THE WHEAT-MEAL-FERMENTATION TIME TEST

by

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Numerous attempts have been made to find a single and adequate method to describe the baking potentialities of a wheat or the "strength" or "quality" of wheat as determined by the baking test. The methods used for determining the value of wheat for baking purposes are generally divided into: chemical tests; physical tests; physico-chemical tests and the baking test. The connotation of quality is often viewed differently by the wheat breeder, the farmer, the miller or baker. No one test has been found to be adequate for the evaluation of wheat quality. Miller and Johnson (1954) state that only in the baking test are the composite effects of overall quality factors brought into action simultaneously. Although the baking test can be carried out on a micro scale (Shellenberger et al., 1958), the procedure has not been used extensively by plant breeders. Any baking test is affected by a certain amount of subjective evaluation, and is much influenced by a variety of factors; therefore it is sometimes difficult to determine the one factor responsible for low quality.

It is generally accepted by many workers that the quantity and quality of protein in wheat are the best indicators of "flour strength". Protein is highly correlated with loaf volume. The quantity of protein-gluten is no doubt the most important single factor which determines the bread baking quality of wheat and wheat flour. The most accepted method of determining total protein is the Kjeldahl method, which, although the most reliable, fairly rapid and well accepted of the tests, has a number of shortcomings. These include: the need for a trained chemist, a well equipped laboratory,

use of large amounts of acid and base, noxious fumes, and the generation of excessive heat. The drawbacks have given rise to numerous simpler methods of determining protein, mostly methods such as those by Zeleny (1941) in which he measured colorimetrically the turbidity of the suspension produced by reacting a phosphate buffer with alkali extracts of wheat flour. Pinckney (1949) proposed to evaluate the protein content by employing the principle of the Biuret reaction. Another method was proposed by Udy (1956) in which he measured by means of a photocolorimeter the dye binding power of ground wheat, using a disulfonic acid dye, Orange G, at pH 2.2. Feinstein and Hart (1959) extracted wheat flour with alkali and reacted it with sulfosalicylic acid for the measurement of protein. They measured optical density with a Klett-Summerson photoelectric colorimeter at 540 millimicrons. A sedimentation test had been proposed by Zeleny (1947) where results are correlated with the quantity (and to some extent the quality) of flour proteins by measuring the extent of hydration of the gluten in a dilute lactic acid solution. A similar method was proposed by Berliner and Koopman (1929).

Many methods had been proposed to determine the quality of protein-gluten directly by testing the quality of washed gluten itself as mentioned in the review made by Miller and Johnson (1954). These quality tests for gluten are classified into four different methods: expansion by heat; recovery from compression, obtained by measuring the force required to inflate a bubble of gluten and also the volume of the bubble at the time it is ruptured; crude gluten extension, where gluten quality is expressed by its resistance to rupture; and the crude gluten relaxation methods.

Despite the numerous evidences supporting the theory that many

properties of the dough are due to the gluten component of flour, there is the problem of separating the gluten from the flour without altering the gluten to any extent, even when mechanical washing devices are used. Assuming that the gluten is separated from the flour without any alteration at all in its physical and chemical configuration, there still is the possibility of the other components of flour contributing to the properties of the dough to a certain degree. Such components include starch and lipids which are believed to form complexes with protein and affect its properties. Furthermore, no objective test of gluten quality has been devised that provides results that correlate highly with the baking results (Miller and Johnson, 1954). Sullivan (1954) stated that quality can not be due to amino acid composition alone because gluten obtained from flours varying widely in dough characteristics and baking quality show no significant differences. Cereal chemists in the U.S.A. are, therefore, more inclined to study the properties of the dough itself in evaluating the quality of flour.

The wheat-meal-fermentation time test (W-M-F-T) originally proposed by Saunders and Humphries (1928), is based on the length of time elapsing before a dough ball made from wheat meal, water and yeast, disintegrates after it is placed in water. The "doughballs" made from weak wheats disintegrate quickly, while those made from strong wheats retain their shape for a long time; therefore, the distintegration time has been employed as an index of wheat quality. Swanson (1937, 1939) found that disintegration time was influenced by many factors, such as proteases, fineness of meal, amount of mixing, temperature, length of storage between grinding and testing, and concentration of CO_2 in the water where the dough floats. Saunders

and Humphries (1928) used 10 g. flour, 6 ml. of water and 0.5 g. yeast at 80°F. during mixing. Pelshenke (1933) used 5 g. wheat meal, 0.25 g. of yeast, and enough distilled water to make a doughball of medium stiffness, at 88°F. to 91°F. Cutler and Worzella (1933) modified the test by using a 10 g. sample, and mixing it with 10 ml. of a 10 per cent yeast suspension, maintained at 80°F. They also tried using a 3.4 - 5 g. sample per dough with a proportionately reduced yeast suspension of the same concentration. Bayfield (1935) tested the method of Cutler and Worzella. Using a 150 ml. beaker, he observed that for high protein wheats, sticking at the sides was an interfering factor which did not occur with wheats of low protein content. However, in addition to the sticking to the sides of the container, he observed that there were additional factors interfering with the relationship of "time" and "strength". He further suggested that in determining dough strength, two principal factors must be taken into consideration, namely, gas-production and gas-retention. He emphasized the effect of protein quality and quantity, proteolytic enzymes, sugar content of wheat and its maltose producing ability (diastatic activity). Pelshenke (1933) found that the gas-holding power of a dough is dependent upon both the gluten quality and quantity and other properties such as content of mineral material, and fat content. Swanson and Dines (1939) studied the effect of protease, protease activators and protease inhibitors on the time test. They reported that pepsin reduced the time of Tenmarq wheat (a long time wheat) significantly but not the time of Clarkan which is normally a "short time" wheat. Adding a few milligrams of ascorbic acid increased the time of Clarkan to almost the same time as that of Tenmarq. Swanson and Kroeker (1932) suggested that enzymatic activity was an environmental factor largely determined by

harvest and milling conditions, and was not an inherent variety factor.

Swanson (1935) found that tempering hard wheat increased the "time", probably because of finer granulation. According to Swanson (1937), the coarser the grinding, the shorter the time and the finer the meal, the longer the time. He observed that the coarser meal endosperm was in chunks and did not contribute much to the dough mass. Granulation did not affect so much the time test with "short time" wheats, but "long time" varieties and the intermediate ones were affected by differences in particle size. Bushuk (1961) reported that increases in accessible sulfhydryl groups were obtained for doughs prepared from flours of decreasing particle size or increasing in specific surface area, and those doughs subjected to prolong mixing. Granulation studies by Finney et al., (1949) have shown that flours milled on the Buhler and Hobart mills have about the same average flour particle size and are appreciably coarser than the commercially milled flour, the latter giving a higher gassing power at the end of four hour fermentation time. McCluggage (1943) in studying samples milled by a Hobart mill found the flours to contain a higher percentage of ash than the ash content of commercially milled flours. Sibbitt, et al., (1943) comparing flours from micro experimental mills and Allis-Chalmers mills, found that the ash in micro-milled flour was higher than the ash in Allis-Chalmers milled flours. This aspect of the wheat-meal-fermentation time test is very important if flour is used, since time is affected by the presence of high amounts of ash and bran particles in the flour. Coleman et al., (1934) suggests that uniformity in fineness of grinding must be maintained because the maltose value is directly proportional to the degree of fineness to which the sample is ground. Pool et al., (1958) observed a significant increase in kernel

softness and increase in amount of fine flour without changes in total flour yield from wheats during a delayed harvest of 46 days. Protein and ash percentages were unchanged. Cutler and Worzella (1933) found that ground wheat increased in "time" more rapidly in short storage than did unground wheat. Changes which occur during the first three or four days after grinding are so small that they apparently fall within the experimental error of the test. Swanson (1937) found similar effects of aging and suggested that wheats to be used should at least be stored for 6 weeks after harvest and ground a day before the determination of the "time". He further states that in wheats having a W-M-F-T times above 200 minutes, the results are affected by factors unrelated to strength, namely insufficient supply of yeast food.

Pool and Patterson (1958) observed a decrease in the "time" with increasing levels of sugar in soft wheats. Swanson (1940) observed an increase in both hard wheat and semi-hard wheats, as a result of added sugar in the mixture. Cutler and Worzella (1933) found that as the amount of the "longer time" high protein wheats is reduced in a blend, the time or quality of mixture is correspondingly reduced or vice versa but found no direct mathematical relation.

Bayfield (1935) found that individually mixed doughballs gave W-M-F-T times which were different (usually shorter) from "twin" doughballs, made by dividing a single larger dough, such as a 5 g. dough into two 2.5 g. doughballs.

Pool et al., (1958) found that rain and sunshine affected the W-M-F-T times of wheat meal of three varieties of soft red winter wheat during a delayed harvest for 46 days. They offered the hypothesis that these effects

were the result of an activation by rain and the inactivation by sunshine of glutathione or other sulfhydryl compounds.

Miller et al., (1951) and Winter and Gustafson (1934) modified the W-M-F-T test. Instead of waiting for the ball to disintegrate in the water, they measured the water displaced by the material as it started to expand and used this amount of water displaced as a measure of the strength of the meal. They stated that the expansion was correlated highly with loaf volume. Maes (1952) reported the development of a test similar to the one described by Miller et al., (1951), and obtained positive correlations between fermentation time and loaf volume, and the total baking score, using both winter and spring wheats. Wilson et al., (1933) comparing two mills, the Arcade and the Wiley mill, got a correlation coefficient between the two mills of $r = 0.6387$ for spring wheat and $r = 0.877$ for winter wheat. They also found a positive correlation between time and per cent protein, loaf volume and total strength score. Correlation coefficients obtained by Cutler and Worzella (1933) for the relation between time and protein and between time and loaf volume were as high as 0.82 and 0.84 respectively.

Kellenberger and Swenson (1948), Wilson et al., (1933), Pool and Patterson (1958), and others outlined the difficulties in determining the "end point" or the time the ball is supposed to be considered "disintegrated".

Any test without standardized procedure will never give reproducible and reasonably accurate results. The W-M-F-T test has not so far met the requirements of a standardized method of testing quality of wheat. It has been used extensively for breeding work as a tool for culling out undesirable strains as early as F_2 by relative comparisons with the original stock. Bayfield (1936) conducted a collaborative study of the W-M-F-T

test using hard and soft wheats. Ground and unground wheats were sent to different laboratories and workers were asked to use the method by Cutler and Worzella without any modifications. The results were still erratic.

A major part of this current study deals with proposed modifications in the W-M-F-T test to minimize as much as possible steps in the analytical procedure where differences in results may occur due to the operator. The factors which merit consideration include: the manner in which the wheat must be kept before grinding and the manner in which it must be ground; uniform mixing of the dough; dough consistency and dough development time; molding of the doughballs; and the control of humidity at the surface above the floating doughball.

The Contribution of Amylases to the Production of Sugars Fermentable in Panary Fermentation

The Role of Mechanically Damaged Starch. Diastatic activity in bread doughs is the result of two factors, enzyme content and starch susceptibility.

Several studies have established that the quantity of available or susceptible starch, rather than the concentration of beta-amylase is the limiting factor governing the results of autolytic tests on sound wheat flours. In the flours released at various stages in the commercial milling process there is a high correlation between maltose value and the percentage of starch granules over 20 u in diameter which are stainable with congo red (Geddes, 1950).

Flour produced from Turkey wheat showed a reducing power equivalent to 1.2 per cent maltose but the same flour ground for 53 hours showed a

reducing power equivalent to 5.4 per cent maltose (Alsberg and Griffing, 1925). According to Karacsonyi and Bailey (1930) overgrinding wheat flours resulted in substantial increases in diastatic activity as measured by the Rumsey autolytic method (Rumsey, 1922). Gas production and gas retention in freshly mixed doughs as well as in doughs previously fermented for 3 hours were not substantially modified by overgrinding. This suggests that the Rumsey method for measuring diastatic activity may fail at times to afford an adequate basis for estimating the fermentation potentialities of a wheat flour when measured in terms of gas production, gas retention or fermentation tolerance. According to Markley and Bailey (1934), the condition of the surface of the flour particles as affected by the humidity of the air during the grinding and bolting of the middlings, rather than the particle size in itself was found to be of major importance in determining the diastatic activity of flour. Fisher et al., (1938) have concluded on the basis of their observations, that the routine maltose test cannot be used as an adequate substitute for the gassing determinations. Bottomley (1938) reported a low correlation between diastatic activity and gassing power. Since diastatic measurements did not account for all the factors which influenced gas production, the results obtained were less informative than methods which actually measured gas production. Jones (1940) suggested that the enzymes responsible for diastasis in sound wheat products must be regarded as uniformly distributed throughout the wheat endosperm. Diastasis in a water suspension is not affected by particle size per se, and in flours and intermediate stocks variously milled from a given wheat the maltose figure is a measure of the number of "ghosts" present. "Ghosts" are present in flours to very different extents according to the

conditions of milling. The type of damage to the granule in "ghosts" depends both on shearing and crushing action encountered in roller milling. Dadswell and Wragge (1940), found that injury to the starch granules in milling was correlated with variety but not with place of growth. The maltose formed during the autolytic digestion of flour was positively correlated with the extent of injury to the starch granules. The smoother the reduction rolls, the more heat they develop in milling. Ziegler (1940) reported that roll temperatures below 20°C. had more effect on diastatic activity per degree increase, than temperatures above 20°C. From about 30°C. to 80°C. the increase in maltose figure seemed to be a linear function. The higher maltose figures of commercially milled flours versus experimentally milled flours was in part, at least, the result of the higher roll temperatures involved in the former process. Grinding flour with steel balls for 30 hours in a porcelain ball mill resulted according to Selman and Sumner (1947) in a drop of maximum Amylograph viscosity from 595 to 440, increased pressuremeter values from 553 to 642, and increased maltose values from 304 to 502.

The Contribution of Alpha- and Beta-Amylases to Gassing Power. Both alpha- and beta-amylase are involved in the breadmaking process. Sound wheats contain relatively large amounts of beta-amylase whereas their content of alpha-amylase is extremely low. Beta-amylase is acid stable, and thermolabile, but it is unable to attack raw, mechanically-uninjured starch and it hydrolyzes damaged starch slowly. No beta-amylase is present in fungal concentrates. Much of the beta-amylase is present in flour in an inactive or "bound" condition which is liberated and becomes active or "free" when the grain is germinated. The initial action of beta-amylase is on the non-reducing chain end of the starch molecules thus liberating maltose.

An amylose molecule containing an even number of glucose units is hydrolyzed to maltose, while one containing an odd number is broken down to maltose plus one molecule of maltotriose. The latter is very slowly converted to maltose and glucose by beta-amylase. Beta-amylase action on amylopectin ceases when the alpha (1 - 6) linkage is reached thus forming beta-amylase limit dextrin. It is recognized that beta-amylase rapidly saccharifies available starch to an extent of some 60 per cent conversion to maltose; in contrast, alpha-amylase has a low capacity for the production of reducing groups, especially in the initial stages of starch conversion. Stamberg and Bailey (1939) found that adding beta-amylase to wheat-flour doughs did not improve the bread and that the enzyme appeared to be present in the flour in sufficient quantities. Kneen et al., (1941) estimated after comparing the starch degrading properties of 12 barley malts that over three quarters of the degree of saccharification was due to beta-amylase. But they also pointed out that several malts high in alpha-amylase showed greater saccharogenic activity than would be predicted on the basis of the beta-amylase content. There was, however, no correlation between alpha-amylase and saccharogenic activity. Lee and Geddes (1959) described experiments which were said to justify the conclusion that the very rapid increase in the maltose content of regular sponges and doughs during mixing was due to the action of beta-amylase on the susceptible starch in the flour. The consensus seems to be (Silberstein, 1961) that beta-amylase acts primarily during the fermentation and proof time; when the dough reaches the baking stage the heat-labile enzyme is rapidly inactivated.

Alpha-amylases from different sources are capable of liquefying, dextrinizing and saccharifying starch which is not sufficiently damaged to be

susceptible to beta-amylase. The initial action is a very rapid liquefaction of starch paste caused by the rupture of the more centrally located alpha-1, 4-glucosidic linkages. In contrast to the beta-amylases, the alpha-amylases are able to cleave branched substrate molecules between the branch points. In place of the high molecular weight limit dextrin produced by beta-amylase, the alpha-amylase "limit-dextrins" are low molecular weight branched oligosaccharides containing only a few glucose units. Concomitant with dextrinization, the starch molecule loses its capacity to form colors with iodine. Cleavage of only 0.1 per cent of the alpha-1-4-glucosidic links in the starch substrate reduces the viscosity of a paste to 50 per cent, liquefaction is complete when about 0.5 per cent of the bonds are broken (Hopkins, 1946); dextrinization requires the cleavage of about 7 per cent of links. Alpha-amylases of different origins differ in addition to the optimal and stability characteristics regarding pH and temperature, also in the types of end products which they form beyond the stage of the achroic point (Redfern, 1950). These differences are mainly to be accounted for by varying affinities of amylases for their various substrates. Action of amylases has generally been considered to be random such that the long chain is attacked indiscriminately with the production of shorter chains; production of reducing and fermentable sugars is relatively slow but may be eventually extensive (French, 1957). This picture seems to hold in part only. Even in the initial stages of amylolysis there is simultaneous production of high molecular weight dextrins and low molecular weight oligosaccharides. Pazur and Sandstedt (1954) found that as a result of a 4-hour action of malt alpha-amylase on starch a series of sugars of low molecular weight including glucose, maltose, amylotriose, amylotetraose and

amylopentaose was formed. This is in contrast to the action pattern of salivary amylase which produces only three reducing sugars of low molecular weight, maltose, amylotriose and amylotetraose (Bird and Hopkins, 1954). Alpha-amylase cannot fragment away 6-substituted glucose units in the form of isomaltose, or one or more 4-substituted glucose units always remaining in the fragment. The smallest limit dextrin formed by malt alpha-amylase is 4-alpha-isomaltosyl-glucose (panose), by animal and fungal amylases 4-alpha-panosylglucose, and by Bacillus subtilis alpha-amylase it is a pentasacchride (Whelan, 1961). Johnson and Miller (1948) have found that fungal alpha-amylase was slightly more effective than malted barley or wheat in increasing gas production and in yielding improvements in bread denoted as malt response. Alfin and Caldwell (1948a) found that the extent of hydrolysis of soluble potato starch, whole potato starch, and linear fractions from corn starch by a highly purified pancreatic amylase, depended in each case upon the concentration of amylase used. Maltose was present from the very early stages both in linear fractions of corn starch and whole potato starch, but in larger concentrations in the hydrolysates from the linear substrate (Alfin and Caldwell, 1948b). Glucose did not appear in the early stages of hydrolysis and was present in slightly larger concentrations in the hydrolysates of potato starch. Waxy maize starch was hydrolyzed more slowly by pancreatic amylase than unfractionated corn starch and much more slowly than the linear fraction from corn starch (Mindell et al., 1949). From the very early stages of the hydrolysis of waxy maize starch by purified pancreatic amylase, maltose was present in significant concentrations. Glucose also was liberated but in smaller concentrations and not in the very early stages of the hydrolysis. Hanrahan and Caldwell (1950) reported that increases

in the concentrations of purified crystalline Taka-amylase effected an increase in the rate and extent of hydrolysis of a linear fraction from corn starch to end products maltose and glucose, as determined by chromatographic and selective fermentation techniques. Although Taka-amylase did not hydrolyze maltose, it hydrolyzed trisaccharides and higher sugars composed of 1,4-alpha-D-glucosidic chains. The linear fraction from corn starch was hydrolyzed more extensively and rapidly than several branched chain substrates. Conn et al., (1950) suggested that the difficulties involved in using bacterial amylases in flour supplementation may be due to their thermostability but also due to the lesser affinity of bacterial amylases for low molecular weight dextrins. Accordingly, bacterial alpha-amylase molecules may be free to split greater numbers of starch molecules with a corresponding increase in dextrin formation and stickiness of bread crumb. Gas production of water extracts of bread was largest (per unit of alpha-amylase) in breads baked with the bacterial enzyme; fungal amylase produced the smallest amounts of fermentable sugars; malted wheat flour preparations were slightly lower than bacterial supplements (Miller et al., 1953). Beck et al., (1957) found that the residual maltose in bread crumb increased much less with increasing quantities of fungal than with comparable quantities of cereal or bacterial alpha-amylase. Ulmann and Seidemann (1958) studied the behavior of amylases on potato and wheat starches by paper chromatographic analysis of the sugars formed. At 40°C. fungal amylase produced maltose and maltotriose in large quantities within 10 min., along with traces of maltotetraose and maltopentaose; after 20-30 min. maltohexaose and maltoheptaose appeared. When compared with different malt preparations and with pancreatic amylase, fungal amylase was found to produce a greater

number of higher saccharides and larger amounts of some of the intermediate sugars. Isomaltose has not been observed in fungal amylolysates of starch. With fungal preparations, glucose appeared at an earlier stage than with malt, though the latter preparation yielded larger amounts of maltose. A temperature of 55°C. was optimal for formation of glucose by fungal amylase and least favorable for production of higher sugars. The greater difficulty in saccharifying wheat starch compared to potato starch was shown by the larger survival of the higher sugars; this effect was observed with fungal, pancreatic and salivary amylases, and beta-amylase. Drapron (1962) reported that when starch was acted upon by bacterial amylases, the formation of glucose and maltose was at the beginning very slow and increases progressively. After 10 minutes the largest moieties were maltoheptose and maltooctaose, both of which disappeared after 15 minutes, concomitant with a rapid formation of maltotriose, maltohexose and maltoheptose. The action of alpha- and beta-amylases was additive at low enzyme concentration, at higher enzyme levels, especially of beta-amylase, the extent of hydrolysis exceeded the calculated one. Fungal amylases were found to produce larger amounts of fermentable sugars than malt amylases produced. According to Hayden (1961) fungal-amylase produced primarily maltose; the main product of cereal amylases was a hexasaccharide. Employing a 2 per cent soluble starch substrate, buffered at pH 4.6, and using the ferricyanide method, Preece and Shadaksharaswamy (1949) found that at 21°C. reducing group production by beta-amylase showed a linear relationship with time up to 15 per cent hydrolysis; the point of action of beta- and alpha-amylases showed a similar relationship up to 20 to 25 per cent hydrolysis. With alpha-amylase there was no true linearity but up to 10 to 15 per cent hydrolysis the

deviation from linearity was small. Sandstedt and Gates (1954) found a wide variation in the relative potential amylase systems from various sources to digest raw starch. A comparison of malt, fungal, bacterial and pancreatic enzyme systems at a constant level of alpha-amylase activity showed that their ability to digest raw starch was not proportional to the alpha-amylase. Used on the alpha-amylase basis, the pancreatic enzyme system was the most effective in digesting raw starch, followed in order by the malt, the bacterial and the fungal; the pancreatic was 20 times as effective as the fungal.

The Gassing Power Test. There are four general methods of measuring alpha-amylase activity: maltose value, gassing power, starch liquefaction and dextrinogenic activity. The first two methods indicate the combined activity of the alpha-amylase added in the form of an enzymatic supplement and the beta-amylase present in the flour. Both measurements are, however, affected also by the presence of damaged starch and its susceptibility to enzymatic attack. The flour mill chemist wants a test which gives an accurate measure of a flour's ability to maintain adequate gas production during panary fermentation. Blish et al., (1932) found that the terms gassing power and diastatic activity were not synonymous. Lack of parallelism between the two properties was attributed due to variations among sugars with respect to original sucrose content. A simple manometric procedure for the estimation of gassing power was proposed. The pressure generated at constant volume was directly proportional, within limits, to the amount of sugar fermented. Improvements in the manometric apparatus were reported (Sandstedt and Blish, 1934) and the method was shown to offer advantages of simplicity of apparatus, economy of space and of equipment, compactness, and minimum of difficulties in temperature control. As the

gassing power test employs measurement of carbon dioxide produced by a yeast-containing fermenting dough, the evaluation is complicated by the necessity of a uniform yeast. Bohn and Favor (1939) have found, however, that yeast variability is usually of very little significance in determining the gassing power of a flour, provided a fresh yeast is used. Doty and Urban (1940) reported that the data obtained with the pressuremeter correlated very well with data obtained by actual test baking. The pressuremeter shows so slight variations in daily shipments of yeast that these variations can be discounted.

According to Hayden (1961) the use of amylases of microbiological origin make the methods, previously employed by the mill chemist, obsolete. Enzymes of fungal origin differ from cereal amylases by virtue of the fact that the former contribute little to the maltose value, despite their pronounced beneficial effect on gas formation during fermentation. Supplementation with fungal amylase would be expected to provide additional substrate for the action of beta-amylase present in wheat flour, and consequently affect the maltose value. This is, however, not the case. The discrepancy is due to the fact that the formation of reducing sugars requires a relatively long time, and cannot be detected by the maltose test. A fermenting dough differs materially from a buffered flour suspension employed in the maltose test. Cell-free yeast extracts have been shown to contain enzymatic systems which provide additional substrate for the action of beta-amylase. Whereas the action of fungal amylases on gas production is affected by yeast-strain variations, no such changes were found on employing equivalent levels of malt-amylases. The existence of yeast-amylase relationships has already been suggested by Preece (1952). Whereas certain

starches in solid granular form are unattacked by a diastatic liquid, they are hydrolyzed if a trace of yeast is added. The mechanism of the complementary action of yeast is as yet obscure. The yeast may activate the malt enzymes, or on the other hand the function of the yeast itself may be favored by the malt enzymes allowing the two together to function more efficiently than either can alone. Whatever the case may be, the gassing power test is better suited than the maltose value to give a dependable picture of the potential contribution of an amylase supplement to gas formation.

The present study deals with a comparison of the relative contributions of fermentable sugars by alpha- and beta-amylases to fermentation employing crystalline enzymes.

MATERIALS AND METHODS

Wheat-Meal-Fermentation Time Test

Grinding of the Wheat Meal. Wheat samples previously kept or stored in a cold room (40°F.) were allowed to equilibrate to room temperature and ground with a Wiley mill (using a No. 20 mesh sieve). Twenty-five g. of wheat were milled a day before the test was to be performed as recommended by Cutler and Worsella (1933) and Swanson (1937). The Hobart laboratory grinder, Tag Happenstall mill and the Wiley mill were first compared. The Wiley mill was chosen because of the following advantages: it is easy and simple to operate; it is quick, the time it takes to grind the sample and to clean the mill for the next sample is only 2 to 2 1/2 minutes.

Mixing of the Dough. A Farinograph (with a special ten-gram mixing bowl) was used to form a dough from the ground wheat. Ten grams of wheat

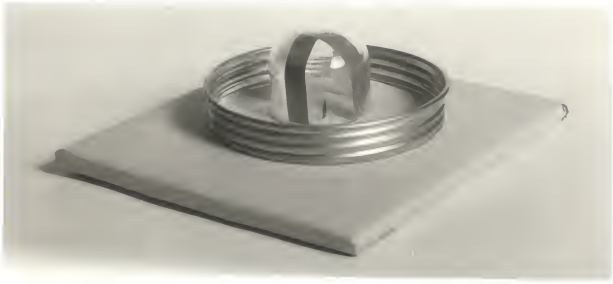
meal were used for each mixing. All types of wheats were mixed to the same consistency in the range of 800-850 Brabender Units. This is the best consistency with which the dough can easily be handled by the operator. Mixing was done at 28 r.p.m. for 10 minutes for hard wheats and 5 minutes for soft wheats. This mixing time was based on the ability of these ground wheats to produce a dough which was not sticky and could easily be handled by the operator.

Yeast Suspension Used. The solution used for making up the dough was composed of 10 g. of Fleischman's baker's yeast suspended in 100 ml. of a 0.8 per cent NaCl solution (at 30°C.). Five ml. of this yeast suspension were used per 10 g. of sample. Enough water was added (when necessary) to the mixture to bring the dough to the desired consistency.

Molding of Doughballs. Three-gram doughs were weighed out from the dough obtained per mixing and molded into doughballs by rounding 15 times with a simple device (Fig. 1) prepared from a smooth metal jar lid (C, 48 mm. diam.) and a nail (B) mounted on a piece of wood (A), and the bottom of a test tube (D; 20 mm. internal diameter, 18 mm. total height). The details of the molding device are given in Fig. 1.

Determination of the W-M-F-T Time. Doughballs were placed in 150 ml. beakers with 50 ml. of distilled water (at 30°C.) and set in a water bath controlled at 30°C. The beakers were covered with watch glasses (convex side up) to maintain a constant humidity inside the beaker. This prevented drying of the unsubmerged portions of the floating doughballs.

Determination of the "End-Point". The test was considered to have reached its "end-point" when:



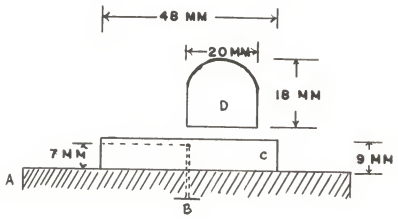


Fig. 1. Doughball molding device.

a) the doughball had disintegrated;

b) a dough with a tiny portion sticking at the sides of the beaker was completely under water, although not necessarily disintegrated;

c) a sagging doughball had its tip touching the bottom of the beaker;

d) a dough had completely sunk.

Effects of Additives. Baking tests, which are considered the ultimate criterion of bread baking potentialities are known to be affected by the strength of wheat flour used, baking procedure, and dough composition. It was therefore decided to determine what effects some of the bread-dough components have on the W-M-F-T times. Four oxidizing agents were used, namely: KIO_3, $Na_3H_2IO_6$, $KBrO_3$, and $(NH_4)_2S_2O_8$. Each oxidant was added at different levels. The effects of reducing agents were also determined by adding 0.5 cc of a 0.001 M solution of a number of reducing agents varying in -SH content and molecular weights (such as cysteine, glutathione and thiolated gelatins). The effect of various levels of sugar (sucrose) on W-M-F-T was studied, both with hard and soft wheats. Various amounts of lactic acid were added to hard and soft wheat meal doughs and the effect on the W-M-F-T test was studied. The effects of increasing amounts of starch and gluten were also studied. The gluten used was a commercial product.

Effect of Storage. Ground and unground wheat was stored at an elevated temperature (105-110°F.) to determine whether the W-M-F-T test could detect changes that took place while on storage. Flour was milled with an experimental Buhler mill from unground wheats that were stored and the baking as well as the sedimentation tests were run (A.A.C.C., 1957). The results were compared.

Wheat Samples Used for Correlation Studies. Nine varieties of hard

red winter wheat from the Central Plains, harvested in 1961 were tested by the modified W-H-F-T method. High-protein samples from Akron, Colorado, intermediate-protein samples grown without irrigation from Garden City, Kansas, low protein wheats from Hays, Kansas, and slightly low protein wheats from Colby, Kansas, were used. The data on wheat protein content, baking test, water absorption, $KBrO_3$ requirement, and mixing time of these wheats were obtained from the Hard Winter Wheat Quality Laboratory, USDA, at Kansas State University, Manhattan, Kansas.

The Contribution of Amylases to the Production of Sugars Fermentable in Panary Fermentation

Reagents: The pure crystalline, Taka-amylase A, used in this study was a gift from Shoji Matsubara, Osaka University, Japan (Matsubara, et al., 1959). The pancreatic, crystalline amylase and the sweet potato crystalline beta-amylase were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio, and the crystalline bacterial enzyme from Worthington, Biochemical Corporation, Freehold, N. J. Additionally, crude amylases from cereal, bacterial and fungal origin were studied. Among the cereal amylases, two samples of wheat, two samples of barley and a sample of sorghum amylase were tested. One of the wheat malt preparations and the sorghum malt were partly purified. The other enzymes were concentrates furnished by three different commercial firms. The dextrinogenic activities of the tested alpha-amylases (expressed as S.K.B. units per gram, Sandstedt et al., 1939) were:

Wheat	19 and 44
Barley	52 and 284
Sorghum	840
Fungal	908;4,590;5,000,7,900;8,140;17,000
Bacterial	5,480;7,950; and 880 respectively.

The crude beta-amylase (essentially free from alpha-amylase) was purchased from Wallerstein Laboratories, Staten Island, N. Y. The starch used as the substrate was pregelatinized wheat starch from Stein, Hall and Co., Inc., New York, N. Y.

The nitrogen base medium for carbon assimilation tests with yeast was prepared in the laboratory according to the formula in the Difco Manual (1953). Yeast suspensions were made by suspending 7.5 g. of Fleischman's wet cake baker's yeast in 95 ml. of a 2 per cent sodium chloride solution. The alpha-amylases were added in the form of a suspension in 0.2 per cent calcium chloride or 0.2 per cent sodium chloride (in case of the pancreatic amylase); the beta-amylases were dissolved in water.

Method: Unless otherwise stated, 10 g. of pregelatinized starch were weighed in one side of the aluminum cup of the Sandstedt-Blish pressuremeter (Sandstedt and Blish, 1934). At 1-minute intervals, 5 ml. of nitrogen base medium, 5 ml. of yeast suspension, 5 ml. of water to give a total volume of 25 ml. of liquid were added. The consistency of the paste was comparable to that of a wheat dough in breadmaking.

The contents were mixed well prior to tightening the lids, and the pressuremeter placed in a water bath at 30°C. After 5 minutes, the pressure was released, and 5 pressure readings were taken at 1-hour intervals.

RESULTS AND DISCUSSIONS

Wheat-Meal-Fermentation Time Test

Mixing of the Dough. Previous studies have emphasized the effects of mixing on the W-M-F-T test, and the necessity of forming a dough of uniform consistency before working it into doughballs. As was already mentioned, a Farinograph (with a special 10-g. bowl) was used in the present study. According to officially recognized procedures (A.A.C.C., 1957; Anonymous, 1958), the dough is prepared by mixing the wheat meal with a yeast suspension in a mortar or in a beaker, employing a pestle or a rod. Under such conditions gluten development probably does not reach its optimum levels failing to give a three-dimensional structure which is believed to be responsible for trapping and retaining the gas formed during fermentation. This may be the reason why the times of all the determinations using the proposed method were relatively longer than the times reported in previous studies. The effect of mixing time can be seen in Table 1.

Table 1. Effect of mixing time (Farinograph) on W-M-F-T.

	W-M-F-T	
Mixing time	Triumph (Hard)	Elmar (Soft)
(min.)	(min.)	(min.)
5	230	47
10	256	sticky
15	sticky-could not be molded	

It is clearly shown that with hard or "long time" wheats the time increases with increase in mixing time up to a point when over-mixing is detrimental to the dough characteristics. Soft or "short time" wheats become sticky beyond 5 minutes of mixing.

Axford and Elton (1960), Sullivan et al., (1961) and Sokol et al., (1960) suggested that during mixing the energy applied to the dough results in some intramolecular -S-S- bonds being changed to intermolecular -S-S- cross linkages through the mediation of very small amounts of thiol groups. This intermolecular disulfide bridge is presumed to confer toughness and greater resistance to extension (Sullivan et al., 1961) and that further mechanical development of the dough causes these bonds to break as a result of the strain on the protein molecules subjected to mechanical shearing forces (Axford and Elton, 1960). Sokol et al., (1960) reported that there was a rapid decrease in sulfhydryl content with time of mixing of a dough. They observed a decrease of as high as 38 per cent to 64 per cent of original sulfhydryl content after 20 minutes mixing. Miller and Johnson (1954) found a higher correlation between breakdown of the Farinogram curve (time elapsed before the curve shows a definite break downward) and loaf volume than between optimum mixing time and protein content. These facts greatly contribute to the picture when a dough is under-oxidized and over-oxidized (either through the addition of oxidants or by atmospheric oxidations).

Mixing the dough with the Farinograph gave some difficulties. Aside from the fact that it takes about 25 minutes to mixadough, (this includes actual mixing of sample and cleaning the mixing bowl and blades for the next sample), it is very difficult to recover or remove most of the dough from the bowl and blades without further modifying the dough through added

pressure by the fingers or hand of the operator. This remains as an undetermined factor in the analytical procedure which can cause differences of results in testing hard red winter wheats by different operators.

Effect of the Method of Molding the Doughballs. The effect of how the doughballs were molded was studied with the use of a molding device and with the palm of the hand. Doughballs made with the palm of the hand had a smoother surface than those molded with the molding device. Comparison of the times of hand (palm) and device molded doughballs is shown in Table 2.

Table 2. Effect of method of molding the doughballs on the W-M-F-T test.

Method	Ave. time (min.)
Hand molded	205
Device molded	256

The times of the hand-molded doughballs were relatively shorter than those molded with the molding device. Varying the number of turns of the glass cup of the molder affected the times of the doughballs. Times increased as the number of turns was varied from five to ten turns, times were consistently uniform at about 15 turns, beyond 25 turns, the times were erratic. It was, therefore, decided to mold the doughballs by turning the glass cup 15 times.

The Effect of Humidity on W-M-F-T. Humidity at the surface of the distilled water at which the doughball floats had an effect in the time test. Previous studies emphasized the need for a constant humidity throughout the test. Cabinets with a controlled humidity or a glass water bath with a cover

on top were used in the previous studies. In the present test, however, the individual beakers were covered with glass covers (with convex side up) as a means of controlling the humidity inside the beaker enough to prevent drying up of the exposed portion of the doughball during the test. Individual covers were used instead of using a cabinet with a controlled humidity because this seemed to be a better way of controlling a uniform humidity in all the beakers throughout the test. Beakers were of the same size and had the same volume of empty space from the surface of the distilled water to the top of the glass cover. In the case of a cabinet with a controlled humidity, however, opening of the cabinet off and on when placing of new samples and taking out of samples which have already reached their "end-point" is likely to cause variations in humidity during the test. The effect of a controlled humidity on the time is shown in Table 3.

Table 3. Effect of humidity on the W-M-F-T.

	W-M-F-T (min.)
Without cover	342
With cover	302

Evaporation in the uncovered beaker is uncontrolled and the exposed portion of the doughball dries up causing a bouyant effect on the doughball as a whole which eventually lengthens the time. One argument against covering of the beaker or any container used is the possibility of trapping so much of CO_2 in the distilled water, since CO_2 is readily soluble in water, thus affecting the time (Swanson, 1937). He found that as the concentration

of CO_2 in the water increased, the time also increased. The relative effect of CO_2 on the "time" seems, however, insignificant as all the wheat samples were treated in the same manner throughout the test (from grinding to molding). Cover glasses were used with their convex side up to prevent droplets of condensation from dropping directly on the doughballs.

Effects of Additives. The effects of various levels of oxidants on the W-M-F-T test have been studied. Four oxidizing agents were used. Their relative effects are shown in Table 4.

Modification of the dough properties due to the presence of oxidants or other maturing agents results from the oxidation of the sulfhydryl groups in the gluten protein. KIO_3 and $Na_3H_2IO_6$ were much more effective oxidizing agents among the four that have been tried. Lee and Samuels (1962) comparing KIO_3 and $KBrO_3$ found the former to be most powerful in oxidizing the sulfhydryls in flour. The W-M-F-T shows an optimum response to oxidizing agents. How well these optimum levels can be used to predict the actual bromate or other oxidizing agent requirement of a corresponding flour for baking is not known. The W-M-F-T test, however, requires more oxidizing agents than does flour. This would be expected from the oxidation requirements of the SH-rich germ and aleurone content of the whole wheat used in the W-M-F-T test.

Adding 0.5 cc of 0.001M solution of a number of reducing agents, varying in their -SH and molecular weights (cysteine, glutathione and thiolated gelatins), had no consistent or significant effect on the W-M-F-T. This is interesting in view of the assumption of Pool and Patterson (1958) on the probable effect of rainfall and sunshine on the W-M-F-T presumably due to the effect on the thiol containing amino acids of the wheat proteins.

The effect of various levels of added sugar (sucrose) on W-M-F-T was

Table 4. Effect of oxidizing agents on W-M-F-T.

Oxidant	Level	W-M-F-T
	(mg./10 g.)	(min.)
KIO_3	0	244
	0.03	259
	0.06	264
	0.12	297
	0.24	316
	0.48	339
	0.77	48
$Na_3H_2IO_6$	0	245
	0.03	265
	0.06	257
	0.12	281
	0.24	332
	0.36	322
$KBrO_3$	0	244
	0.4	249
	0.8	372
	1.6	422
	2.4	398
	3.2	333
	4.0	-
$(NH_4)_2S_2O_8$	0	245
	1.5	280
	3.0	289
	6.0	334
	9.0	397
	15.0	395

also studied. The idea was to supplement the amount of fermentable sugars for long time wheats, and possibly intensify the fermentation and eventually shorten it. Vigorous fermentation, in the presence of added sugar did not, however, shorten the W-M-F-T. This is shown in Table 5.

Table 5. Effect of added sucrose on W-M-F-T.

Amount of Sugar	W-M-F-T	
	Triumph	Omar
(mg.)	(min.)	(min.)
0	253	37
100	289	41
250	372	43.5
500	over 400	sticky (cannot be molded)

These results are comparable to those observed by Swanson (1940) who observed an increase in W-M-F-T, both for soft and hard wheats. Pool and Patterson (1958), however, observed a decrease in time when sugar was added to soft red winter wheats.

A number of studies deal with the role of lactic acid in baking. Additionally, lactic acid is used in a number of wheat-strength tests such as the sedimentation test and the Berliner-Koopman test. The effect of lactic acid on W-M-F-T was therefore studied. Table 6 shows the response of soft and hard wheats to different levels of lactic acid.

Lactic acid at the 0.2 per cent level increased the "time", higher levels of lactic acid decreased the W-M-F-T of hard wheat. In the case of the soft wheat Omar, however, the W-M-F-T increased up to 0.80 per cent of lactic acid. It is possible that in the case of soft wheats, lactic acid

Table 6. Effect of lactic acid on W-M-F-T.

Lactic Acid Added	W-M-F-T	
	Triumph	Omar
(%)	(min.)	(min.)
0	239	40
0.20	289	45
0.40	210	63
0.60	180	112
0.80	173	307

had an effect similar to the one shown in the MacMichaele's viscosity test, where increased levels of lactic acic increases the viscosity of flour suspension, as a result of protein hydration and swelling. In the case of Triumph, normally a long time wheat, the same effect seems to be shown only to a limited extent, beyond which other factors seem to overcome the improving effect caused by lactic acid.

The W-M-F-T test has been shown to be affected by dilution with starch. As shown in Table 7, the time decreases as the amount of starch is increased in a mixture of wheat meal and starch.

Table 7. Effect of diluting wheat meal with starch using the present method.

Proportion in Mixture		W-M-F-T
Wheat meal	Starch	Triumph
(gm.)	(gm.)	(min.)
10	0	254
9	1	223
8	2	216
6	4	156
5	5	133

The results are comparable to those found by Swanson (1940). However, when the latter added starch to flour, the time was not reduced as much as when wheat meal was used.

The effect of proteinacious material on the W-M-F-T was also studied. Wheat gluten at different levels was added to the wheat meal. The results are shown in Table 8.

Table 8. Effect of commercial wheat gluten on W-M-F-T.

Proportion in Mixture		W-M-F-T
Wheat meal	Gluten	Triumph
(gm.)	(gm.)	(min.)
10	0	254
9	1	185
8	2	86
6	4	67
4	6	57

It is surprising that the addition of wheat gluten to the meal decreased the time. This might be due to the quality of the gluten used or due to the fact that the gluten was in some way denatured. The gluten used was a commercial product. The effect observed was essentially that of dilution.

Blending of wheats before milling to obtain a desired type of flour is commercially done. The W-M-F-T test was therefore used to determine whether it could detect changes resulting from blending two types of wheat. A hard and soft wheat was blended and their fermentation time determined.

It is seen that as the amount of hard wheat meal in a mixture is decreased, the time also decreases. The relation, however, is not linear.

The effect of storing ground and unground wheat at an elevated

temperature (105-110°F.) on W-M-F-T has been studied. Triumph 1960 was used.

Table 9. Effect of blending two types of wheat on fermentation time.

Proportion in Blend		W-M-F-T
Triumph (Hard)	Omar (Soft)	
(gm.)	(gm.)	(min.)
10	0	252
8	2	159
6	4	100
5	5	57
2	8	39
0	10	36

Table 10. Effect of storing whole wheat and wheat meal at 105-110°F. on the W-M-F-T of Triumph.

Length of Storage	W-M-F-T	
	Ground	Unground*
(days)	(min.)	(min.)
1	343	260
6	464	269
22	463	391
27	547	412
29	714	427

*Ground just before the determination is made.

Storing ground wheat or wheat meal changed or increased the fermentation time more rapidly than did storing the whole wheat. This can be expected since the ground wheat has a greater surface area exposed and therefore oxidation is more enhanced resulting in a greater maturing effect.

This study on storage was repeated. The wheat was milled and the flour was baked. Sedimentation values were also determined. Results are shown

in Table 11.

Table 11. Effect of storing whole wheat at 105-110°F. on quality scores of Triumph.

Length of Storage	Total Baking W-M-F-T	Score	Sed. Value
Control (Cold)	275	90	65
1 week	316	93	65
2 weeks	344	93	66
4 weeks	371	90	65
6 weeks	358	88	62.4
8 weeks	321	88	56.4

While both the W-M-F-T and the Sedimentation tests parallel the results from actual baking, the W-M-F-T and sedimentation tests over-emphasize the changes which took place. It is feasible that in the actual baking test, the interplay between a number of factors tends to reduce the effects of certain variables observed by the other tests.

The results of correlation studies of nine varieties of hard red winter wheat from the Central Plains, harvested in 1961 are shown in Table 12 and Figs. 2-5.

Table 12. Correlation data.

	Correlation Coefficient
Loaf vol. vs. protein	+0.850 XXX
Loaf vol. vs. sedimentation value	+0.856 XXX
W-M-F-T vs. sedimentation value	+0.717 XXX
W-M-F-T vs. loaf volume	+0.757 XXX
W-M-F-T vs. percent absorption	+0.34
W-M-F-T vs. percent wheat protein	+0.15
W-M-F-T vs. $KBrO_3$ Reg.	-0.113
W-M-F-T vs. Mixing time	0.09

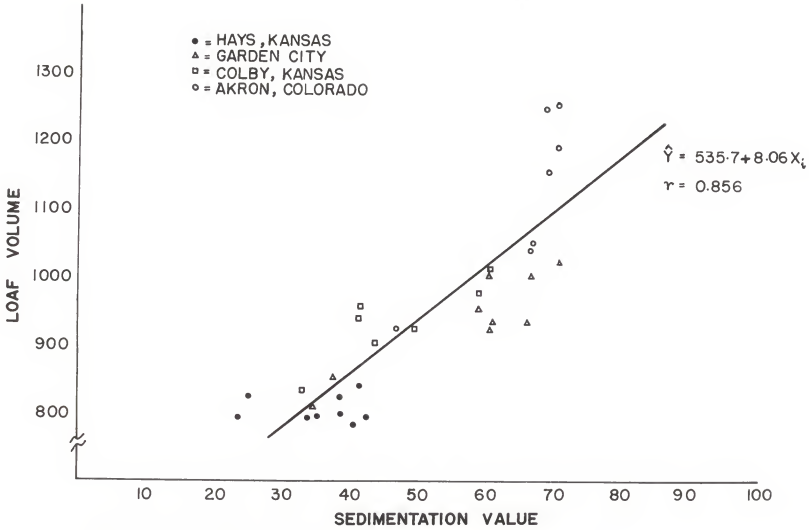


Fig. 2. The relation of loaf volume and sedimentation value.

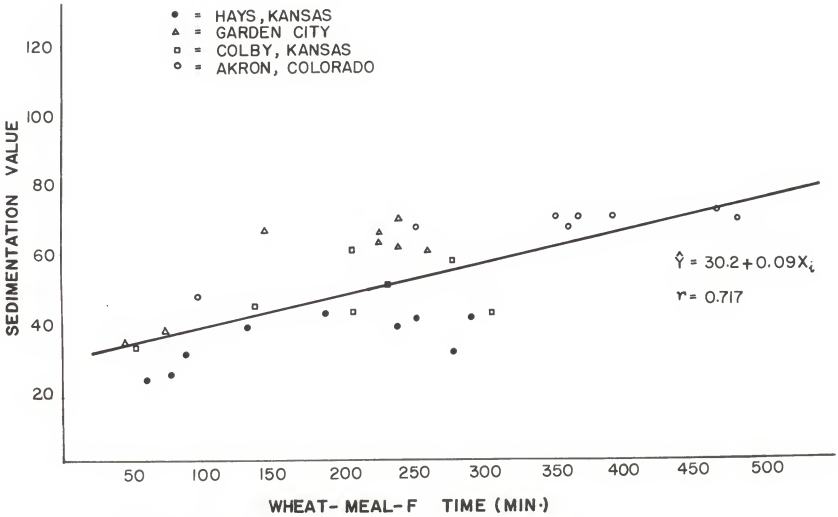


Fig. 3. The relation of sedimentation value and W-M-F-T.

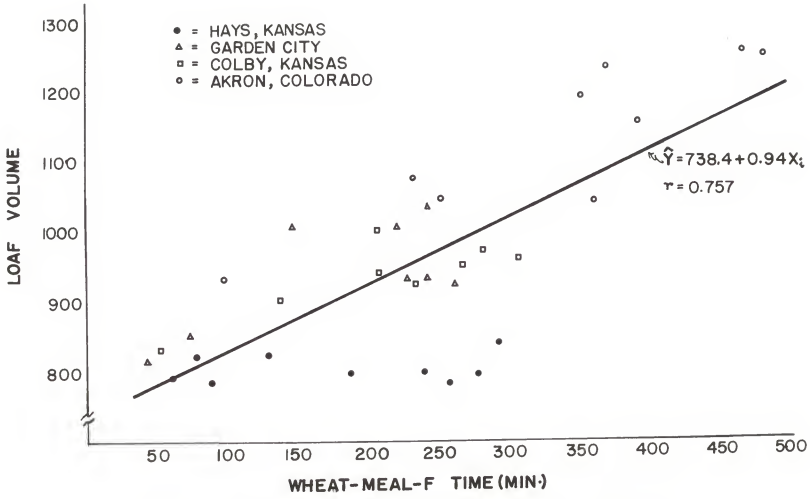


Fig. 4. The relation of loaf volume and W-M-F-T.

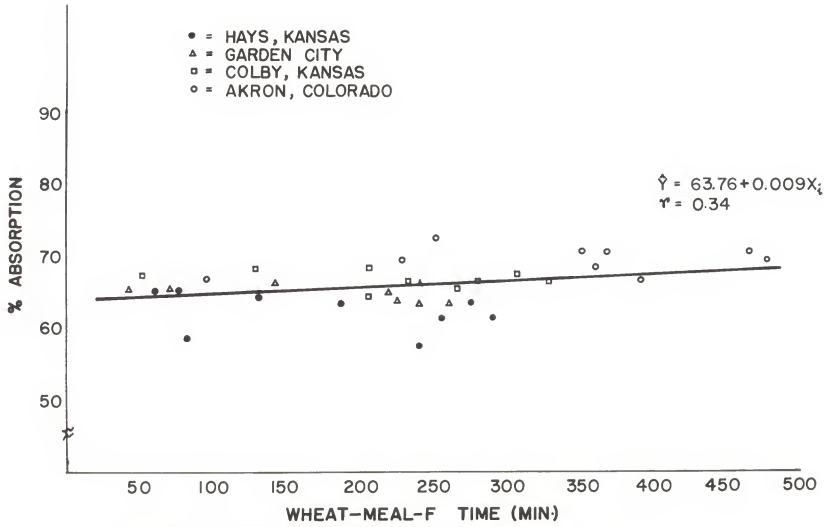


Fig. 5. The relation of percent water absorption and W-M-F-T.

The Contribution of Amylases to the Production of Sugars Fermentable in Panary Fermentation

Comparison of gassing power as a result of action of crystalline amylases on pregelatinized wheat starch is summarized in Figs. 6-9. While these results show that on an equal weight basis, beta-amylase is at least 10 times as potent in providing fermentable sugars for baker's yeast, the contribution of alpha-amylases during the 5-hour fermentation period is quite extensive. A comparison among the amylases from three sources shows the bacterial amylase most potent, the fungal least active, and the enzyme of pancreatic origin intermediate. In case of the pancreatic amylase the low activity is likely to be due to the low pH of the paste (French and Robyt, 1961).

Employing mixtures of alpha- and beta-amylases, the results shown in Table 13 were obtained.

The beta-amylase used in this series was a crude preparation free from alpha-amylase; the alpha-amylases were pure crystalline enzymes. Mixtures of bacterial amylase and beta-amylase or of Taka-amylase and beta-amylase resulted in the formation of fermentable sugars which were, on the average, 14 and 19 per cent respectively lower than the amounts computed from adding the contributions of each enzyme separately. In the case of pancreatic amylase, however, the addition of beta-amylase had practically no effect on the amounts of fermentable sugars by beta-amylase. It seems that in the case of the enzyme of pancreatic origin, the amylase not only severes long chains, but also breaks them down to small, fermentable units, leaving little, if any, substrate for the action of beta-amylase.

A comparison between the action of crude amylase preparations, together

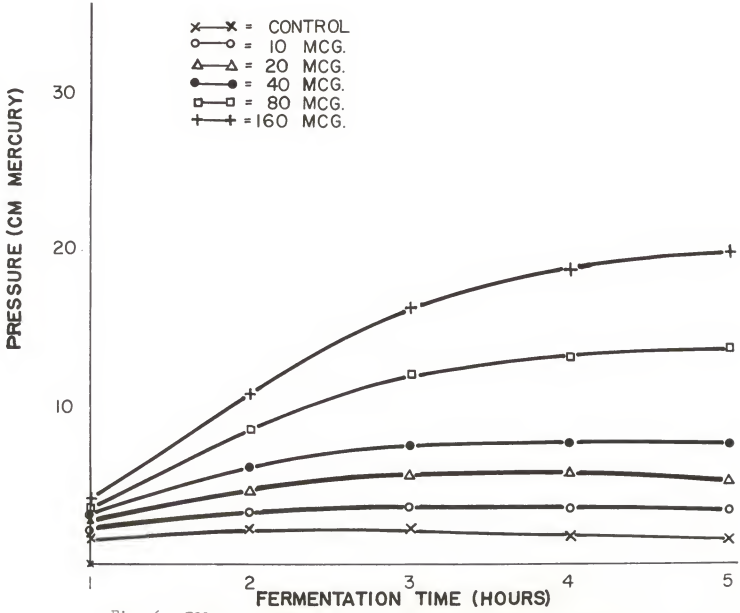


Fig. 6. Effect of crystalline bacterial alpha-amylase on gassing power of pregelatinized wheat starch.

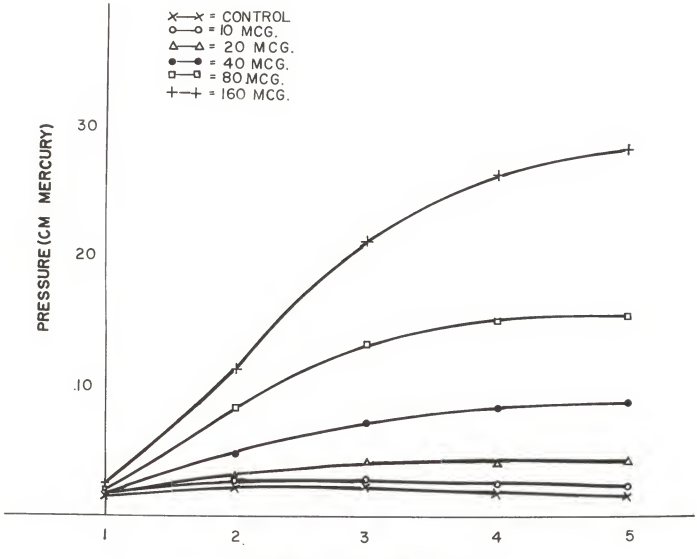


Fig. 7. Effect of crystalline pancreatic alpha-amylase on gassing power of pregelatinized wheat starch.

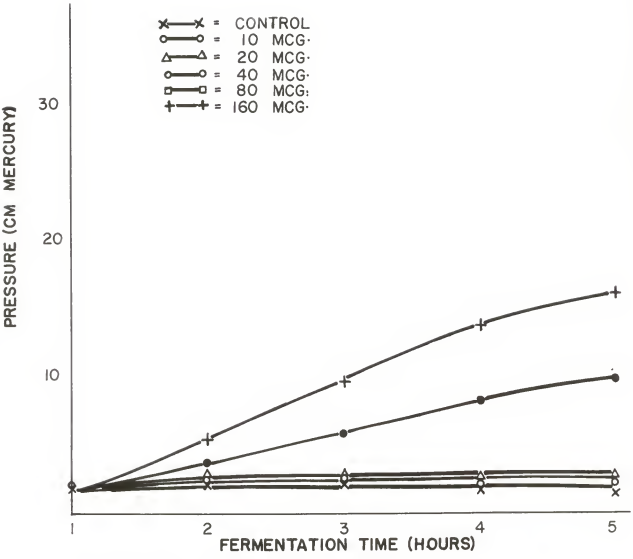


Fig. 8. Effect of crystalline Taka-amylase on gassing power of pregelatinized wheat starch.

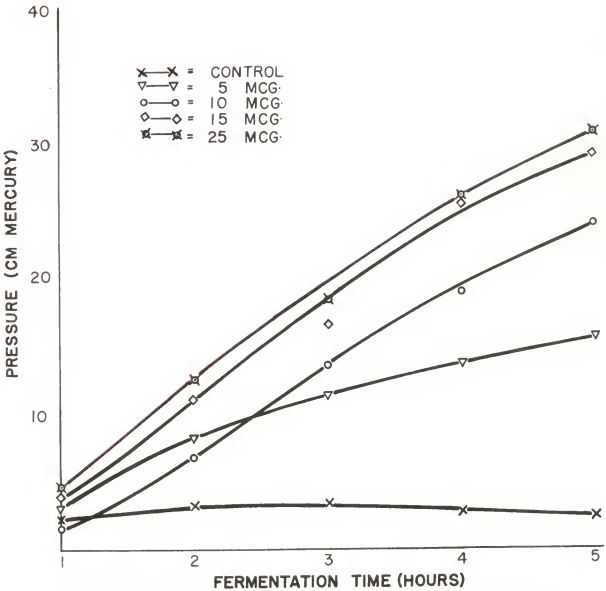


Fig. 9. Effect of crystalline beta-amylase on gassing power of pregelatinized wheat starch.

Table 13. Effect of mixtures of alpha- and beta-amylases on formation of fermentable sugars.

	α-amylase	β-amylase	Pressure (cm. mercury) after fermentation for 2 hrs.	3 hrs.	4 hrs.	5 hrs.
	(mcq.)	(mcq.)				
	-	100	3.8	5.1	5.7	5.8
	-	300	7.1	11.1	12.5	13.2
	-	500	8.5	14.9	17.6	18.5
Bacterial	20	-	3.1	4.3	4.2	4.3
	40	-	4.9	7.1	8.4	8.8
	80	-	8.3	13.1	15.0	15.6
	20	100	4.3	6.4	7.8	8.6
	20	300	7.2	11.0	12.9	13.8
	20	500	10.0	16.5	21.0	25.6
	40	100	5.5	8.0	11.4	12.3
	40	300	8.4	13.4	17.0	18.3
	40	500	11.3	18.8	23.3	27.3
	80	100	7.7	14.0	18.6	21.0
	80	300	10.4	17.5	20.8	27.0
	80	500	11.4	19.8	26.5	30.1
Pancreatic	20	-	4.7	5.6	5.7	5.7
	40	-	6.1	7.6	7.8	7.9
	80	-	8.7	12.2	13.2	13.8
	20	300	3.3	4.9	5.8	6.5
	20	500	4.6	5.8	6.9	7.4
	40	100	3.8	5.6	6.4	6.8
	40	300	5.2	7.4	8.6	8.6
	40	500	4.3	8.3	8.3	9.4
	80	100	6.4	9.4	11.0	11.9
	80	300	6.4	9.7	11.8	16.0
	80	500	7.4	11.2	13.5	14.7
Fungal	20	-	2.4	2.5	2.6	2.6
	40	-	2.7	3.6	4.2	4.4
	80	-	3.6	5.9	8.2	9.8
	20	300	6.5	10.1	13.0	14.7
	20	500	8.2	13.1	16.7	18.7
	40	100	3.8	5.8	7.5	9.0
	40	300	6.1	10.6	13.0	16.5
	40	500	6.3	11.7	15.8	19.1
	80	100	4.3	7.6	10.4	13.3
	80	300	6.2	11.1	14.8	18.0
	80	500	7.3	13.4	18.2	22.6

with the crude beta-amylase is given in Table 14.

Whereas the beta-amylase was free from alpha-amylase, the alpha-amylase must be considered to contain various amounts of beta-amylase (in case of enzymes of cereal origin) or amyloglucosidase (in case of enzymes of microbial origin). The additive effect of beta-amylase has been found in many of the microbial amylases tested, and corresponds to that observed with crystalline preparations. The effect of mixtures of alpha-amylases and beta-amylases is smaller than that calculated from adding the contribution separately. The two low alpha-amylase samples showed no increase in formation of fermentable sugars as a result of supplementation with additional beta-amylase. This seems to be due to their original, relatively high content of free and active beta-amylase. The purified wheat and sorghum alpha-amylases show a beta-amylase response, apparently due to their low beta-amylase content.

SUMMARY AND CONCLUSIONS

Wheat-Meal-Fermentation Time Test

The W-M-F-T test is affected by the following analytical factors: the fineness of the wheat meal, storage of wheat sample before and after grinding, extent of mixing, dough consistency, method of molding the doughball, and control of humidity at the surface of the distilled water where the doughball floats.

Adding sugar at increasing levels increased the W-M-F-T. Oxidants increased the W-M-F-T up to an optimum which varied with the oxidant employed; reducing agents had no consistent effect on the "time" test. Addition of starch and commercial, dry gluten to wheat meal decreased the time presumably due to

Table 14. Effects of mixtures of crude preparations of amylases on gassing power on pregelatinized wheat starch.

	α-amylase (SKB)	β-amylase (mcg.)	Pressure (cm. mercury) after 2 hrs.	3 hrs.	4 hrs.	5 hrs.
FUNGAL:						
Rhozyme 33	-	300	2.7	6.7	10.1	12.5
	4	-	0	1.2	2.3	2.7
	4	100	2.0	4.4	6.8	8.4
	4	300	4.9	9.1	12.7	14.7
	4	500	5.2	12.4	17.4	20.5
F-fungal	-	300	4.2	9.6	10.7	11.9
	4	-	1.0	3.2	5.3	7.2
	4	100	2.5	6.0	9.1	11.4
	4	300	3.3	9.0	14.1	17.7
	4	500	4.9	12.1	17.6	20.9
Mylase	-	300	2.4	5.7	8.6	11.0
	4	-	1.5	3.1	5.1	7.8
	4	100	1.9	3.6	7.2	9.9
	4	300	4.4	9.4	14.4	17.2
	4	500	5.0	11.3	15.8	19.5
Takamine	-	300	2.9	5.7	8.6	11.0
	4	-	0.2	0.9	1.6	2.4
	4	100	1.2	3.7	5.8	7.5
	4	300	3.5	8.1	11.2	13.4
	4	500	3.8	9.8	13.9	16.7
Wallerstein (low-protein)	-	300	3.0	7.1	-	11.6
	4	-	1.1	2.3	3.4	4.4
	4	100	1.7	4.4	6.4	8.1
	4	300	5.1	10.6	14.0	16.4
	4	500	4.6	10.7	14.6	17.1
Wallerstein	-	300	3.0	7.1	-	11.6
	4	-	1.3	3.1	4.4	-
	4	100	4.5	7.4	9.7	11.6
	4	300	3.6	8.3	11.6	14.0
	4	500	3.9	9.3	13.3	16.3
	4	1000	6.2	14.3	20.5	24.8
	4	2000	6.3	15.4	23.6	30.2

Table 14. (cont.)

	α-amylase (SKB)	β-amylase (mcg.)	Pressure (cm. mercury) after 2 hrs.	3 hrs.	4 hrs.	5 hrs.
CEREAL:						
Sorghum	-	300	4.0	8.7	12.3	15.0
	4	-	1.0	2.2	3.7	4.9
	4	100	2.4	5.7	8.9	11.0
	4	300	5.0	10.7	15.0	16.1
	4	500	6.2	13.5	19.8	24.7
	4	1000	7.8	17.1	25.7	32.4
	4	2000	7.3	15.7	24.3	31.5
Wheat	-	300	4.0	8.7	12.3	15.0
	4	-	2.8	6.5	9.5	11.6
	4	100	3.4	8.1	11.0	14.4
	4	300	5.8	13.7	19.6	23.7
	4	500	5.2	12.7	20.1	26.1
Cereal (Rohn & Haas)	-	300	3.1	9.8	14.6	-
	4	-	8.6	17.8	27.9	35.3
	4	100	6.4	15.1	24.5	31.7
	4	300	7.7	17.8	27.9	35.8
	4	500	7.4	16.6	26.2	33.4
	4	1000	7.3	16.2	25.8	33.1
	4	2000	6.9	16.2	26.4	34.0
Barley (Rohn & Haas)	-	300	3.1	9.8	14.6	-
	4	-	8.9	18.9	29.8	37.7
	4	100	7.8	17.7	28.1	36.0
	4	300	8.9	19.3	30.2	38.3
	4	500	6.9	16.7	27.0	35.0
Wheat (Rohn & Haas)	-	300	5.1	9.9	13.3	15.6
	4	-	11.9	22.6	32.4	42.4
	4	100	10.5	21.7	29.6	38.0
	4	300	10.7	22.0	30.5	39.0
	4	500	10.2	20.8	29.2	37.5
BACTERIAL:						
Wallerstein	-	300	3.7	6.8	10.8	11.9
	4	-	1.5	3.3	4.8	5.3
	4	100	2.2	4.8	7.0	8.4
	4	300	4.0	9.1	14.1	16.7
	4	500	4.0	9.1	14.4	17.5

Table 14. (concl.)

	α-amylase (SKB)	β-amylase (mcg.)	Pressure (cm. mercury) after 2 hrs.	3 hrs.	4 hrs.	5 hrs.
Bacterial	-	300	3.5	7.5	10.9	12.7
	4	-	1.2	3.1	4.6	5.3
	4	100	1.7	4.4	7.4	9.0
	4	300	4.3	9.7	14.1	16.1
	4	500	3.0	7.5	12.7	15.3
Rhozyme H-39 (Rohn & Haas)	-	300	4.2	9.6	10.7	11.9
	4	-	1.8	3.9	4.5	5.5
	4	100	2.1	4.3	7.6	9.2
	4	300	4.1	9.7	13.6	16.5
	4	500	4.3	11.0	16.7	20.7
PANCREATIC:						
	-	300	5.1	9.9	13.3	15.6
	4	-	2.4	4.5	5.9	6.8
	4	100	3.6	7.1	9.6	11.5
	4	300	5.8	11.9	15.3	18.0
	4	500	5.3	12.3	17.2	21.6
	4	1000	6.4	14.7	21.1	27.0
	4	2000	7.3	16.5	23.9	30.6

dilution of the functional wheat proteins. Lactic acid increased considerably the "times" of soft wheats and only slightly increased the "times" of hard wheats.

As shown by the correlation studies, no single test has been successful in giving a full picture of the quality of a wheat as assessed by the baking test, where the composite effects of several quality factors are brought into the picture simultaneously. While the protein content seems to be the major constituent governing wheat quality from the bread baking standpoint, additional factors seem to be important. These factors seem to include the presence and liability of sulfhydryl-containing amino acids in the protein molecule. According to Sullivan (1954) amino acid composition has proved disappointing in explaining the rheological (dough handling), physical or bread baking properties of wheat flour doughs. One tends therefore to agree with the view that until we know more of the structure, the configuration and the secondary valence forces of the amino acids, the unique properties of wheat gluten shall not be fully explained.

With these complexities arising, one single test (outside of baking) is unlikely to describe adequately the bread baking potentialities of a wheat. It is therefore likely that combining the information provided by two or more tests will give a better overall appraisal of the quality of a wheat. In Germany, for example, a combination of more than one test is used. The evaluation of wheat quality is calculated from the following formula:

$$WQ = X_1 \text{ gluten} + X_2 \text{ B K} + X_3 \text{ WMFT}$$

where WQ = wheat quality score
BK = Berliner-Koopman test
WMFT = Wheat-Meal-Fermentation Time values

In the United States one would tend to substitute the protein determination for the gluten test, as the latter is not precise, and is more laborious. The Berliner-Koopman value, being essentially a test of gluten swelling in lactic acid could be replaced by the sedimentation test, and the standardization of the W-M-F-T test along the lines reported could enable its inclusion in the above formula.

The coefficients X_1, X_2, X_3 were determined in Germany arbitrarily; therefore a statistical re-evaluation would give more significant results.

Another possibility would be the use of specific sedimentation or specific W-M-F-T test. This is obtained by dividing the results (time or sedimentation values) by the protein content.

The Contribution of Amylases to the Production of Sugars Fermentable in Panary Fermentation

When using crystalline amylases, beta-amylase contributed at least 10 times as much fermentable sugars as did equal amounts of bacterial, pancreatic or fungal amylases. Among the alpha-amylases the enzyme of bacterial origin was the most potent, the fungal the least, and the pancreatic intermediate. Mixtures of alpha- and beta-amylases gave fermentable sugars in amounts slightly smaller than calculated, in case of bacterial and fungal amylases. The addition of beta-amylase to pancreatic amylase resulted in no increase in fermentable sugar produced by the enzyme of animal origin. Mixtures of crude preparations from cereals showed a beta-amylase response, provided the plant alpha-amylase was low in beta-amylase.

SUGGESTIONS FOR FUTURE WORK

Wheat-Meal-Fermentation Time Test

The purpose of this work was primarily to minimize variations of results due to undetermined factors introduced by the operator while performing the test. Recovery and handling of the dough from the Farinograph bowl and blades may affect the results despite a uniform mixing. It seems, therefore, desirable to use a kneader which could mold a mixed dough into a doughball and convey this directly to a beaker with distilled water. It is also suggested to mix only individual doughballs. To prevent sticking at the sides of the beaker, applying a "very slight" and uniform vibration to all the container (beaker) might give a slight stirring of the water, a uniform concentration of CO_2 in the solution, and a slight motion of the doughball. A means of accelerating the test to make it more useful and practical, seems highly desirable. To eliminate human factors, the determination of "end point" with an electronic eye can be used. This can be done with the use of a deeper or taller beaker (say 6 inches) where an electric eye is focused at the center between the bottom and the surface of water where the doughball floats. As the doughball sags beyond this point, or if the doughball disintegrates or sinks, it passes between the electronic eye and can cause an alarm bell to ring through a relay. Only limited number of samples has been tested by the proposed method. Testing a larger number of wheat samples, from several crops, and by a number of collaborators seems highly desirable.

The Contribution of Amylases to the Production of Sugars Fermentable in Panary Fermentation

To obtain a better picture of the pattern of the action of the amylases tested, identification of the products of enzymatic action by paper chromatography, column separation and molecular sieves would add valuable information. The use of purified crystalline amylases of plant origin would supplement and augment the information obtained in this study.

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THE WHEAT-MEAL-FERMENTATION TIME TEST

by

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Wheat-Meal-Fermentation Time Test

The wheat-meal-fermentation time test has been modified by mixing wheat meal with a yeast suspension in a micro-farinograph bowl to optimum consistency and molding the dough balls in a simple specially designed mechanical device. The "times" of wheats were affected by length and speed of mixing of the dough and efficiency of shaping the dough balls. Oxidants increased the W-M-F time; the increase depended on the level and type of oxidant employed. Lactic acid increased the "times" of soft wheat more than it increased the times of strong wheat; the reverse was true as a result of adding fermentable sugar. Adding starch or soft wheat to a strong wheat had a diluting effect. Storage of wheat had a slight improving effect which was shown by both bread baking and W-M-F-T times. The W-M-F-T times of 36 samples of hard red winter wheat were highly correlated with loaf volume ($r = 0.76$, significant at the 0.1% level). This correlation coefficient was significantly higher than the correlation between W-M-F-T and wheat protein.

The Contribution of Amylases to the Production of Sugars Fermentable in Panary Fermentation

The contribution of alpha- and beta-amylases to the production of sugars in bread fermentation has been followed by measuring the gassing power of pregelatinized wheat starch mixed with a buffered solution of yeast nutrients and a yeast suspension. When crystalline amylases are added to pregelatinized starch, beta-amylase contributed at least 10 times as much fermentable sugar as did equal amounts (SKB units) of bacterial, pancreatic or fungal amylases. Alpha-amylase of bacterial origin was the

most potent, fungal alpha-amylase the least, and pancreatic alpha-amylase was intermediate. Mixtures of alpha- and beta-amylases gave fermentable sugars in amounts slightly less than calculated, in case of bacterial and fungal amylases. The addition of beta-amylase to pancreatic amylase resulted in no increase in fermentable sugar. Mixtures of crude malt preparations from cereals showed a beta-amylase response, provided the plant alpha-amylase was low in beta-amylase.