FERMENTATION STUDIES WITH SOFT WHEAT FLOURS

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INTRODUCTION

With the development of the biscuit, cracker, and breakfast food industries, has come a demand for soft wheat flours of a known high quality. Michigan farmers grow annually between 800,000 and 900,000 acres of wheat most of which is soft winter wheat. Both red and white varieties are grown, the red being favored by certain millers who are making bread flours, and the white by others who are interested in special lines of the pastry flour trade.

Quality in wheat is a rather intangible thing, and may have any of a number of different meanings, depending upon who is using the term. By the producer, quality is considered ordinarily from the standpoint of such factors as plumpness of grain, weight per bushel, freedom from diseases, purity and other factors which go to make up market grade. For the miller, quality also includes flour yield, flour texture, color, etc. From the baking standpoint it has reference to strength of flour, which is closely associated with the amount and nature of the proteins, but is also influenced by the starch content, diastatic activity, and possibly the fats, ash constituents and other factors.

Cereal chemists have been interested for years in the development of a satisfactory measure of baking quality in the various types of wheat, but have as yet achieved only a partial success. This is especially true when only small lots are available for testing, as in the case with new varieties developed in plant breeding work. There is no single chemical test yet available which will give the desired information as to the baking value of a flour. The finding of such a test presents unusual difficulties because of the many factors involved.

Tests in general use include the determination of protein content, viscosity, hydrogen ion concentration, and probably most important of all, the loaf volume, as shown by the experimental baking test. Several of these tests are used generally, one alone not being sufficient for judging the baking value of a flour. These tests were designed principally for the stronger flours, with the idea in mind of producing a good loaf of bread. This is not necessarily an exact criterion of quality in soft wheat flours, since such flours are used largely in the manufacture of pastry products.

A strong highly extensible gluten is desired in bread making, but for the processes involving no fermentation or only a short fermentation period, such a flour would not produce the soft tender flakiness desired in pastry products. On the other hand, it cannot be said that the weaker the gluten the better the flour for pastry use. Eventually some point would be reached at which there would be a lowering in quality. It can be seen then, that high quality in a soft wheat flour may be something different from that in a hard wheat flour, although there is no doubt that there are certain elements in common.

Of all single chemical tests, the protein test is considered generally to be the best indicator of baking quality, especially with the hard wheat or bread flours. In Europe, however, the protein test does not find the general favor that it does in America. Kent-Jones (1929) says that there is a tendency to neglect the protein test, and to place more emphasis upon the diastatic activity of flours. The "maltose figure" described by Kent-Jones (1927) which is used to a considerable extent in England, and is based on the work of Rumsey (1922), is a measure of the reducing sugars (expressed as maltose or as dextrose) originally present and that produced in one hour of diastase activity at a temperature of 27° C. The difference in the views held on the merits of the protein test may be due to the fact that in many European countries, several types of wheats are used quite often in making up the blends for bread making, locally grown soft wheats being mixed with the imported wheats, which are largely bread wheats, from the United States, Canada, Australia, Argentina, or the Union of Socialist and Soviet Republics.

The protein test does not give very satisfactory results in indicating quality in the case of the soft wheats. There are two soft white wheats in Michigan, for example, both of which are relatively low and about equal in protein content. However, flours from these two varieties react quite differently in the bakeshop. The one produces a flour very desirable for the pastry trade while the flour from the other is said to act much like that from fairly strong soft red wheats. It would be very desirable to be able to measure this difference before the flour goes to the bakeshops, or even before it goes to the mill, for a uniform flour of known quality means much in the production of a highly specialized and standardized product for which a great deal of this type of wheat is used.

In 1929 the National Milling Company, of Toledo, Ohio, established fellowships at Michigan State College, Ohio State University and Purdue University, for the purpose of studying quality problems in the production and handling of soft wheats. At this time a fermentation test or expansion test was being used in the National Milling Company's laboratory, in which the expansion of doughs upon fermentation by yeast was measured, under controlled conditions of temperature and humidity. The results obtained indicated that the method might be of considerable value in determining quality in soft wheats. It was to be used for comparing and classifying existing varieties and for testing new varieties developed by plant breeders. The variation of varieties grown under different conditions of environment was to be determined as well. By plotting the expansion of the dough in cubic centimeters against the time of fermentation, "fermentation curves" were obtained, which indicated the strength of the flour. It was found that to some extent, varieties could be classified according to their fermentation curves; also that some varieties showed much greater uniformity in results than others. There seemed to be, however, no explanation for some of the results obtained, particularly the dropping off in volume of expansion of certain samples of a variety which ordinarily showed uniformity. It seemed probable then, that the factors which caused the differences in expansion among the different soft wheat flours were not thoroughly understood.

Accordingly, an investigation of some of the factors influencing the test was made, in an effort to make the expansion test more accurate and usable. The carbon dioxide and oxygen relations in the fermentation of flour suspensions and doughs by baker's yeast were also studied. It was felt that these studies would add to our knowledge and understanding of the actual processes taking place in the fermentation and expansion of doughs, and that the information obtained might aid materially in establishing a more adequate measure of some of the factors which go to make up "baking quality" in the soft wheat flours.

HISTORICAL

The literature on quality of wheat and flour is voluminous and no attempt will be made to present a complete review here. Only a brief background will be given for the work taken up in the present investigation, with citations of some of the work which bears directly on the problem at hand. Bailey (1925) in his book on The Chemistry of Wheat Flour gives a rather complete bibliography on the subject up to 1925. Since then, many papers have appeared and several of these have good literature reviews on different phases of flour strength and its determination. Pasco, Gortner, and Sherwood (1930) give an excellent review of work, particularly in reference to experimental milling and baking. Herd (1931) presents a rather definite picture of the development of ideas concerning strength of flour, in his paper on effect of heat on flour proteins.

Strength of Flour.

The strength of flour has been considered for a long time to be closely associated with the amount and physical nature of the gluten, that gummy portion of the wheat kernel that makes it different and more suitable for making bread than are the other cereal grains. Jago (1895) defined flour strength as "the capacity for producing a bold, large-volumed, well risen loaf". Another definition quite similar is that given by Humphries (1907) as adopted by the National Association of British and Irish Millers, which said that "a strong wheat is one which yields flour capable of making large well-piled loaves". It is easy, from these definitions, to distinguish between hard and soft wheats on the basis of baking quality, for the soft wheats do not in general produce flour which makes such large "well-piled" loaves as do the hard wheats. In this study, however, we are concerned with differences in strength of flours from different soft wheat varieties only, and not from both hard and soft wheats.

The proteins in wheat may be roughly classified into three groups: the gluten proteins of which gliadin is the most important, the non-gluten proteins such as glutenin, and the soluble proteins. It was supposed formerly that the relative amounts of these proteins in a certain flour determined its strength. Ratios of gliadin to glutenin of about 65:35 or of 2:1 were often given as representative of flours which could produce good loaves. Later investigations have shown that the importance of these different protein portions is not very definitely known. Blish (1916) found that the proteins from strong and weak flours seemed to be identical in composition as determined by Van Slykes method. In a later paper Blish (1930) states that high protein wheats usually contain more gluten proteins in proportion to non-gluten proteins than do the low protein wheats. He also says that at the present time gliadin is the only wheat protein whose chemical nature has been established. He believes that glutenin may be a type of protein or perhaps a derived protein, but its individuality is somewhat doubted. Others do not quite agree on the nature of the proteins, for Herd (1931) says that "glutenin has not yet been shown to be as ill-defined as is gliadin". In spite of the lack of agreement as to the nature of the differences in wheat proteins from different flours, it is generally recognized that some difference in quality of proteins does exist, whether it is in the chemical composition or in the colloidal state.

A theory of the colloidal state of doughs is described by Swanson (1925) in which he says, "We have at least two continuous phases, water and the gluten meshwork. In this double continuous phase system are held the starch grains as well as the non-gluten proteins, all covered by a film of water. In this water are found molecularly dispersed salt, sugar and simpler organic compounds, such as amino acids or soluble proteins". He believes that the protein particles form chains or strands which become matted together in the mass known as gluten. The quality of the dough is largely determined by the inherent structure and the environment of this gluten meshwork.

The hydration capacity of the proteins has been thought an important factor in flour strength. It is known that the stronger flours absorb more water when they are made into dough than do the soft flours. However, Newton and Cook (1930) in studying the bound water of flour suspensions found very little difference between soft and hard wheat flours in the hydration capacity of the proteins. They concluded that the starch present in flour bound as much water as did the protein. Alsberg (1927) believed that the starch played an important part in flour strength. Viscosity of doughs and flour suspensions was thoroughly studied by Gortner and Sharp (1924) and they found the colloidal properties of the glutenin to be of extreme importance. In an earlier paper (1923) they showed that viscosity could be used as a measure of hydration capacity, and showed how the physical state of the gluten influenced loaf volume.

Tests for Flour Strength.

The ultimate criterion for baking strength is without doubt the behavior of the flour in the bakeshop. The experimental baking test has been used to predict this but it has some well known disadvantages and limitations, such as, the time required to complete the test, the difficulty in obtaining uniform results, etc. Other tests have been devised for the purpose of supplementing or partially replacing the baking test, and some of these will be mentioned.

Harvey and Wood (1911) described an apparatus for determining baking strength of single ears of wheat, in which they made up flour suspensions and observed their turbidities. The distance through which a small light filament could be seen through a water suspension of the flour was measured and a classification of varieties was made on this basis. Chopin (1927) devised a method for measuring the extensibility of doughs by determining the pressure required to burst a bubble formed by air pressure operating through a small opening against a thin layer of dough. Vilmorin and Chopin (1929) later used this method with considerable success in getting indications of baking quality in small lots of wheat, particularly with wheat obtained from single plants in breeding experiments. Chopin considered the extensibility of dough a measure of gluten quality, and therefore of strength of flour. Dusseau (1930) classified wheat varieties and strains into soft, medium and hard by means of an examination of the alcoholic chlorophyll extract of the fresh wheat leaves. The chlorophyll extracts from the soft wheats were found to absorb more light rays than the extracts from the hard wheats.

Bailey (1916) used an expansion test for determining flour strength in which he measured the expansion of doughs in glass jars under controlled conditions. He found a decided difference between strong and weak flours in the amount of expansion of their doughs. A 4 1/2 hour previous fermentation was given to the strong flours and this period was shortened 30 minutes with the weak flours. Elion (1930) described a fermentation test which he used principally for testing different kinds of yeasts. In fermenting doughs for three or more hours he found that the carbon dioxide given off, corresponded very closely in amount and rate of evolution, to the volume of expansion of the dough, if the dough was punched or kneaded down every half hour. He says that it should be punched just before it starts to lag or slow up in expansion, which usually takes place after the dough has been fermenting for about one hour or a little longer. Schweizer (1930), using a very similar method, obtained results much in accord with Elion. Schweizer concluded that the expansion method could be used to determine the initial speed of fermentation, and with half hour punches to determine the total expanding power of a given yeast, when a flour of definitely known quality was used and the experiment was carried out under carefully controlled conditions.

Fermentation and Yeast Metabolism.

The fermentation of doughs is brought about principally by baker's yeast. Wild yeasts and bacteria are present in flours but when a sufficient amount of baker's yeast is added the reactions are mainly produced by that particular organism. The active principle in causing fermentation is the presence of the fermenting enzyme, zymase. This enzyme was first recognized by Buchner (1897).

He demonstrated that fermentation could be brought about without any living yeast cells if he had present this yeast juice extracted from ground yeast cells. The starch grains present in the flours are broken down to glucose through the action of the enzymes diastase and maltase. The hexoses are readily split by zymase and oxidized to carbon dioxide and water under complete aerobic conditions - under anaerobic conditions alcoholic fermentation takes place - and it is under these conditions that fermentation takes place most rapidly. Stephenson (1930) has expressed a series of reactions to show the breakdown of a hexose as follows:

Complete oxidation

(1) $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ + 674 K.Cals.

Partial oxidation

(2) $2C_6H_{12}O_6 + 9O_2 \rightarrow 6C_2H_2O_4 + 6H_2O$ + 2x493 K.Cals.

Anaerobic break down

(3) $C_6H_{12}O_6 \rightarrow 2C_3H_6O_3$ + 22.5 K.Cals.

$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ + 22 K.Cals.

$C_6H_{12}O_6 \rightarrow 3C_2H_4O_2$ +15 K.Cals.

One of the necessary steps in fermentation as stated by Gortner (1929), is enolization of the hexose. The enol form then breaks down to carbon dioxide and ethyl alcohol, the break coming at the double bond of the enol formula. The important feature in our consideration is that large a-amounts of carbon dioxide are given off and under aerobic conditions large amounts of oxygen are consumed. Brown

(1892) believed that the rate of fermentation was independent of the sugar concentration within wide limits (5-20%), but proportional to the number of yeast cells present. Warden (1921) further showed that the rate of fermentation was independent of sugar concentration between 1 and 10%. He believed also that the action of yeast in fermentation was catalytic - the yeast itself playing a passive role, and the surfaces present acting as catalysts. He used artificial surfaces made of fibrin and sodium oleate, and produced a very similar but slower fermentation effect. He found that under aerobic conditions, yeast uses some sugar and grows rapidly, while under anaerobic conditions it causes a rapid fermentation of sugar solutions, remains viable, but unchanged for considerable time, finally undergoing autolysis and disappearing.

Meyerhof (1925) using the Warburg manometric method, and working with well-washed yeast suspensions of pure culture in a phosphate buffer, measured the oxygen consumed per mg. yeast per hour, and the carbon dioxide given out per mg. yeast per hour. The oxygen consumed was determined in buffer solution and in buffer sugar, first in nitrogen gas and then in air. He showed that the rate of fermentation as measured by the carbon dioxide evolved does not proceed as rapidly in oxygen as it does in anaerobic conditions.

Oxygen and Carbon Dioxide Relations in Doughs.

Maurizio (1902) stated that the volume of the loaf depended not upon the total gases evolved in fermentation but upon the capacity of the dough to retain carbon dioxide to make the bread rise. Using different yeasts he got only slight differences in the carbon dioxide produced even when different flours were used. Wood (1907) believed that the carbon dioxide given off in the later stages of fermentation was what influenced the size of the loaf. Carbon dioxide is lost from doughs at varying rates depending upon the type of flour used. Bailey and Weigley (1922) showed this when strong and weak flours were compared. The weak flours lost carbon dioxide at a greater rate than did the strong flours, the ratio of gas lost to gas produced being 1:6 in strong flours, and 2:6 in weak flours. The carbon dioxide diffusion ratio was studied by Bailey and Johnson (1924) as a measure of the fermentation period. They found that after a lapse of 100 to 180 minutes there was a sudden increase in the loss of carbon dioxide from the fermenting dougs. The time required for this change to take place was correlated with the optimum fermentation period for the flours concerned. In a later paper, Johnson and Bailey (1925) noted that starch added to flours impaired the gas retaining power, but the gas producing power was not lowered. An estimation of the gas produced was made by adding the carbon dioxide lost from the dough to the increase in volume of the dough.

The question then arises as to whether the different soft wheat flours are fermented at different rates. If so, what would be the effect of this difference upon the expansion of the dough in such tests as the expansion test? Would the flour which had the fastest rate of fermentation have the shortest total fermentation period? Also, would there be a difference in the capacity of different soft wheat flours for holding carbon dioxide, combined with the protein, or as bicarbonate, or dissolved in the liquid films surrounding the flour particles? Possibly a difference in the capacity for the retention of carbon dioxide might cause a difference in the expansion rate and the total expansion of different doughs. Bailey and Weigley (1922) suggest that as fermentation progresses, a part of the carbon dioxide becomes dissolved in the dough without effecting expansion, and that such carbon dioxide may constitute a reserve of gas, which would be available later for causing expansion in baking. It would seem likely that a more rapid rate of fermentation would mean a shorter period of fermentation. These questions bring up the idea that there may be other things besides the nature of the gluten which may be equally important in determining the behavior of a dough upon its fermentation. It was in an endeavor to answer such questions more satisfactorily that the studies were made on the carbon dioxide and oxygen relations, as reported later in this manuscript.

The rate of fermentation of different flours may be of considerable importance in determining the expansion of doughs. James and Huber (1928) studied rates of fermentation with clear and patent flours of different types. In working with flour suspensions they found that clear flours fermented more rapidly than did patent flours. They also found that alkalinity decreased yeast growth and added acidity increased the rate of fermentation. The effect of acidity, however, varied considerably with the type of flour used.

The amount of starch and the condition in which the starch granules exist in a flour may have a marked effect upon the rate of fermentation, especially in flour-water suspensions where there is little effect of the gluten quality on the amount of carbon dioxide given off in fermentation. Whymper (1909) in a microscopic study of starch granules, found that large granules were more easily attacked by diastase than were the small granules. The softer flours are considered to have starch granules which are larger, and less intimately associated with the gluten particles. It might be expected then that the softer or weaker flour used, would have a faster rate of fermentation than would the stronger flour. The question arose as to whether such a difference could be shown in the flours under investigation, American Banner and Red Rock.

EXPERIMENTAL

Materials Used.

Flours. The flours used in this study were from two varieties of soft winter wheat common to Michigan - Red Rock and American Banner. These flours show considerable difference in flour strength, the Red Rock being considered as one of the better soft wheat flours for breadmaking, the American Banner being in particular demand for the pastry flour trade.

A ninety pound lot of each variety, which was grown during the 1930 season on the Michigan State College farm, was milled in the Farm Crops Laboratory in an Allis-Chalmers experimental mill. The milling yield, calculated on the basis of the products taken out of the mill, was 70% for the Red Rock and 72% for the American Banner. After milling, each variety was thoroughly mixed and stored in tightly covered tin cans. The results of the analyses of the two flours are shown in Table I, A. O. A. C. methods being used as far as possible.

Table I. Analyses of Flours used.

Determination	American Banner	Red Rock
Moisture	11.55%	11.90%
Protein, N x 5.7 (13.5% H_2O)	9.4 "	10.1 "
Ash	0.43"	0.43"
Wet Gluten	32.4 "	35.5 "
Dry Gluten	11.4 "	12.3 "
Absorption of Water	58.0 "	60.0 "
H ion conc. (pH of water suspension) det'd by hydrogen electrode	5.88	5.71
Loaf Volume*	1630 cc.	1925 cc.

Yeast. A one-pound cake of Fleischman's yeast was delivered to the laboratory once each week, and was kept in the ice box until used. Samples used were weighed directly from the pound cake each day as needed.

The Expansion Test.

Methods.

The basic formula and the procedure used in the study of the expansion of doughs were essentially those used by the National Milling Company in their laboratory at Toledo, Ohio, in what is called the "fermentation test".

*Baking tests by National Milling Company laboratory, Toledo, Ohio.

Several modifications were made, however, and the following descriptions of the formula used and the procedure followed, show the method which was adopted in the study:

Formula.

Flour	150 grams
Yeast	6 "
Sugar	5.25 "
Salt	1.8 "
Water	(According to absorption, making all doughs to approximately the same consistency).

The absorption value is taken as that percentage of water which, when added to a flour in a dough mix, gives to the dough what appeared to be the proper consistency for baking a loaf. It was determined by trial, making up samples with various percentages of water, and judging their consistency by feeling of them, and noting how the samples of dough settled or spread out upon standing. It is a value which may be influenced to some extent by the personal factor.

Procedure. The flour samples were weighed out and kept at 30° C. in the fermentation cabinet for several hours before the test was conducted. Salt and sugar were weighed out for all samples to be run in a test, and dissolved in enough water, so that 50 cc. of this solution would contain the proper amounts of sugar and salt for one flour sample. Six grams of yeast were weighed out for each sample of flour and separate suspensions were made up with 25 cc. of water each. The yeast suspensions were placed in the fermentation cabinet for thirty minutes before using. In mixing, the flour was put into the bowl, then the sugar and salt solution, then the yeast suspension; finally water was added, to make a total water content equal to the absorption value of the flour. A small Hobart electric mixer was used. The ingredients were mixed at low speed for one minute and at medium speed for three minutes, after which the dough was removed, kneaded slightly by hand and placed in a Chidlow expansion jar. It was firmly pressed into the bottom, making sure that all large air bubbles were excluded. A cover glass was placed over the top of the jar, and the jar put into the fermentation cabinet at a temperature of 30° C., and a relative humidity of 80%. During the course of several trials the exact relative humidity did not seem to be of great importance, as long as it was high enough so that the dough remained moist on top, and did not tend to crust over. The cover glass also aided in keeping a uniform moisture condition present. Twelve samples were run at one time, each dough being put into the cabinet as soon as mixed.

The volumes of the doughs were read and recorded every 15 minutes, as fermentation progressed. After one hour the doughs were punched and kneaded down to about their original volume, and again volumes were recorded every 15 minutes during a second rise until a maximum expansion was reached, shortly after which the doughs fell. The volumes reached upon expansion in the second rise were plotted against the time of fermentation, producing "fermentation curves" for the flours or different treatments being compared. Both varieties of flour, Red Rock and American Banner, were used each day. Two samples of each were run as controls using the standard procedure. The other eight samples represented different treatments or procedures, according to just what factor was being studied, four being of each of the two varieties. The results were recorded and calculated in the following manner: Each day the mean of the control samples of each variety was taken as 100% for that variety, and all other samples expressed as a percentage of the controls. After a series of tests were made, extending over a period of several days or weeks, the mean of all the controls for one variety in that series in cubic centimeters was taken as 100% for that variety and all other samples were recalculated back to cubic centimeters from their values which had been expressed as percentages of the controls. In this way the different procedures or treatments were compared, taking into account the variations from day to day which did occur, as shown by the results secured from the control samples themselves.

Results.

Temperature. Tests were run at temperatures of 27°, 30°, and 35° C. obtaining the results given in Table II, the figures given being an average of results from six to nine determinations with each variety used.

Table II. Effect of Temperature on the Rate of Expansion of Fermenting Doughs.

Temp. in degrees C.	Time of previous fermentation	Time required to reach maximum expansion in second rise.	
		American Banner	Red Rock
27	1 hour	2 hrs. 9 min.	3 hrs. 2 min.
30	1 "	1 " 49 "	2 " 18 "
35	1 "	1 " 12 "	1 " 33 "

It will be noted that as the temperature was increased, the time required for the dough to reach its maximum volume decreased. The time of previous fermentation has reference to a preliminary fermentation, or as it is sometimes called, the first rise. This first rise was always terminated by punching and kneading the dough back into almost its original form and volume, in the bottom of the jar, care being taken to exclude all large air bubbles.

The differences in maximum volume which might be reached during fermentation of the two doughs, seemed of importance, so a comparison was made of these differences when the tests were conducted at different temperatures.

Table III shows the results obtained, the values being an average of eighteen determinations for each variety.

Table III. Effect of Temperature on the Difference Between the Maximum Expansion of Red Rock and American Banner Doughs.

Temp. in degreex C.	Difference in cc. between varieties	Max. Volume Red Rock	Max. Volume American Banner
27	261	1114	853
30	284	1140	856
35	248	1136	888

A slightly greater difference between the two flours was obtained when the test was run at 30° C., over that obtained at either of the other temperatures. At 35° C. the fermentation was very rapid, and more difficulty was encountered in maintaining this temperature accurately, due to opening the doors which was necessary in placing the jars into and removing them from the cabinet. At 27° C. the results seemed to be no better than at 30° C., and the time required to complete a test was considerably more, so 30° was adopted as the standard temperature for all succeeding tests.

Moisture Content of Doughs. The influence of the moisture content of doughs on the expansion test was determined by making up doughs to several different moisture contents, and recording the expansion which occurred upon fermentation. In these determinations 150 grams of flour were used and the amount of water added ranged from 54% to 62% of the flour weight in the case of the American Banner, and 56% to 64% in the case of the Red Rock. The absorption value for the American Banner flour was considered to be 58% and for the Red Rock flour 60%. These amounts of water (58% and 60% respectively) added in the dough mix made doughs of the proper consistency for handling, and were used in all other determinations of the expansion test. When less water was used, the doughs tended to be dry and lacked smoothness; when very much more was used the doughs were so wet as to make handling very difficult. The results obtained are given in Table IV.

Table IV. Effect of Moisture Content of Dough on Expansion During Fermentation.

Kind of Flour	Per Cent H_2O Added	Time of First Rise	Time of Second Rise	Corrected Maximum Volume
American Banner	54	1 hr. 15 min.	2 hr. 0 min.	897 cc.
" "	56	" "	2 " 0 "	943 "
" "	58	" "	1 " 20 "	952 "
" "	60	" "	2 " 0 "	952 "
" "	62	" "	1 " 15 "	952 "
Red Rock	56	" "	2 " 15 "	1111 "
"	58	" "	2 " 0 "	1195 "
"	60	" "	2 " 0 "	1195 "
"	62	" "	2 " 0 "	1215 "
"	64	" "	1 " 45 "	1205 "

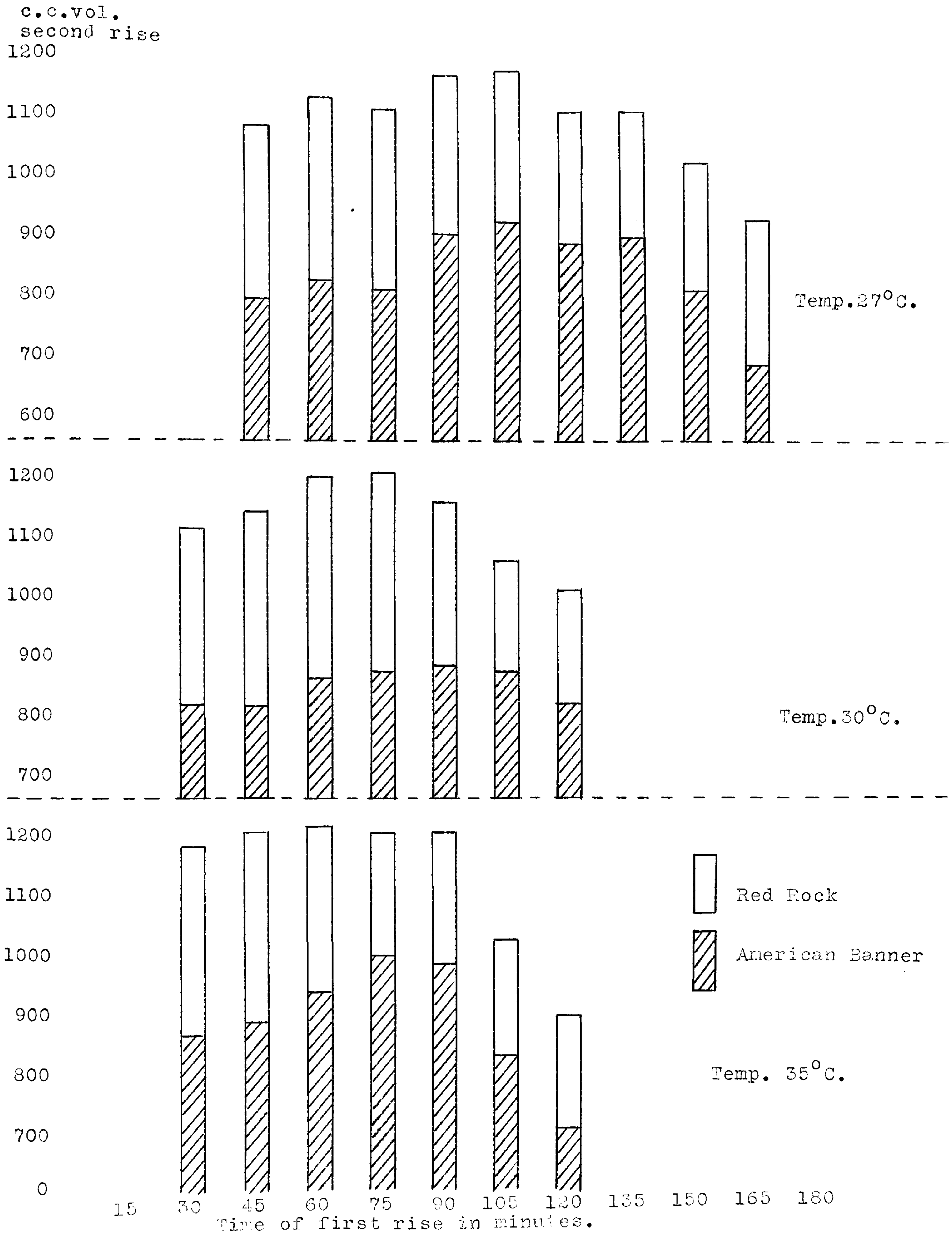
When 4% less water than the absorption value were used, the expansion was reduced considerably. An increase in water added, above 56% in the case of American Banner and 58% in the case of Red Rock, did not materially affect the maximum volume reached by the dough in the second rise. The rate of fermentation, however, appeared to be faster as the moisture content was increased.

Time of First and Second Rise of Doughs. As was mentioned before, the doughs were allowed one hour of fermentation and then punched, terminating what is known as the first rise. The expansion from that point on until the dough reached its maximum volume, is called the second rise. The effect of variations in the time of the first rise of doughs, upon the maximum volume reached during the second rise was determined at the three temperatures afore mentioned. The data are shown in Table V, the values representing the maximum volume reached during the second rise. It is shown in this table that there was a definite first rise period for each of the temperatures used, with which the maximum expansion during the second rise could be obtained. This is also shown graphically in Plate I. The varieties differ somewhat, as might be expected, in respect to the first rise period which produced the maximum expansion in the second rise. The Red Rock had the maximum second rise at 27° C. with a first rise period

Table V. Expansion of Doughs at Different Temperatures as Affected by Time of First Rise.

Time of First Rise	American Banner			Red Rock		
	cc. vol. at 27°C.	cc. vol. at 30°C.	cc. vol. at 35°C.	cc. vol. at 27°C.	cc. vol. at 30°C.	cc. vol. at 35°C.
30 min.		827	871		1124	1187
45 "	810	819	891	1101	1149	1207
60 "	844	871	940	1154	1209	<u>1217</u>
75 "	825	880	<u>1001</u>	1134	<u>1221</u>	1207
90 "	920	<u>888</u>	986	1189	1173	1207
105 "	<u>937</u>	880	830	<u>1196</u>	1076	1028
120 "	903	827	700	1131	1028	898
135 "	913			1131		
150 "	825			1044		
165 "	699			945		

Plate I. Effect Of Time Of First Rise On Expansion (second rise).



of 105 minutes; at 30° C. with a first rise of 75 minutes; and at 35° C. with a first rise of 60 minutes. The American Banner produced its maximum second rise at 27° C. when given a first rise of 105 minutes; at 30° C. when given a first rise of 90 minutes; and at 35° C. when given a first rise of 75 minutes. If the dough was punched at these definite periods a maximum expansion during the second rise was obtained. When this first rise was allowed to continue longer the second rise was not as great, as is shown in the graphic representation.

Rate of Expansion of Doughs at 30° C. The rate of expansion of the two flour doughs was determined and Tables VI and VII show the results when a first rise of one hour, and one hour and fifteen minutes respectively were previously given. The values given are an average of 6 - 9 determinations. It will be noted that the American Banner reached its maximum volume in 105 minutes when a previous fermentation of one hour was given, and in 90 minutes when a previous fermentation of 75 minutes was given. The Red Rock, however, reached its maximum volume in 120 minutes with a previous fermentation of one hour, and in 165 minutes when a previous fermentation of 75 minutes was given to the dough. The rates of expansion of the two flours are shown in graphic form in Plates II and III. The striking feature was that the American Banner reached its maximum volume much sooner

Table VI. Rate of Expansion of Doughs at 30°C.
Doughs Previously Fermented One Hour.

Time of Fermentation Second Rise	Total Volume		Increase in Volume		% Increase in Volume	
	Red Rock	American Banner	Red Rock	American Banner	Red Rock	American Banner
0 min.	200	200				
15 "	393	380	193	180	96.5	90.0
30 "	576	551	376	351	188.0	175.5
45 "	740	703	540	503	270.0	251.5
60 "	901	817	701	617	350.5	308.5
75 "	1028	828	828	628	414.0	314.0
90 "	1116	842	916	642	458.0	321.0
105 "	1159	858	959	658	479.5	329.0
120 "	1191	851	991	651	495.5	325.5
135 "	1184	834	984	634	492.0	317.0
150 "	1150		950		475.0	

Table VII. Rate of Expansion of Doughs at 30°C.
Previous Fermentation 75 Minutes.

Time of Fermentation	Total Volume		Increase in Volume		% Increase in Volume	
	Red Rock	American Banner	Red Rock	American Banner	Red Rock	American Banner
0 min.	200	200				
15 "	383	382	183	182	91.5	91.0
30 "	558	553	358	353	179.0	176.5
45 "	713	710	513	510	256.5	255.0
60 "	860	810	660	610	330.0	305.0
75 "	997	845	797	645	398.5	322.5
90 "	1077	873	877	673	438.5	336.5
105 "	1113	868	913	668	456.5	334.0
120 "	1165	857	965	657	482.5	328.5
135 "	1172		972		486.0	
150 "	1172		972		486.0	
165 "	1182		982		491.0	
180 "	1157		957		478.5	
195 "	1145		945		472.5	
210 "	957		757		378.5	

Plate II. Expansion Of Doughs During Fermentation.

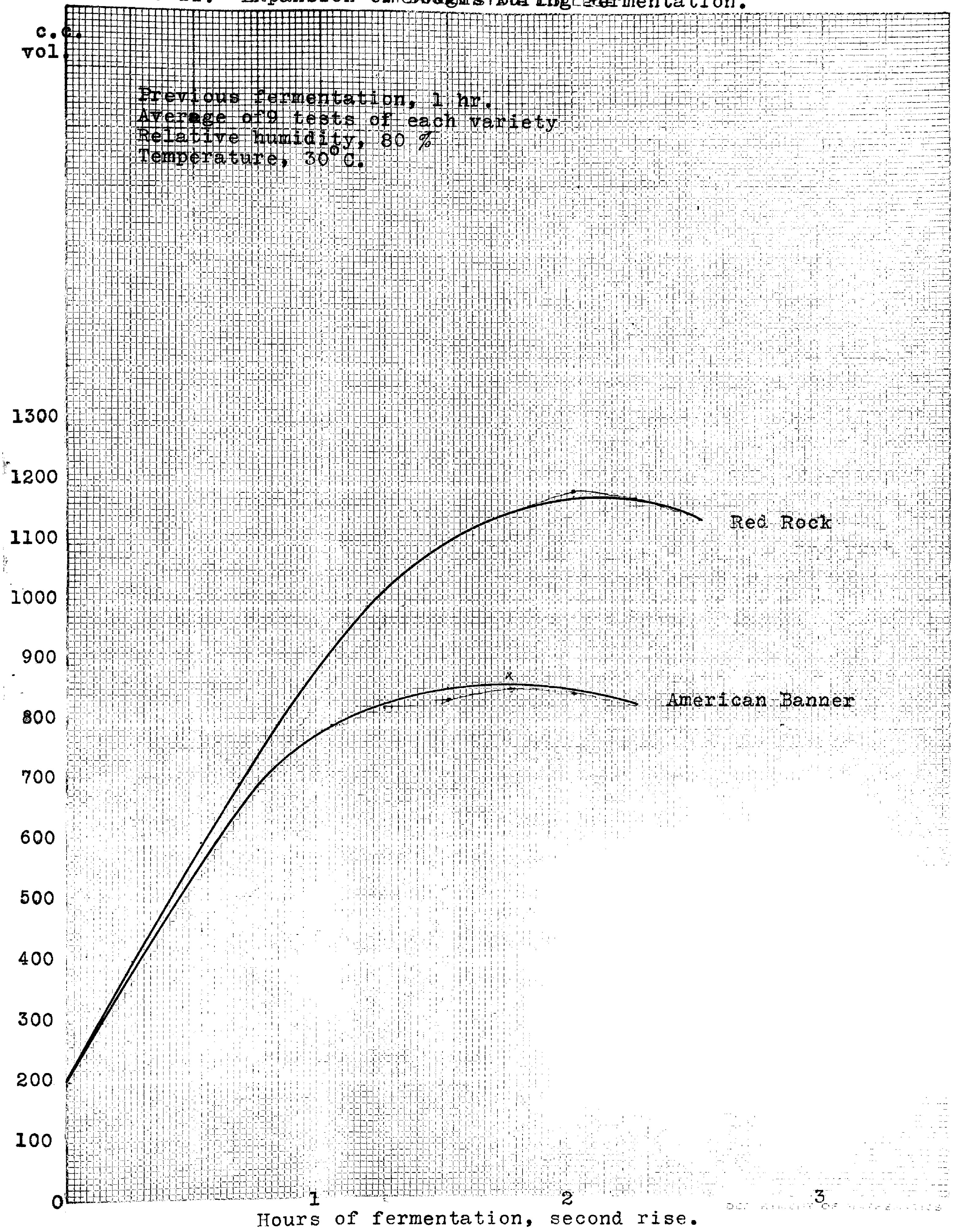
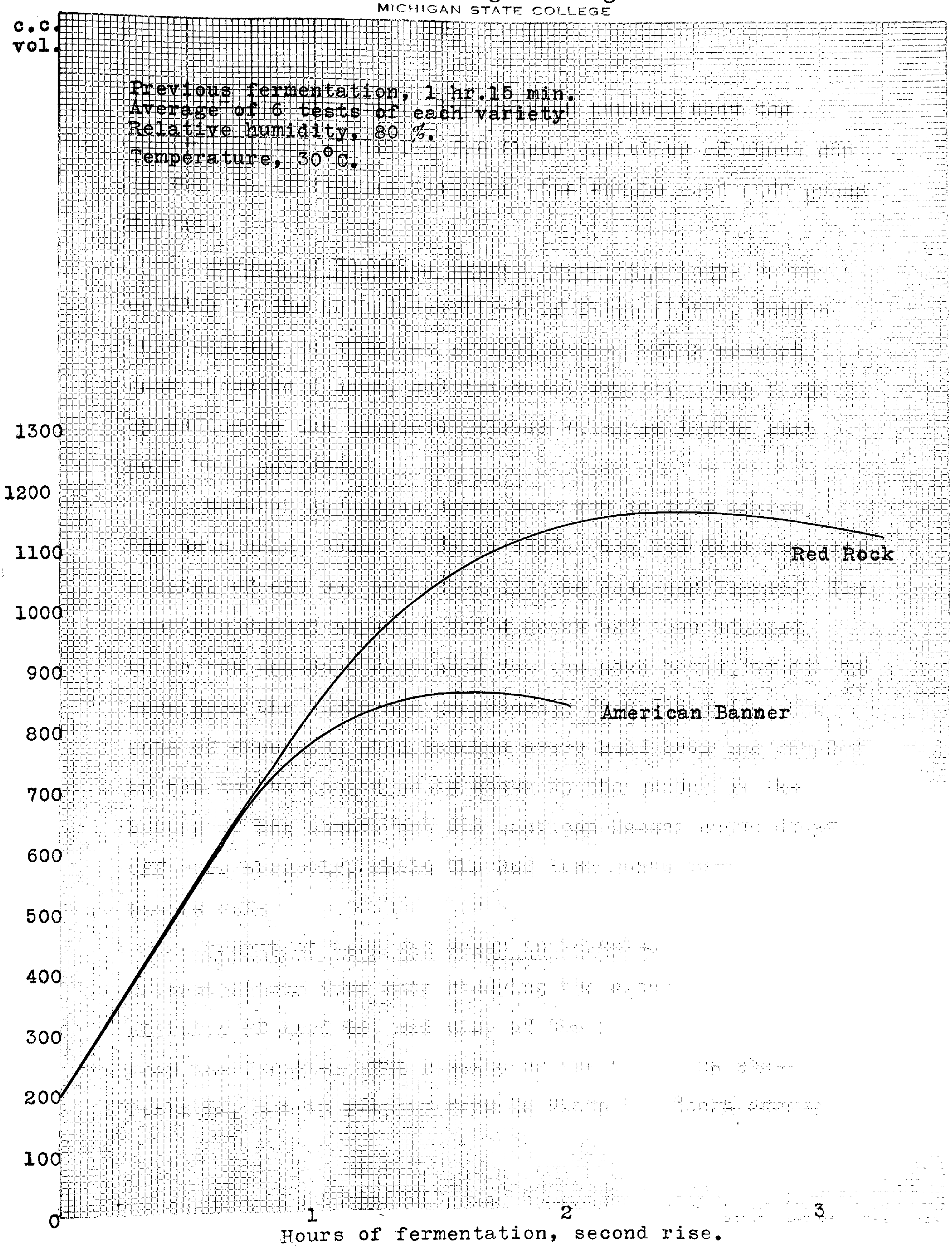


Plate III. Expansion Of Doughs During Fermentation.



than did the Red Rock. The volumes reached show the characteristic variation for these varieties of about 250 to 300 cc. difference with the size sample used (150 grams flour).

Effect of Punching Doughs Every Half Hour. — According to the method described by Elion (1930), doughs were allowed to rise for several hours, being punched down every half hour, and the total expansion was found by adding up the separate volumes obtained during such half hour periods.

Several expansion tests were run in this manner, the data being shown in Table VIII. The Red Rock expanded a total of 420 cc. more than did the American Banner. The American Banner expanded for 4 hours and then stopped, while the Red Rock continued for two more hours, as can be seen from the table and graphically from Plate IV. The rate of expansion when punched every half hour was similar in the two varieties as is shown by the curves at the bottom of the sheet, but the American Banner curve drops off more abruptly, while the Red Rock curve very gradually tapers off.

Effect of Lard and Sugar in Formula. —— Several determinations were made studying the effect of the addition of lard to, and also of the omission of sugar from the formula. The results of the tests are shown in Table IX, and in graphic form in Plate V. There seemed

Table VIII. Expansion of Doughs Punched Every Half Hour. Temp. 30° C.

Half Hours Fermentation	Expansion of American Banner in cc.	Expansion of Red Rock in cc.
1	210	210
2	340	360
3	380	390
4	380	390
5	330	360
6	260	290
7	140	230
8	30	120
9	0	60
10		40
11		25
12		15
13		0
Total increase in volume	2070 cc.	2490 cc.
Increase in Red Rock over American Banner		420 cc.

American Banner Expanded for 4 hours, Red Rock 6 hours.

Plate IV. Expansion Of Doughs During Fermentation.

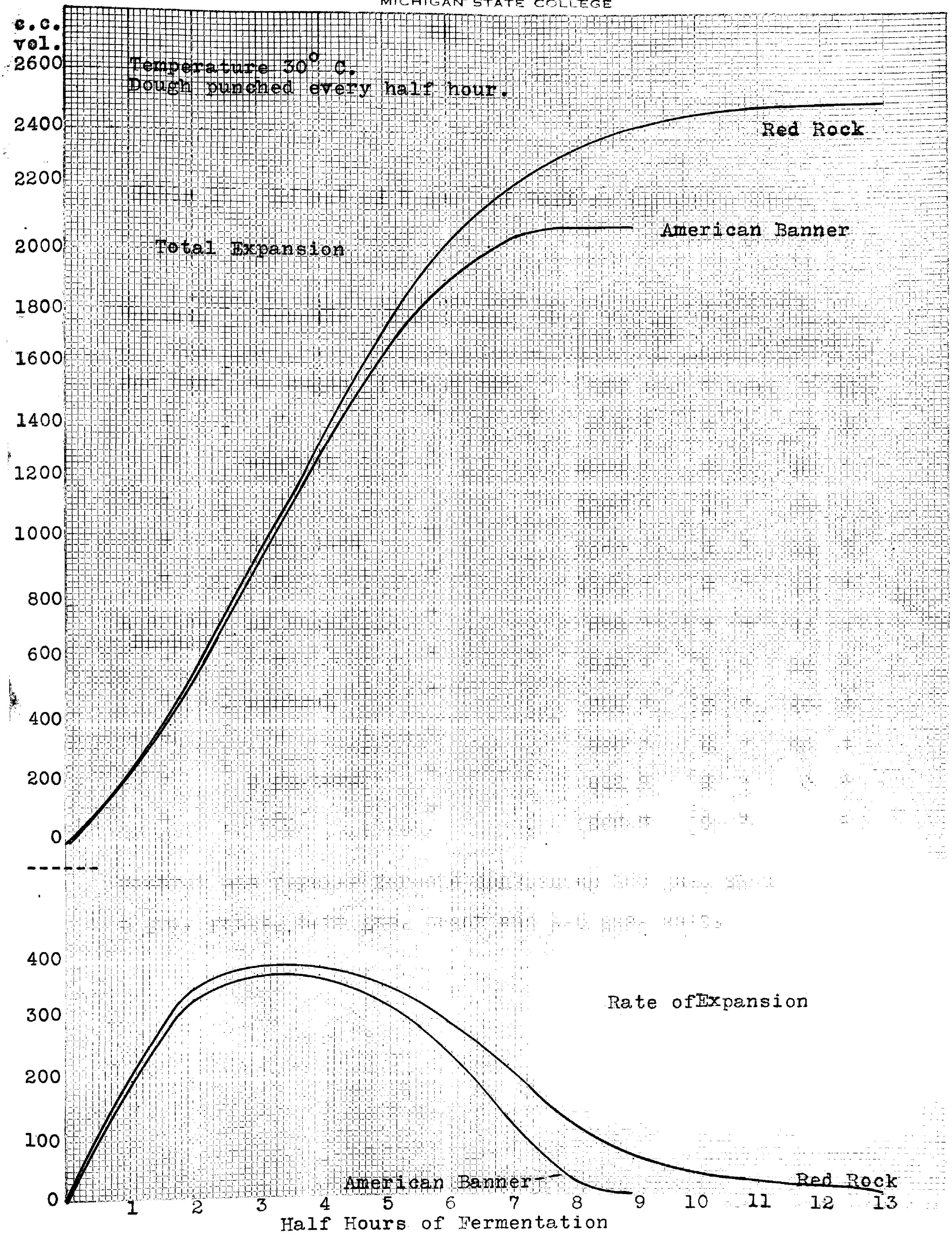
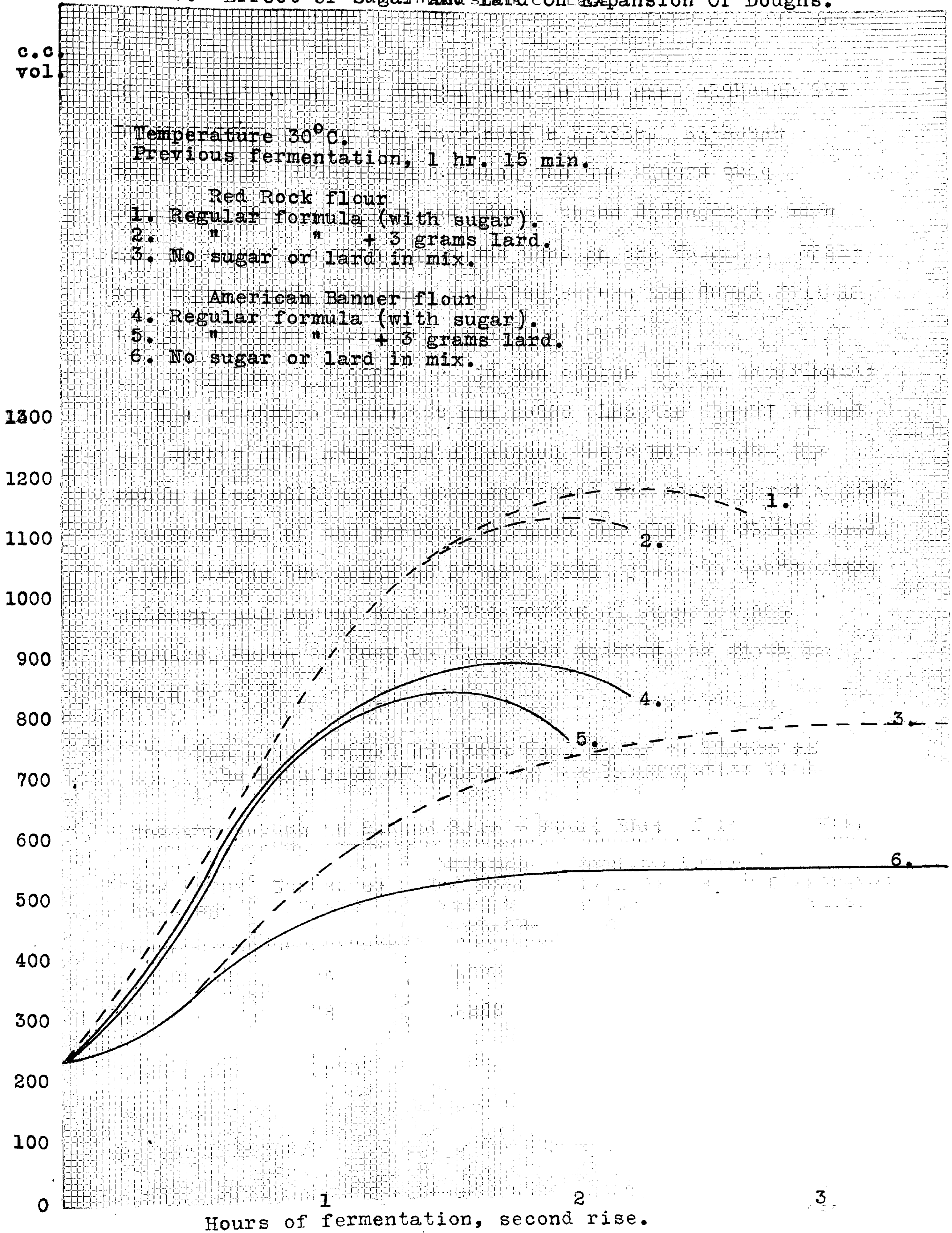


Table IX. Effect of Sugar and Lard on Expansion Test.

Variety	Formula	First rise	Max. volume second rise	Total time of fermentation
Red Rock	no sugar	1 hr.15 min.	800 cc.	7 hrs. 0 min.
"	"	"	820 "	7 " 0 "
"	control	"	1200 "	4 " 40 "
"	"	"	1180 "	4 " 40 "
"	3 gm.lard	"	1140 "	4 " 20 "
"	"	"	1150 "	4 " 15 "
American Banner	no sugar	"	560 "	7 " 15 "
"	"	"	560 "	7 " 10 "
"	control	"	900 "	3 " 40 "
"	"	"	880 "	3 " 45 "
"	3 gm.lard	"	820 "	5 " 0 "
"	"	"	860 "	5 " 0 "

Control was regular formula containing 150 gms. flour, 6 gms. yeast, 5.25 gms. sugar and 1.8 gms. salt.

Plate V. Effect Of Sugar And Lard On Expansion Of Doughs.



to be no advantage in adding lard to the mix, although its fermentation period was shortened a little. Although fairly large differences between the two flours were obtained, when no sugar was added, these differences were not as marked as when sugar was used in the formula. Without sugar added, the time required before the dough fell in the second rise increased considerably.

Aging of Flours. — In the course of the experiments on the expansion tests, it was noted that the flours seemed to improve with age. The expansion tests were begun one month after milling and were continued for about three months. A comparison of the results obtained for the two flours used, first during the month of October 1930, just one month after milling, and second during the months of December and January, three to four months after milling, is given in Table X.

Table X. Effect of Short Time Aging of Flours on the Expansion of Doughs in the Fermentation Test.

Maximum Volume in Second-Rise - First Rise of 1-1 1/4 Hrs.

Time after milling	Number of tests	Maximum Red Rock volume in cu.cm.	Maximum American Banner volume in cu.cm.	Difference in cu.cm.
1 month	15	1208	876	332
3-4 "	12	1232	974	258

The data indicate that after the first month the American Banner flour improved considerably more than did the Red Rock flour. This is evidenced by the greater proportionate volume increase obtained in the tests, by the American Banner after 3-4 months of storage as compared with the volumes obtained one month after milling. The difference between the volumes obtained with the two varieties dropped from 332 cu.cm. to 258 cu.cm., the tests being run as nearly alike as possible in all cases. The temperature for all tests was 30° C.

Yeast Metabolism Studies.

Methods.

In these studies the methods used were those described by Warburg (1924, 1925, 1926). He used the Barcroft Warburg Manometric apparatus for determining the respiration and glycolysis of cells and tissues, obtaining accurate measurements of cell metabolic processes. It seemed likely that the method could be adapted to the measurement of the carbon dioxide and oxygen relations in the fermentation of flour suspensions and doughs by yeasts. The principle of the method briefly is as follows: a closed vessel, partially filled with a suspension of yeast cells and flour (furnishing carbohydrates for the energy source for the yeast), is connected with a manometer. The manometer shows a change in pressure due to the evolution or disappearance of a gas. The vessel is kept at constant temperature in a water thermostat, by being moved back and forth through the water. The amount of gas evolved or taken up can be calculated by means of the following equation:

$$x = h\left[\frac{V_G \frac{273}{T} + V_F \cdot \alpha}{P_o}\right]$$

h = manometric reading in mm. at constant volume.

P_o = normal pressure of the confining liquid (Brodie's solution used, 10, 000 = 760 mm. of mercury).

V_G = volume of gas space to meniscus of the liquid in the manometer.

T = absolute temperature of thermostat.

V_F = volume of suspension in vessel.

α = Bunsen absorption coefficient of the gas which arises or disappears.

The volumes V_G and V_F are in cu.mm. so that the value for x is obtained in cu.mm. The enclosed expression K is always positive, so that when a gas arises, the x is positive and when a gas disappears the x is negative. The value for K will be influenced by the amount of suspension used, the initial volume of the vessel and the nature of the gas present, the α for different gases being different.

When x has been determined, if the dry weights of yeast and flour are known, the rate of gas evolution or disappearance can be calculated in cu.mm. per hour per unit dry weight of yeast and flour. The oxygen consumption in respiration of yeast in different flour suspensions, the carbon dioxide evolved in anaerobic fermentation, the combined carbon dioxide retained by flour suspensions, and the combined effect of the oxygen consumption and carbon dioxide evolution or metabolism of the yeast can thus be determined by the use of this method.

<u>Preparation of yeast</u>. — The yeast used in these studies was obtained as described under "Materials used". A small amount of yeast 0.15 or 0.30 grams (wet weight) depending upon the particular experiment, was washed with a buffer solution (M/20 KH_2PO_4 having a pH of 5.6 - 5.8) twice and centrifuged twice. It was then made up to 50 cc. in a volumetric flask. One cc. portions of this suspension were generally used, always taking portions of the same suspension for comparative experiments conducted on any one day. At the same time an aliquot portion was dried in the oven to obtain the oven dry weight of the yeast used.

<u>Flour suspensions</u>. — Flour suspensions were made up freshly each day, by thoroughly mixing 15 grams of flour with 50 cc. of distilled water. The suspensions were pipetted into the vessels of the apparatus, and it was found that rather uniform samples were obtained by pipetting if extreme care was taken in stirring the suspensions well during the withdrawal of the sample. Dry weights on the flour in the samples used were determined by drying the vessels in the oven after the test was completed.

Gases used. — Oxygen, nitrogen, and carbon dioxide were used to secure the desired conditions in the apparatus: the suspensions being saturated in the vessels by passing gas through them for from 5 to 10 minutes. The carbon dioxide and oxygen were taken directly from pressure tanks by means of a rubber tube attached to the stop cock on the upper part of the manometer. The nitrogen was de-oxygenated by passing it over red hot reduced copper just before it entered the manometric apparatus. In determining yeast metabolism or the combined carbon dioxide and oxygen relations, nitrogen and oxygen containing 5% (by volume) of carbon dioxide were used. The mixtures were made up in gasometers and analysed for carbon dioxide by means of the Haldane apparatus.

For the carbon dioxide retention experiments several air-carbon dioxide mixtures were prepared - also made up in the gasometer and analysed for carbon dioxide content.

Procedure.

Oxygen Consumption in Yeast Respiration in Flour Suspensions.

In the determination of the comparative rates of oxygen consumption by yeast in flour suspensions, four Warburg vessels were used as follows:

1. 2 cc. water only - used as a thermobarometer.
2. Control. 2 cc. of yeast suspension in buffer. 0.2 cc. of 5% NaOH in center well.

3. 1 cc. American Banner flour suspension + 1 cc. yeast suspension. NaOH in well.

4. 1 cc. Red Rock flour suspension + 1 cc. yeast suspension. NaOH in well.

In this determination, 0.3 gm. of yeast was used to make up the yeast suspension, preparing it as was previous described. The 5% NaOH solution was placed in the center wells of the vessels to absorb the carbon dioxide evolved in the respiration process. After the vessels had been partially filled with the proper suspensions, pure oxygen gas was run through the apparatus for 5 minutes, saturating the suspensions. Then the vessels with manometers attached were placed in position in the water thermostat at 30° C. and set in motion and the contents allowed to come to equilibrium, which took about 15 minutes. Zero readings were taken, and the vessels again set in motion. Readings were then taken at 5 and later at 10 minute intervals, until there was a constant rate of oxygen consumption as observed by the change in pressure in the manometers. Readings were always taken with the volume V_G constant. This was made possible by an adjusting screw which could be used to raise or lower the liquid level in the manometer. The consumption of oxygen was calculated from the following equations as given by Warburg (1926):

$$x_{O_2} = h_{O_2} \cdot K_{O_2}$$

where $$K_{O_2} = \left[\frac{V_G \frac{273}{T} + V_F \cdot \alpha}{P_o} \right]$$

The oxidation quotient in cu.mm. per hour in oxygen,

$$(Q_{O_2}) = \frac{xO_2}{\text{yeast wt.}\cdot\text{flour wt.}\cdot\text{time in hours}}$$

<u>Carbon Dioxide Evolution in Fermentation</u>. — The rate of fermentation of flour suspensions by yeast was determined both with and without sugar added. Six vessels were used as follows:

1. Water only - as a thermobarometer.
2. 1 cc. of yeast suspension + 1 cc. water.
3. 1 cc. of yeast suspension + 1 cc. of American Banner flour suspension + 0.2 cc. of 10% glucose solution.
4. 1 cc. of yeast suspension + 1 cc. of Red Rock flour suspension + 0.2 cc. of 10% glucose solution.
5. 1 cc. of yeast suspension + 1 cc. American Banner flour suspension. No glucose.
6. 1 cc. of yeast suspension + 1 cc. Red Rock flour suspension. No glucose.

The suspensions were saturated with nitrogen gas which was de-oxygenated by passing it over red hot reduced copper just before it entered the apparatus, so that anaerobic conditions would be secured. The vessels were placed in position in the thermostat at 30° C. and when equilibrium had been reached, readings were taken as was described for the oxygen consumption determination. The amount of carbon dioxide evolved was computed from the following equations given by Warburg (1926):

$$x_{CO_2} = h_{CO_2} \cdot K_{CO_2}$$

where $$K_{CO_2} = \left[\frac{V_G \frac{273}{T} + V_F \cdot \alpha}{P_0} \right]$$

The carbon dioxide quotient in nitrogen, in cu.mm. per hour,

$$Q^{N_2}_{CO_2} = \frac{x_{CO_2}}{\text{Yeast wt.} \cdot \text{flour wt.} \cdot \text{time in hrs.}}$$

Combined Carbon Dioxide in Flour Suspensions. — In the determination of the combined carbon dioxide held by flour suspensions and liberated by citric acid, 1.0 cc. of flour suspension was placed in each vessel and 0.4 cc. of 5% citric acid was put into the side bulb of each vessel. Carbon dioxide was run through the apparatus for 10 minutes and the manometers placed in position on the thermostat at 30° C. When equilibrium had been reached and no more carbon dioxide was absorbed by the suspensions, the acid was mixed with them, liberating the combined carbon dioxide present. When the manometers showed no further change, readings were taken and the barometric pressure noted. The cu.mm. of carbon dioxide liberated per gram of flour used, was calculated, correcting the readings to standard barometric pressure and multiplying by the vessel constant.

$$CO_2 \; \frac{h \cdot \text{Bar. pressure} \cdot KCO_2}{76.0 \cdot \text{flour weight}}$$

Pure carbon dioxide and several air-carbon dioxide mixtures were used in saturating the suspensions, the reason for this being that it is not known just what the percentage of carbon dioxide in the gases given off in fermenting flour suspensions or doughs is, and it was desired to know if the percentage of carbon dioxide used, influenced the comparative amounts of carbon dioxide held by the respective flours.

Yeast Metabolism in Flour Suspensions. — Determinations were made of the metabolism of yeast in flour suspensions, or in other words, the combined effect of the oxygen consumption and carbon dioxide evolution in the presence of oxygen. Five vessels were used as follows:

1. water only - as a thermobarometer.
2. 1 cc. flour suspension + 1 cc. yeast suspension + 0.2 cc. NaOH in well (fill with pure O_2).
3. 1 cc. flour suspension + 1 cc. yeast suspension (fill with 5% CO_2 in O_2).
4. 3 cc. flour suspension + 3 cc. yeast suspension (fill with 5% CO_2 in O_2).
5. 1 cc. flour suspension + 1 cc. yeast suspension (fill with 5% CO_2 in N_2).

This determination is based upon the difference in solubilities of carbon dioxide and oxygen in water. When two vessels are used, such as vessels 3 and 4 above, with different volumes of suspension present, it is possible to calculate the oxygen consumed and the carbon dioxide evolved by means of a series of equations as follows, (Warburg 1926):

(1) $H = H' \frac{a}{b}$

H = reading of manometer of vessel containing large volume of suspension.

a = weight of yeast and flour in vessel containing small volume of suspension.

b = weight of yeast and flour in vessel containing large amount of suspension.

(2) $$x_{O_2} = \frac{h \cdot k_{CO_2} - H \cdot K_{CO_2}}{\frac{k_{CO_2}}{k_{O_2}} - \frac{K_{CO_2}}{K_{O_2}}}$$

k_{CO_2} = vessel constant for CO_2 (small amt. of suspension).

K_{CO_2} = vessel constant for CO_2 (large amt. of suspension).

k_{O_2} = vessel constant for O_2 (small amt. of suspension).

K_{O_2} = vessel constant for O_2 (large amt. of suspension).

(3) $$x_{CO_2} = \frac{h \cdot k_{O_2} - H \cdot K_{O_2}}{\frac{k_{O_2}}{k_{CO_2}} - \frac{K_{O_2}}{K_{CO_2}}}$$

This method is referred to by Meyerhof (1925) as the "Kästchen" method and will be called method K. As a check on the results obtained for the Q_{O_2} and $Q_{CO_2}^{O_2}$ by method K, values for these gas quotients were calculated in a different manner. From vessel 2, the Q_{O_2} was obtained as a separate determination, using pure oxygen to saturate the suspension and NaOH in the well to absorb the carbon dioxide given off. When the same yeast suspension is used and the tests are run the same day, it may be assumed that the oxygen consumption in vessel 2 is approximately the same as in vessel 3. Vessel 3 has no NaOH present to absorb the carbon dioxide given off so the result obtained from this vessel represents the combined affect of the oxygen consumption and carbon dioxide evolution or $Q_{O_2} + CO_2$. From the results obtained in these two vessels then (vessel 2 and 3), the $Q_{CO_2}^{O_2}$ may be determined by subtracting algebraically the

Q_{O_2} (vessel 2) from the $Q_{O_2} + CO_2$ (vessel 3). This method is known as the old method and will be designated as method A. Thus there are two methods of calculation by which the Q_{O_2} and $Q_{CO_2}^{O_2}$ may be determined, as it is very convenient to have such a check on the results obtained.

<u>Carbon Dioxide Evolved by Fermenting Doughs</u>. — Experiments were conducted with doughs, using the Warburg method, in a similar manner to that used in studying flour suspensions. Certain modifications were necessary in adapting the method to its use with doughs, because of the different nature of the doughs and suspensions. A suspension was made up, using 0.15 grams of wet yeast, 2.9 grams of sugar, 1.0 grams of salt, and water to make the volume up to 50 cc. Twenty five grams of flour were used and the doughs were made up by mixing this amount of flour with the suspension of yeast, salt and sugar, using 14.5 cc. of suspension for the American Banner flour and 15 cc. for the Red Rock. This made doughs of the same consistency used in the expansion test, all ingredients being in the same proportion with the exception of the yeast. It was necessary to use a much smaller proportionate amount of yeast so as to secure a gas evolution which could be measured accurately with the manometers. After the doughs were mixed, they were put into the vessels, the apparatus filled with nitrogen, and the test run as with flour suspensions. Some difficulty was encountered in placing the dough into the small vessels, be-

cause it was so sticky. A small glass tube was fitted with a plunger and calibrated so that it held a definite volume of dough when filled. One cc. of dough was used, and it was transferred to the vessel by inserting the tube into the mouth of the vessel and forcing the dough out with the plunger. It was then spread out in a thin layer on the bottom of the vessel, exposing a rather large surface. It is believed that not very much saturation took place when the nitrogen was run through the apparatus for a 10 minute period. In the calculation of results, the same values for α used in tests with suspensions was used. This was not strictly correct, of course, for doughs. However, when small volumes are used, the effect of α is rather small, and would not change the results to a great extent.

Combined Carbon Dioxide Retained by Doughs. — Determinations of the combined carbon dioxide liberated upon acidification of doughs with a 5% solution of citric acid were made, the doughs being previously saturated with carbon dioxide. It was found necessary to leave the vessels in the water thermostat for about two hours before equilibrium had been reached, and no more carbon dioxide was being absorbed by the doughs. The results were calculated as for combined carbon dioxide retention by flour suspensions.

Results.

Oxygen Consumption by Yeast in Flour Suspensions. — The effect of varying the flour content on the respiration of yeast was determined, the results being shown in Table XI.

Table XI. Effect of Varying the Flour Content on the Respiration of Yeast in Flour Suspensions.

Q_{O_2} in Cu. mm. per hour. 30° C.

Yeast in mgs.	Flour in mgs.	Q_{O_2} per mg. yeast	Q_{O_2} per 200 mg. flour	Q_{O_2} per mg. yeast per 200 mgs. flour	Flour Used
0.88	233.9	- 144	- 108	- 123	Amer. Banner
0.88	145.2	- 139	- 168	- 191	" "
0.79	228.5	- 135	- 94	- 118	Red Rock
0.79	135.7	- 116	- 135	- 171	"

When the yeast content was constant and the flour content decreased from 233.9 mgs. to 145.2 mgs. the oxidation quotient per mg. yeast dropped from -144 to -139 cu. mm. per hour. This took place with the American Banner flour, but with the Red Rock flour even greater effects were obtained by varying the flour content as the table shows. In calculating succeeding results, a dry flour basis of 200 mgs. was used, which was very close in amount to the actual weight of flour used, in the majority of cases.

In comparing the two flours, American Banner and Red Rock, as to oxygen consumption by yeast in their suspensions, the results shown in Table XII were obtained. The values represent a series of determinations, an average being given as well. The oxidation quotients were determined, using the formulas given in "Methods" and are shown as in the preceeding table in three ways: (1) per mg. of yeast, (2) per 200 mgs. flour, and (3) per mg. yeast per 200 mgs. flour.

Table XII. Oxygen Consumption in Respiration of Yeast in Flour Suspensions.

Q_{O_2} - Oxygen Quotient in Cu. mm. per Hour, at 30° C.

American Banner Flour			Red Rock Flour			Buffer
Per mg. yeast	Per 200 mg. flour	Per mg. yeast per 200 mg. flour	Per mg. yeast	Per 200 mg. flour	Per mg. yeast per 200 mg. flour	Per mg. yeast
- 168	- 222	- 148	- 148	- 195	- 131	- 7.8
- 170	- 224	- 150	- 143	- 190	- 126	- 7.0
- 175	- 232	- 154	- 155	- 204	- 137	- 8.5
- 178	- 224	- 157	- 161	- 203	- 142	- 9.5
- 151	- 202	- 133	- 124	- 167	- 109	- 6.4
average - 168	- 221	- 148	- 146	- 192	- 129	- 7.8

It will be noted from the table that the Q_{O_2} was much higher in flour suspensions than in buffer alone, and also that the Q_{O_2} values in American Banner suspensions were higher than those in Red Rock suspensions. The application of Student's method to the data showed odds of 10,000 to 1 that the rate of yeast respiration in the American Banner suspensions was higher than in the Red Rock suspensions.

Carbon Dioxide Evolved in Fermentation of Flour Suspensions. — The effect of varying the flour content upon the rate of fermentation in flour suspensions was studied, and the results obtained are given in Table XIII.

Table XIII. Effect of Varying the Flour Content on the Rate of Fermentation of Flour Suspensions by Yeast at 30° C.

Mgs. of yeast	Mgs. of flour	$Q_{CO_2}^{N_2}$ per mg. yeast	$Q_{CO_2}^{N_2}$ per 200 mgs. flour	$Q_{CO_2}^{N_2}$ per mg. yeast per 200 mg. flour	Flour Used
0.88	134.9	228	298	338	American Banner
0.88	220.9	395	315	385	" "
0.88	427.9	605	249	283	" "
0.79	129.2	218	266	337	Red Rock
0.79	218.8	404	292	369	"
0.79	363.1	579	252	319	"

The rate of fermentation varied with the flour content when the amount of yeast was constant. With small amounts of flour, up to 250 mgs., the rate varied almost directly as did the flour content, but as the weight of flour was increased beyond this, there was a falling off in the rate of fermentation, in proportion to the amount of flour used.

The results obtained in comparing the carbon dioxide evolved in fermentation of the two kinds of flour suspensions are given in Table XIV.

The data show that the American Banner flour suspensions were fermented at a faster rate than were the Red Rock flour suspensions. The application of Student's method showed significant odds that the rate in the American Banner suspension was the higher of the two. The addition of small amounts of glucose, either 0.91% or 1.82%, seemed to have no appreciable effect in the rate of fermentation.

Table XIV. Carbon Dioxide Evolved in Fermentation of Flour Suspensions by Yeast.

Q_{CO_2} in Nitrogen - in Cu. mm. per Hour at 30° C.

American Banner Flour			Red Rock Flour			
Per mg. yeast	Per 200 mgs. flour	Per mg. yeast per 200 gms. flour	Per mg. yeast	Per 200 mgs. flour	Per mg. yeast per 200 mgs. flour	% Glucose added
372	240	328	304	196	268	0.91
456	246	402	352	190	311	0.91
370	222	327	284	171	252	1.82
390	234	344	338	203	298	0.00
416	246	367	341	202	301	0.91
394	233	348	339	200	299	0.00
395	251	349	---	---	---	1.82
382	243	337	333	212	294	0.00
average 396	239	350	327	196	289	

Retention of Combined Carbon Dioxide by Flour Suspensions. — Table XV shows the comparative amounts of combined carbon dioxide held by the American Banner and Red Rock flour suspensions. The table gives the various percentages of carbon dioxide used in saturating the suspensions, and the effect on the amount of combined carbon dioxide liberated by acidification with citric acid.

Table XV. Retention of CO_2 by Flour Suspensions.

Cu.mm. combined CO_2 liberated by citric acid at 30° C.

American Banner				Red Rock			
100% CO_2 used	51% CO_2 used	22.7% CO_2 used	14.9% CO_2 used	100% CO_2 used	51% CO_2 used	22.7% CO_2 used	14.9% CO_2 used
224	173	133	107	184	155	104	79
225	179	139	113	164	130	102	84
227	172			158	143		
232				181	126		
248				176			
250							
average 234	175	136	110	173	138	103	82

It may be noted that regardless of the percentage of carbon dioxide used in saturating the suspensions, the American Banner had more combined carbon dioxide present than had the Red Rock. Curves showing the relative amounts of carbon dioxide held as combined carbon dioxide by the two flours, are given in Plate VI.

Plate VI. Combined Carbon Dioxide Held By Flour Suspensions.

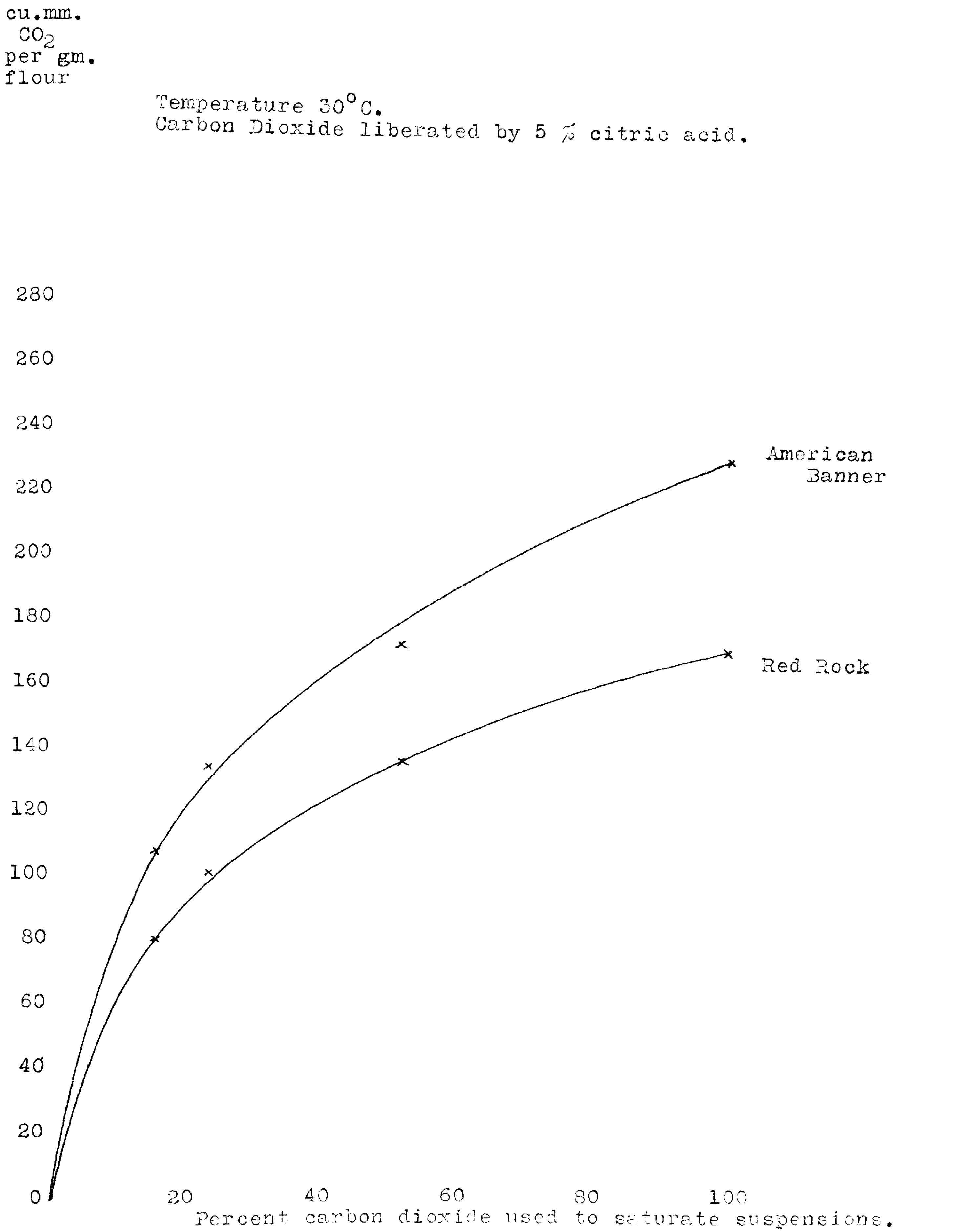


Table XVI. Metabolism of Yeast in Flour Suspensions.*

Q_{O_2} in O_2 (5% CO_2)			Q_{CO_2} in O_2 (5% CO_2)			Q_{CO_2} in N_2 (5% CO_2)
Per mg. Yeast	Per 200 mg. flour	Per mg. yeast per 200 mg. flour	Per mg. yeast	Per 200 mg. flour	Per mg. yeast per 200 mg. flour	Per mg. yeast per 200 mg. flour
- 195	- 119	- 165	392	239	333	399
- 200	- 129	- 180	404	261	363	454
- 172	- 119	- 147	345	238	294	373
- 201	- 131	- 177	398	259	351	384
- 185	- 111	- 161	394	237	344	
- 204	- 127	- 182	423	264	378	
Average - 193	- 123	- 169	393	250	344	402
			Red Rock			
- 160	- 100	- 137	339	212	290	409
- 163	- 104	- 140	346	221	299	336
- 125	- 80	- 116	291	187	270	402
- 141	- 90	- 130	319	204	296	
- 174	- 108	- 155	372	232	331	
- 173	- 108	- 154	421	262	375	
Average - 156	- 98	- 139	348	220	310	382

*Calculated by method K, ("Kastchen Method").

<u>Metabolism of Yeast in Flour Suspensions.</u> — A comparison of the rates of yeast metabolism in Red Rock and American Banner flour suspensions is given in Table XVI.

The data show a higher rate of metabolism of yeast in the case of the American Banner suspensions, with a $Q_{CO_2}^{O_2}$ of 344 as compared to 310 in Red Rock suspensions. The $Q_{CO_2}^{N_2}$ determined at the time of these metabolism experiments are higher than the values for $Q_{CO_2}^{O_2}$. A higher rate of fermentation is usually secured under anaerobic conditions than when oxygen is present. As a check on the results in Table XVI, some determinations of the Q_{O_2} and $Q_{CO_2}^{O_2}$ were made, using the old method (Method A) as was previously described. These comparisons are made in Table XVII, showing a typical test calculated by each method.

Table XVII. Metabolism of Yeast in Flour Suspensions.

Cu.mm. per hour per mg. yeast per 200 mg. flour.

Flour	Q_{O_2}		$Q_{CO_2}^{O_2}$	
	Method K	Method A	Method K	Method A.
American Banner	-147	-150	294	284
Red Rock	-137	-143	290	284

The results as obtained by the two methods check fairly closely, the variations not being any greater than variations ordinarily obtained from various samples when calculated by the same method.

Carbon Dioxide Evolved in Fermentation of Doughs. — The data obtained for the rate of carbon dioxide given off in nitrogen by fermenting doughs was calculated, using 1.0 gram of flour as the basis of flour weight, instead of 200 mgs. of flour as has been previously used. The oven dry weight of flour used in each sample was nearly 1.0 gram. The results obtained are given in Table XVIII.

Table XVIII. Carbon Dioxide Evolved in Fermentation of Doughs.

American Banner Dough			Red Rock Dough		
Per mg. yeast	Per gm. flour	Per mg. yeast per gm. flour	Per mg. yeast	Per gm. flour	Per mg. yeast per gm. flour
576	260	807	510	238	737
584	263	852	522	244	698
596	269	820	637	299	807
646	291	828	618	288	878
561	253	696	614	286	814
569	257	738	588	275	811
595	277	750	567	264	704
Average 590	267	784	580	271	778

The data indicate that the rate of carbon dioxide evolved from American Banner doughs was slightly higher than from Red Rock doughs. Statistical analysis of the data by the use of Student's method, however, showed little significance in this higher rate. The moisture content of the doughs was different for the two varieties, however, which might explain the similarity of results. American Banner doughs were made up using 58% water and Red Rock using 60% water. An experiment in which the moisture content of American Banner doughs was varied, showed the results given in Table XIX.

Table XIX. Carbon Dioxide Evolved in Fermenting Doughs - Moisture Content Varied.

Flour	H_2O added in % of flour	$Q_{CO_2}^{N_2}$ per mg. yeast	$Q_{CO_2}^{N_2}$ per gm. flour	$Q_{CO_2}^{N_2}$ per mg. yeast per gm. flour
American Banner	56	473	289	587
" "	60	481	314	628
" "	60	496	325	663
Red Rock	60	449	293	574
"	60	470	308	605

When the moisture content of the two kinds of doughs was the same, a higher rate of carbon dioxide was evolved in the American Banner than in the Red Rock. For two determinations the $Q_{CO_2}^{N_2}$ per mg. yeast per gm. flour was 645.5 c.mm. per hour for American Banner and 589.5 c.mm. per hour for Red Rock. This is a smaller difference, however, than was obtained between the two varieties in the $Q_{CO_2}^{N_2}$ for yeast in flour suspensions. When the moisture content of the American Banner dough was lowered to 56%, the $Q_{CO_2}^{N_2}$ was lowered from 645.5 to 587.0 cu.mm.

<u>Combined Carbon Dioxide Held by Doughs.</u> — Results of a series of determinations in the combined carbon dioxide held by doughs and liberated upon acidification with 5% citric acid are given in Table XX.

Table XX. Retention of Carbon Dioxide by Doughs at 30° C.

Cu.mm. combined CO_2 liberated by citric acid, after doughs had been saturated with CO_2 (per gram of flour).

Trial No.	American Banner	Red Rock
1	98.8	77.4
2	107.8	96.5
3	113.8	76.5
4	89.7	67.7
5	76.7	
Average	97.3	79.5

The results show considerable variation, which was possibly due to the difficulty in getting the doughs completely saturated with carbon dioxide, before the acid was mixed with them. In general, however, the data show a greater amount of combined carbon dioxide held by American Banner doughs. This was also shown in the case of the flour suspensions.

DISCUSSION

The Expansion Test

The expansion test with doughs was studied in order to secure more accurate information as to the factors which affect the results, the interpretation of results, and the reliability of the method for evaluating soft wheats on the basis of their flour strength.

Within the temperature range of 27° - 35° C., an increase in temperature caused a more rapid expansion and consequently a shorter fermentation period. This was undoubtedly due to the more optimum conditions for carbon dioxide production by the yeast cells secured at the higher temperatures. At 35° C. the expansion of the doughs was rapid, and the test was completed in considerably less time than when run at 27° or 30° C. The fermentation cabinet was maintained at 35° C. with a little difficulty, due to the opening of the doors necessary in placing jars into and removing them from the cabinet. The tests at 30° C. showed the greatest differences between the varieties, and the test was completed with some saving in time over that required at 27° C.

The time of first rise was found to bear a relation to the maximum volume which was reached by the dough during the second rise. After a certain point (in the time of first rise) was reached, any further increase in the first rise period, caused a decrease in the expansion during the second rise. (Table V and Plate I). It seems probable that if the first rise is too long, the physical properties of the doughs are changed so as to prevent a normal expansion in the second rise. A weakening of the gluten quality perhaps takes place, or possibly a condition in the dough caused by the accumulation of by-products of fermentation in the immediate vicinity of the yeast cells hinders the normal fermentation when the dough is finally punched or kneaded down.

During the first rise period a steady and rapid expansion was obtained for the first hour, with both varieties of flour. Soon after that, or usually between one and one and one-half hours, the expansion began to lag or drop off. According to Working (1929), this lag in expansion is probably due to the accumulation of end-products of fermentation. Punching the dough at this stage brings about a renewed expansion at a normal rate, undoubtedly because the yeast cells are brought into contact with new foods, and the end-products of fermentation are more evenly distributed throughout the dough mass. This idea and that of Elion (1930) who suggests punching doughs every half hour, bring up an interesting possibility as to the expansion test.

The data show that when the doughs were punched every half hour a greater total expansion and a more rapid rate of expansion was obtained than when two rises only were given. The length of the fermentation period as determined by this method showed that the American Banner expanded for only 4 hours while the Red Rock expanded for 6 hours. (Table VIII). This method of conducting the expansion test is suggested as a means of determining the possible expanding power of a flour, and it is possible that the results would be of more value than those derived by other methods of procedure with the test.

It is believed that the moisture content of doughs in the expansion test may be made up by either of two methods. The one method is to make up the doughs to a definite percentage of moisture by weight, taking into account the moisture content of the flour; the other is to determine a figure for the absorption of water for each flour to be used, and make up the doughs each time by adding the proper amount of water as determined by this absorption figure. The latter method appears to be the better of the two, because all doughs are made up to approximately the same consistency, which is a great convenience in handling the doughs during the course of a test. The absorption values of different soft wheat flours vary considerably, and if the doughs are made up to a definite moisture content by weight, no importance is given to these differences in absorption.

This point should not be over-emphasized, however, for it was found that variations in moisture content up to 2 or 3% did not materially affect the volume of expansion in the fermentation test, (Table IV). The rate of fermentation, however, increased with the higher moisture contents as was shown by the shorter fermentation period, and more rapid expansion of the doughs. It seems probable that this higher metabolism rate of yeast, in doughs containing higher moisture contents, might be caused by the more anaerobic conditions existing when more moisture is present.

The question of the reliability of the expansion tests is of much importance. Differences in flour strength as great as are shown between Red Rock and American Banner can easily be measured, for by the method outlined with a first rise of one to one and one quarter hours, differences of from 250 to 300 cu. mm. in the expansion during the second rise were obtained. When varieties showing less difference in flour strength than these two are tested together what will be the accuracy in determining this difference by the expansion method?

An analysis of the data shows that for 33 determinations, using a standard procedure, and a first rise of 1 - 1 1/4 hours, the mean volume reached during the second rise for the American Banner was 992 ± 34.7 cc. (Probable Error of a single determination). If it could be assumed that other varieties would show a similar variation from day to day in a series of tests with the same general sample of flour, the difference necessary to show a significant variation from American Banner could be calculated. The P. E. diff. = $\pm\sqrt{a^2+b^2}$ where "a" and "b" equal the respective errors. Than if "a" is assumed equal to "b", the P. E. diff. = $\pm\sqrt{2a^2}$ or $a(\pm\sqrt{2})$. The limit of equality = $3.3(\pm\sqrt{2}\cdot)a = \pm 4.67$ a. When a = $\pm$ 34.7 cc., the limit of equality = $(\pm 4.57) \times (\pm 34.7)$ cc. = $\pm$ 162 cc. With a single determination, if an unknown sample varies as much as $\pm$ 162 cc. from the control sample of American Banner, it may be considered to be different from the American Banner in flour strength as shown by the expansion test. For a duplicate determination, which is always recommended, the limit of equality would be $\pm 162 \div \sqrt{2} = \pm$ 114.5 cc.

This limit of equality is probably too high, for the determinations used to obtain the Probable Error, extended over a period of three months and it has been shown that the flour improved with age, which accounts for some of the variation as shown by the P. E.s of $\pm$ 34.7 cc. If tests were conducted during a short period of time, this value for the limit of equality would probably be smaller. (Table X).

With the Red Rock variety a mean of 1214 $\pm$ 19.6 cc. (P.E. single determination) was obtained with 33 determinations.

The limit of equality as determined before, was $\pm$ 4.67 a. In this case "a" equals $\pm$ 19.6 cc., so the limit of equality equals $(\pm 4.67) \times (\pm 19.6)$ cc. = $\pm$ 91.5 cc. A variety being compared with Red Rock, showing a variation of $\pm$ 91.5 cc. from Red Rock, when a single determination was made, could be considered different from Red Rock in regard to flour strength, as measured by the expansion test. For a determination in duplicate, the limit of equality = $\pm 91.5 \div \sqrt{2} = \pm 64.7$ cc. thus, within reasonably wide limits, from 75 - 125 cc. perhaps, the expansion test can be expected to give reliable results, if a careful technique is followed. The procedure used and recommended includes these points: 150 grams of flour used, temperature of 30° C. for fermentation, relative humidity of 80%, first rise of 1 - 1 1/4 hours, and the second rise to maximum volume reached. The results tend to indicate that differences of much less than 75 cc. between varieties would not be of significance, unless more than two trials of each were made.

Yeast Metabolism Studies

Using the Warburg manometric method, oxygen and carbon dioxide relations in the fermentation of flour suspensions were considered. In respiration studies, the Q_{O_2} in buffer averaged 7.8 c.mm. per hour per mg. yeast. In flour suspensions it was much higher, averaging 148 c.mm. in American Banner and 128 c.mm. in Red Rock on the basis of both yeast and flour. (Table XII).

The $Q_{CO_2}^{N_2}$ measured in buffer sugar (0.91 to 1.82% glucose) was 273.4 c.mm. per hour per mg. yeast. In American Banner flour suspensions the $Q_{CO_2}^{N_2}$ for several determinations averaged 350 c.mm. per hour per mg. yeast per 200 mg. flour, while in Red Rock suspensions it was 289 c.mm. per hour. (Table XIV). The addition of small amounts of glucose, 0.91 to 1.82%, had no appreciable effect on the rate of carbon dioxide produced with either flour. This shows that the difference obtained between the two varieties in rates of oxygen consumption and carbon dioxide production, was not due alone to the available sugar content. There was probably plenty of sugar available in either case for the amount of yeast present. It is probable, however, that from the standpoint of the yeast, the difference was due to a nutritional factor. The exact chemical nature of flours is not very definitely known and it is entirely possible that the American Banner suspension presents an environment more favorable for rapid yeast metabolism than does the Red Rock. The possibility of there being a yeast accelerator, such as a phosphate, a difference in vitamine content or other chemical differences which would speed up the yeast action in the one flour is suggested.

The rate of fermentation was found to be higher in an atmosphere of nitrogen than in oxygen (Table XVI). This tends to show that the oxygen used under aerobic conditions is not essential for the yeast. If it is not supplied as free oxygen, the oxygen used for energy by the yeast is readily obtained from the sugar itself, causing more carbon dioxide to be evolved under anaerobic conditions.

Meyerhof (1925), in his yeast studies, obtained values for Q_{O_2} and Q_{CO_2} with several strains of baker's yeast as follows:

Q_{O_2} in buffer = 10

Q_{O_2} in 5% sugar = 90 - 100

Q_{CO_2} in 5% sugar = 250 -300 in nitrogen

Q_{CO_2} in 5% sugar = 60 - 100 in air

The results obtained in the present investigation (Tables XII and XIV) agree quite closely with these results of Meyerhof as far as the yeast itself is concerned. He did not work with flours.

The Warburg method was applied to the study of dough fermentation with less definite results than were obtained using flour suspensions. The conditions present, comparing the two systems, were of course quite different. In a suspension, after it has become saturated with a gas, the gas escapes freely from the surface while in a dough the quality of gluten enters into the problem. In the suspensions the pH was controlled to considerable extent with the buffer, while in doughs no attempt was made to regulate the pH. It was noted that whereas a constant rate of gas evolution could be secured with suspensions, this was almost impossible with doughs. This might be due partly to a change in pH as fermentation progresses, partly perhaps, to the accumulation of end-products of fermentation in the immediate vicinity of the yeast cells, and possibly to the more or less unequal distribution of moisture in the dough, affecting enzyme activity and other reactions.

<u>Relationship of the Yeast Metabolism Studies to the Expansion Test.</u> — In considering the possible relation between the results obtained in the study of the expansion test, and those obtained in the yeast metabolism study, several points will be noted. The shorter fermentation period obtained in the expansion test might be explained on the basis of the more rapid rate of fermentation. The end of the fermentation is probably caused either by an exhaustion of available sugar or other yeast nutrients, or by the accumulation of by-products of fermentation which inhibits yeast metabolism, preventing further fermentation or possibly partly by both causes. The softer, weaker flours are usually considered to have larger and more distinct starch granules, which would be more easily attached by diastase. Rumsey (1922) stated that a more easily digested starch increases the rate of maltose fermentation. It might appear then that the more readily available sugar being used up more rapidly would be gone in a shorter time, thus causing the end of the fermentation period to come earlier, in the softer flour - American Banner. This hardly seems to explain the facts fully though, for a change in sugar concentration did not materially affect the rate of fermentation in flour suspensions. With the relatively large amounts of yeast used in an expansion test, which are multiplying as well as metabolizing under partially aerobic conditions, slight variations in the sugar

concentration might be of more importance.

Simple experiments with flour suspensions showed that American Banner flour settled out more rapidly than did the Red Rock. This indicates that Red Rock flour is composed of finer particles and American Banner coarser ones. Suspensions which had been centrifuged, showed little or no starch present in the supernatant liquid. This liquid, however, was more or less opalescant and that from Red Rock suspensions showed more turbidity and colloidal properties than the liquid from American Banner suspensions. This would indicate that not only are the starch particles smaller in Red Rock flour, but also the protein left in suspension was more colloidal. It may be that this difference in size of particles is the cause of the different amounts of combined carbon dioxide liberated, when the flour suspensions were first saturated with carbon dioxide, and then acidified. There seemed to be little difference in the amount of carbon dioxide left in acidified suspensions, comparing the two varieties. The water content seemed to be the most important factor in determining the carbon dioxide held in acidified and non-acidified suspensions as experiments showed. Samples of both kinds were taken and analysed for carbon dioxide with the Van Slyke gas machine and no significant difference could be shown between the varieties of flour. The indications are that the carbon dioxide liberated with citric acid then, was held combined as a bicarbonate. The retention of carbon dioxide in doughs after acidification might show very different results. It seems likely that more carbon dioxide might be held as such by Red Rock doughs, only in so far as the spaces are smaller and that there are more of them in the dough, and as the volume increases these smaller spaces do not break and free the carbon dioxide as readily as do the larger spaces in American Banner dough.

This factor of size of particles, naturally occuring with the same milling process may, to a great extent, be the cause of the differences in expansion exhibited by these two flours. Along with finer particles in Red Rock, there is a more elastic gluten, higher water absorption, finer vesicles for holding carbon dioxide, all of which help to make the dough hold up better when expanding. The American Banner, on the other hand, with larger particles, less surface for water absorption, larger vesicles for holding carbon dioxide, and a softer gluten, probably cannot, regardless of the rate of fermentation, expand into as large and finely textured, well risen a mass as does the Red Rock.

Yeast is added to doughs to produce carbon dioxide in order to expand them into light, fine textured loaves when baked. When no sugar was added in the expansion test, less volume of expansion was obtained, but the relative volumes of the two doughs were about the same. With the manometric method, using very small quantities of yeast, the addition of small amounts of sugar to flour suspensions, did not increase the rate of fermentation. It seems likely then that sugar is not the deciding factor in determining the differences in the two flours. When yeast and sugar are added to the dough mix, in the quantities as given in the expansion test formula, it is probable that the rate of fermentation has little influence on the volume of expansion reached by the dough. Plenty of carbon dioxide to obtain a large volume, is probably available in either dough, if the proper physical condition for its retention exists. The physical state of the dough must be such that it can utilize this carbon dioxide to best advantage, holding it in a finely divided network of vesicles, the gluten films being strong and elastic enough to hold these vesicles in place as the dough rises.

Application to Soft Wheat Quality Studies

The expansion test offers a method of comparing wheats on the basis of their flour strength. It seems to be reasonably accurate in judging differences between the various types of soft wheat flours. In order to use the test with any degree of success, a careful procedure must be followed, controlling all factors possible. The American Banner and Red Rock flours each show a characteristic expansion of dough upon fermentation. Other varieties which give a fermentation curve corresponding to American Banner, for example, might be expected to be of about the same flour strength. If a variety grown under different environmental conditions show uniformity in this test, it might be expected to be uniform in its behavior in the bake shop, within reasonable limits.

The faster rate of fermentation, or greater ease of fermentation, shown by the American Banner flour may be of significance in classifying soft wheats as to their baking quality. Whatever the reason may be, that causes the flour to have this characteristic rapid fermentation rate, whether it is larger particles, more easily digested starch, a yeast accelerator or other substance causing a more rapid yeast metabolism, the question of practical importance is, does this characteristic hold true for other flours of like strength? If so, the Warburg Manometric method might be used with success as a test of flour quality. Even if there is no particular relation between the rate of fermentation and the volume of expansion of a dough, it is well to have the added information concerning the characteristic fermentation which takes place in the different flours. It is hoped that this study has at least suggested a few points of importance in the complicated problem presented in trying to determine more specifically the nature of flour strength, and better and simpler ways of testing wheats for baking quality.

SUMMARY AND CONCLUSIONS

1. A study of the fermentation of experimentally milled flours from two soft wheat varieties, Red Rock and American Banner, was made. The Red Rock is a soft red winter wheat which produces large well-risen loaves in the experimental baking test, while the American Banner is a soft white winter wheat which produces rather poor loaves, but is favored for the pastry flour trade.

2. In the expansion test with doughs using 150 grams of flour, a temperature of 30° C., a first rise of 1 - 1 1/4 hours, and a second rise to maximum volume reached, the Red Rock expanded about 300 cc. more than the American Banner.

3. At 30° C. larger differences in expansion were obtained between the two flours than at 27° C. or 35° C. When these temperatures were used, the time required to complete an expansion test varied inversely as the temperature.

4. Variations in moisture content of doughs as much as 2 - 4% above that required for good consistency of dough, did not materially change the total volume reached upon expansion. Reducing the moisture content much below that required for good dough consistency reduced the volume of expansion. The absorption method of adding water was considered satisfactory for the

expansion test.

5. The addition of 3 grams of lard to the dough mix reduced the total expansion slightly and shortened the fermentation period. It is not recommended as possessing any advantages in the test.

6. The omission of sugar from the dough mix greatly reduced the expansion of the doughs and increased the time required to complete a test.

7. When the doughs were punched every half hour, the Red Rock expanded for two hours after the American Banner had stopped. This method is suggested as having good possibilities.

8. After the first month, from the date of milling of the flours, the American Banner improved more with age than did Red Rock over a period of four months, as shown by an increase in the expansion of doughs upon fermentation.

9. Using the expansion test, differences of 100 - 125 cc. in volume of doughs when a duplicate determination is made, show a significance in comparing two varieties or strains. Differences between two flours of less than 75 cc., when duplicate determinations are made, are probably of little significance. The ultimate value of the test, however, mainly depends upon how well these volume differences actually measure differences in flour strength.

10. The Warburg Manometric Method gave satisfactory results in determining the rates of yeast metabolism in

flour suspensions. Oxidation quotients were obtained for yeast respiration in buffer and in flour suspensions; carbon dioxide quotients were obtained for yeast fermentation in nitrogen, first in buffer sugar and then in flour suspensions, with and without sugar added; oxidation and carbon dioxide quotients were obtained at the same time in flour suspensions in oxygen, using different volumes of suspensions; combined carbon dioxide liberated upon acidification of suspensions previously saturated with carbon dioxide, was determined; and carbon dioxide quotients were obtained for yeast fermentation in doughs.

11. In the Warburg studies, the yeast in American Banner flour suspensions showed a more rapid rate of respiration in oxygen, a more rapid rate of fermentation in nitrogen and a faster rate of metabolism in oxygen than was shown in Red Rock flour suspensions. More combined carbon dioxide was given out by American Banner suspensions and doughs when they were previously saturated with carbon dioxide, and then acidified with 5% citric acid.

12. In flour suspensions an addition of 0.91 to 1.82% glucose did not affect the rate of fermentation by yeast as measured by the Warburg method.

13. In flour suspensions, the amount of flour present influenced the rate of fermentation if the yeast content was constant, a larger amount of flour producing a more rapid fermentation rate. The rate, however, did not

vary directly with the flour content, although with small amounts of flour, (100 - 400 mgs.), it approximated a direct relationship.

14. The significance of the difference in the rates of fermentation, as shown in comparing the two flours studied, is not definitely known. It seems likely that a faster rate of fermentation would mean a shorter fermentation period, which was the case at least with the American Banner flour. It is possible that the carbon dioxide given off in the later stages of fermentation would be too small in quantity to effect a good rise in the dough, as the work of Wood (1907) suggests. This would probably be the case, if a very long fermentation period were given to such a weak flour. It seems likely, however, that with the temperature and period of fermentation used, plenty of carbon dioxide was evolved with either flour to effect a large volume of expansion, if the proper physical condition of the dough was present, to enable it to be used most effectively.

15. American Banner flour in water suspensions settled out more rapidly than did Red Rock. The supernatent liquid from Red Rock suspensions, after the starch grains had settled out, was more colloidal in nature. Indications point to a difference in the physical condition of doughs from the two flours, the Red Rock being made up of the finer particles. The difference

in size of particles is suggested as being a factor of importance in determining the size of the volume reached by a dough upon fermentation.

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