GLUTEN QUALITY AND THE EFFECT OF DILUTION
OF WHEAT FLOURS WITH STARCH

John William Hopkins
Department of Field Crops

University of Alberta
Edmonton, Alberta
August, 1930.

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John William Hopkins

Department of Field Crops

A THESIS

submitted to the University of Alberta in
partial fulfilment of the requirements for
the degree of
MASTER OF SCIENCE

Edmonton, Alberta

August, 1930

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GLUTEN QUALITY AND THE EFFECT OF DILUTION OF WHEAT FLOURS WITH STARCH.

By John William Hopkins.

INTRODUCTION

Ever since the discovery that the peculiar suitability of wheat flour for bread-making was the result of the characteristic protein mixture known as gluten, this fraction of the flour has been subject to extensive examination by cereal chemists.

It was early recognized that gluten content must play an important part in determining the baking "strength" of any particular flour, but during the first decade of the present century it became apparent, through the observations of numerous workers in this field, that although the best bread-making flours were, in general, those containing the greatest proportion of gluten, nevertheless variations in the "quality" or physical condition of the gluten from different flours often occurred. Gluten quality was, in many cases, just as important as gluten quantity. More recent and extended investigations have only served to strengthen this conclusion; thus Bailey (1, pp. 258-60) gives an account of baking tests carried out by different workers, involving in the aggregate

several hundred samples of flour grouped according to protein content. The results indicate that when the averages of a sufficient number of samples are considered, the order of gluten content is the same as that of loaf volume, although individual flours were found in each group which varied in baking quality more or less widely from the mean of their group. The results also show, however, that comparisons must be restricted to flours of the same grade, since equal amounts of protein in flours of different grade were not in general of equal value in loaf production; in other words, the quality of the protein in the lower grades of flour was inferior. It is, therefore, obvious that some method whereby the quality of the gluten in any particular flour might be estimated would be of the greatest value.

"Quality" of course is not an absolute characteristic of any material, but is a purely relative term denoting the suitability of the material under consideration for some particular purpose. For this reason actual baking tests must always remain the final court of appeal in the matter of gluten quality. Such tests are, however, laborious, time-consuming, and require a relatively large amount of material. More serious objections are the possible complication of comparisons due to variations in other factors, especially diastatic activity, and the difficulty of separating effects

several hundred samples of these groups according to protein content. The results indicate that when the averages of a sufficient number of samples are considered, the order of gluten content is the same as that of loaf volume, although individual flours were found in each group which varied in baking quality more or less widely from the mean of their group. The results also show, however, that comparisons must be restricted to flours of the same grade, since equal amounts of protein in flours of different grades were not in general of equal value in loaf production; in other words, the quality of the protein in the lower grades of flour was inferior. It is, therefore, obvious that some method whereby the quality of the gluten in any particular flour might be estimated would be of the greatest value.

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produced by the quality of the gluten from those the result of its concentration, since in practice both quantity and quality usually vary simultaneously. There has, therefore, been a constant search on the part of cereal chemists for some comparatively simple determination which would be an infallible indication of gluten quality: an ideal which has not yet been attained.

The investigation now to be reported represents an attempt to modify the usual baking test in such a way as to render it capable of revealing those differences in the baking value of different flours which are due to quality, as opposed to quantity, of gluten. It was proposed to dilute portions of the flour with starch as required to bring all flours in a test series to definite and comparable protein contents, and to bake the original flours and several such dilutions. There seemed then to be two possible ways of interpreting the results: (1) by the falling off in loaf volume per unit decrease in protein content; (2) by the absolute loaf volume at given protein contents. The first way being independent of absolute volume, it seemed at the time justifiable to neglect diastatic activity. The second way, however, called for methods of stimulating gas production such as would ensure all loaves reaching the maximum volume which the gluten was capable of sustaining.

It was realized that the extra labour involved in the proposed modifications of the baking test would render

produced by the activity of the system from those the
results of the concentrated, which in practice would
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described in detail the usual activity and in such a way as
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the behavior of different forms which are due to
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required to bring all films in a test series to definite
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called for methods of estimation and production such as
which create all forms reaching the maximum volume which
the system was capable of containing.

It was realized that the entire system involved in the
proposed modifications of the system would require

the method unsuitable for ordinary routine determinations. This objection, however, would not be an insuperable one in the case of plant breeders and others to whom the estimation of gluten quality in a restricted number of samples is often of great importance, and here, it was felt, the method, if successful, would be of considerable service.

REVIEW

The various investigations into the nature and measurement of gluten quality which have from time to time been undertaken or proposed fall naturally into five main groups: chemical, immunological, physico-chemical, mechanical, and actual baking.

It is not necessary for our purpose to consider all these aspects of the problem in detail, though had more satisfactory results been obtained elsewhere the need for the present investigation might not have arisen. Suffice it to say that it has not so far been found possible to differentiate by chemical means between the corresponding proteins of "strong" and "weak" flours, (3, 4, 9) or to demonstrate differences in the relative amounts of gliadin and glutenin, the two main gluten constituents, in such flours, (15, 16).

Immunological reactions, which have so often proved valuable in differentiating between proteins, appear to be capable of yielding even less information in this

connection than the ordinary chemical methods. Thus Wells and Osborne (25) found that, using the anaphylaxis reaction, the gliadins of wheat and rye could not be distinguished, and also (26) that wheat gliadin and glutenin actually reacted with each other, though not so strongly as with themselves.

Physico-chemical studies of dough have led to the suggestion (13) that gluten from a "weak" flour has a lower rate of hydration and a much lower maximum hydration capacity than that from a "strong" flour. Attempts to utilize the viscosity of flour-in-water suspensions, which varies with the degree of hydration, as a measure of gluten quality have not, however, been successful (5, 11). The bound-water method has also been applied to the measurement of hydration in flour suspensions (8), but any differences found were too small to be used as an index to gluten quality. Negative correlations have been found (14) between loaf-volume and the ease with which the gluten proteins are peptised by certain salt solutions, but the results from a larger series of samples (12) indicate that the correlation is not great enough to make ease of peptisation a reliable index of gluten quality.

Of the various mechanical dough-testers which have been devised, the Chopin extensimeter (6) is undoubtedly the most successful. This machine measures the ability of a dough to extend its area without rupturing. A good

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Of the various mechanical dough-instruments which have
been devised, the Chopin extensometer (3) is undoubtedly
the most successful. This machine measures the elasticity
of a dough to extend its area without rupturing. A good

relationship has been found to hold between the results thus obtained and the loaf-volume from baking tests. Unfortunately, however, the apparatus is quite expensive, and in most laboratories not available.

Coming now to modifications of the baking test involving the use of starch, we find that Bailey (1) gives an account of experiments carried out by Jago in which varying percentages of corn starch were added to a sample of Canadian patent flour containing 16.1 per cent of dry crude gluten, and the resulting mixtures baked. Each successive addition of starch brought about a diminution in loaf volume. Bailey had himself carried out a somewhat similar experiment and noted a steady decrease in loaf volume and deterioration in texture as the proportion of starch was increased. Bailey and Le Vesconte (2) determined the effect of admixture of starch upon the extensibility of dough, as determined by the Chopin apparatus. Mixtures containing 0, 10, 20, 30 and 40 per cent of starch exhibited a continuously decreasing extensibility, indicating that gas-retaining capacity had been impaired by the added starch. That this was actually the case was demonstrated by Johnson and Bailey (17), who added to five portions of a patent flour containing 10.32 per cent protein, starch sufficient to give mixtures containing 10, 20, 30, 40 and 50 per cent starch respectively. Doughs made from these mixtures fell into a regular sequence as far as gas production and loss of gas

were concerned, gas retention decreasing with increasing proportion of starch.

Larmour and Macleod (19), and Larmour (18) have used a blend of forty per cent soft wheat flour and sixty per cent of the flour to be tested as a supplement to the ordinary baking test in order to obtain a measure of "reserve strength" or blending capacity. This may afford a satisfactory means of comparing different flours as such, but makes no provision for estimating with any degree of exactitude what part of the results obtained is due to variations in the quantity of protein, and what to variations in its quality.

DETAILS OF PROPOSED INVESTIGATION

From the foregoing review it will be seen that no satisfactory chemical or physico-chemical method of estimating gluten quality has so far been found. The experiments there referred to involving the use of starch were all designed to demonstrate the effects of variation in gluten concentration; but the question arose as to whether information concerning the quality of the gluten could not also be obtained in this way. In particular it was thought that if several flours were diluted with starch, in such a way as to reduce the protein content by successive steps, the relation between decrease in protein content and decrease in loaf volume might prove to be a linear one. The decrease in loaf

volume per unit decrease in protein content might, however, vary from flour to flour, the effects of starch dilution being most marked in those flours whose gluten was "weak" or of poor quality. When it is considered that the greater part of the Western Canadian wheat exported to Europe is used to blend with home-grown or imported wheat of inferior quality in order to improve the resulting flour, the practical significance of any test of quality based on blending or "carrying-power" becomes obvious.

It is, as a matter of fact, known that some British millers habitually test the quality of Canadian wheat by mixing flour milled from it with home-grown wheat flour in proportions ranging from 10 to 90 per cent Canadian wheat flour, and noting the resulting improvement. As a quantitative measure this is open to the criticism that no two home-grown wheat flours would necessarily yield the same results. The use of a standard starch, as in the method now proposed would not however be subject to this criticism, all results thus obtained being truly comparable. It was proposed, therefore, to investigate the effects of starch dilution on flour, and if possible to establish a relation between such effects and gluten quality, in the hope that a method for the estimation of the latter might thus be provided.

It is to be noted that the British miller estimates the quality of Canadian wheat flour by the improvement it effects when added to his home-grown wheat flour,

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whereas in the proposed method quality would be estimated rather by resistance to deterioration on dilution with starch. There is a fundamental difference in these two methods, the importance of which will appear more fully in later discussion.

EXPERIMENTAL

Preliminary Experiments

Preliminary experiments were first carried out to determine the best type of starch to use as a diluent. Jago used corn starch. Bailey and Le Vesconte (2) and Johnson and Bailey (17) do not state what kind they used. Inasmuch, however, as the starches from different species of plants often vary considerably in their physical properties, it was thought that perhaps actual wheat starch would be the most suitable. A quantity of a commercial patent flour, found to contain 12.6 per cent of protein, was obtained, and diluted to a series of lower protein contents with five different starches. These were commercial corn starch, starch prepared from the original flour by the method of Rask and Alsberg (22), one lot washed with distilled water and one lot with 70 per cent alcohol, and technical and c.p. wheat starches supplied by Eimer and Amend. The nitrogen content of these starches was determined, with the following results, which indicate a satisfactory degree of purity.

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Table 1. Nitrogen content of starches.

Kind of starch	Nitrogen content
	%
Corn starch	0.09
Starch from flour (by distilled water)	0.06
Starch from flour (by 70% alcohol)	0.05
E. and A. wheat starch (tech.)	0.05
E. and A. wheat starch (c.p.)	0.06

It was assumed that in the processes incidental to the preparation of these starches, any protein present would be denatured; hence no allowance was made for protein in starches when calculating the amount to be used in dilutions.

Sufficient starch was added to portions of the flour to reduce the protein to 12.0, 11.0, 10.0 and 9.0 (dry basis), series of this nature being prepared using each of the five starches. The mixtures obtained, together with the original flour, were then baked according to the following formula (all bakings being performed in duplicate).

Flour..... 100 grams (at 13.5% moisture)

Yeast..... 3 "

Salt..... 1 "

Sugar..... 2.5 "

Water..... sufficient

The resulting loaf volumes are shown in Table 2.

Table 2. Loaf volumes obtained with flour diluted with various starches.

Protein content	Loaf volume				
	Corn starch	Starch from flour (dist. water)	Starch from flour (70% alc.)	E. and A. wheat starch (tech.)	E. and A. wheat starch (c.p.)
%	cc.	cc.	cc.	cc.	cc.
12.6	498	498	498	498	498
12.0	475	458	458	460	456
11.0	433	444	432	425	422
10.0	427	406	410	376	395
9.0	380	382	369	342	371

These results have also been plotted (Figure 1).

In the case of some of the starches the points thus obtained are rather irregularly distributed, but in each instance a straight line could be fitted to them. It will be seen that the rate of decrease of loaf volume with dilution is not the same for the different diluents. The technical wheat starch supplied by Eimer and Amend gave the line of steepest slope and also showed the least scattering of points. It was, therefore, decided to use this in all future experiments.

Determinations of the resistance of certain of these starches to diastatic action was made by the method of Malloch (21). Similar determinations were also performed in the case of the flour and the dilutions of it made with Eimer and Amend technical wheat starch.

Table 2. Lost volumes obtained with three different starches.

Protein content	Lost volume			
	Corn starch	Green from flour (dry weight)	Starch from flour (70% alc.)	R. and A. wheat starch (2.5%)
%	55	55	55	55
12.5	498	498	498	498
12.0	473	473	473	473
11.0	448	448	448	448
10.0	423	423	423	423
9.0	398	398	398	398

These results have also been plotted (Figure 1). In the case of some of the starches the points thus obtained are rather irregularly distributed, but in each instance a straight line could be fitted to them. It will be seen that the rate of decrease of lost volume with dilution is not the same for the different starches. The technical wheat starch supplied by Hiner and Almond gave the line of steepest slope and also showed the least scattering of points. It was, therefore, decided to use this in all future experiments.

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Figure 1. Loaf volumes obtained with flour diluted with various starches.

Table 3. Starch resistance of flour, starches and starch dilutions.

Material	Starch resistance
Starch from flour (washed by distilled water)	40
Starch from flour (washed by 70 per cent alcohol)	39
Wheat starch, E. and A. (tech.)	151
Flour, 12.6 per cent protein	38
Flour, 12.0 per cent protein (diluted with E. and A. wheat starch (tech.))	34
Flour, 11.0 per cent protein (diluted with E. and A. wheat starch (tech.))	33
Flour, 10.0 per cent protein (diluted with E. and A. wheat starch (tech.))	37
Flour, 9.0 per cent protein (diluted with E. and A. wheat starch (tech.))	38

The figures for starch resistance represent one thousand times the reciprocal of the number of milligrams of maltose produced by 10 grams of dry material when digested for one hour at 27° C. with 0.03 grams of taka-diastase. It will be noted the starch prepared by washing out from the flour had the same resistance, within the limits of error of the method, as had the original flour. The Eimer and Amend wheat starch had a much higher resistance, but the resistance of the mixtures in which it was used as a diluent did not differ significantly from that of the original flour. This seeming anomaly

Table 2. Effect of various concentrations of glucose, sucrose and fructose on the growth of *Aspergillus niger*.

Concentration of sugar (g/l)	Effect on growth
0	Control (no sugar)
10	Glucose 10 g/l, sucrose 10 g/l, fructose 10 g/l
20	Glucose 20 g/l, sucrose 20 g/l, fructose 20 g/l
30	Glucose 30 g/l, sucrose 30 g/l, fructose 30 g/l
40	Glucose 40 g/l, sucrose 40 g/l, fructose 40 g/l
50	Glucose 50 g/l, sucrose 50 g/l, fructose 50 g/l
60	Glucose 60 g/l, sucrose 60 g/l, fructose 60 g/l
70	Glucose 70 g/l, sucrose 70 g/l, fructose 70 g/l
80	Glucose 80 g/l, sucrose 80 g/l, fructose 80 g/l
90	Glucose 90 g/l, sucrose 90 g/l, fructose 90 g/l
100	Glucose 100 g/l, sucrose 100 g/l, fructose 100 g/l

The results for the effect of various concentrations of glucose, sucrose and fructose on the growth of *Aspergillus niger* are shown in Table 2. It will be noted that the growth of the fungus was not significantly affected by the presence of any of the sugars at the concentrations tested. This is in contrast to the results obtained with *Aspergillus niger* by other workers, who have reported that the growth of this fungus is inhibited by high concentrations of glucose, sucrose and fructose. The reason for this difference is not clear, but it may be due to the fact that the fungus used in this work was a more resistant strain than the one used by the other workers. It will be noted that the growth of the fungus was not significantly affected by the presence of any of the sugars at the concentrations tested. This is in contrast to the results obtained with *Aspergillus niger* by other workers, who have reported that the growth of this fungus is inhibited by high concentrations of glucose, sucrose and fructose. The reason for this difference is not clear, but it may be due to the fact that the fungus used in this work was a more resistant strain than the one used by the other workers.

may be explained by assuming that, since only a small proportion of the total starch available is converted into sugar, there was present, even in the highest dilutions, an excess of the more easily hydrolyzed flour starch over the total amount capable of being converted into sugar by the enzyme. This flour starch might then be supposed to be attacked by the enzyme in preference to the more resistant starch used as a diluent.

The diastatic activity of these mixtures was also determined, using Malloch's (20) modification of Rumsey's method.

Table 4. Diastatic activity of flour and starch dilutions.

Protein content	Diastatic activity
%	
12.6	188
12.0	178
11.0	195
10.0	192
9.0	168

These results, whilst subject to some fluctuation, do not indicate any significant downward trend with increasing dilution. This seemed rather surprising, for in the starch dilutions the amount of diastase present would

may be explained by assuming that, since only a small proportion of the total atoms available is converted into water, there was present, even in the highest dilutions, an amount of the more easily oxidizable inert gases over the total amount capable of being converted into water by the oxygen. This theory seems to be supported by the fact that the amount of the inert gases is the same whether there was or was not a reaction.

The dissociative activity of these elements was also determined, using Williams' (28) modification of Ramsay's method.

Table 4. Dissociative activity of fluorine and oxygen.

Fluorine converted	Dissociative activity
9.9	100
10.4	100
11.2	100
12.0	100
12.4	100

These results, which are subject to some discussion, do not indicate any significant downward trend with increasing dilution. The second factor mentioned, that in the above diagram the amount of available oxygen was

presumably decrease in the same ratio as the proportion of flour. The added starch was shown to have no diastatic activity, and was in addition much more resistant to diastatic action than the starch which occurred in the flour. A decrease in diastatic activity with dilution was therefore certainly ~~not~~ to be expected.

Gluten Quality and Decrease in Loaf Volume
per Unit Decrease in Protein Content.

Having established a linear relationship between protein content and loaf volume for this particular flour, using E. and A. technical wheat starch as a diluent, the next step was to apply the dilution procedure to a selected series of flours. This consisted of flour from three lots of Marquis wheat, grown at the University of Alberta and having an identical protein content but showing varying degrees of frost injury, and also, for comparison, Quaker flour (a commercial patent), and flour milled from Marquis wheat grown in Southern Alberta. All but the commercial flour were milled in the laboratory. The following is a description of the series:

No. 332. Marquis U. of A., after fallow. Injured by frost, grade No. 6+. Protein content of wheat 16.3 per cent, of flour 14.5 per cent.

No. 333. Marquis U. of A., after fallow. Injured by frost, grade No. 4-. Protein content of wheat 16.3 per cent, of flour 14.4 per cent.

presumably decrease in the same ratio as the proportion of flour. The added starch was shown to have no diastatic activity, and was in addition much more resistant to diastatic action than the starch which occurred in the flour. A decrease in diastatic activity with dilution was therefore certainly not to be expected.

Gluten Quality and Increase in Loaf Volume
per Unit Decrease in Protein Content.

Having established a linear relationship between protein content and loaf volume for this particular flour, using E. and A. technical wheat starch as a diluent, the next step was to apply the dilution procedure to a selected series of flours. This consisted of flour from three lots of Marquis wheat, grown at the University of Alberta and having an identical protein content but showing varying degrees of frost injury, and also, for comparison, another flour (a commercial patent), and flour milled from Marquis wheat grown in Southern Alberta. All but the commercial flour were milled in the laboratory. The following is a description of the series No. 755. Marquis U. of A., after follow. Injured by frost, grade No. 5-1. Protein content of wheat 15.7 per cent, of flour 14.5 per cent. No. 756. Marquis U. of A., after follow. Injured by frost, grade No. 4-1. Protein content of wheat 15.7 per cent, of flour 14.4 per cent.

No. 335. Marquis U. of A., after fallow. Injured by frost, grade "Feed". Protein content of wheat 16.3 per cent, of flour 14.4 per cent.

Quaker. Used as standard in baking laboratory. Protein content of flour 13.0 per cent.

Southern Alberta Marquis. Protein content of flour 13.9 per cent.

Three dilutions of each sample were made, using E. and A. technical wheat starch, all percentages being calculated on the dry basis. Bakings were performed as before with the results shown in Table 5 and Figure 2.

Table 5. Bakings of starch dilutions of selected series of flour samples.

Flour	Protein content	Loaf volume	Flour	Protein content	Loaf volume	Flour	Protein content	Loaf volume
	%	cc.		%	cc.		%	cc.
No. 332	14.5	598	No. 333	14.4	577	No. 335	14.1	597
	13.0	521		13.0	531		13.0	553
	11.5	475		11.5	466		11.5	494
	10.0	428		10.0	404		10.0	413
Quaker	13.0	539	Southern Alberta Marquis	13.9	479			
	12.0	511		13.0	456			
	11.0	470		11.5	425			
	11.5	425		10.0	405

Figure 2. Decrease in loaf volume with dilution of selected series of flours.

Table 6 shows a single-figure estimate¹ of the baking quality of the five original flours, together with dV/dP , the slope of the line in Figure 2 showing the rate of falling-off in loaf-volume with decrease in protein content.

Table 6. Baking quality of flours, and decrease in loaf volume per unit decrease in protein content.

Flour	Baking Quality	dV/dP
No. 332	101	38.0
No. 333	98	39.1
No. 335	92	44.1
Quaker	87	33.0
S. Alberta Marquis	57	19.0

It will be observed (from Fig. 2) that in each case the falling-off in loaf volume with dilution was directly proportional to the decrease in protein content, or so nearly so as to make the fitting of a straight line quite justifiable. In the first three cases (the frost-damaged samples), the decrease in loaf volume per unit decrease in protein content was in the inverse order of the baking

¹ The single-figure estimate of baking quality was computed as follows:

Loaf volume - 400×0.2
Texture score (possible 10) $\times 3$
Crumb color score (10) $\times 2$
Gen. appearance score (10) $\times 1$
Per cent absorption - 60×1
Total = estimate of baking quality

quality of the original flour, though not of the grade of the wheat from which it was milled. The remaining two samples, however, although inferior in baking quality, showed a smaller decrease in lost-volume on dilution. The Southern Alberta samples, the poorest flour of the whole series, the wheat from which it was milled being undoubtedly deteriorated owing to ageing, showed by far the least decrease of m.f.

Dilution tests were also performed on a series of samples available from an investigation of nutrition under various grasses and alfalfa which was being carried on in the Department of Field Crops. This consisted of flour milled from Northern wheat grown after Western Rye Grass, Timothy, Bromo Grass and Alfalfa respectively. The results shown in Table V and Figure 7, again reveal a linear decrease in lost-volume with dilution. The rate of decrease is in the ascending order: wheat after Timothy, Western Rye Grass, Bromo Grass and Alfalfa. At the time it was thought that this might be an indication of one possibly inferior quality of the increased protein obtained after such crops as alfalfa. (Although the actual percentage of protein was lowest in wheat after alfalfa, the yield and hence the nitrogen removed per acre was higher than in the case of the other three).

Figure 3. Decrease in loaf volume with dilution of flour from wheat grown after Timothy, Western Rye Grass, Brome Grass and Alfalfa.

Table 7. Dilution bakings of flour from wheat grown after Timothy, Western Rye Grass, Brome Grass and Alfalfa.

Flour	Protein content	Loaf volume	dV/dP	Flour	Protein content	Loaf volume	dV/dP
	%	cc.			%	cc.	
Wheat after Timothy	17.7	498	22.7	Wheat after W. Rye Grass	17.8	503	23.8
	13.0	386			13.0	382	
	10.0	315			10.0	319	
Wheat after Brome Grass	17.0	512	26.3	Wheat after Alfalfa	16.9	515	28.4
	13.0	401			13.0	404	
	10.0	328			10.0	319	

Starch Dilution and Diastatic Activity.

Although the preliminary experiments indicated that diastatic activity was unaffected by dilution, it was suspected that these results were not typical. It certainly seemed unlikely in the extreme that when, as in some of the mixtures worked with, over forty per cent of the flour had been replaced by starch which contained no diastase at all and in addition was quite resistant to enzyme hydrolysis, the amount of sugar produced would be unchanged. Accordingly, the influence of dilution on diastatic activity was studied in three additional cases;

Table 7. Analysis of variance of linear trends from
linear regression, between the groups, between
groups and between

Linear	Quadratic	Cubic	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic
10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0

Linear regression and quadratic regression

Although the preliminary experiments indicated that
discrete activity was unaffected by dilution, it was
suggested that these results were not typical. It
certainly seemed unlikely in the extreme that when, as in
some of the above work with, over forty per cent of
the linear and non-linear activity was contained in
discrete activity and in addition was quite resistant to
dilution. Accordingly, the influence of dilution on
discrete activity was studied in three separate series.

the Quaker and Southern Alberta Marquis flours already referred to, and a third, designated "Flour A", which was available at the time in the laboratory and consisted of a mixture of residues from trial millings, etc.

The diastatic activity of the original and diluted flours was determined by the method used before (20), and in each case a definite downward trend with dilution was established. In Table 8 the actual decrease in diastatic activity on dilution is shown, together with a "theoretical" diastatic activity calculated on the assumption that the diastatic activity was directly proportional to the amount of flour (and hence of diastase) in the mixtures. Although the agreement is far from perfect, there is, nevertheless, some indication that the diastatic activity is roughly proportional to the amount of flour in the mixture.

Table 8. Starch dilution and diastatic activity.

Flour	Protein content	Diastatic activity as determined	Diastatic activity calculated from dilution
	%		
Flour A	12.9	155	---
	12.0	131	144
	11.0	121	132
	10.0	118	120
Quaker	13.0	282	---
	12.0	198	260
	11.0	204	239
	10.0	179	217
Southern Alberta Marquis	13.9	149	---
	11.5	136	126
	10.0	121	107

has passed and somewhat altered under these conditions. It is a white, crystalline solid, melting at 110°C. It is soluble in water, alcohol, and ether. It is a mixture of isomers, the main component being the 1,2-isomer.

The dissociation constant of the 1,2-isomer is 1.5 x 10⁻⁴. It is a weak acid, and its dissociation is reversible. In the presence of a catalyst, it can be converted into a more stable form. The reaction is as follows:

$$\text{C}_6\text{H}_5\text{COOH} \rightleftharpoons \text{C}_6\text{H}_5\text{COO}^- + \text{H}^+$$

The equilibrium constant for this reaction is 1.5 x 10⁻⁴. The reaction is reversible, and the equilibrium can be shifted in either direction by changing the concentration of the reactants or products.

Table 1. Dissociation constants and dissociation constants.

Flow	Dissociation constant	Dissociation constant	Dissociation constant
Flow A	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴
Flow B	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴
Flow C	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴
Flow D	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴
Flow E	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴
Flow F	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴
Flow G	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴
Flow H	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴
Flow I	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴
Flow J	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴	1.5 x 10 ⁻⁴

From the foregoing work the following facts emerge:-

1. When flour is diluted with starch, a linear relation between protein content and loaf volume is obtained.
2. In one case at least (the three frost-damaged samples) dV/dP , the decrease in loaf volume per unit decrease in protein content, seems to be related to gluten *quality*.

It was now thought that possibly the diastatic activity factor should not be ignored after all, even in method (1) (see page 3). In the case of the Quaker and Southern Alberta Marquis flours, wide differences in diastatic activity (Table 8), and dV/dP (Table 6) occurred, these differences being in inverse directions. It seemed possible therefore that the anomalous behaviour of the Southern Alberta Marquis might be due to its gas production being so low as to be the limiting factor in loaf volume, protein scarcely coming into question at all.

In method (2) of course there was no doubt that differences in diastatic activity would have to be eliminated. Sherwood and Bailey (24) concluded from their own and Rumsey's (23) work that a diastatic activity of about 250 was the optimum for bread-making purposes, but for high protein Canadian wheat flours, forming doughs of greater extensibility, this would be a somewhat conservative estimate. It seemed fairly safe to assume, therefore, that in the majority (if not all) of the flours here dealt with, gas production was a limiting factor in loaf volume. The gluten was not being extended to the full amount of which it was capable, and there

from the foregoing with the following facts emerged:-

1. When there is dilution with water, a linear relation between protein content and leaf volume is obtained.

2. In one case at least (the above first-mentioned analysis)

4V/3P, the difference in leaf volume per unit decrease in

protein content, seems to be related to nitrogen content.

It was now thought that possibly the diastatic activity

factor should not be ignored either at all, even in method (2).

(see page 4). In the case of the present and previous analyses

Marquis flour, wide differences in diastatic activity

(Table 3), and 4V/3P (Table 3) occurred, these differences

being in inverse directions. It seemed possible therefore

that the anomalous behaviour of the Marquis flour might

might be due to its gas production being so low as to be

limiting factor in leaf volume, protein actually coming into

question at all.

In method (2) of course there was no doubt that diastatic

enoses in diastatic activity would have to be eliminated.

Shorwood and Bailey (22) concluded from their own and

Rumsey's (23) work that a diastatic activity of about 200 was

the optimum for bread-making purposes, but for high protein

Canadian wheat flours, turning doughs of greater extensibility,

this would be a somewhat conservative estimate. It seemed

fairly safe to assume, therefore, that in the majority of

not all of the flours have gas limit, gas production was a

limiting factor in leaf volume. The writer was not being

extended to the full amount of which it was capable, and there

was no reason to suppose that in a series of flours the ratio of actual to maximum possible loaf volume would be the same in any two cases.

Further complications became apparent owing to the fact that on dilution with starch, both the diastatic activity of a flour and the maximum loaf volume that it could sustain would be reduced. There seemed to be three possibilities with regard to the relationship between actual gas production (governed by diastatic activity) and the gas production necessary for maximum loaf volume:

- (a) both might decrease with dilution by the same relative amount;
- (b) the gas production necessary for maximum loaf volume might decrease relatively more rapidly than actual gas production;
- (c) actual gas production might decrease relatively more rapidly than the gas production necessary for maximum volume.

It seemed quite possible that (c) could be the case in flours of high diastatic activity, whilst (b) held in those of very low diastatic activity. In view of these possibilities and the above mentioned facts it seemed that perhaps the best way to obtain true comparisons might be to adjust the diastatic activity of all flours and their dilutions, so that the maximum possible loaf volume was produced in every case.

was no reason to suppose that in a series of times the
ratio of actual to maximum possible total volume would be
the same in any two cases.

Further complications become apparent when in the
last stage on division with respect to the division
activity at a time and the maximum total volume that it
would generate would be reached. There seemed to be large
possibilities with regard to the relationship between
actual and potential (expressed by division activity)
and the gas production necessary for maximum total volume:
(a) both might decrease with division by the same
relative amount:

(b) the gas production necessary for maximum total volume
might decrease relatively more rapidly than actual gas
production;

(c) actual gas production might decrease relatively more
rapidly than the gas production necessary for maximum
volume.

It seemed quite possible that (a) could be the case in
flows of high diastolic activity, while (b) held in cases
of very low diastolic activity. In view of these
possibilities and the above mentioned facts it seemed that
perhaps the best way to obtain true comparisons might be
to follow the diastolic activity of all flows and finally
divisions, so that the maximum possible total volume was
produced in every case.

Experiments Involving the Stimulation of Gas
Production.

In the attempt to eliminate diastatic activity as a complicating factor, and definitely to establish, if such existed, the relationship between gluten quality and behaviour on starch dilution, further experiments were now designed. In these experiments it was planned to use flour milled from wheat of four standard varieties, chosen so as to form a graded series in respect of baking quality. Wheat of these four varieties, Marquis, Red Bobs No. 222, Huron and Vermilion, had been grown in adjacent plots at Clover Bar, near Edmonton, during the summer of 1929. A considerable quantity of each variety was milled, the protein content of the flour determined, and dilutions made.

As a first step the original and diluted flours were baked in the ordinary way and also with the addition of 0.001 per cent of potassium bromate. This substance acts as a flour improver, its effect as a rule being more pronounced the higher the protein content of the flour. The precise nature of its action is not yet known, ~~but~~ ~~certain workers~~, Working (27, 28) and Geddes (10), believe that it affects the physical condition of the gluten, possibly as the result of a dispersing action on certain phosphatides intimately admixed with the gluten constituents, but it still remains to be proved that this effect alone explains all the changes resulting from its use. Table 9 and Figures 4, 5 and 6 show the results

Experimental Investigation of the

Properties

In the attempt to eliminate systematic errors in the
measuring device, and determine its sensitivity, it was
checked, the relationship between the quantity and
behavior on a fixed distance, the first experiment was not
satisfactory. In these experiments it was found that the
value of the force varied with the distance, and was not
constant. To form a fixed series in respect of the quantity, three
of these four variables, namely, the mass of the body, the
and velocity, had been given in adjacent order of discovery
but, near the end of the series of 1922, a considerable
quantity of each variety was added, the system consisted of
the first determined, and different ways.
As a first step the original and added forces were
added in the ordinary way and also were the addition of
0.001 per cent of potential energy. This substance was
as a first experiment, the effect of a very small force
produced the higher the force, and the more of the force.
The precise nature of the effect is not yet known, but
certainly, the effect is not yet known, but
that it affects the physical condition of the system,
possibly as the result of a disturbance in the system
organization, and is also related with the system and
structure, and is also related to the system and
effect of the system, and the system is also related to the
use. The system is also related to the system and the system

thus obtained.

Table 9. Dilution bakes, with and without bromate, of flour from Marquis, Red Bobs No. 222, Huron and Vermilion wheat.

Variety	Protein content	Loaf volume, standard method	dV/dP	Loaf volume, bromate method	Response to bromate
	%	cc.		cc.	cc.
Marquis	18.5	573	24.1	748	175
	16.0	516		573	57
	14.0	470		518	48
	12.0	421		462	41
	10.0	371		397	28
Red Bobs No. 222	17.0	529	24.8	644	115
	14.0	450		492	42
	12.0	403		420	17
	10.0	370		400	30
Huron	15.7	480	30.4	590	110
	14.0	417		477	60
	12.0	372		406	34
	10.0	312		358	56
Vermilion	14.8	337	16.8	417	80
	13.0	302		332	30
	11.5	287		290	3
	10.0	267		269	2

times obtained.

Table 9. Dilution series, with and without bromate, of flour from Marquis, Red Bob No. 882, Huron and Vermilion wheat.

Variety	Protein content	Loaf volume, standard method	DV/45	Loaf volume, bromate method	Response to bromate
Marquis	10.0	471	22.1	497	26
	12.0	481		482	41
	14.0	470		515	43
	16.0	516		575	57
	18.5	573		748	175
	20	60		60	60
Red Bob No. 882	10.0	470	24.2	460	20
	12.0	467		480	17
	14.0	480		493	48
	17.0	523		505	115
Huron	10.0	512	20.4	528	28
	12.0	572		468	41
	14.0	477		477	60
	15.7	480		530	110
Vermilion	10.0	527	18.8	522	2
	11.5	527		590	5
	17.0	502		575	70
	14.8	507		417	30

Figure 4. Decrease in loaf volume with dilution of flour from Marquis, Red Bobs No. 222, Huron and Vermilion wheat. Baked by ordinary method.

Figure 5. Decrease in loaf volume with dilution of flour from Marquis, Red Bobs No. 222, Huron and Vermilion wheat. Baked with the addition of 0.001 per cent potassium bromate.

Figure 6. Variation in response to bromate with dilution of flour from Marquis, Red Bobs No. 222, Huron and Vermilion wheat.

With the ordinary baking procedure, the falling-off in loaf-volume was again proportional to the decrease in protein content, and in the case of three of the varieties, namely Marquis, Red Bobs No. 222 and Huron, the slopes of the curves obtained (Fig. 4), by plotting the loaf volume against protein content were in the inverse order of baking quality, although the difference in slope between Marquis and Red Bobs No. 222 was but slight. Vermilion, however, which is known to be very inferior in this respect, although giving a small loaf volume at first did not fall off to any great extent on dilution, thus behaving in a similar manner to the Southern Alberta Marquis of a previous experiment.

It is apparent from Fig. 5, that when these bakings were performed with the addition of potassium bromate, the linear relation between protein content and loaf volume disappeared. The resulting curves do not appear to be related to gluten quality in any simple manner. The response to bromate (i.e. the difference between loaf volume with and without bromate), plotted in Fig. 6, also fails to reveal any helpful differences between the varieties.

Since it was felt that in all probability these results were complicated by variations in diastatic activity, an effort was now made to control this factor. If some fairly wide range existed over which, owing to its no longer being a limiting factor, such variations had no appreciable effect on loaf volume, then it was thought that the problem

might be fairly readily solved. It should be possible, by means of a relatively few experiments, to determine the amount of some diastatic preparation to add to any flour of known diastatic activity in order to bring its sugar production within the desired range.

With this end in view, varying amounts of each of two highly diastatic preparations, takadiastase supplied by the Parke-Davis Co. and malt extract of 200 degrees Lintner, supplied by Standard Brands Ltd. were added to each of the four experimental flours and the resulting changes in diastatic activity determined. (In the case of the malt, 0.05 per cent of mono-ammonium phosphate, $\text{NH}_4\text{H}_2\text{PO}_4$, which acts as a yeast stimulant, was also added). The results are summarized in Table 10 and Figures 7 and 8. With takadiastase the increase in sugar production in each flour follows a similar law, although the actual amount of the increase varies in each case, owing probably to differences in starch resistance. The results obtained by the use of malt are more irregular. This may be accounted for, in part at least, by the difficulties involved in manipulating small amounts of this substance, it being of a thick syrupy nature. There is also the possibility of non-homogeneity of the malt, thorough mixing being impossible. In general, however, the results seem to be of a somewhat similar nature to those obtained with takadiastase.

It was also planned to bake loaves containing the same proportions of these preparations, in order to

determine the region of optimum diastatic activity, as judged by the attainment of maximum loaf volumes. When this was attempted, however, it was found that in addition to being highly diastatic, both the takadiastase and malt evidently contained proteolytic enzymes. These adversely affected the gluten during fermentation, resulting in sticky, "runny" doughs which could not be handled. By reducing the amount of water added in mixing the dough (in certain instances by as much as 12 cc.), some loaves were obtained, and the volumes of these have been included in Table 10.

Table 10. Results of adding takadiastase and malt extract to experimental flours.

Flour	Amount of preparation added	Taka-diastase		Malt extract + 0.05% ammonium phosphate	
		Diastatic activity	Loaf volume	Diastatic activity	Loaf volume
	%		cc.		cc.
Marquis	--	140	554	140	554
	0.05	273	745	187	642
	0.10	318	---	199	661
	0.15	350	340	305	687
Red Bobs No. 222	--	184	541	184	541
	0.05	358	660	283	570
	0.10	431	---	322	618
	0.15	455	---	385	650
Huron	--	131	498	131	498
	0.05	268	638	165	544
	0.10	301	---	221	584
	0.15	332	616	279	582
Vermilion	--	95	450	95	450
	0.05	154	538	149	512
	0.10	172	520	166	522
	0.15	208	456	190	520

determine the region of optimum distillate activity, as judged by the attainment of maximum distillate volume. When this was attempted, however, it was found that no distillate was being highly distillate. Both the vaporizers and still evidently contained protective emulsion. These emulsions affected the system during fermentation, resulting in atypical "runny" distillate which could not be handled. By reducing the amount of water added in making the mash (in certain instances by as much as 12 oz.), some losses were obtained, and the volumes of these have been included in Table 10.

Table 10. Results of certain distillations and stills extracted at experimental times.

Time	Amount of distillate obtained	Still-1000		Still-1000 activity	Still-1000 volume
		Still-1000 activity	Still-1000 volume		
Malt	0.00	140	140	140	140
	0.05	200	200	200	200
	0.10	210	210	210	210
	0.15	220	220	220	220
	0.20	230	230	230	230
Red Bobs No. 200	0.00	180	180	180	180
	0.05	220	220	220	220
	0.10	240	240	240	240
	0.15	250	250	250	250
	0.20	260	260	260	260
Baron	0.00	170	170	170	170
	0.05	200	200	200	200
	0.10	210	210	210	210
	0.15	220	220	220	220
	0.20	230	230	230	230
Vermilion	0.00	100	100	100	100
	0.05	120	120	120	120
	0.10	130	130	130	130
	0.15	140	140	140	140
	0.20	150	150	150	150

Figure 7. Effect of addition of varying amounts of takadiastase on the diastatic activity of the four experimental flours.

Figure 8. Effect of addition of varying amounts of malt extract (200 deg. Lintner) on the diastatic activity of the four experimental flours.

It was realized, however, that owing to their proteolytic action, as a result of which the physical condition of the gluten was affected, these preparations were unsuitable for use in the present studies, and so some other method of augmenting sugar production was sought. The addition of starch which had been finely ground, and was hence very susceptible to enzyme hydrolysis, in constant amount in each dilution, although this would make impossible comparison with the loaf volume of the original flour, seemed to be a possible line of attack. A portion of wheat starch was therefore ground for forty hours in a ball mill, mixed with some of the Marquis flour, and the mixtures baked. The results given in Table 11 show that the increase obtained by the use of the finely-ground starch alone is obviously not of the required order.

Table 11. Use of wheat starch of low resistance in the endeavour to stimulate gas production.

Mixture	Loaf volume
	cc.
1. Marquis diluted to 16.0% protein with unground wheat starch	516
2. Marquis diluted to 16.0% protein with finely ground wheat starch	548
3. As in (1) but with addition of 0.05% Taka-diastrase	650
4. As in (2) but with addition of 0.05% Taka-diastrase	670

These attempts to increase the production of sugar in the dough by natural means (i.e. enzyme hydrolysis) having proved impracticable, it was thought that possibly the desired result might be achieved by the addition, not of diastatic preparations or readily hydrolysed starch, but of the product which was actually used by the yeast, namely maltose. It is true that this might appear to entail a departure from the conditions usually occurring in fermenting dough, in which the amount of maltose produced by starch-splitting is negligible at the commencement of the fermentation period, but has become fairly large in the later stages, when it is most needed. Inasmuch, however, as a considerable quantity of sucrose (2.5 per cent), which must be in excess of the early needs of the yeast, is added in the ordinary baking procedure, it was thought that probably the added maltose would not lead to over-stimulation of the yeast in the early stages but would instead ensure the presence of an adequate supply of sugar during the important final period of fermentation.

A few preliminary experiments demonstrated that considerable increases in loaf volume could be obtained with maltose, added in solution to aid in thorough mixing. In addition, the texture of the loaves thus obtained was superior to that of the original flour, whereas those resulting from the use of Taka-diastase or malt were impaired in texture. Maltose and 0.05 per cent ammonium phosphate, it was found, gave better results than maltose alone. Thus Marquis flour baked with the addition of

These attempts to increase the production of sugar in the dough by natural means (i.e. enzyme hydrolysis) having proved unsuccessful, it was thought that possibly the desired result might be achieved by the addition, not of diastatic preparations or specially hydrolyzed starch, but of the product which was actually used by the yeast, namely maltose. It is true that this might appear to entail a departure from the conditions normally occurring in fermenting dough, in which the amount of maltose produced by starch-splitting is negligible at the commencement of the fermentation period, but has become fairly large in the later stages, when it is most needed. Inasmuch, however, as a considerable quantity of sucrose (2.5 per cent), which must be in excess of the early needs of the yeast, is added in the ordinary baking procedure, it was thought that probably the added maltose would not lead to over-stimulation of the yeast in the early stages but would instead ensure the presence of an adequate supply of sugar during the important final period of fermentation. A few preliminary experiments demonstrated that considerable increases in loaf volume could be obtained with maltose, added in solution to aid in thorough mixing. In addition, the texture of the loaves thus obtained was superior to that of the original flour, whereas those resulting from the use of 0.5 per cent ammonium phosphate, it was found, gave rather results than maltose alone. The results flour baked with the addition of

2.0 per cent maltose gave a loaf volume of 642 cc., whereas with 2.0 per cent maltose and 0.05 per cent ammonium phosphate, a volume of 668 cc. was obtained.

It was decided, therefore, to investigate the effect of adding varying proportions of maltose to flour, in order to determine whether, with increasing concentration, the loaf volume rose to a sharp maximum and then fell off again, or whether any considerable region of "optimum concentration" existed. A number of bakings of the four experimental flours (Marquis, Red Bobs No. 22, Huron and Vermilion) were made with the addition of varying amounts of this sugar and 0.05 per cent of ammonium phosphate, the results being included with those of subsequent work in Table 12 and Figs. 9, 10, 11 and 12. In each case a maximum loaf volume was obtained, but whilst with Red Bobs No. 222 no significant change in volume occurred over a range of concentration extending from four to six per cent, the volumes given by the other three flours increased to well defined maxima and then fell off again with varying degrees of abruptness.

As no general "optimum zone" of maltose concentration had been established for the undiluted flours, the foregoing procedure of baking with the addition of varying amounts of maltose was applied to the various starch dilutions. The loaf volumes obtained are shown in Table 12 and the accompanying Figures 9, 10, 11 and 12.

Table 12. Loaf volumes obtained by the addition of varying amounts of maltose + 0.05 per cent ammonium phosphate.

Flour	Protein content	Maltose added	Loaf volume	Flour	Protein content	Maltose added	Loaf volume
	%	%	cc.		%	%	cc.
Marquis	18.5	0	573	Red Bobs No. 222	17.0	0	529
		2	627			3	571
		3	648			4	620
		4	663			5	623
		5	639			6	616
Marquis	16.0	0	516	Red Bobs No. 222	14.0	0	450
		4	604			4	517
		5	600			5	565
		6	628			6	567
		7	614			7	565
Marquis	14.0	0	470	Red Bobs No. 222	12.0	0	403
		4	578			3	480
		5	615			4	494
		6	577			5	508
						6	507
Marquis	12.0	0	421	Red Bobs No. 222	10.0	0	370
		4	455			5	404
		5	546			6	460
		6	480			7	453
		7	452			8	390
Marquis	10.0	0	371				
		3	370				
		4	384				
		5	370				
		6	423				
Huron	15.7	7	400	Vermilion	14.8	0	337
		8	402			2	489
						3	534
						4	510

Table 12 (cont'd.)

Flour	Protein content	Maltose added	Loaf volume	Flour	Protein content	Maltose added	Loaf volume
	%	%	cc.		%	%	cc.
Huron	14.0	0	417	Vermilion	13.0	0	302
		4	520			3	472
		5	518			4	472
		6	536			5	468
		7	551			6	471
		8	552				
Huron	12.0	0	372	Vermilion	11.5	0	287
		4	479			4	451
		5	470			5	462
		6	497			6	460
		7	477			7	466
						8	452
Huron	10.0	0	312	Vermilion	10.0	0	267
		4	397			3	420
		5	450			4	423
		6	435			5	430
		7	435			6	452
						7	438

The curves obtained by plotting loaf volume against concentration of added maltose are of some interest. In general there is an increase, with dilution, in the amount of added sugar necessary to produce maximum loaf volume. There are also marked differences in the types of the curves themselves. In the case of the Marquis, for example, the loaf volume of the three highest dilutions (lowest protein contents) rises sharply to a maximum, and then falls off again; with the original flour and the first dilution, however, there is no such peak.

Table 12 (cont'd.)

Flour content	Maltese added	Flour	Flour content	Maltese added	Flour
14.0	0 4 8 8 7 8	Vermilion	14.0	0 4 8 8 7 8	Vermilion
12.0	0 4 8 8 7 7	Vermilion	12.0	0 4 8 8 7 7	Vermilion
10.0	0 4 5 5 5 7	Vermilion	10.0	0 4 5 5 5 7	Vermilion

The curves obtained by plotting loaf volume against concentration of added maltose are of some interest. In general there is an increase, with dilution, in the amount of added sugar necessary to produce maximum loaf volume. There are also marked differences in the types of the curves themselves. In the case of the Verapula, for example, the loaf volume of the three highest dilutions (lowest protein contents) rises sharply to a maximum, and then falls off again; with the original flour and the first dilution, however, there is no such peak.

Figure 9. Loaf volumes obtained by the addition of varying amounts of maltose + 0.05 per cent ammonium phosphate to the original and diluted flour - Marquis.

Figure 10. Loaf volumes obtained by the addition of varying amounts of maltose + 0.05 per cent ammonium phosphate to the original and diluted flour - Red Bobs No. 222.

Figure 11. Loaf volumes obtained by the addition of varying amounts of maltose + 0.05 per cent ammonium phosphate to the original and diluted flour - Huron.

Figure 12. Loaf volumes obtained by the addition of varying amounts of maltose + 0.05 per cent ammonium phosphate to the original and diluted flour - Vermilion.

Figure 13. Maximum loaf volumes obtained by the addition of maltose + 0.05 per cent ammonium phosphate to the original and diluted experimental flours, plotted against protein content.

This "peak" in the loaf-volume curve occurs to a varying degree in the higher dilutions of all the varieties. The fact that a similar type of curve was obtained with the undiluted Vermilion flour suggests that it is possibly an indication of "weakness", although the other curves furnish several exceptions to such a generalisation.

The maximum loaf volumes of the various flours and their dilutions obtained with the addition of maltose and ammonium phosphate have been plotted against protein content in Fig. 13. Whilst gas production as a limiting factor has now undoubtedly been eliminated, the resulting curves do not appear to lend themselves to any simple quantitative estimation of gluten quality. As a matter of fact three different types of curves are obtained from the four varieties. The Marquis curve is concave downward, Red Bobs No. 222 and Huron give practically straight lines, and Vermilion a curve concave upward. The Marquis curve may perhaps be explained on the assumption that, owing to the good quality of the gluten, considerable quantities of starch can be added to the flour without causing any great decrease in loaf volume, but that when a certain critical decrease in protein content has been effected, the cumulative effect of the added starch results in a sudden large diminution. Similarly the relatively large initial decrease with dilution in the loaf volume of Vermilion may be taken as evidence of inferior gluten quality. The subsequent "flattening-out" of this curve is not, however, so easy to explain.

This "peak" in the low-frequency curve occurs at a
 varying degree in the range of 10 to 20 Hz. The
 low-frequency curve is a typical type of curve and is
 indicated by the term "peak". Although the peak
 indicates a resonance, it is not a resonance.
 The maximum low-frequency value of the curve is
 their difference between the low-frequency value and
 maximum frequency have been determined. The
 in Fig. 1. The curve is a typical type of curve
 has not been determined. The curve is
 do not appear to be a resonance. The curve is
 estimation of the curve. The curve is
 different types of curves and are shown in Fig. 1.
 varied. The curve is a typical type of curve
 some 10, 20 and 30 Hz. The curve is a typical
 and variation of the curve is shown in Fig. 1.
 any curve is shown in the curve. The curve
 to the peak value is the curve. The curve
 quantities of which can be added to the low-frequency
 causing the curve to be in the low-frequency
 a certain critical frequency in the low-frequency
 effect, the curve is a typical type of curve
 in a wide range of frequencies. The curve
 large initial decrease with change in the low-frequency
 variation may be seen as a resonance in the low-frequency
 The subsequent "dip" in the curve is not
 however, as seen in Fig. 1.

Figure 14. Loaf volume obtained by the bromate method, minus maximum loaf volume obtained by the addition of maltose and ammonium phosphate, plotted against protein content.

The differences between the loaf volume obtained by the bromate method (Table 9) and the maximum obtained by the addition of maltose and ammonium phosphate (Table 12) are plotted for the various flours and their dilutions in Figure 14. There seems to be no systematic difference in the curves given by the different varieties. It is worthy of note, however, that in the case of the four undiluted flours, Marquis, Red Bobs No. 222 and Huron gave a greater, and Vermilion a lesser, loaf volume by the bromate method than with the addition of maltose. The magnitude of the differences, it will be noted, is precisely in the order of baking quality of the original flours. The ability to give a greater loaf volume with bromate than can be obtained by the addition of maltose and ammonium phosphate would therefore appear to be correlated with baking quality. Some confirmation of this hypothesis was obtained by baking a sample of pastry flour, known to be of inferior bread-making quality, by the ordinary method, with the addition of bromate, and with the addition of maltose. By the ordinary method a volume of 430 cc. was obtained. This was only increased to 440 cc. by the addition of 0.001 per cent potassium bromate, but with 5 per cent maltose a loaf of 502 cc. resulted.

From the nature of the curves in Figure 14, however, it appears that this relation does not necessarily hold when a number of flours are brought to some similar protein content by the addition of starch.

The difference between the two values obtained in the previous method (Table 2) and the values obtained in the addition of malic acid (Table 1) are plotted for the various times and acid additions in Figure 1. There seems to be no systematic difference in

the curves given by the different methods. It is worthy of note, however, that in the case of the four modified times, namely, 10, 20, 30, and 40 min, the values are a greater, and therefore a lesser, than values of the previous method with the addition of malic acid. The magnitude of the difference, it will be noted, is

precisely in the order of being equal to the original times. The values to give a greater last volume with acetate acid can be obtained by the addition of malic acid and ammonium chloride which therefore must be so correlated with malic acid. A more detailed

of this hypothesis was obtained by using a series of acetic times, known to be of interest in acid-making quality, by the ordinary method, with the addition of malic acid, and with the addition of malic acid. If the ordinary method is used at 10, 20, 30, and 40 min, the values are only increased to 10, 20, 30, and 40 min, but with a few more minutes per cent acetate produced, but with a few more minutes a loss of 50% or less is observed.

From the nature of the curves in Figure 1, however, it appears that this relation does not necessarily hold when a number of times are present in some relation. Protein content of the addition of acetate.

DISCUSSION OF RESULTS, AND CONCLUSIONS.

In the preliminary discussion, attention was directed to the fundamental difference between the method here employed and that used by some British millers as an index of flour strength. In the latter, the greater the difference between the strong Canadian flour and the weak flour with which it is being mixed, the greater is the improvement effected. In the former, on the other hand, it was assumed that the greater the difference in properties between the flour to be tested and the starch added to it, the smaller would be the resulting impairment.

Emphasizing this fundamental difference, two opposing tendencies seem to be discernible in the results of those experiments in which no attempt was made to stimulate gas production. These are:

(a) The better the quality of the gluten, the greater the resistance of the flour to reduction in loaf-volume by starch dilution.

(b) The poorer the gluten, the less the difference in properties between flour and added starch, and hence the less the change on dilution.

It would appear that in flours with good quality gluten, tendency (a) is dominant, and that differences in gluten quality can be detected by the starch dilution method. In those of a markedly inferior nature, however,

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(a) The better the quality of the gluten, the greater the resistance of the flour to reduction in loaf-volume by starch addition.

(b) The poorer the gluten, the less the difference in properties between flour and added starch, and hence the less the change in loaf-volume.

It would appear that in flours with good quality gluten, tendency (a) is dominant, and that differences in gluten quality can be detected by the starch dilution method. In those of a markedly inferior nature, however,

(b) seems to become increasingly dominant, rendering comparison with superior flours impossible. On this account the method cannot be capable of any general application in the estimation of gluten quality.

The stimulation of gas production, in order to obtain the maximum possible loaf volumes, has led to more complicated, rather than simpler, results. No explanation of these, other than that already suggested, can be offered here, though in view of the fact that loaf volume is largely governed by physical factors, the possible effect of starch dilution on the physical properties of the dough (apart from mere reduction in protein content) should not be overlooked. Certain facts of interest have emerged however.

(i) The unsuitability of at least two commercial diastatic preparations as agents for the stimulation of gas production in flours has been demonstrated. These results, together with those of Collatz and Racke (7) and Sherwood and Bailey (24) indicate the necessity of devising some method for the purification of preparations of this enzyme. On the other hand, it has been shown that by the addition of maltose and 0.05 per cent of ammonium phosphate, loaf volume could be increased to a maximum.

(ii) As already suggested, one way of interpreting the results might be to compare the maximum loaf volume which the various flours in a test series were capable of

attaining when all had been reduced to some similar protein content. From Figure 13 it will be seen that there is a considerable range of protein contents over which the loaf-volumes at any given protein content would be in the order Marquis, Red Bobs No. 222, Huron and Vermilion; that is, in the order of gluten quality. Owing, however, to the differing rates of decrease in loaf volume with dilution exhibited by the four varieties, the quantitative results obtained from such a procedure would vary with the protein content chosen. The loaf volumes though they fell in the order of gluten quality, could not be said to be proportional to it.

(iii) The variations in the response to potassium bromate and maltose are of considerable interest. It is believed that the response due to bromate is related to both quantity and quality of gluten. The discovery that in "strong" flours the loaf-volume obtained with the addition of 0.001 per cent potassium bromate was greater than the maximum obtained with the addition of maltose, whilst in "weak" flours the reverse was true seems further corroboration of this view, since the maltose presumably acts solely as a yeast stimulant. It is disappointing, however, to observe the irregular nature of the results obtained when this procedure is applied to the diluted flours.

(iv) The nature of the curves obtained by plotting loaf volume against concentration of added maltose has

already been noted. A sharp maximum is, in general, associated with low concentration or inferior quality of gluten. This is analagous to the well-known lack of stability and narrow range of fermentation tolerance in weak flours.

SUMMARY.

1. The purpose of this investigation has been to ascertain whether the baking behaviour of flours when brought to a series of definite and comparable protein contents by dilution with starch could be used to estimate gluten quality.
2. When flours are diluted with starch, the rate of decrease in loaf volume per unit decrease in protein content has no general application as a measure of gluten quality. This is true whether the baking is performed with or without the stimulation of gas production.
3. Diastatic activity is reduced by starch dilution to an extent roughly proportional to the decrease in the amount of flour in the mixture.
4. The addition of maltose to increase gas production has proved more satisfactory than the use of diastatic preparations, owing to proteolytic enzymes being contained in the latter.

already been moved. A sharp reaction is, in general, associated with low concentration or higher quality of glucose. This is analogous to the well-known lack of activity and narrow range of fermentation tolerance in weak fibrous.

SUMMARY.

1. The purpose of this investigation was to ascertain whether the feeding behavior of flies was related to a series of definite and objective physical factors by dilution with starch could be used to separate glucose quality.
2. When flies are diluted with starch, the rate of decrease in food volume per unit decrease in glucose content had no general application as a measure of glucose quality. This is true whether the feeding is performed with or without the stimulation of gas production.
3. Histidine activity is reduced by starch dilution to an extent roughly proportional to the decrease in the amount of flour in the mixture.
4. The addition of histidine to increase food production has proved more satisfactory than the use of histidine preparations, owing to previously unknown feeding conditions in the latter.

5. The maximum volumes which a series of flours are capable of attaining when all have been brought to the same protein content by starch dilution seem to be in the same order as, though not necessarily proportional to, gluten quality, provided that the degree of dilution is not too high.

6. "Strong" flours give a greater loaf volume when baked with the addition of 0.001 per cent potassium bromate than can be obtained by the use of maltose to increase gas production. With "weak" flours the reverse is true. When a number of flours are reduced to the same protein content by the addition of starch, however, the differences between loaf-volume by the bromate and maltose methods are not necessarily in the order of gluten quality.

7. When successive increments of maltose are added to a "weak" flour, in general the loaf volume increases to a well-defined maximum and then falls off again. With "strong" flours on the other hand, the maximum is less pronounced, and tends to broaden out into an "optimum zone".

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The author wishes to thank Dr. Robert Newton, who suggested the problem, for his constantly helpful criticism and advice, and Mr. G. D. Macmillan for his valuable technical assistance.

5. The maximum volumes which a series of flasks are capable of attaining when all have been brought to the same protein content of serum dilution seem to be in the same order as, though not necessarily proportional to, flasks density, provided that the degree of dilution is not too high.

6. "Strong" flasks give a greater final volume when mixed with the addition of 0.01% per cent potassium bromate than can be obtained by the use of maltose to increase gas production. With "weak" flasks the reverse is true. When a number of flasks are referred to the same protein content by the addition of serum, however, the differences between final volume by the bromate and maltose methods are not necessarily in the order of flasks quality.

7. When successive increments of maltose are added to a "weak" flask, in general, the final volume increases to a well-defined maximum and then falls off again. With "strong" flasks on the other hand, the maximum is less pronounced, and tends to broaden out into an "optimum zone".

DISCUSSION

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