QUANTITATIVE CHANGES IN VARIOUS SUGAR CONCENTRATIONS DURING FERMENTATION OF DOUGHS, SPONGES AND BREWS

by

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INTRODUCTION

Yeast-leavened wheat bread has been one of the most important foods of mankind for centuries. However, it was not until the second half of 19th century that scientific investigation on the nature of yeast fermentation was conducted. Extensive research work concerning the nature of yeast fermentation has revealed most of the chemical mechanisms involved. Most of the early knowledge of yeast physiology and fermentation was obtained in nutrient solutions rather than in bread dough. The first study of the changes in concentration of various sugars in a fermenting dough was reported by Koch et al. (53) in 1954. They used an analytical method developed by Dubois et al. (27), in which sugar mixtures were separated by paper chromatography and quantitatively determined by phenol-sulfuric acid method. Using the same technique, Lee et al. (67) studied sugar changes in fermenting sponges and doughs. Recently, Lee and Chen (63) and Lee and Liao (64) studied the change of some sugars in a fermenting straight dough by use of radioactive sucrose-^{14}C. However, limited studies concerning the fate of various sugars during continuous breadmaking processes have been reported.

It was the objective of this research project to study the following:

1) The changes in concentration of various sugars during fermentation of straight doughs, sponges and brews with different levels of flour content.

2) The rate of fermentation of the straight doughs, sponges and brews.

3) The residual sugars in the bread crumb of breads made by different procedures.

4) The techniques necessary to develop simplified methods for routine analysis of sugars in doughs, sponges, brews and bread crumb.

REVIEW OF LITERATURE

I. The Carbohydrates of Wheat and Wheat Flour

The total carbohydrate content of wheat varies with variety, weather, soil and other factors, but usually falls in the range of 63 to 73% (on 14% moisture basis) (5, 50, 51). A large percentage of the carbohydrate is starch, and simple sugars are present only in trace amounts.

Wheat starch, which comprises from 54 to 74% of the dry weight of whole grain and 95.8% of total carbohydrate of the endosperm, occurs in granules in endosperm cells (5, 30, 35, 73). A large percentage of the wheat starch granules range in size from less than 2 to 35 μ in diameter, while the largest ones range in about 50 μ (99, 107, 127). Generally, most of these granules are either less than 7.4 μ or greater than 14.8 μ in diameter. The small granules are sperical in shape, average 81.6% of total granule number but only 4.1% of total weight. The larger ones are lenticular in shape and average 12.5% of total number of 93% by weight (111). Wheat starch is composed of at least two fractions: the unbranched amylose and branched amylopectin (101). The ratio of these two fractions varies, and the amylose fraction ranges between 17 and 29% (24).

Some starch granules are severed during the milling operations and these "damaged" starch granules swell and gelatinize much more rapidly than the intact granules. They are readily hydrolyzed to maltose and other malto-oligosaccharides by the combined action of α-

and β- amylases. Since the enzymatic activity of the sound flour is more or less constant, therefore, the rate of sugar formation during and after dough-mixing is in proportion to the percentage of the damaged starch granules present in flour (48). Indirectly, the extent of starch damage affects fermentation rate and also the quality of bread (56).

The presence of a small amount of sucrose, maltose, fructose, glucose and raffinose have been reported in flour by a number of workers (6, 11, 52, 57, 83, 132). Positive identification and quantitative determination of these sugars have been reported only recently with various chromatographic methods (29, 40, 55, 77, 113, 134). There appears to be some lack of agreement in the results reported by different workers. This is at least partly due to the existence of great variations in variety and condition of the wheat studied and the methods of determination employed by the different workers. The mono- and di-saccharides contents, as reported by different workers, are summarized in TABLE I.

TABLE I: The Mono- and Di-saccharides Content of Flour[1]

Sugar	Flour					
	Hard Red Spring	Durum	English Wheat	German Wheat	Hard Red Spring	Amber Durum
Glucose	0.01-0.09	0.02-0.04	0.09	----	0.06	0.03
Fructose	0.02-0.08	0.04-0.09	0.06	----	0.08	----
Sucrose	0.19-0.26	0.26-0.57	0.84	0.88	0.54	0.74
Maltose	0.06-0.10	0.10-0.15	trace	0.04	0.05	0.18
Galactose	----	----	----	----	0.02	0.02
Data Source	125	125	77	121	72	80

[1]Percent of flour.

Galactose was found in wheat flour by some workers, and probably arose from the hydrolysis of galactosyl glycerides and/or raffinose during storage (72, 80). Other disaccharides reported to be found in wheat flours are melibiose and difructose (28, 94). They are probably the products of partial hydrolysis of raffinose and some glucofructans.

Raffinose has been known to exist in wheat for a long time (6). It exists mainly in the germ and represents 37.6% to 45.3% of the total sugar of germ, while the remainder is mostly sucrose (28, 35, 70). The level of raffinose in flour was reported between 0.19 and 0.68% (77, 80, 121). Stachyose, a non-reducing tetrasaccharide related to raffinose, was also reported to be found in flour (131).

There exists a whole series of oligosaccharides composed of glucose and fructose in wheat and other cereals. The presence of these oligosaccharides, or glucofructans, was first reported at as early as 1891 and they have been known as "levosine", since they yield levoluse upon acid hydrolysis (120). The percentage of this "levosine" fraction in wheat flour was reported to be between 0.4 and 1.5% of flour (6, 52, 83, 84). This fraction was sub-fractioned (131, 133), and structures studied (84). Evidence indicated there are probably two homologous series of glucofructans in wheat flour (84).

The simplest glucofructan is glucodifructose, which composes from 0.11 to 0.41% of wheat flour (35, 77, 121), and like other glucofructans, is readily hydrolyzed by yeast enzymes during fermentation, though this hydrolysis is not complete in all cases. In general, the members of this group of oligosaccharides are non-reducing and water soluble. Since their solutions have no viscous characteristics, their presence in dough will probably not affect dough properties (84).

Other carbohydrates include cellulose and various pentose-containing polysaccharides. The endosperm of wheat grain contains only a trace of cellulose (about 0.3%), and this fraction is undigestable for man and unfermentable by yeast (42, 43, 89). Pentosan and hemicellulose are all complicated pentose-containing carbohydrates (3), which also include "xylan" (3, 12, 84), "amylan" (6), and "wheat gum" (37, 84). The water-soluble pentosans give a viscous solution and may play an important role in the rheology of doughs and quality of bread (8, 78, 92, 107).

II. Quantitative Determination of Sugars in Natural Materials

Boiling or cold water is used to extract sugars from biological materials (100). However, pure water also frequently extracts interfering substances such as acids, salts, and colloidal substances which are dispersed or peptized in it (23, 123). For the extraction of sugars from flour or dough, pure water is undesirable, the amylases present will rapidly change the original sugar content upon wetting (66), while hot water will rapidly gelatinize the starch.

Hot aqueous ethanol is widely used for extraction of sugar from biological materials for its rapid inactivating effect on enzymes present in these materials. Hot ethanol also precipitates many macromolecules and reduced the contamination of ionic substances in the extracts (14, 23, 49, 102, 123).

To eliminate the substances that may interfere with quantitative determination and chromatography, the crude extracts are frequently subjected to one or more of the following treatments: filtration, centrifugation, decolorization, dialysis, ion-exchange and clarification by treatment with various precipitating agents. Ion-exchange by strongly

acidic resins like Amberlite IR-120 and Dowex 50 are very frequently used and are effective in removal of the ionic substances (40, 86).

The extent and type of treatment required will depend on the nature of interfering substances, materials, and method used for determination and of its sensitivity to non-sugar substances in the extract. Since loss of sugar and introduction of new errors may be caused by any unnecessary additional treatment, the dispensibility of this treatment can be determined by comparing the sugar recovery of extracts with and without treatment (49).

Hundreds of different methods for the quantitative determination of sugar have been reported. In general, these methods can be classified into the following three groups:

A. Methods based on the reducing nature of sugar.

A very large number of quantitative methods are based on the oxidation of sugars with a reducing keto or aldehyde group. The oxidizing agents commonly used are cupric, silver or ferricyanide ions, and the reaction readily takes place in an alkaline solution at elavated temperatures. Cupric ions are reduced on Cu_2O, and can be determined gravimetrically, eletrolytically, or indirectly by reoxidation by persulfate, permanganate or ferric ions, followed by determination of the excess by titration (1, 2, 26, 112, 113). Oxidation-reduction titration, using methylene blue as the indicator, is also frequently used. For micro determinations, iodometric and spectrophotometric methods are more often used (32, 41, 88, 115, 116). Ferricyanide is reduced to ferrocyanide and is determined by iodometric titration (20) or spectrophotometry (124).

Although widely used, both the cupric and ferricyanide oxidation

of reducing sugars do not proceed stoichiometrically. Nonetheless, by carefully selecting the method, and controlling the conditions and adjusting the concentration of sugars in the solution so that it falls within the optimum range, highly accurate and reproducible results can be obtained (112).

The traditional methods of measuring the reducing power of sugar extracts before and after acid or enzyme hydrolysis (19, 87) was neither specific nor very informative. The reporting of results in "maltose" equivalents for total reducing sugars and "sucrose" equivalents for total non-reducing sugars is both arbitrary and misleading. Great caution must be taken to interpret these results (22, 85).

Other quantitative methods of this group include color reaction with tetrazonium salts, various nitro compounds, iodine, cerium salts, etc.

B. Methods based on reaction with furfural or furfural derivatives.

Upon heating with strong acids all aldoses and ketoses with five carbon atoms or more give rise to furfural or its derivatives, which readily undergo color reactions with phenols and amines. The reaction is more specific for reducing sugars. The acids most often used are sulfuric, hydrochloric, phosphoric and various organic acid (40, 112). The phenols most frequently used for general sugar determination are anthrone (54, 74), 1-naphthol and 1-naphtholsulphonate (25), and phenol (29). For specific determinations, resorcinol, orcinol, naphthoresorcinol and phloroglucinol are used. The amines used for sugar determinations of this type are mostly aromatic amines, such as aniline, benzidine, diphenyl-amine, p-bromo-aniline, p-anisidine,

3,3-dimethylbenzidine, p- and o-aminophenol, and o-phenylenediamine. Organic acids, such as acetic, oxalic, trichloroacetic and phthalic acids, are frequently used in connection with these amines (40, 91, 112).

C. Other Methods.

Different strains of baker's yeast have been used to determine individual sugars in the presence of other sugars (103, 106, 114), but their applications are limited. Enzymatic determination of glucose by using glucose-oxidase was reported to be very accurate and sensitive (98). A number of classical reactions of sugars, such as periodate cleavage, cyanohydrin reaction and phenylhydrazine reaction, have been also used for quantitative determination (34, 112, 128).

With the extracts of natural materials, there usually exist more than one kind of sugar, as well as various non-sugar components. It is, therefore, necessary to separate the sugar components of interest from each other before the quantity of each can be determined. Of all the separation techniques available to chemists, none surpasses the chromatographic methods in specificity and applicability. They are especially suitable for studying complicated systems, such as extracts of various biological materials.

A. Paper Chromatography.

The advantages of paper chromatography are the following: 1) satisfactory separation can usually be obtained; 2) sample size required is small; 3) equipment required is relatively unsophisticated and inexpensive; 4) the techniques involved are relatively simple (112).

Separation on coarse papers, such as Whatman No. 54 or 31 ET, requires less time but gives larger, and more diffusion of spots, while that on smooth papers, such as Whatman No. 1 or 3 MM, requires

a longer time, but gives more concentrated spots; these results are more desirable for quantitative determinations (40).

The most commonly used developing techniques for sugars is one-dimensional on an ascending, descending, horizontal or circular arrangement. Hundreds of solvent systems have been studied and reported, but the results will not be included in this thesis. The general rules in selection of a solvent system are 1) the sugars will exhibit low R_f values (0.2-0.3) in such a system; 2) the system should not cause any chemical change of the substances being analyzed; and 3) should not react with the detection reagent nor reduces its sensitivity (55, 112).

For quantitative determination, the separated sugars on the chromatograms are either determined in situ, or eluted from paper and determined by the various quantitative methods mentioned in the previous section. In situ determinations are usually based on either the relationship between the area of spot and logarithm of concentration of sugar or the relationship between the intensity of the spot and concentration of sugar. All these methods are rapid and have an accuracy between $\pm 5\%$ and $\pm 10\%$. They are nonetheless suitable for routine analyses (16, 40, 75). The accuracy can be improved by employing the densitometer to determine the intensity of spots and standardization of conditions. Accuracy of $\pm 2\%$ may be obtained (31, 75).

B. Thin-layer Chromatography.

Although TLC separation of sugars was first reported in 1961 (109), the techniques are developing rapidly and a large number of established methods are now available. The chief advantages of TLC are its

rapidity, sensitivity and high resolving power.

The adsorbent or partition materials usually used in TLC analyses of sugars are silica gel-G, kieselguhr, gypsum, cellulose powder, and powdered glass (15, 90, 110, 135). In the last few years, various kinds of salt-impregnated layers have been studied and they are reported to give better resolution than do ordinary layers (46, 71, 90, 119).

Quantitative determinations can be made in situ, or by elution methods. Densitometry and fluorometry are most frequently used for in situ determination of sugars.

C. Other Types of Chromatographic Methods.

Column chromatography is usually used for sugar separation on a larger scale, although it is also possible to use it in the analysis of sugar mixtures in small quantities (21, 129). The adsorbent most commonly used is activated carbon which is mixed with a porous material, such as Celite (44). By washing with water and gradually increasing the concentration of ethanol in washing solution, different sugars and oligosaccharides can be successfully separated (130).

Electrophoresis study of sugar on filter paper or glass fibre has been reported to give good separations with some sugar mixtures (33).

Sugar analysis by gas-liquid partition chromatography has been under extensive investigation in recent years. Sugars are rendered volatile for analysis by methylation, acylation, acetalation or other reactions. The distinct advantages of gas-liquid partition chromatography are its great resolving power, rapid separation and high precision for quantitative analysis (10).

III. The Fate of Sugars in Breadmaking

The directly fermentable materials in a bread dough are some simple sugars which are either directly utilized by the yeast, such as glucose and fructose, or readily hydrolyzable on the action of the enzymes of yeast which yields glucose and/or fructose, such as maltose and sucrose. Indirectly fermentable materials in dough include malto-oligosaccharides, dextrins, starch, etc. These substances can be hydrolyzed by amylases and eventually yield maltose. Some trisaccharides, such as raffinose and glucodifructose, can be hydrolyzed by the invertase of yeast and yield fermentable sugars (76, 84). The raffinose can not be completely hydrolyzed and the millibiose produced can not be utilized by the yeast (76). Glucofructans can also be partially hydrolyzed upon the action of invertase and will yield fructose (9, 84). Lactose, which composes about 50% of the total weight of non-fat milk solids, is not hydrolyzed by yeast enzymes and is completely unfermentable (4).

Although the flour contains only a very limited amount of directly fermentable sugars, a substantial percentage of the fermentable sugars present in dough is produced indirectly through the hydrolyses of some components of flour. The combined actions of α- and β-amylases, as shown in some classic tests (17, 18), play an important role in the production of maltose during fermentation. Glucofructan content varies considerably from one wheat to another (9, 84), and so do other indirectly fermentable sugars.

The conditions of grain affect the composition and performance of flour tremendously. The flour milled from steeped or germinated wheat not only has a much higher sugar content, but also has a much higher

enzymatic activity (4). The effects of steeping and sprouting on the flour are presented in TABLE II (121):

TABLE II: Changes of Sugar Content in Steeped and Germinated Wheat

Treatment	Time Elapsed (days)	Oligosaccharides, dry matter %			
		Sucrose	Maltose	Glucodifructose	Raffinose
None		0.80	0.06	0.11	0.28
Steeping	1	0.79	0.14	0.08	0.21
	2	0.70	0.24	0.10	0.17
Germinating	3	0.71	0.51	0.08	0.10
	4	0.64	0.93	0.16	0.33
	5	0.81	1.03	0.32	0.57
	6	1.00	2.69	0.58	0.64
	7	1.74	3.79	0.49	0.94

The carbohydrate compositions of sick wheat and deteriorating wheat were shown to be quite different from those of sound wheat (38, 82). Lynch et al. (72) has reported that in wheat stored under anaerobic conditions for eight weeks, the sucrose and maltose decreased. The glucose and fructose increased by three fold, and galactose increased by 4 to 5-fold, presumably from the hydrolyses of raffinose and glyco-lipids.

Early workers conducted extensive studies on the fermentability of different sugars and their fermentation rates, the adaptation of baker's yeast to maltose fermentation, and factors that influence the rate of fermentation (4). However, there had been limited detailed studies concerning the fermentation of different sugars in dough, owing to the

unavailability of a proper analytical method to study such a complex system.

Hopkins and Roberts (45) reported that glucose was fermented at a slightly faster rate than fructose in the range of 2 to 8% sugar concentration and much faster at lower concentrations. Geddes and Winkler (36) reported that sucrose was hydrolyzed much faster than it was fermented, and the rate of its fermentation was equivalent to that of an equimolecular mixture of glucose and fructose. The fermentations of glucose, fructose and sucrose are almost instantaneous; however, the fermentation of maltose was found to require a considerably longer induction period (96). It has been reported that in the absence of the "activators", this induction period was extremely long. These activators were found to be glucose and maltase (68, 104), sucrose and oxygen (105). Maltose was found to be the last sugar fermented (59, 62) in the dough. The observation was supported by the gas production curves of the sucrose containing dough, which exhibited two maxima (58, 81).

Koch et al. (53) used paper chromatography-elution determination methods to study the change of concentrations of different sugars in dough. They found the sucrose was inverted completely in a very short time and concentration of glucose decreased at a uniform rate throughout the fermentation. The concentration of fructose, however, did not start to decline until the glucose became quite low. When fructose was the only fermentable sugar present in the dough, it is also fermented at a constant and uniform rate. This observation was confirmed by Griff and Johnson (39). The maltose concentration in straight dough was found to increase as long as there was sufficient hexose to support the fermentation; but in those doughs to which no sugar was added maltose began to decrease

about one hour after the mixing when glucose and fructose in dough became depleted. Lee et al. (67), in their study with sponge and doughs, reported that once the yeast became well adapted to rapid maltose fermentation, glucose and fructose no longer had a sparing effect on maltose. They found that after the remix of sponge, the dough that contained maltose produced more gas than doughs containing glucose or sucrose during the first 40 minutes. Johnson et al. (47) studied brew with no flour and 6% sucrose and found that 50% of the sugars were fermented within 1½ hour, and only 10% of sugar was found at the end of 5 hours of fermentation.

Studies of the change of sugar in dough during breadmaking with radioactive tracers were recently reported (63, 64, 65). In general, the findings were in agreement with those of earlier workers. The inversion of sucrose was found to be incomplete, and 0.5% of the original amount added to the formula was found in the bread. Evidence was also found indicating monosaccharides derived from sucrose could undergo degradation as well as condensation or polymerization to produce disaccharides and oligosaccharides during baking (64).

As with all living organisms, the growth and activities of yeast is to a great extent influenced by environmental factors. Temperature was found to affect the rate of fermentation of the various kinds of sugars differently (108). Baker's yeast produces more CO_2 at 34°C than at 18°C and lower or at 39°C and still higher degrees; and the optimum temperature seems to lie between 25° and 35°C (79, 93, 117). Retardation of yeast activities at high sugar concentrations due to osmotic effect have been studied by many workers (4, 45, 61, 79). Different strains of yeasts were found to have different tolerances under osmotic pressure conditions (122). Salt, as a completely ionized electrolyte; its osmotic effect on

fermentation rate is expectedly much higher than that of sugars (122, 126). There seems to be a fairly broad optimum pH range for yeast fermentation with a peak on the acid side of pH 5.0, which also coincides with the optimum range of pH for α- and β-amylase activities (4, 69, 95). In an unbuffered dough, the pH decreased steadily during the fermentation period from 6.0 to about 5.0 (93). When non-fat milk solids are added to the dough or brew, the decrease of pH is stopped or slowed down (47). Sometimes, it becomes necessary to add certain acid salts to brew to lower the pH to maintain an active fermentation and the quality of the product (7, 60, 118).

Few investigations of the residue sugars were reported by the early workers. By use of a modified copper reduction technique, Rice (97) found a higher content of fructose than glucose in bread made from a formula containing sucrose. A lower maltose content in bread made by sponge and dough process was also reported. Employing special strains of yeast which fermented glucose, fructose and sucrose but not maltose, Bohn (13) found no hexose in breads made from no-sugar or 1% sucrose-straight doughs. With 2% and more sucrose, the hexose content of bread increased with the sugar level in the formula. The analyses of 18 commercial breads revealed great variations in total and specific sugar contents. The analyses showed the hexoses content of bread crumb varies from 0 to 3.54%, with maltose from 0.15% to 2.85% and with total sugar from 2.36 to 5.56%, based on crumb moisture. Koch et al. (53) found that, in general, the sugar composition of dough and corresponding bread were very similar; however, the bread will contain slightly less glucose and fructose. Bread made by the sponge dough process was found to have a much lower maltose content than that made by straight dough process. Lee et al. (67) reported similar

results. C. C. Lee and co-workers studied change of sugars with ^{14}C -labeled starch and sucrose (63, 64, 65). With 5% sucrose used in a straight dough, 42% of the activity was recovered in the finished bread. After separation by paper chromatography, the activities of glucose and fructose were found to be 1:3.5. The decrease of hexose content during baking was explained by the polymerization and the degradation of these sugars at high temperature. Glucose was reported to go readily to polymerization of this type (64).

METHOD AND MATERIALS

I. Flour Samples

The flours used were obtained from samples milled at the Kansas State University pilot mill. The results of the milling studies were as the follows:

A. Code No. 9008---Hard Red Winter, 1965 Crop. Moisture: 12.0%; protein: 11.0%; ash: 0.40%. Extraction: 74.5%. Water absorption: 62.0%.

B. Code No. 9003---Hard Red Spring, 1965 Crop. (Pembina var., N. Dakota) Moisture: 11.1%; protein: 13.4%; ash: 0.41%. Extraction: 74.68%. Water absorption: 63.8%.

II. Formulation, Dough Make-up and Baking

The following general formula was used throughout this experiment:

Ingredient	%, flour basis
Flour	100.0
Sucrose	5.0
NaCl	2.0
Yeast, compressed	2.5

Ingredient	%, flour basis
Yeast food	0.5
Malt flour	0.5
Shortening	3.0
Water	62.0 (9008) 63.8 (9003)

In all five treatments, regardless of the distribution of amount of ingredients in sponge (or brew) and dough, the total of each ingredient remained constant as indicated in the above formula.

A. Straight Dough.

The general formula, based on 400 grams of flour, was used and was mixed in a Hobart Model A. 200 Mixer in a MacDuffy mixing bowl. The dry ingredients were placed in the mixing bowl first and the yeast was added after having been suspended in a portion of the liquid (temperature of water: 25°C). The ingredients were mixed at 1st speed for 30 seconds, and then at 2nd speed until mixture was fully developed (8 min. for 9008 and 10 min. for 9003). A modified Standard AACC fermentation and make-up schedule was followed in the experiment. The National fermentation cabinet was used, with a control temperature at 30°C and relative humidity at 75%. The first punch was made at 105 min. (after the dough was mixed), the second punch and dividing were made at 155 min., and the molding at 180 min., and started the proofing immediately, and baking at 240 min. Pup loaves were baked at 230°C for 25 min.

B. Seventy-five (75) percent Flour Sponge and Dough.

The general formula was divided into 2 parts. To the sponge, no sugar, salt or shortening were added. Seventy-five (75) percent of the total flour (300 g) was mixed with 10 g of yeast, 2 g malt, 2 g yeast food

and 75% of the total water. The ingredients were mixed at 1st speed for 30 sec. and the sponge was allowed to ferment for 4 hours. The sponge was remixed with the remaining ingredients at the end of 4 hours until developed (about 7 to 9 min.). The dough was allowed to rest for 30 min. before it was divided and molded. Proofing, and baking were the same as those for the straight dough.

C. Fifty (50) percent Flour Brew and Dough.

Fifty (50) percent of the total flour in basic formula (200 g), 40% of total sugar (8 g), all the yeast, yeast food, malt and all but 20 ml of water were mixed in a 2-liter stainless steel beaker with a large spatula until a reasonably homogeneous mixture was obtained (in about 3 min.). The brew was allowed to ferment at 30°C for 150 min. and remixed with the rest of the ingredients at the end of the period. The dough was mixed until developed (in 7 to 9 min.) and immediately divided and molded. The dough was allowed to proof for 75 min. before the pup loaves were baked at 230°C for 25 min.

D. Twenty-five (25) percent Flour Brew and Dough.

Twenty-five (25) percent of the total flour in the basic formula (100 g) was mixed with all the sugar, salt, yeast, yeast food, malt flour and all but 8 ml of water in a 2-liter stainless steel beaker with a large spatula until reasonably homogeneous (in about 2 min.). The brew was allowed to ferment at 30°C for 150 min. and then was remixed with the rest of ingredients. The dough was divided, molded, proofed and baked as the 50% flour brew and dough.

E. No Flour Brew and Dough.

The brew was a mixture of all the sucrose, salt, yeast, yeast food, malt flour and all but 8 ml of water. The ingredients were mixed well

in a stainless steel beaker with a large spatula. The brew was fermented at 30°C for 150 min. before being remixed with the rest of ingredients. The remaining operations were the same as those for the 50% flour brew and dough.

III. Determination of Gas Production Rates

Gas pressures of different doughs, brews and sponges were determined on an equal yeast cell number basis. The sizes of samples for all five treatments of the different stages are presented in TABLE III:

TABLE III: Size of Samples Used in Determining Gas Production Rates

Treatment	Weight of sample (g)	
	Sponge of brew	Dough
Straight Dough	---	17.00
75% Flour	12.15	17.00
50% Flour	10.95	17.00
25% Flour	9.30	17.00
No Flour	6.90	17.00

The samples were weighed in pressure jar on a Mettler balance; the manometers were screwed on, tightened and the whole units were placed in a water bath (temperature controlled at 30°C). The gas was released after 5 minutes, and manometers were adjusted to 0. Pressure readings were recorded every 30 min.

Straight dough gas pressure readings were taken at (min. after dough was mixed) 5, 35, 65, 96, 125, 155, 185, 215 and 245. The dough sample was punched down by a spatula immediately after the reading was taken at 185, and manometers were readjusted to 0. (This operation corresponded

to molding and panning.)

Seventy-five (75) percent flour sponge and dough gas pressure readings were taken at (min. after sponge was mixed) 5, 35, 65, 95, 125, 155, 185, 215, 245, 255, 285, 315 and 345. Immediately after the reading at 245 was taken, which corresponded to the ending of sponge fermentation, the original sponge sample was discarded. A new sample from the just remixed dough was placed in jar and the manometer readjusted to 0 at 255.

The gas pressure readings of liquid brew with different levels of flour and the resulting doughs of these brews were taken at (min. after brew mixed) 5, 35, 65, 95, 125, 155, 165, 195 and 225. The first brew samples were discarded immediately after the pressure readings were taken at 155, which corresponded to the time of dough remixing, dividing and panning. A new sample from the just remixed dough was placed in the jar, and the manometer readjusted to 0 at 165.

IV. Schedule of Sample Collection for Sugar Analyses

Straight dough samples were taken at (min. after dough mixing) 0, 30, 60, 90, 120, 150, 180, 210, and 240. (Designated by No. 1 through 9). The bread crumb sample was taken 15 min. after the bread came out of oven, and was designated by No. 10.

Sponge samples were taken at (min. after mixed) 0, 30, 60, 90, 120, 150, 180, 210 and 240, designated by No. 1 through 9 respectively. After remixing, dough samples were taken at 250, 280, 310 and 340 (No. 10 through 13). The bread sample was designated by No. 14.

Brew samples were taken at (min. after mixed) 0, 30, 60, 90 and 150 (No. 1 through 6). After remixing, dough samples were taken at 180, and 210, and, finally, the bread crumb sample (No. 7 through 9).

V. Method of Extraction of Sugars from Dough, Brew, Sponge and Bread Crumb

A 10 gram sample of dough, brew, sponge or bread crumb was taken and placed in Waring Blender (steel sup) and about 70 ml of boiling 70% (w/v) aqueous ethanol was added. The mixture was allowed to be homogenized for 3 minutes. Then, the mixture was transferred into a 125 ml Erlenmeyer flask, and two 10 ml portions of boiling 70% ethanol were used to wash out the residue materials in the blending cup, pooled with mixture. The flasks containing the dough homogenates were placed in a water-bath shaker, and the temperature controlled at 70°C, for one hour. The mixture was cooled to room temperature, and volume accurately adjusted to 100 ml. The mixtures were centrifuged at 1800 x g at 5°C for 20 minutes. A 50 ml aliquot of supernatant was taken from each tube after it had returned to room temperature. The extracts were kept in sample bottles and stored at -10°C for future analyses.

A portion of the solvent was lost through evaporation during centrifugation; this loss was found to be about 5% of the total volume of liquid in the tube. To bring the extracts to their original concentration, 2.5 ml of the solvent was added to each 50 ml of supernatant collected.

VI. Method of Analysis

It has been a general practice for cereal chemists to measure the reducing power of a sugar extract. The results are usually expressed in either the equivalent of maltose or glucose. However, there are usually more than one sugar present in the extract and their reducing powers may be greatly different from one another. Therefore, such a results may be ambiguous and misleading, and should always be carefully interpretated.

In this experiment, the total reducing sugar values of extracts were taken, since in this particular case, these values are quite reliable indexes of the readily fermentable sugars present in the dough. Since no milk was included in the formula, therefore, no reduction due to lactose would occur. Moreover, examination by thin-layer chromatography, it was showed that little maltotetraose and nearly no maltopentaose nor any higher malto-oligosaccharides were extracted by the methods used. The reducing power was mainly attributed to fructose, glucose and maltose--three principal fermentable sugars of baker's yeast.

The Folin-Wu blood sugar determination method (32) was chosen for the following reasons: 1) excellent reproducibility with linear relationship between sugar concentration and absorbance when sugar concentration is under 0.03%; 2) highly sensitive, suitable for micro-analysis; 3) reagents are relatively easy to prepare and are stable, frequent caliberation of the standard curve became unnecessary; 4) nearly stoichiometric oxidation of sugar under the optimum conditions. The reducing power of glucose was found to be very close of that of fructose and twice that of maltose if a longer reaction time was given.

REAGENTS:

Alkaline copper solution--Dissolve 40 g anhydrous Na_2CO_3 in 400 ml water, add 7.5 tartaric acid. When the crystals were dissolved, add 4.5 g $CuSO_4$-$5H_2O$, make up 1 liter.

Phosphomolybdic acid solution--To 35 g molybdic acid and 5 g Na_2WO_4, add 200 ml of 10% NaOH and 200 ml water. Boil vigorously for 20-40 min. Cool, dilute to ca. 350 ml and add 125 ml of conc. (85%) H_3PO_4. Dilute to 500 ml.

PROCEDURE: Transfer 2 ml of standard sugar solution or sugar solution to be determined to a Folin-Wu sugar tube. Add 2 ml of alkaline copper solution.

Transfer the tube to a boiling water bath. After boiling, cool in running water without shaking. Then, add 2 ml phosphomolybdic acid reagent. Dilute to the mark (25 ml) after 1 min., mix thoroughly. Measure absorbance at 420 mμ with reference to a reagent blank setting to 0.

CALCULATION OF THE RESULTS: The total reducing sugars in doughs, sponges, brews and bread crumb extracts were calculated based on the following formula (the results were all reported in a glucose equivalent):

Total Reducing Sugars (mg glucose in 1 g dough, sponge, brew or crumb) =

$$\frac{\text{Absorbance of the Standard (concn: 0.1 mg/ml) X Times of Dilution}}{\text{Absorbance of the Unknown}}$$

The component sugars in dough, sponge, or brew extracts were separated by paper chromatography, and then, determined in situ by direct photometry in transmitted light.

Chromatographic separation was conducted by using Whatman No. 1 filter paper, because it gave good resolution and concentrated spots. The paper was cut into 8" X 18" strips. Seven samples or standard mixtures can be spotted on each sheet at 1" interval. They were spotted by using a micropipette and dried in a hot air stream. The amount of sample for each spot was determined by theoretical calculation and test-chromatography. The important points are these: a) the separated sugar spots should have the density as close to standard as possible, and b) to deliver too small or too large a volume of liquid through a micropipette may result in introduction of error and should be avoided. Therefore, proper dilution of extracts prior to spotting is as important as accurate measurement and delivery. In this experiment, at least two spots of different volumes were spotted for each sample. A standard mixture, which contained 5 μg each of glucose and fructose an 10μg of maltose in each 5μl alquot, was

spotted on each chromatogram as the reference for quantitative determination.

The descending developing technique was used. The solvent system, which is constituted of n-propanol-ethyl acetate-water in a 6:2:2 ratio, gave satisfactory separation to all sugars studied in the experiment. The spotted paper strips were developed for 24 hours at room temperature before visualization.

The chromatograms were air-dried for at least 2 hours to remove all the organic solvents and water before being treated with detecting reagents. The silver nitrate-methanolic NaOH-sodium thiosulfate detecting system was used in the experiment. This system yielded black sugar spots on a white or slightly grayish background chromatographic paper.

REAGENTS:

$AgNO_3$ in acetone--0.5 ml saturated $AgNO_3$ solution in water was added to 100 ml acetone. Add the least amount of water required to dissolve all the $AgNO_3$ crystals.

Methanolic NaOH--A 0.4% solution of NaOH is prepared in methanol. Add least amount of water required.

%5 $Na_2S_2O_3$ solution--50 g crystalline $Na_2S_2O_3$ is dissolved in water, to make up to 1 liter.

PROCEDURE: Dip dry chromatogram in $AgNO_3$ reagents. Air dry for an hour. Then, dip in methanolic NaOH solution. After development of color, remove the background brown color by dipping in thiosulfate solution. The chromatogram is then washed with tap water and air-dried.

Direct photometric determination of sugars on paper chromatograms was conducted by use of Photvolt Multiplier Photometer (Model 520-A) with Varicord Variable-Response Recorder (Model 42-A), manufactured by Photovolt Company, New York. For this experiment, the measuring unit of

the instrument was slightly modified. The original 6 mm-wide upper slit was removed, and a 19 mm X 1 mm lower slit was installed. A 526.5 mμ filter was also used.

Detected, dried chromatogram was placed between two pieces of clear, 20 X 20 cm glass plates and mounted on the moving table. The instrument, as well as the continuous recording unit, was adjusted to 100% transmittance by selecting an area of background. Each series of the separated sugars from individual sample was recorded continuously on a strip of chart paper. The area under each curve on the chart, which corresponded to a separated sugar, was integrated by using a Gelman High Precision Planimeter. Each curve was read twice and the average value recorded.

CALCULATION OF THE RESULTS: Each component sugar in each extract was calculated based on the following formula:

Concentration of Individual Sugar in Dough, Sponge, Brew, or Bread Crumb (percent of dough, etc. by weight) $= \frac{D \times A_u \times S}{V \times A_s}$

D : Times of dilution of the extract

A_u: The integrated area of the unknown

S : Number of g of sugar in the standard spot on the same chromatogram

V : The volume of the diluted extract spotted

A_s: The integrated area of the standard

RESULTS AND DISCUSSION

I. Discussion of the Experimental Methods

In the development of methods to conduct this research project it was found necessary to modify and evaluate some of the methodology.

The effectiveness of different extracting solvents had been compared by use of tungstic acid solution, 70% (v/v) and 70% (w/v) aqueous ethanols by paper and thin-layer chromatographies. As far as glucose and fructose recoveries are concerned, no difference between the three extracts was found. However, tungstic acid solution seemed to extract a little more maltose, as indicated by the darkness of sugar spots when they were compared on chromatograms. It also appeared the maltose in tungstic acid extract was increased if a longer standing time was allowed. Upon examination of the chromatograms under UV light after separation of the component sugars on borate-impregnated silica gel thin layer, and detected by aniline phthalate reagent, the tungstic acid extract was found to have a large amount of malto-oligosaccharides (up to G_8); however, in both ethanolic extracts, very little oligosaccharides beyond G_5 was found. The tungstic acid extract had a turbid appearance; but the ethanolic extracts were reasonably clear.

No change of sugar concentration or distribution was found in the same ethanolic extracts during a period of eight weeks at the room temperature based on weekly chromatographic comparisons. Precipitation of some white substances from the extracts was observed in both extracts a few days after the extraction. The presence of this precipitate, presumably lipids and/or proteins, did not seem to be accompanied with any change of the sugar components in the extract.

Deionization of extracts of biological materials by ion-exchange has been extensively used as a means to eliminate the ions which otherwise may interfere with subsequent chromatography and/or sugar determination. Two cation exchangers, Amberlite IR-120 and Dowex 50, were tested in the experiment. The ethanolic extracts were passed through a 10 cm column of

either exchanger at the rate of 1 cm/min. and the column was washed with 25 ml of 70% ethanol. Then, the extracts were concentrated by evaporation and adjusted to original volumes and compared with the untreated extracts. However, no significant difference was found with both chromatographic separation or total reducing sugar determined by Folin-Wu Method. This operation, therefore, was omitted from all subsequent extractions.

The extraction method employed in this experiment was partly based on the assumption that all sugars were evenly distributed in all phases in the mixture after homogenization. However, it is not so in a strict sense. The fact that ten times as much extractant was used to dilute the sample, which contains on an average 50% or more moisture, the errors introduced by this operation should be reasonably small. Empirical data presented in TABLE IV showed that the errors due to the uneven distribution of sugars between the liquid and solid phases was quite insignificant.

TABLE IV: Determination of Sugar Recovery by Folin-Wu Method

Dough[1] + % glucose	Reducing sugars (% glucose) found	Glucose recovered	% recovered	Mean deviation
0	1.40 1.40			
1%	2.42 2.44	1.02 1.04	103	±0.01
2%	3.52 3.49	2.12 2.09	105	±0.01
5%	6.50 6.54	5.10 5.14	102.5	±0.005

[1]The dough used in this particular test was obtained from the baking laboratory. Its formula and make-up methods were unknown.

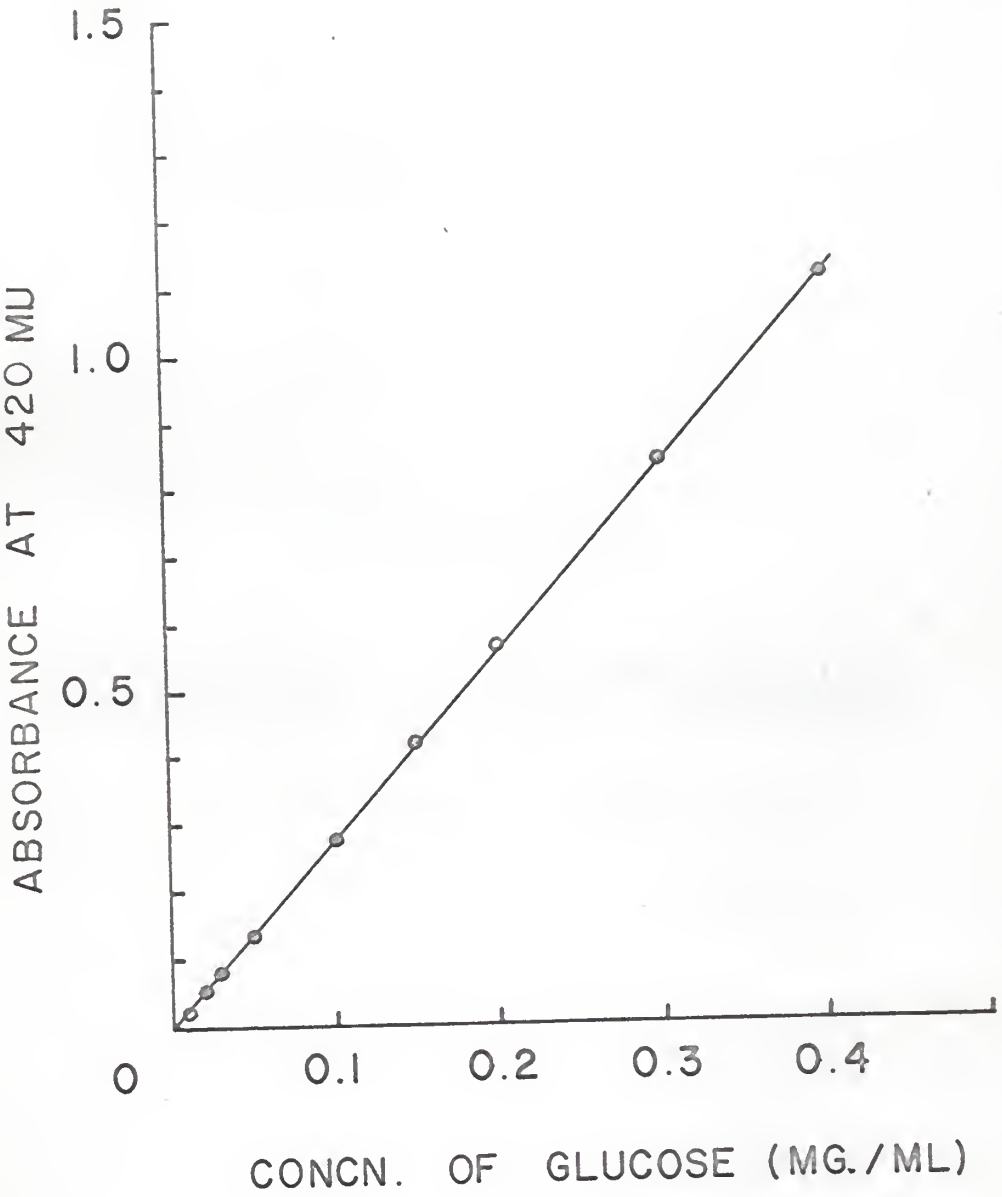


Figure 1: Standard curve of Folin-Wu method for reducing sugars determination

The Folin-Wu method for the determination of reducing sugars appeared to be highly accurate and reproducible. With standard glucose solutions, it was found that the color reaction between 0 and 0.4 mg/ml followed Beer's Law. The standard curves for glucose using the Folin-Wu method is presented in Figure 1.

The boiling time suggested by the literature was eight min., but it was found that 20 min. of boiling was required to oxidize maltose stoichiometrically. The oxidation of glucose and fructose were not affected by the longer boiling time.

For accurate measurement, it is essential to dilute the extracts properly so that the final sugar concentration will give an absorbance within the optimum range, that corresponds to 0.005 to 0.30 mg of glucose per ml. The absorbance was directly read at lower and medium sugar concentrations. However, at higher concentrations, the absorbance values were calculated from the transmittance readings.

In the quantitative method used in this experiment to determine the individual component sugars, a number of factors were found to affect the accuracy and precision of the determination. It was found that in order to obtain round and concentrated spots, which are more desirable for this type of <u>in situ</u> determination, the water on chromatograms and in reagents should be as low as possible. If the chromatograms were not thoroughly dried or the concentration of water in reagents were too high, diffuse, larger and elongated spots would be the result. The time for sugar spots to develop color was also critical. Different sugars were found to develop color at quite different rates. In this experiment, exactly 5 min. was allowed for chromatograms to develop color before they were fixed by washing in thiosulfate solution. This standardization of

"color developing time" was found to improve the reproducibility of the subsequent photometric determination significantly.

Within the optimum range, the reproducibility of result was quite satisfactory and linear relationship between the integrated area under the curve and sugar content in spot was observed. In this particular case, the optimum range of sugar concentrations per each separated spot were as follows: Glucose: 2 to 10 μg; fructose: 3 to 12 μg; maltose: 5 to 15 μg (Figure 2).

Reproducibility of results with maltose appeared to be more easily affected by the change of reagent concentration, temperature, and other factors, however, the results with glucose reproduced best and were least affected by environmental changes

The reproducibility of sugar spots containing the same amount of sugar on different chromatograms was extremely poor. 5 μg of glucose had given a corresponding area of as high as 385 units in one chromatogram and as low as 155 in another. Even when all the humanly possible cautions were taken, a deviation up to 40% was not at all uncommon. However, the reproducibility on the same chromatogram were normally within $\pm$5% or better. Therefore, a standard mixture containing 5 μg each fructose and glucose and 10 μg maltose was always spotted with the extract samples; sometimes, more than one such standard mixture spots were applied to each chromatogram.

The irreproducibility of results with different chromatograms appeared to be mainly due to the change of reagent strength, especially that of NaOH, which changes considerably after the dipping of the first one or two chromatograms. It has been observed that among a set of four chromatograms, the first one had the highest density, while the same standard mixture on the chromatogram that was last dipped in the same reagent had the lowest density.

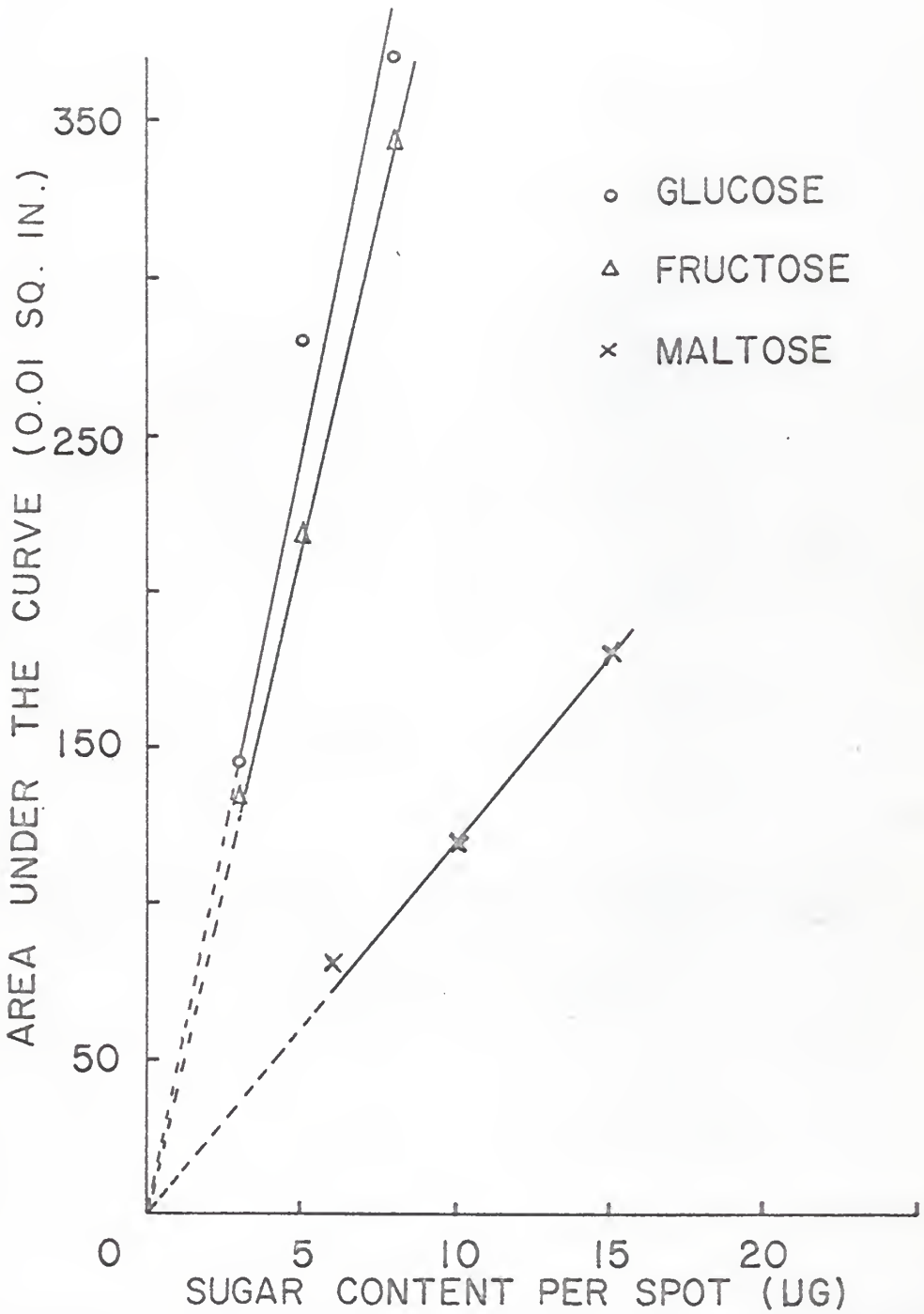


Figure 2: Relationship between the quantity of sugar per spot and the integrated area under the curve by direct photometry in transmitted light at 526.5 um. (Spots in each series are on the same chromatogram.)

TABLE V: Examples of Results Obtained by In Situ Determination

Chromatogram No.	Sugar	µg	Area (0.01 sq.in.)	Average Area	Mean Deviation
5GF-1	Glucose	5	265		
	"	5	259	262.75	±4.25
	"	5	258		
	"	5	269		
5GF-1	Glucose	3	163		
	"	3	148	153.33	±5.44
	"	3	149		
5GF-1	Fructose	5	191		
	"	5	203	193.75	±4.75
	"	5	187		
	"	5	194		
5GF-1	Fructose	3	106		
	"	3	118	112.00	±4.00
	"	3	112		
5M-2	Maltose	10	123		
	"	10	114	116.00	±4.67
	"	10	111		
5M-2	Maltose	5	72		
	"	5	60	65.00	±4.67
	"	5	63		

The fact that the optimum range for determination of each sugar in this particular case is quite narrow. It is essential to make proper dilution of the extracts and/or to apply a proper volume of sample. These should be carefully calculated in advance and checked by test-chromatography. Throughout this experiment, two different levels of the same unknown were spotted separately so that the concentration of a particular sugar in the extract can be calculated by comparing the standard spot with the unknown which has the nearest density, if the other spot gives a different value. (In fact, deviations of this type was usually reasonably small.) However, if an unknown spot is way out of the optimum measuring range (usually when

the concentration was too low), calculated results based on extrapolation of the standard were also recorded, but with a (*) mark. The reliability of the results with such a mark is expectedly lower than those which are calculated from spots whose densities lie within the optimum range.

II. Change in Total Reducing Sugars of Extracts

The changes in total reducing power of extracts of various doughs, sponges and brews are presented in Figures 3, 4, 5 and 6.

A steady decrease of the reducing power of dough, sponge and brew extracts was observed in all treatments from 30 min. after initial mixing to the end of the fermentation period. In almost all the cases, however, an increase in reducing power during the first 30 min. was observed. This increase was probably due to the following reasons: 1) a rapid increase of maltose due to the combined action of α- and β-amylases; 2) inversion of the remaining sucrose that was not dissolved and/or hydrolyzed during the initial mixing period; 3) low yeast activities during this initial period; 4) enzymatic hydrolyses of certain oligosaccharides, such as glucofructans.

In the discussion of determination of blood sugars by this method, Folin and Wu reported the presence of "non-saccharoid" substances in the extract were also responsible for part of the total reducing power. The same situation was expected in this experiment. However, the amount of such non-saccharoid reducing substances present in the extracts of doughs, sponges and brews was found to be quite small and was estimated to be in the order of 0.10% of dough. The chemical nature of this fraction is unknown, presumably it includes amino acids, peptides and certain lipoid substances.

Fig. 3

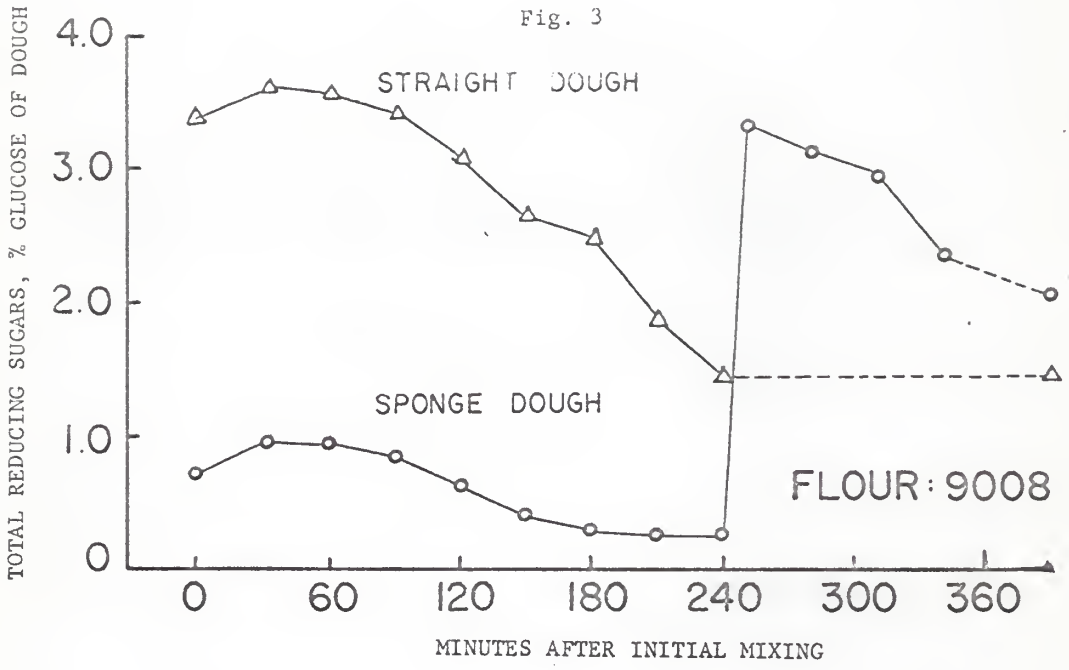
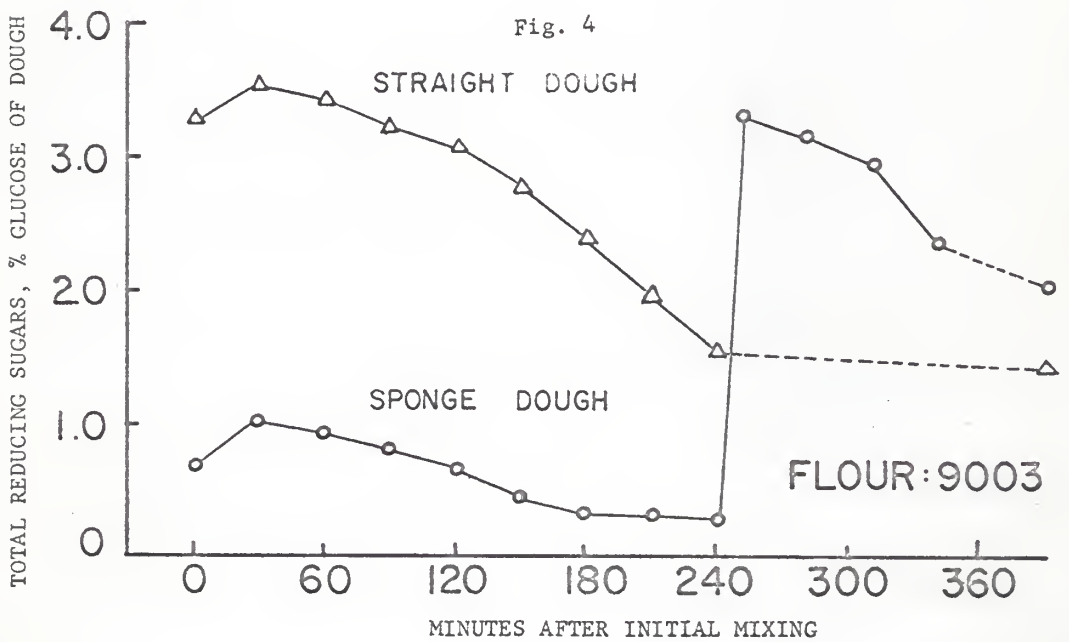


Fig. 4



Figs. 3 and 4: Changes of total reducing sugars during fermentation of straight and sponge doughs. The last reading in each series is that of the crumb of resulting bread.

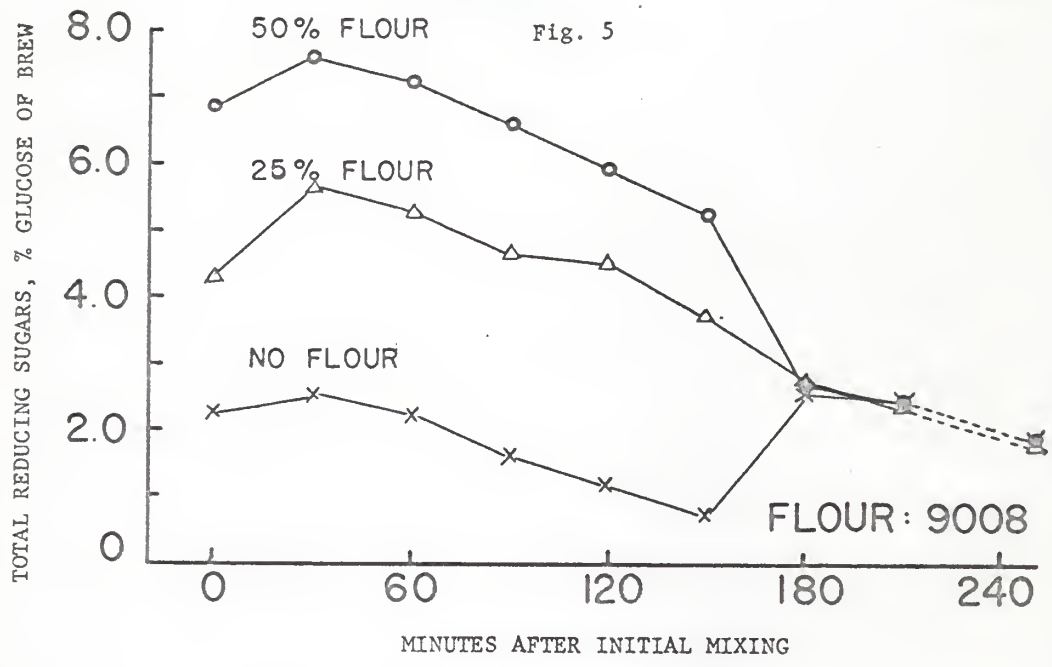


Fig. 5

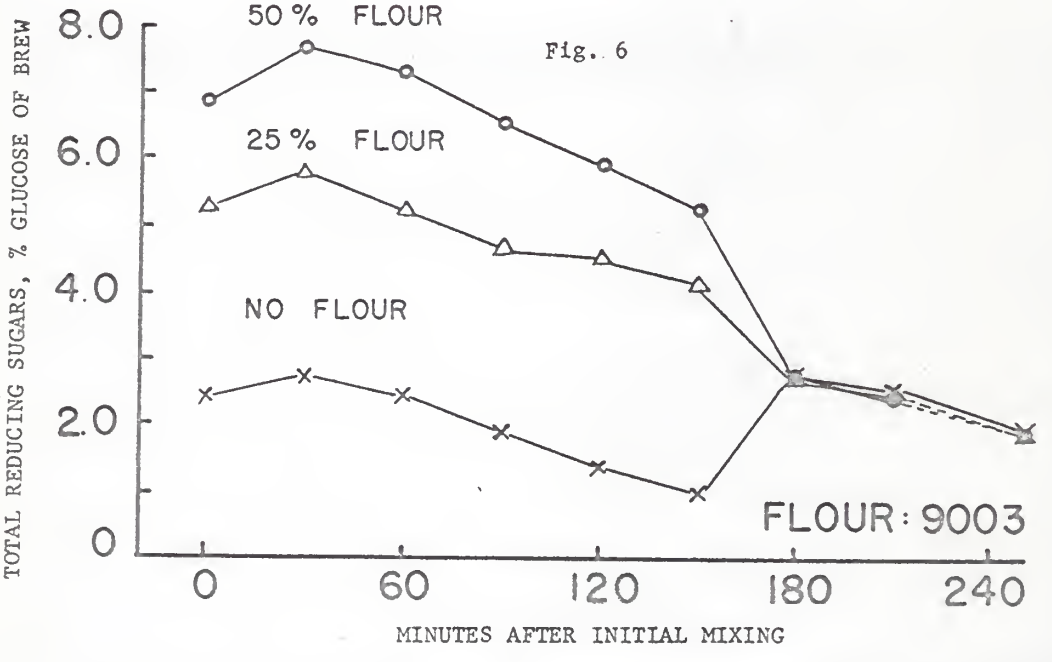


Fig. 6

Figs. 5 and 6: Changes of total reducing sugars during fermentation of liquid brews with different levels of flour and doughs after remix. The last readings are that of bread crumb.

III. Change of Concentration of Individual Sugars During Fermentation

The changes of the concentration of glucose, fructose and maltose in fermenting doughs, sponges and brews are presented in Figures 7 through 16.

In the straight doughs, a steady decrease of glucose concentration throughout the whole fermentation period was observed. There was no significant consumption of fructose during the first $1\frac{1}{2}$ hour of fermentation. In fact, at its maximum concentration, at least 0.20% (dough basis) more fructose than that theoretically expected from the hydrolysis of sucrose was recovered. Apparently, this increase was due to the hydrolyses of various fructose-containing oligosaccharides. The decrease of fructose did not become significant before the concentration of glucose dropped to about 0.90% of dough. After that, the two sugars decreased at approximately the same rate. The maltose increased rapidly soon after the dough was mixed. The increase was slowed down considerably soon after the available substrate for amylases became depleted. Toward the end of fermentation, the level of maltose was nearly constant and appeared to be on the way to decline.

In sponge doughs (Figs. 9 and 10), not only maltose, but also glucose and fructose were found immediately after the sponge was mixed. A substantial percentage of glucose and fructose, which are present in flour only in trace amounts, were produced by the hydrolyses of sucrose, raffinose, gluco-fructans and other oligosaccharides. These hexoses soon became depleted, and during the next 3 hours, maltose played the role as the sole sugar supporting the sponge fermentation. Nevertheless, the presence of a very small amount of hexose was still detectable by paper and thin-layer chromatography even at the end of 4 hours of sponge fermentation. This hexose was found to have a R_f value comparable to that of glucose, but

no positive identification was made. Reducing oligosaccharides with R_f values comparable to maltotriose, maltotetraose and 2 other low R_f sugars were also found. At the end of 4 hours of fermentation, the maltose concentration in sponge was very low, yet, not completely depleted. After remixing, although much hexoses now became available, but the fermentation rate of maltose appeared to be as high as before. These observations were in agreement with the findings by Lee et al. (67). Little fructose was consumed after remixing and most of it was recovered from the crumb of the resulting bread.

Unlike the other two brews, the 50% flour brews did not contain all the sucrose in the formula. Only 40% of the total sucrose was added during the initial mixing of brew. Glucose was nearly depleted in $1\frac{1}{2}$ hour. After $2\frac{1}{2}$ hours of brew fermentation, and there was only a trace amount of fructose left in the brew. The fermentation of maltose appeared to start at about two hours after mixing, when the concentration of glucose and fructose became very low (Figs. 11 and 12).

Except for the flour content in brew, the 25% flour-brew (Figs. 13 and 14) and no-flour brew (Figs. 15 and 16) showed the same compositions of all the other ingredients. However, the rates of fermentation in these two brews significantly differed from each other. In both the brews, glucose was the only sugar consumed in significant amount, but the rate of decrease of glucose concentration in no-flour brew was considerably slower than that of the 25% flour brews. At the end of $2\frac{1}{2}$ hours brew fermentation, the average glucose concentration in 25% flour brews was 46% of that at 30 minutes after the initial mixing. In those brews to which no flour was added, the glucose concentration after $2\frac{1}{2}$ hours of fermentation was 64% of that at 30 minutes after initial mixing. In both cases, the concentration

Fig. 7

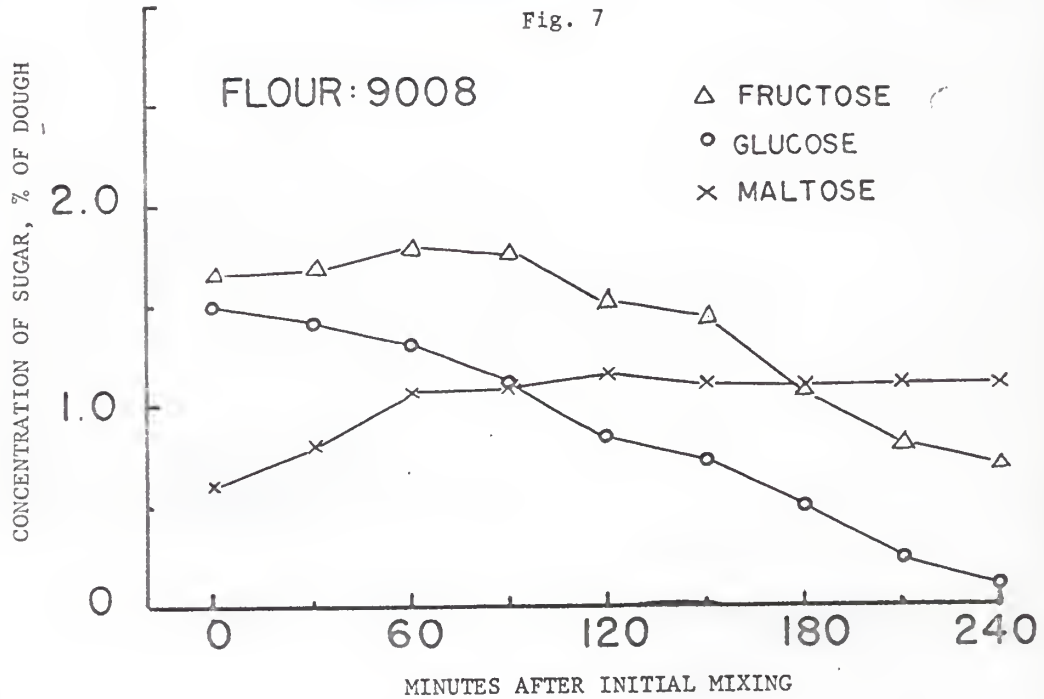
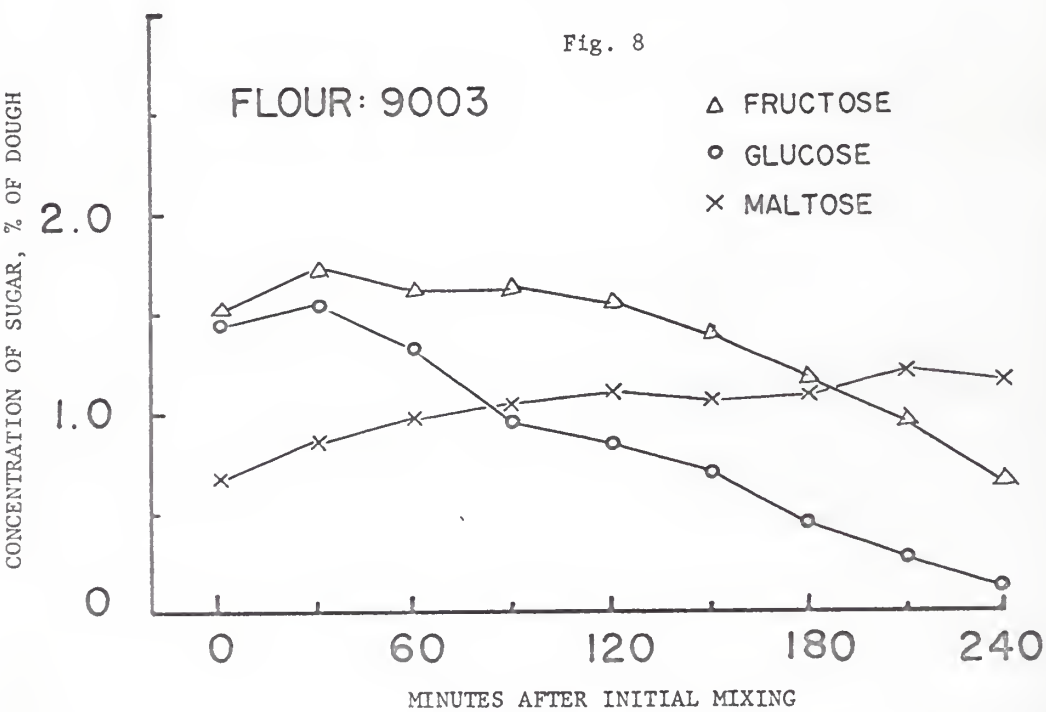


Fig. 8



Figs. 7 and 8: Changes of sugar concentrations during fermentation of straight doughs.

Fig. 9

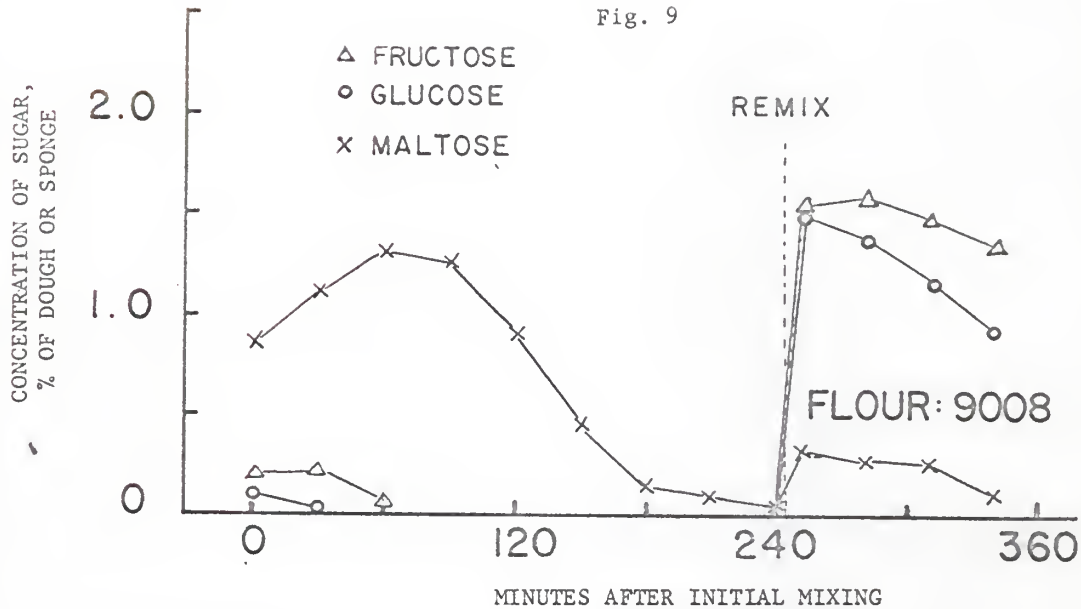
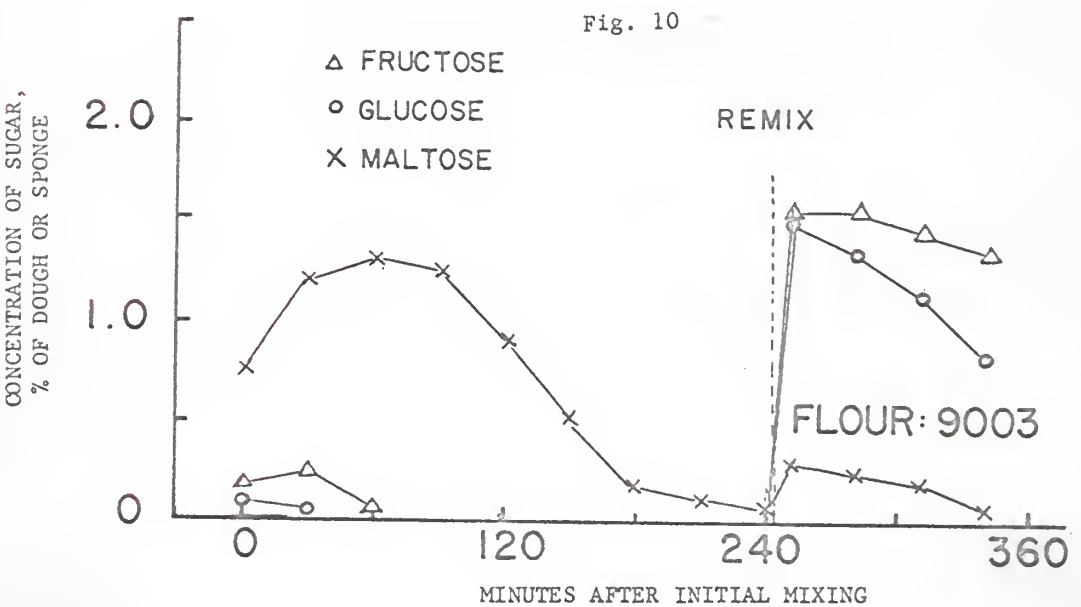


Fig. 10



Figs. 9 and 10: Changes of sugar concentrations during fermentation of 75%-flour sponge and doughs.

Fig. 11

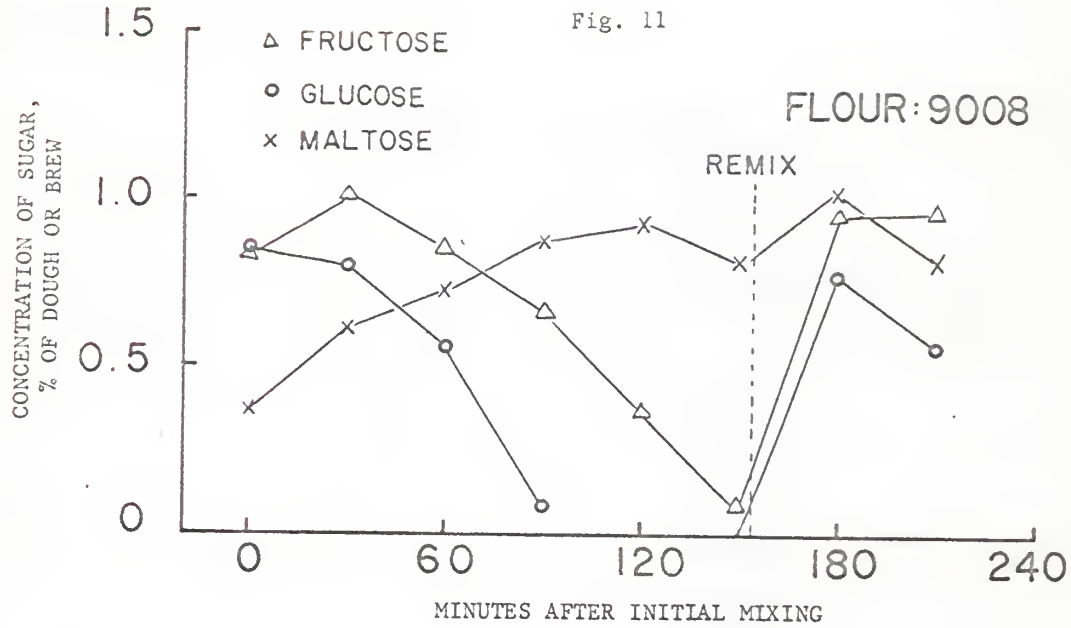
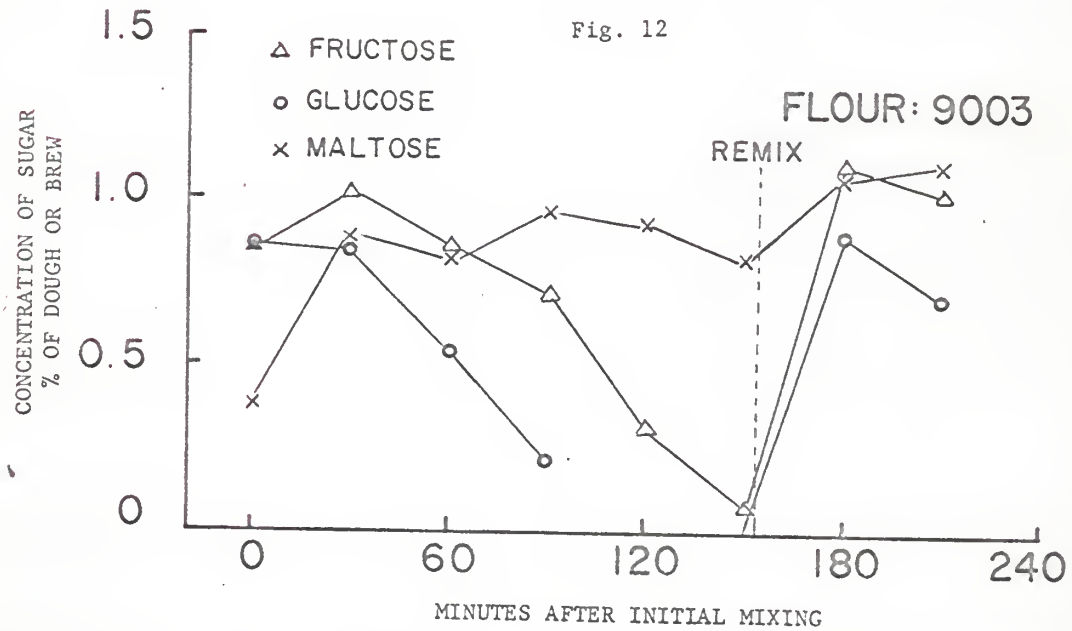
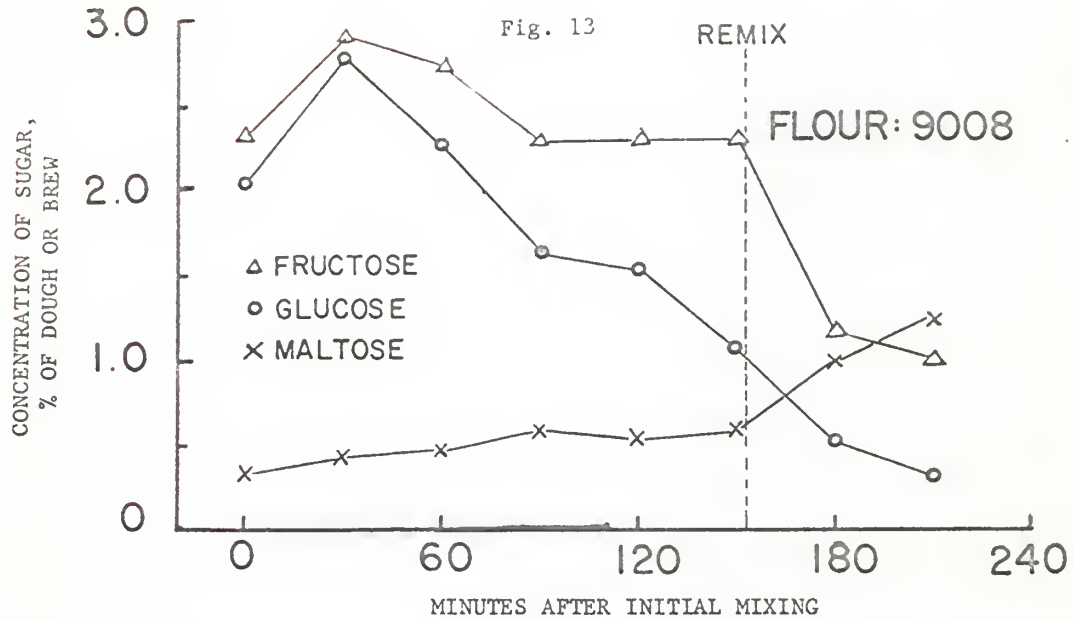
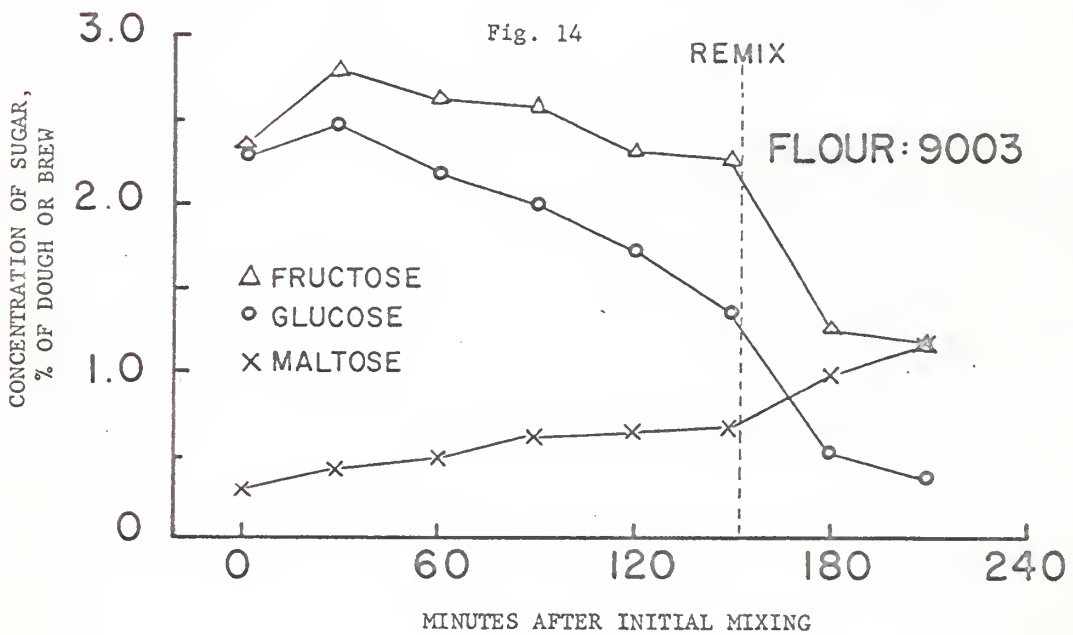


Fig. 12

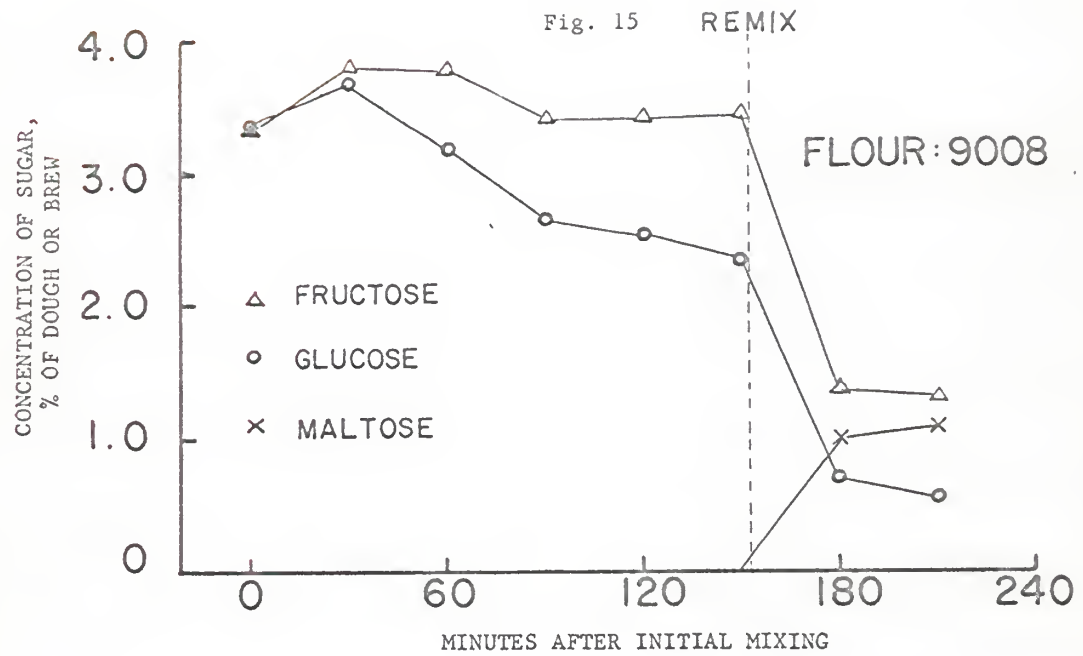


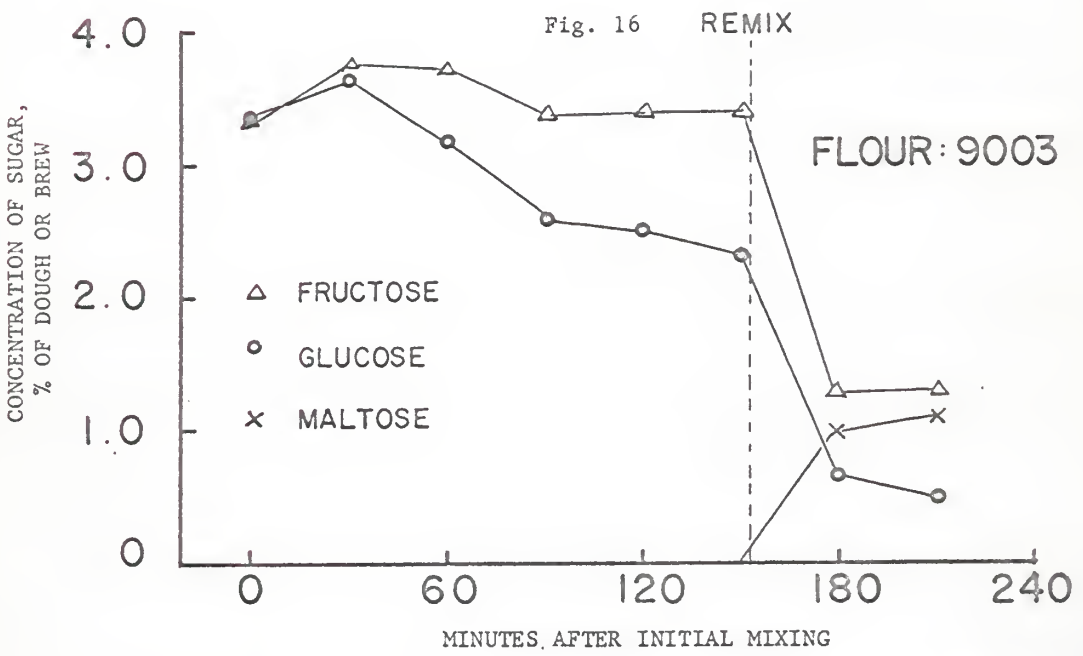
Figs. 11 and 12: Changes of sugar concentrations during fermentation of 50%-flour brews and doughs.





Figs. 13 and 14: Changes of sugar concentrations during fermentation of 25%-flour brews and doughs.





Figs. 15 and 16: Changes of sugar concentrations during fermentation of no-flour brews and doughs.

of fructose decreased only slightly during this period. After remixing, the concentration of maltose in both doughs increased rapidly and soon reached maximum. No decrease of maltose in either case was found.

IV. Rate of Gas Production

The results of the rate of gas production of all doughs, sponges, and brews (means of quadruplicate determinations) are presented in Fig. 17, 18, 19 and 20. All the results were reported in change of gas pressure in mm-Hg in 30 minutes, on a equal yeast number basis.

The gas production rate of sponge during the first 30 min. of fermentation was found to be more than twice that of straight doughs, but fell off sharply during the next 30 min. The absence of salt and added sugar in the sponge apparently provided yeast with an environment from which the assimilation of nutrients was not slowed down by the high osmotic pressure, as is the case of the straight doughs. The sugars for supporting this initial fermentation in sponges are glucose and fructose originally present in flours and that formed from sucrose, glucofructans and other easily-hydrolyzed oligosaccharides. Because of the fact that the total amount of these hexoses and oligosaccharides that existed in flour is small and can sustain the fermentation only for a short time, the exhaustion of these carbohydrates and the inability of yeast to start maltose fermentation immediately caused a sharp drop in the rate of gas production during the second 30-minute period. The drop was less pronounced in sponge of 9003 flour than in that of 9008. This difference was explained by the fact that more total hexose was recovered from the 9003-flour sponge than from that of the 9008 flour. Gas production in sponges reached their peak at 155 minutes after mixing. After that, there was a sharp decrease in gas

production rate, as a result of drastic decrease of maltose concentration in the sponge.

In straight doughs, the gas production rate increased with time at a slower but steady rate. After 150 minutes of fermentation, the rate became more or less constant and dropped slightly at the end of the fermentation period. No sharp dipping in gas production rate, as in that of sponge dough, was observed. This situation was explained by the fact that the more readily fermentable hexoses had never really become exhausted, and that the maltose had not been fermented to any significant extent.

On the same yeast cell number basis, it was found that the brews with higher flour in them produced more gas than those containing less or no flour. Even after remixing, the no-flour brew dough still produced gas at a considerably slower rate than the others. The 50% flour brew produced gas at the highest rate among the liquid brews. As in the case of 75% flour sponge, a dip of gas production at 120 min. after mixing, followed by a sharp recovery, was observed. The dipping of gas production rate corresponded well with the sharp decrease of concentration of total hexoses and the subsequent decline of that of maltose, as shown in Figs. 11 and 12.

The fact that glucose was fermented at a considerably faster rate in brew containing 25% flour than in no-flour brew was discussed in the previous section. Again, the different fermentation rates were shown in the gas production of these two brews. The initial rates did not show much significant difference until about 90 minutes after mixing. The no-flour brews started to decline in their gas production rate, while that of 25% flour brews were still on the increase until about 120 minutes after mixing. The difference continued after dough remixing; however,

Fig. 17

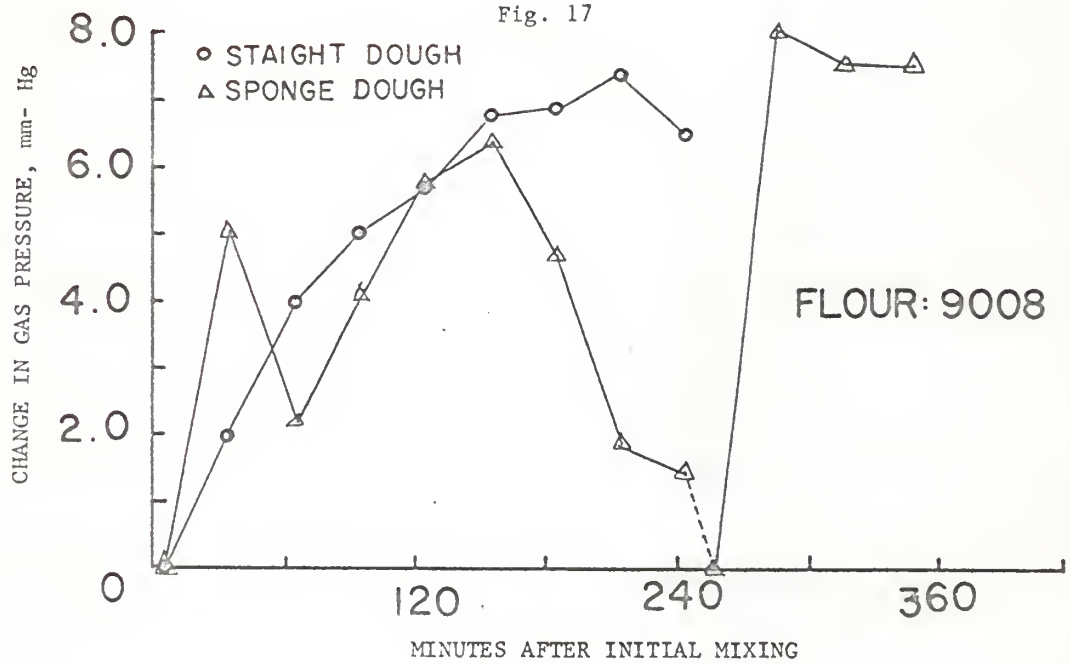
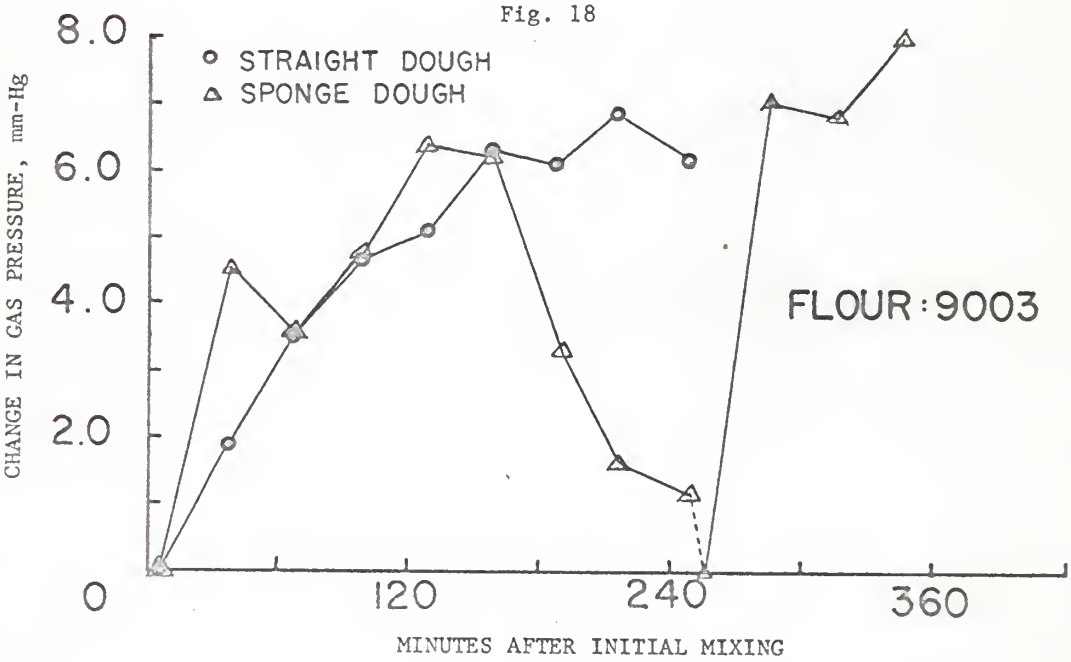


Fig. 18



Figs. 17 and 18: Changes in the rate of gas production during fermentation of straight and sponge doughs, on equal yeast number basis.

Fig. 19

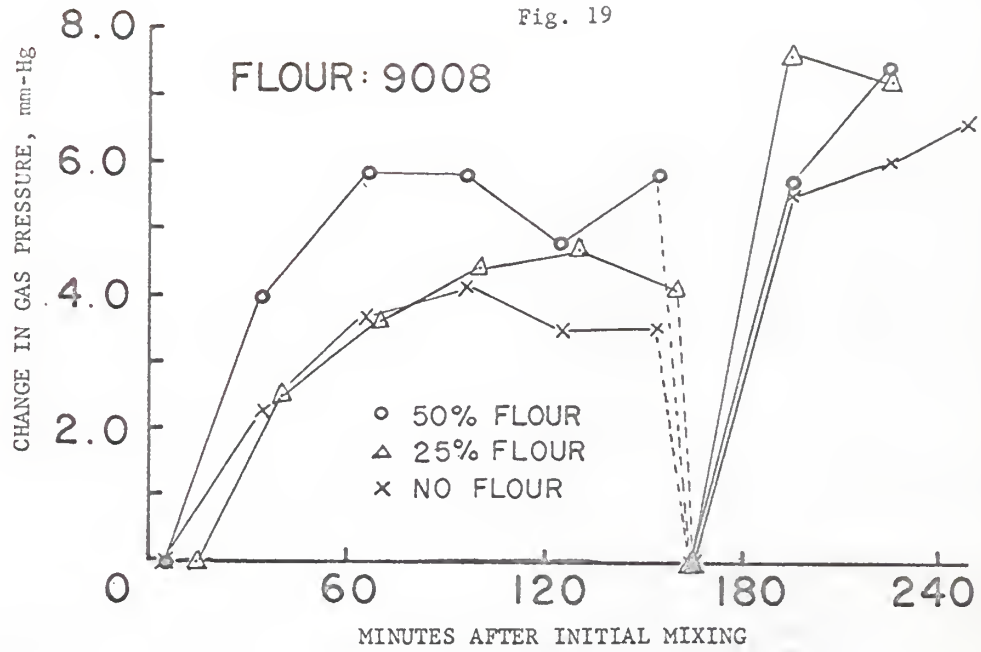
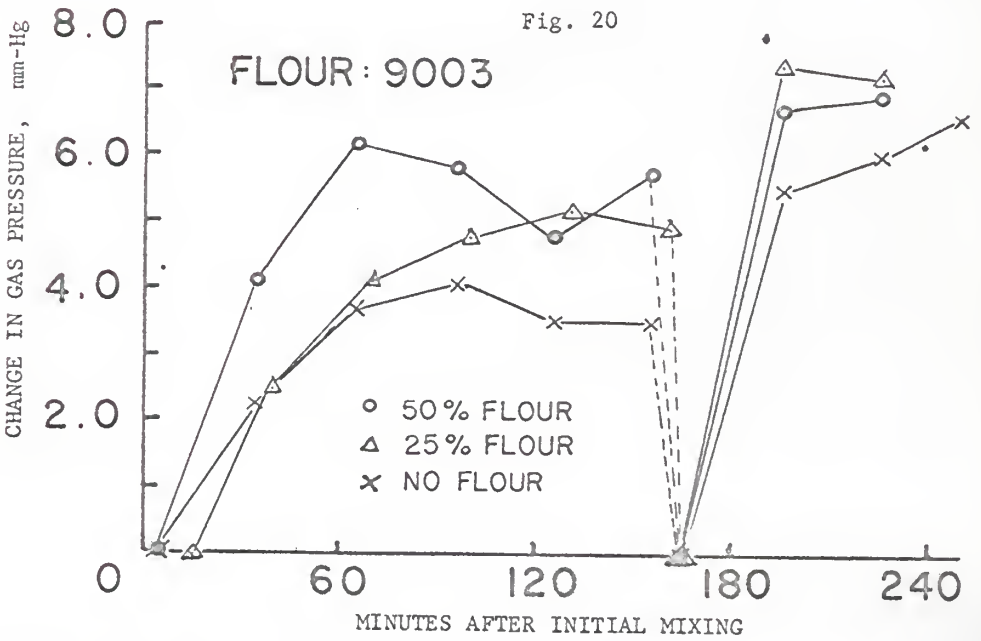


Fig. 20



Figs. 19 and 20: Changes in the rate of gas production during the fermentation of liquid brews and doughs, on equal yeast basis.

the dough from no-flour brew showed a clear trend of rapid increase in rate of gas production.

The different fermentation rates between brews with different flour content are probably due to the differences in osmotic pressure, vitamin content in brew, pH value, etc. Differences in osmotic pressures against yeast in 3 brews tested in the experiment did not seem great enough to explain the difference between the fermentation rates of 25% and no-flour brews. Vitamins, especially thiamine, which are normally present in flour, have been known to have a stimulating effect of yeast fermentation (4). The absence of flour might result in the deficiency of vitamins and/or other growth factors and decreased yeast activities. The pH values of the brews, although not taken in the experiment, were known to be affected by the presence and amount of flour and other buffering materials, e.g. non-fat milk solids, in the brew (7, 60, 118). Johnson et al. (47) has shown that the pH value of a non-buffered sugar brew dropped rapidly after mixing to 3.0 and lower, and a 10% flour (total weight basis) containing brew would maintain a pH at 5.0 for about one hour, and then, decreased sharply to 3.0 or lower. Since low pH, especially at 3.0 or lower, will seriously slow down yeast fermentation, this situation was probably the explanation for the apparently reduced activities of yeast in low or no-flour brew.

V. Residual Sugars in Bread

The amount of principal residual sugars found in crumbs of bread made by different processes are presented in Figs. 21 and 22.

Great variations in content of different sugars in the breads studied were noted. The straight dough bread had a very high maltose content and

Fig. 21

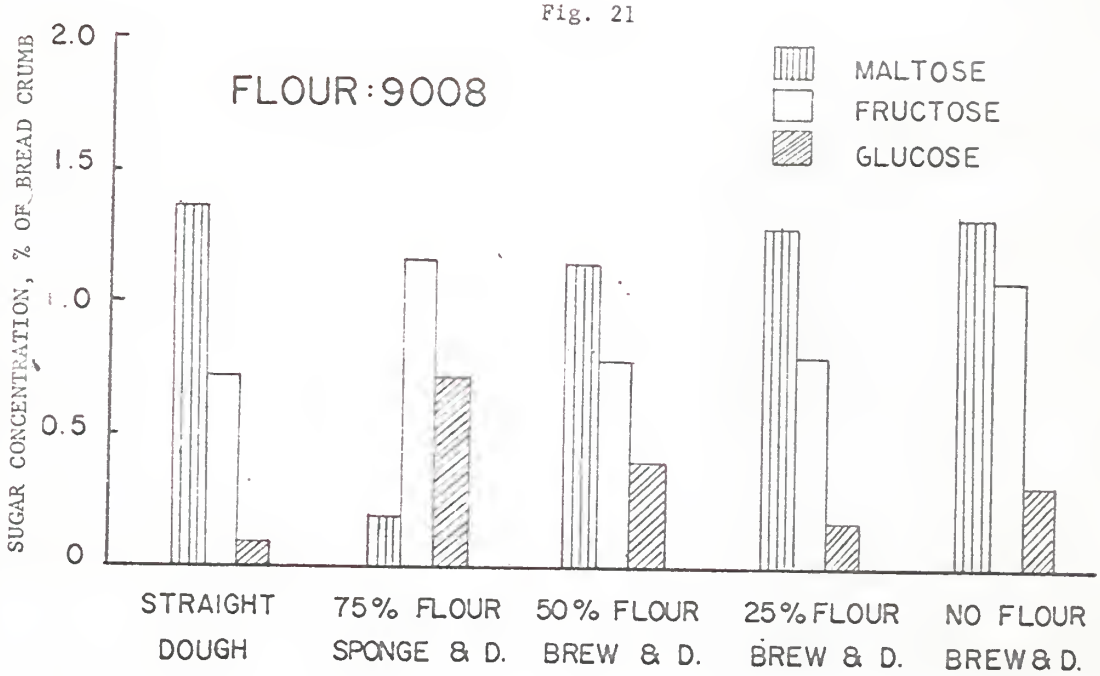
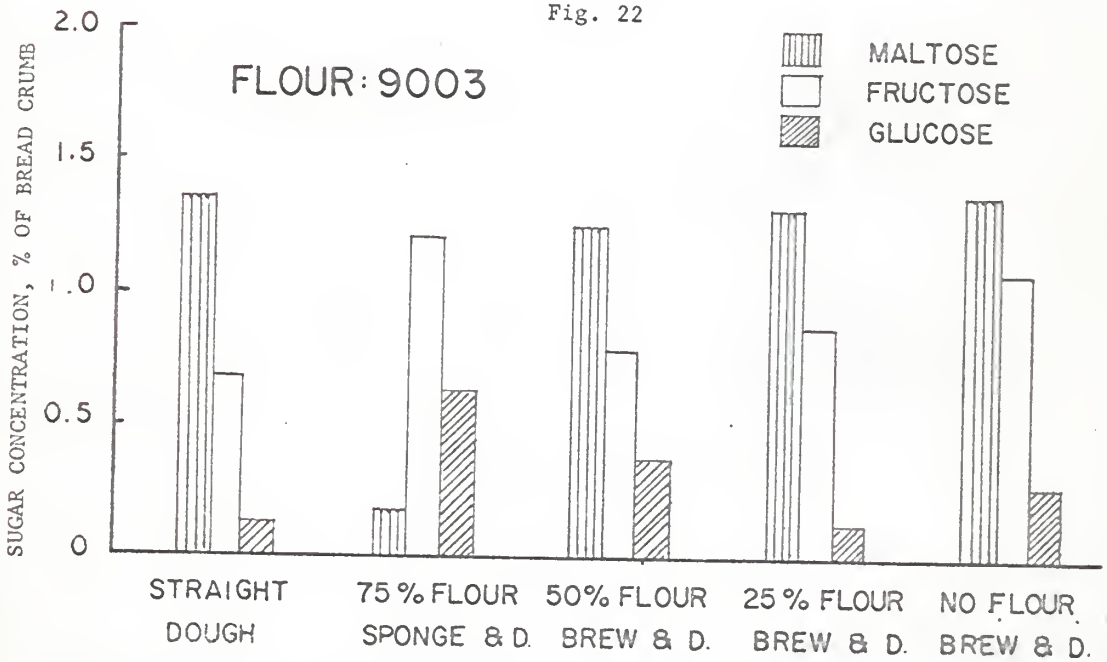


Fig. 22



Figs. 21 and 22: Residue sugars in crumb of breads made by different procedures on the same general formula, calculated or crumb moisture basis.

the lowest total hexose content. On the other hand, bread made from 75% flour sponge and dough process contained the least amount of maltose and had the highest level of total hexoses.

Bread made from brews containing different levels of flour also showed some distinct differences, though they are not as drastic as those between the straight and sponge dough breads, in their variations of residual sugar contents. The no-flour brew bread had the highest total hexoses, as well as maltose, content, a fact which reflects the fact that there was less sugar loss during fermentation. The 25% flour-brew bread contained slightly more maltose, and less total hexoses, than the 50% flour-brew bread.

By and large, maltose content increased during baking, while hexoses decreased in the procedure. The reduction of glucose content during baking was found to be slightly higher than that of fructose.

SUMMARY AND CONCLUSION

A simplified direct photometric method for quantitative determination of sugars in paper chromatograms has been described. The method has an accuracy of ±5% or better for glucose and fructose when the proper amounts of sugar samples are applied. The accuracy for maltose determination is about ±8%. Further improvement of accuracy by standardization of the developing and improving detecting techniques is possible. The main advantages of this method is its simplicity in operation and its reasonable reliability. It is especially suitable for routine analyses.

Determination of pure reducing sugar by Folin-Wu method is highly reproducible, accurate and simple in operation. In the determination of the mixture of reducing sugars, the method appeared to be more sensitive

and reproducible than the ferricyanide oxidation method. The total reducing sugar value per se, however is not always meaningful, especially when there are sugars with different reducing powers present in the same solution. Interpretation of this value, which is usually expressed in terms of maltose or glucose, should be very carefully applied and always with the knowledge of the kinds of sugars which are present in the solution.

The extracting method used in this experiment is simple and effective for routine analysis. Sugar recovery rate is satisfactory, and few interfering substances was extracted.

No significant difference was found between the behaviors of the two different flours in doughs, sponges, and brews. Nevertheless, 9003 flour appeared to have a slightly higher content of either fermentable hexoses or oligosaccharides which are readily hydrolyzed and which yield those hexoses, or both.

Inversion of sucrose during and after the initial mixing was fast, as reported by Koch et al. (52). Nonetheless, the inversion of sucrose was not complete at the end of mixing period as was claimed, and the hydrolysis seemed to proceed more slowly in brews with little or no flour.

The fermentation rates of different doughs, sponges and brews were studied by reducing power, change of concentration of individual sugars and gas production. The results of those different determinations appeared to be in satisfactory agreement with each other.

In liquid brews, the flour level was found to be related to fermentation rate. The fermentation rate of brews with higher flour content was found to be higher than those with less or no flour.

The composition of residual sugars in bread crumb varied tremendously, but was closely associated with the manufacturing procedure. Straight

dough bread was found to have a high maltose content and the lowest total hexose content. Sponge dough bread contains the least maltose and the highest amount of total hexoses among the bread studied. Bread made by brew process contains more total sugar in general, especially in the bread made from no-flour brew. The difference in sugar compositions can be easily explained by the behaviors of individual sugars in different doughs, sponges or brews during the fermentation period.

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QUANTITATIVE CHANGES IN VARIOUS SUGAR CONCENTRATIONS DURING FERMENTATION OF DOUGHS, SPONGES AND BREWS

by

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AN ABSTRACT OF A MASTER'S THESIS

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requirements for the degree

MASTER OF SCIENCE

Food Science
Department of Grain Science and Industry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1968

The changes of the concentration of various sugars during breadmaking in five different procedures were studied and compared in the experiment. The procedures studied were straight dough, sponge dough and liquid brews with three different flour levels, (50%, 25% and no flour). All treatments were based on the same general formula which contained 5.0% sucrose, 2.5% yeast, 2% salt, 3% shortening, 0.5% each of yeast food and malt flour, and water. Gas production measurements were taken every 30 min. throughout the fermentation period. Samples of dough, sponge, or brew were also collected at a 30 min. interval. The sugars in these samples were extracted by boiling aqueous ethanol. Total reducing sugars were determined by the Folin-Wu method and the individual sugars were separated by paper chromatography. The concentration of sugars were determined in situ by photometry in transmitted light. These analytical methods were rapid, simple and reasonably accurate and appeared to be suitable for routine analysis.

Shortly after dough mixing, most of the sucrose was found hydrolyzed into fructose and glucose. Glucose was utilized by the yeast first, a rapid and steady decrease in its concentration was observed in all doughs and brews. Fermentation of fructose proceeded rapidly when the concentration of glucose was lowered. The concentration of maltose increase rapidly immediately after dough mixing and increased during the entire fermentation period, except in the sponge doughs. A noticeable increase in fructose was observed in all flour-containing doughs and brews. This increase was apparently due to the hydrolyses of glucofructans and other oligosaccharides in flour. The fermentation rate of liquid brew was found to be closely related to the amount of flour present. On an equal yeast basis, the brew containing 50% flour produced more gas than the brew containing 25% flour.

The brew containing no flour had the lowest gas production rate.

The kind and amount of the residue sugars recovered from the bread crumb was found to be closely related to the manufacture procedures. The straight dough bread had a high maltose content, a low fructose content, and only a trace of glucose. The sponge dough bread had a trace of maltose and the highest amount of glucose and fructose of all the breads. The sugar composition of the breads produced from liquid brews followed the same trend as noted in the fermentation rate. The no-flour brew bread had the highest content of total residue sugars, and 50%-flour brew bread had the lowest. In general, liquid brew breads contained more residue sugars than both the straight dough and the sponge dough breads.