	17	15	10	77
G_5	0.5	813	16	6	7	5	17	15	10	76
	1.0	750	15	6	7	5	16	15	10	74
G_6	0.5	740	14	6	7	5	15	15	10	72
	1.0	713	14	6	7	5	14	14	10	70
G_7	0.5	775	15	6	6	5	16	15	10	73
	1.0	755	15	6	8	8	17	16	10	80
Dextrin	0.5	755	15	5	7	7	17	16	10	77
	1.0	760	15	5	7	7	17	16	10	77

Measurement of crumb firmness, Table 6, showed that the change in firmness was affected by both sugar type and days of storage. The analysis of variance is presented in Table 7.

Table 6. The effect of addition of oligosaccharides on crumb firming.

Oligosaccharide	Conc.	Compressibility 0	24	72 (hours)
	%		grams	
Control		60	163	254
G_1	0.5	61	137	313
	1.0	57	152	347
G_2	0.5	67	126	291
	1.0	67	151	292
G_3	0.5	73	148	247
	1.0	80	206	333
G_4	0.5	72	166	359
	1.0	58	179	316
G_5	0.5	60	148	352
	1.0	92	159	345
G_6	0.5	78	197	292
	1.0	71	226	333
G_7	0.5	67	191	289
	1.0	69	160	339
Dextrin	0.5	72	207	326
	1.0	64	186	354

Firmness of bread crumb was significantly affected by sugar type, concentration, and time of storage. A comparison of the compressibility of the means of various sugars with that of the control by least significant difference showed that the first 24 hours of storage did not significantly affect the compressibility except for bread containing maltose and malto-hexaose. This can be seen more clearly in Figs. 8 and 9 where the crumb from all

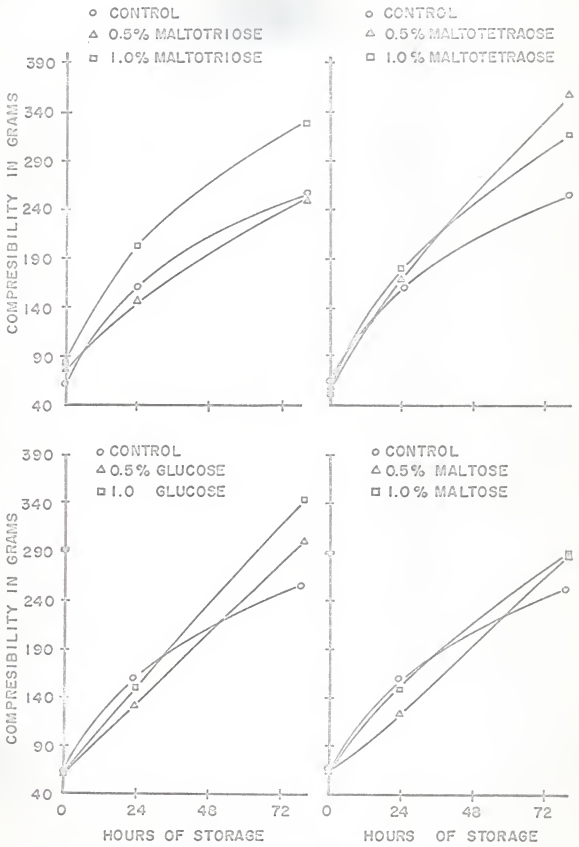

Fig. 8. Compressibility of bread crumb on storage.

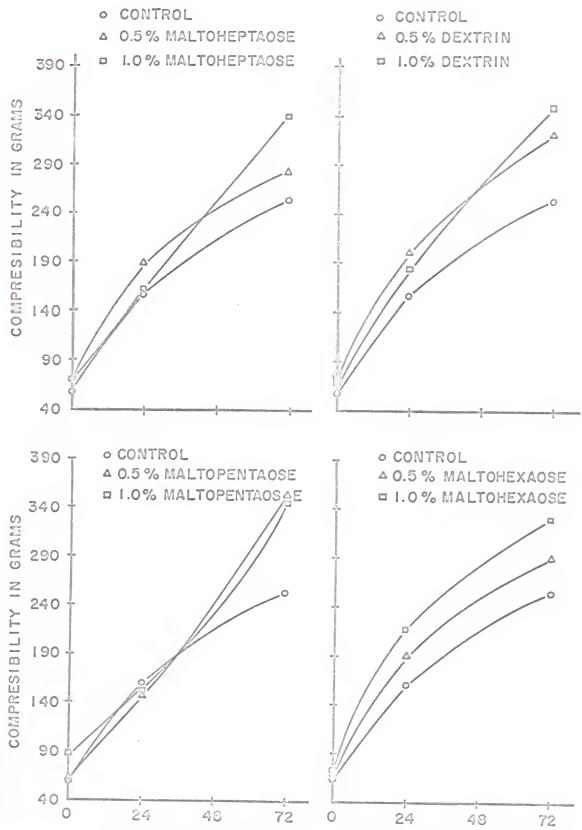

Fig. 9. Compressibility of bread crumb on storage.

Table 7. Analysis of variance of crumb compressibility.

Source of variation	Degree of freedom	Mean square	F[1]
Sugar	7	3496.35	4.29**
Concentration (17.5)	1	5753.13	7.05**
Time	2	66834.94	81.91***
Rep.	3	1238.48	1.52
Sugar x concentration	7	1442.62	1.77
Sugar x time	14	3097.09	3.80
Concentration x time	2	1585.07	1.94
Sugar x concentration x time	14	946.68	1.16
Remainder	141	815.94	

[1]5% level of significance

treatments have the same degree of compressibility in fresh bread but as the time of storage increased, the differences became significant. After storage for 48 additional hours, the crumb firming increased significantly except for maltotriose. The interaction between time of storage and concentration was non-significant. The large error in the compressibility measurements could not be avoided because of the use of "pup" loaves. The interaction between the type of sugar and the time of storage was significant.

The addition of these oligosaccharides appeared to enhance the retrogradation of starch which in turn affected the firming of the bread crumb. This effect may be explained assuming that the oligosaccharides serves as plastizing agents of starch causing rapid retrogradation. Moreover the higher the concentration, the higher the rate of firming. However the production of these oligosaccharides during starch degradation by amylase supplements in the flour do not decrease crumb compressibility. Therefore,

the results suggested that presence of these sugars does not inhibit the firming of the bread crumb but rather that retarding of the process, in the presence of enzymes, is caused by the hydrolysis of the complex starch molecules which normally would aggregate.

It can be concluded, however, that corn syrup for breadmaking should be those with high dextrose equivalent in order to decrease the amounts of oligosaccharides present and to provide more fermentable carbohydrates.

SUMMARY

The oligosaccharides for crumb compressibility studies were isolated from corn syrup by adsorption column chromatography. Thirty gms. of corn syrup placed on 60 x 550 mm carbon-celite column were eluted with aqueous ethanol solutions. Seven liters of 5% ethanol were required to remove D-glucose and D-maltose. Higher ethanol concentrations, including 7.5%, 15%, 20%, 23%, and 30% were required for removal of the trisaccharides, tetrasaccharides, pentasaccharides, hexasaccharides, and heptasaccharides, respectively. The progress of elution was followed by orcinol test, ferricyanide oxidation method and paper chromatography. The combined pure fractions of each were evaporated to dryness in vacuum and gave a white to light yellow amorphous powder. An absolute separation of the first three sugars was achieved but some mutual contamination occurred in the succeeding higher oligosaccharides ($< 5\%$) in spite of rejecting the extreme fractions in which some tailing took place.

Characterization and identification of these oligosaccharides by their relative mobilities compared to malto-dextrins from amylase hydrolyzates by paper chromatography, optical rotation and beta-amylolysis showed that

the isolated sugars were members of the homologous series of malto-dextrin.

The individual oligosaccharides isolated were added to the bread formula using two levels (0.5 and 1.0 percent). Four replicates were used. There were slight decreases in crust color, symmetry, break and shred, grain and texture scores when the higher oligosaccharides were used. Statistical analysis of crumb compressibility measurement showed significant increase in crumb firming with time. The addition of the oligosaccharides caused an increase in the crumb firming rate. Therefore, there appears to be no advantage to use such sugars in bread. The production of these sugars as a result of wheat starch degradation by amylases would appear to decrease crumb firming. The beneficial effects of amylases on retarding of crumb firming must be associated with the degradation of the large starch molecules which normally would retrograte rapidly. The use of corn syrup with high dextrose equivalent in bread making would appear beneficial.

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SEPARATION OF OLIGOSACCHARIDES FROM CORN SYRUP AND THEIR EFFECT ON BREAD

by

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The present investigation was undertaken to separate and identify the oligosaccharides present in corn syrup and to study their effect on baking quality and bread staling.

Columns (60 x 550 mm) were packed with a mixture of equal weights of activated carbon (Darco G 60) and Celite 545 by slurry method. Thirty grams of corn syrup diluted to 100 ml. were chromatographed using stepwise elution technique. Glucose and maltose were eluted with 5 percent ethanol in water. Tri-, tetra-, penta-, hexa-, and hepta-saccharide were eluted from the column with 7.5, 15, 20, 25, and 30 percent ethanol, respectively. Dextrine (D P, > 7) was desorbed from the carbon-celite column with 70 percent ethanol. The progress of fractionation was followed by the orcinol test, ferricyanide oxidation, and paper chromatography. The pure fractions from several trials were combined and evaporated to dryness at 50°C. under vacuum. It was finally dried over phosphorus pentoxide under vacuum. A white or light yellow amorphous powder was obtained for each fraction. An absolute separation of the first three sugars was achieved but contamination with the next higher member (< 5%) occurred in the succeeding higher oligosaccharides. Relative mobilities on the papergram, optical rotation, and beta-amylolysis identified these oligosaccharides as members of a homologous series of the malto-oligosaccharides.

The second part of this work involved study of the effect of the addition of individual oligosaccharides on bread quality. Two levels, 0.5 and 1.0 percent, were used. Slight decrease in crust color, break and shred, symmetry, grain, and texture were observed with the higher oligosaccharides. While there was no differences in firmness in the fresh bread crumb on the addition of oligosaccharides from that of the control, there was a significant increase in the rate of crumb firming as the bread was stored. Moreover, the

larger the amount of oligosaccharide, the faster the crumb firming occurred. Therefore, the addition of these oligosaccharides enhance, rather than retard staling as reported for alpha-amylase supplementation. This may be explained assuming that the oligosaccharides serve as an aggregating agent causing complexing of the starch, while alpha-amylase causes starch to be split into small units which will retrogradate to a lesser extent. Considering these results, corn syrup with high dextrose equivalent and low oligosaccharide content are to be preferred for breadbaking.