A STUDY OF

DIASTATIC ACTIVITY AND AMYLOCLASTIC SUSCEPTIBILITY OF STARCH IN WHEAT FLOUR

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A C K N O W L E D G M E N T S

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DIASTATIC ACTIVITY AND STARCH RESISTANCE IN WHEAT FLOUR

INTRODUCTION

The heterogeneity of the starch granule has long been an accepted fact. The existence of two fractions variously termed α amylose and β amylose, amylopectin and amylose etc., was known long before their isolation by Ling and Nonji[1] in 1923. Further the presence of a third fraction somewhat in the nature of a hemicellulose was also ascertained by its isolation by the same workers[2] in 1925. The amylohemicellulose was found to be absent in potato and arrowroot but present in most cereals in varying proportions. Also a certain variability in the proportions of the other constituents in different starches was observed. The great differences in the properties of these constituents makes their variation of concentration in starch granules, from different sources, of particular importance. Undoubtedly this variation is an important factor contributing to the wide differences in physical and chemical properties which characterize starches of different origin.

Differences in chemical constitution of starches have been known for some time, and tend to emphasize the fact that the term "starch" designates not a compound but a class of compounds. It can

no longer be doubted that non-carbohydrate material in chemical combination with the predominant carbohydrate portion comprises a small but definite fraction of the starch complex. Phosphate phosphorus and in some cases silican constitute an integral part of at least one of the starch fractions, also the presence of long chain fatty acids closely associated with the starch granule has been indicated by Taylor and Lehrman(3).

The relatively great variations in the percentages of these previously unsuspected constituents are of particular interest and importance. Samec(4) has shown that the phosphorus (expressed at P_2O_5) present ranges from .012% in Cassava starch to .112% in potato, a variation of over 900%. This variation takes on additional weight in consideration of the fact that the important property of gelatinization is closely related to the phosphate content. In fact, that the power of gelatinization is directly proportional to phosphate content has been demonstrated by Tiebachy(5).

Obviously the above mentioned differences in constitution must give rise to comparatively great differences in physical properties. Such variations have long been recognized and are still the subject of much investigation. Viscosities, gelatinization, heat of hydration, etc., have all received attention. Alsberg(6) conducted an extensive investigation into the variation with temperature of the viscosity of eleven different wheat starches and noted wide and possibly significant variations. Microscopic gelatinization studies, and gelatinization point determinations have been conducted by various workers, such as La Wall(7), Dox(8), and Alsberg and Rask(9). More recently a detailed study of the effect of starch

gelatinization agents and the responses of the different starches to them have occupied the attention of Mangels[10] and Winkler[11] has investigated the heats of hydration of wheat rice and potatoes and also found marked differences in the starches examined.

In view of the variations noted above, it is not surprising that investigations to determine possible variations in amyloclastic susceptibility have resulted in the demonstration of similar differences. The first indication of this was apparently obtained by Lœberg[12] in 1876. His work showed that potato starch was more easily saccharified than other starches. Carl Lintner[13] also found evidence that resistance of starches from different cereals varied considerably. Confirmation of Lœberg's results was not obtained, however, till 1904 by O. Sullivan[14], whereas Lintner's data has only been subjected to re-examination within the last two decades. More recently Nago[15], Ehrlich[16] and Sherman[17] have valuable contributions to the subject.

Up to this point the discussion has been concerned chiefly with variations in starches from different species. Little mention has been made of those works which have been confined to variation of starch from different varieties of the same species.

Naturally the present work is chiefly concerned with the bearing of the subject on cereal chemistry. Hence variations in starch resistance of different wheats is of prime importance in this discussion.

The first work along these lines was apparently conducted by Whymper[18]. His work indicated that the smaller starch granules had a greater amyloclastic susceptibility than the larger ones. The significance of this discovery lies in the fact that different wheat varieties are more or less characterized by the average size of their

granules. The tendency of investigators at that time, however, to work with gelatinized starches, resulted in evidence that was apparently in contradiction to Whymper's results.

The subject was considerably clarified and systematized by Rumsey's[19] excellent work in 1923. His paper outlined a standardized method for the determination of diastatic activity in wheat flours. In addition an investigation was made of the effect on the diastatic action of malt extract of different varieties of wheat starch. A difference in starch resistances was certainly indicated, but the conclusiveness of the work in this respect was doubtful due to the limited number of samples used. Of extreme importance, however, was the discovery of the futility of attempting to do comparative work on other than raw starches.

Collatz[20] working with several different flours, noted marked differences in the increase of reducing sugar brought about by the same quantities of malt flour.

Mangels[21] using Rumsey's method, determined the diastatic activity in different flours, then using commercial starch as his substrate, determined the activity of the cold water extract of these flours. Only slight differences in the amounts of maltose produced could be detected; hence, the conclusion that variations in diastatic activity were due to variations in the starch and not the diastase.

Hermano and Rask[22] observed the action of malt diastase on starches obtained from several different wheat varieties. Conclusive evidence is furnished by their paper, that variations in diastatic production of sugar are not due to the enzyme alone, and that the character of the starch is an important if not a major factor in sugar production.

Sufficient has been said to show that an undoubted difference in starch resistance to enzymic hydrolysis does exist. Methods of measuring this resistance had not received much attention previous to Rumsey's[19] work in 1923. Rumsey himself devised what is now known as the added diastase method. Essentially the method consists of the addition of a definite quantity of a diastase preparation to a water suspension of the flour under examination. The quantity of reducing sugar produced on digestion under carefully controlled conditions of temperature and time is measured. The result so obtained is corrected for the original activity of the flour, and is then taken as representing the amyloclastic susceptibility. The inverse of the amyloclastic susceptibility is of course a measure of the starch resistance.

Malloch[23] has criticized this method on several grounds. While admitting its effectiveness in demonstrating a variability in starch resistance, its suitability for qualitative work is questioned. His chief objection to the method was the possibility of supplementary actions of two diastases. In order to obviate this difficulty, he devised a method wherein only diastase from one source could act on the substrate. This was done by inactionating the natural wheat diastase present in the flour and then washing out the inactivator by repeated centrifuging from a water suspension. The inactivated flour was then subjected to enzymic hydrolysis by a definite quantity of taka diastase. The ratio of the amounts of maltose produced by autolysis, of the inactivated flour, to the amount of maltose produced by the taka diastase was taken as a measure of the relative diastatic content and starch resistance in different flours.

An important modification introduced by Malloch was the control of pH in diastatic activities determinations as suggested by Sorenson[24], a citrate HCl buffer solution (pH 4.7) being used.

Geddes[25] criticized Malloch's method on the grounds that the laborious procedure made it unsuitable for routine work. In addition it was observed that the washing process used to remove the inactivator entailed the loss of some of the substrate. The latter criticism is particularly pertinent as Malloch himself has shown that maltose production is by no means independent of the concentration of the substrate even under the conditions involved. Geddes, using both methods (Malloch's and "added diastase") to determine starch resistance of different wheat varieties, obtained wide differences in results. His data indicated clearly that no relationship existed between the series of results obtained by Malloch's method and those from the added diastase method. Commenting on the data he expresses the belief that the results obtained by Malloch's method were erroneous, and produces substantiating evidence for his statement. That such results were due to his lack of familiarity with the method, as suggested by him, is inadmissible, as it is highly improbable that checks could have been obtained in some twenty determinations if such was the case.

So far no mention has been made of the importance of a knowledge of starch resistance to the technology of milling and baking. Insofar as the practical baker is concerned, irrespective of the cause, the final gassing power of the doughed flour is an important consideration, but whether this power is the result of a sugar supply obtained by high enzymic content or low starch resistance is immaterial. However, wheats are frequently encountered which though perfect in other respects, yield flours which lack sufficient diastatic activity. Correction of this defect

in the flour can of course be made by addition of malt flours to the finished product. Unfortunately the correction involves the risk of proteolytic activity, a condition which needless to say may result in the development of highly undesirable baking characteristics. It is evident therefore that the problem involved is such as to merit the fullest investigation of every phase.

The preceding discussion has illustrated the lack of any method which may be assumed to give a truly quantitative measure of amyloclastic susceptibility of different starches. In view of what has been stated with regard to the practical side of the question, the need for such a method must be evident. It was therefore chiefly with the object of either evolving such a method or determining the suitability of either of the previous methods, that this work was undertaken.

PROBLEM

The problem, the solution of which has been undertaken in this work, was the development of an improved method for the determining the resistance of wheat starch to diastases. An extension of the problem to a study of variations in starch resistance and a measurement of other starch characteristics, which it seemed might possibly parallel this property was also included.

APPARATUS

For the most part the apparatus used in this work was of the standard type. Hence little description is necessary.

All diastatic activity determinations were made by the Blish-Sandstedt[26] method, the apparatus employed being of the usual type. The thermostatic bath was a Freas Electric water bath.

An Alpine Sun Lamp, Home Model, was employed in that portion of the work in which ultra-violet irradiation was involved.

Wherever centrifuging was necessary an International Centrifuge, size 2, was used.

Descriptive detail of more highly specialized apparatus will be given in those sections in which such apparatus was employed.

EXPERIMENTAL

I. WHEAT DIASTASE INACTIVATION EXPERIMENTS

(a) Inactivation by Ultra-Violet Irradiation

A description of Malloch's method of determining amyloclastic susceptibilities of starches in wheat flours has been briefly sketched in the introduction. As has been indicated this method has proved somewhat unsatisfactory, the fault probably lying in the incomplete removal of the chemical inactivator by the washing process. Hence if the addition of such an inactivator could be avoided a solution to the problem might be achieved.

Several possibilities were indicated by a search of the literature on amylases.

The most promising of these was suggested by the work of Green[27] Bode[28] Agulhon[29] Pincussen[30] and several other investigators. The results of these investigators' work showed that ultra violet irradiation has a definite inactivating effect on amylases. An investigation was therefore projected in which the applicability of this inactivation to wheat flours was to be determined.

In order that inactivating effects might be most pronounced it was decided at the inception of the work to carry out all experiments on a flour, high in diastatic activity, some milled from sprouted wheat, being used.

1. Dry exposure to Ultra-Violet Irradiation.

The first inactivation experiments were carried out on the dry flour. The procedure was to spread out a quantity of the flour in a shallow layer no more than three or four millimeters in depth at any point. The ultra-violet lamp was then placed so that the flour was about twelve inches from the mercury arc. Precautions were taken to ensure complete

exposure of all flour particles by simply sweeping the flour up into a heap with a camel's hair brush and redispersing it again every five minutes.

Exposures over varying periods of time were made, determination of diastatic activity of the exposed flour being made within an hour after exposure. The data obtained is given in Table I, all data given is the average of triplicate determinations.

Table I.

The Diastatic Activity of Sprouted Wheat Flour After Dry Exposure to Ultra-Violet Irradiation.

Time of Exposure in hours.	Diastatic Activity Blish-Sandstedt Units
0 (Control)	476
1	470
2	468
3	473
4	469

From the data given it is obvious that no inactivation resulted from the irradiation. Such variations as do appear are obviously due to experimental error.

It is therefore apparent that no inactivation may be accomplished by ultra-violet irradiation of the dry flour.

2. Irradiation of Flour in Aqueous Suspension.

Inactivation of the dry flour having proved unsuccessful, the next step was to determine the effect of irradiation of the flour in aqueous suspension.

The following is an outline of the procedure followed.

Two five gram samples of the flour were each suspended in 46 cc. of distilled water in two litre beakers. A spoonful of sterile quartz sand was added to each beaker and one of the beakers was then covered with a plate of glass. The beakers were then put in ice baths and placed

under the ultra-violet lamp. Both suspensions were constantly agitated by shaking in order to keep the flour from settling out of suspension.

Two litre beakers were chosen in this experiment in order to expose a maximum surface to irradiation. The sand was introduced so that in shaking the suspensions a more efficient agitation of the flour could be obtained. As during the exposure a certain amount of diastases would take place, the suspensions were kept at low temperature in the ice bath to reduce diastatic activity to a minimum. The plate of glass over one of the beakers served to screen off the ultra violet radiation, thus providing a control which except for the irradiation had been subjected to all the treatment of the irradiated sample.

After the two samples had been subjected to twohhours irradiation, they were removed from the ice baths and placed in the thermostatic bath. The procedure from then on was identical with that prescribed by the Blish Sandstedt method.

The experiment was repeated four times, the data obtained is given in Table II.

Table II.

Inactivation of Flour Diastase by Ultra-Violet Irradiation of an Aqueous Suspension.

Diastatic Activity of Irradiated Suspension	Diastatic Activity of Control
526	601
570	665
500	607
595	655

The data in Table II indicates a definite though partial inactivation. Although every possible precaution was taken to standardize the experiment wide variations in results are noticeable. It is therefore evident that the method was such as to require considerable modification

if easily reproducable results were to be obtained. However, it was apparent that inactivation by ultra-violet radiation merited further investigation. At the same time the necessity of a better method of the procedure than that used was evident, not only that results might be duplicated, but that a more rapid inactivation and hence a smaller interim diastasis might be achieved.

In considering methods by which the required results might be achieved, certain outstanding features had to be emphasized.

The procedure had to be such as to lend itself easily to reproducable results, i.e. easily standardized.

It had to be of a type that could be utilized in series determinations, i.e. requiring a minimum of attention during the procedure.

It was essential that diastasis during the procedure be negligible. This was largely a matter of rapid inactivation. Such rapid inactivation could only be accomplished by the fullest possible exposure of every particle of flour.

After considerable experiment the apparatus represented in diagram (1) was devised. This apparatus appeared to most nearly fulfil the requirements of the procedure specified above.

Essentially the apparatus consists of two concentric cylinders, the outer of celluloid, the inner of glass. A water tight compartment between the two cylinders provides a chamber for the flour in water suspension. The system is cooled by means of ice packed into the central glass tube. Two such units are provided for in this apparatus. They were mounted, and revolved by a pulley system connected to a motor whose speed had been reduced by a rheostat.

The procedure was to put five grams of flour and a spoonful

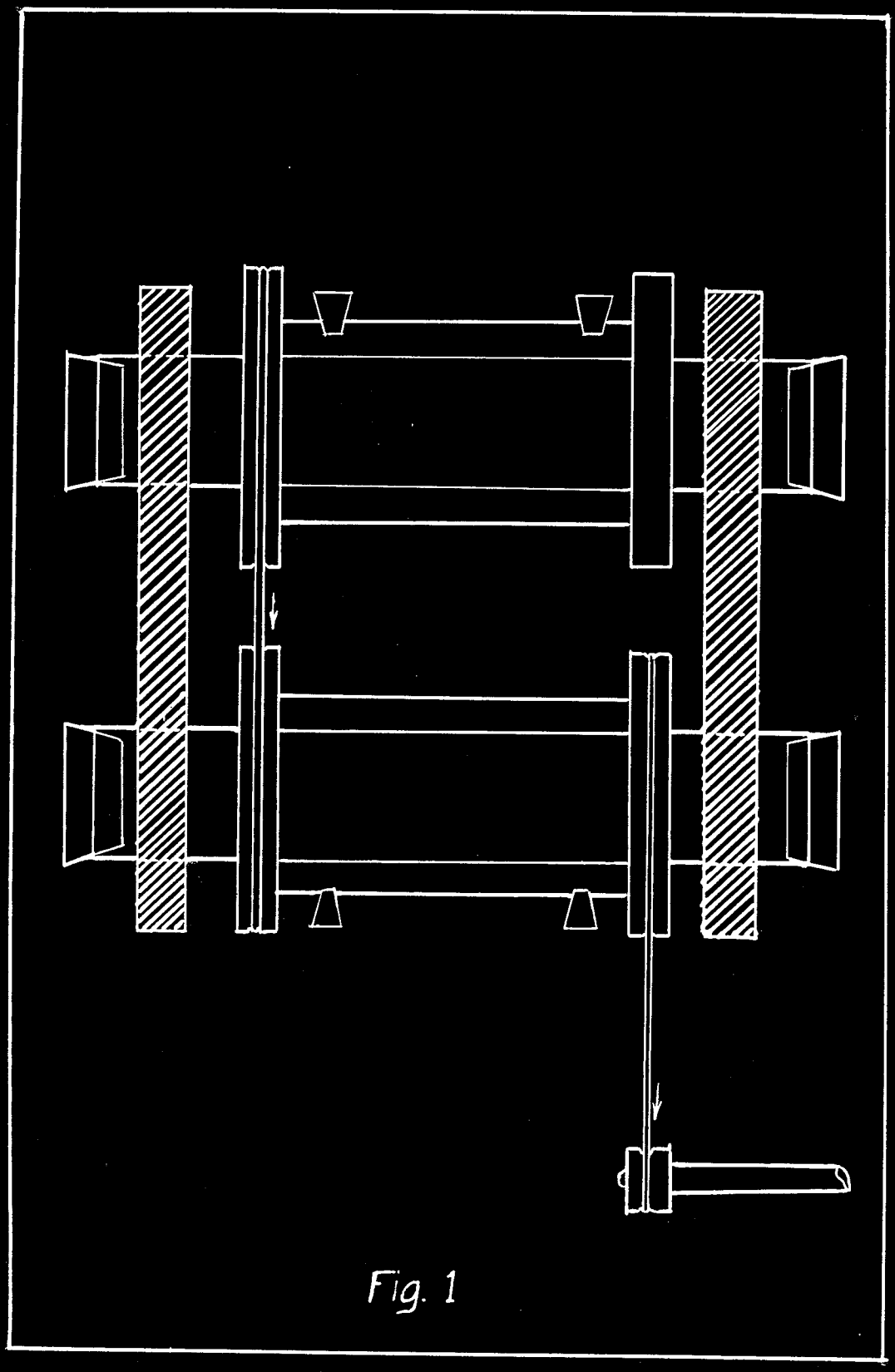

Fig. 1

of quartz into each of the hollow cylindrical chambers, add 46 cc. of distilled water and then reseal the compartments by means of the small corks indicated in the diagram. The inner tubes were then packed with ice and corked, and the units caused to rotate slowly by means of the motor. The mercury arc was arranged so as to irradiate both revolving cylinders.

The outer celluloid cylinders provided fairly free passage to the ultra-violet radiation and the rotation guaranteed an agitation which would provide the most complete exposure.

After irradiation the cylinders were taken from their stands and after removing the ice were placed in the thermostat, and the normal Blish Sandstedt procedure followed.

A series of exposures were made for different lengths of time. As two units were provided it was possible to carry out each exposure in duplicate. In order to provide a check to determine the possibilities of duplication the experiment was performed twice for each time length. As the inactivating effect of ultra violet had already been demonstrated it was not deemed necessary to again use controls in this series of experiments.

The results of the experiment are collected in Table III.

Table III.

Diastatic Activity after Exposure in Aqueous Suspension using Apparatus in Fig.I.

Time of Exposure in hours	Diastatic Activity in Blish Sandstedt Units
1	418 - 425
1	452 - 461
2	547 - 521
2	559 - 550

The double column under diastatic activity represents results of concurrent irradiations. Consideration of the results brings out one fact clearly. Under the conditions imposed the irradiation inactivation is

a slow process, further it is evident that diastasis proceeds at a faster rate than inactivation during the exposure. In addition the results were still unsatisfactory as far as duplication was concerned. This last fault, however, could probably be corrected if a more complete and rapid inactivation could be attained.

3. Irradiation of Flour Suspended in Buffer Solution.

Up to this point, although definite inactivation by ultra-violet irradiation had been demonstrated, the inactivation had been too slow and incomplete to be of any practical application. However, one more possibility of the method still remained to be examined.

Pincussen[31] has shown that the inactivation is greatest at the optimum pH for the amylase. Therefore there was the possibility that inactivation might be accomplished before any great degree of diastasis took place, if instead of distilled water a buffer of pH 4.6 - 4.8 (optimum pH for wheat diastase) was used. The preceding experiment was therefore repeated using an acetic acid sodium acetate buffer of pH 4.7, instead of distilled water. The data tabulated in Table IV gives the results of this experiment.

Table IV

Diastatic Activity after Exposure of Buffer Solution Suspension using Apparatus in Fig.I.

Time of Exposure in hours	Diastatic Activity in Blish Sandstedt Units
1	470
1	452
2	601
2	581

The data given above show clearly that nothing was to be gained by use of the buffer. If anything the figures are higher for the buffered suspension, probably due to the increased diastasis induced by an optimum pH.

4. Discussion and Interpretation of Experiments on Ultra-Violet Irradiation as a Means of Inactivating Diastase in Wheat Flour.

In considering the results of the previous experiments, it should be kept in mind that insofar as this work is concerned, the inactivation is useful only to the extent to which it may be applied to determine amyloclastic susceptibilities of starch in wheat flour. In part 2, a definite inhibiting effect has been demonstrated, but the value of such inactivation as was evidenced is minimized by the succeeding experiments.

The following points are presented as strong arguments against the practical application of ultra-violet irradiation for the desired purposes.

(1) The great difficulty that would be involved in obtaining duplicate results that check would make it unsuitable for routine work.

(2) The inactivation is too slow to make such inactivation either economic or practical, as either a large battery or mercury arc lamps or a long period of time would be necessary should it be desired to apply the method to any extensive work.

(3) The large amounts of maltose produced by diastases during prolonged exposure, even at low temperatures, are such as to make the experimental accuracy of the method when applied highly uncertain.

(4) Even should the above reasons be deemed insufficient and the inactivation be accomplished by irradiation methods, the effect of the large amounts of maltose produced, during the process, on the diastase that must be added to complete the determination, would be extremely indefinite. In all probability a strong inhibitory effect would be produced with the result that amyloclastic susceptibility determinations on this method would be valueless as a true index of starch resistance.

For these reasons ultra violet irradiation as a means of inactivation of wheat diastase was considered inapplicable to the problem and the method was discarded.

(b) Inactivation by Electricity

The inactivating effect of ultra violet radiation has already been discussed at some length. An examination of the literature indicates that the power to change diastatic activity is not limited to ultra-violet portion but extends with varying effects throughout the spectrum, either increasing or decreasing the activity of the diastase. Also experiments by Baly and Semmonds[32] have indicated that specific effects on diastase are produced by plain polarized light. The destructive effect of heat on diastase is another phenomena so familiar that it needs no more detailing here.

From the foregoing considerations, there emerges the fact that diastase is extremely sensitive to many physical phenomena. Hence it would not seem too much to expect that such sensitivity might also be displayed to an alternating electric current. It was therefore decided to investigate the effect of an electric current on the diastatic activity of a suspension of flour in water.

The apparatus used consisted of two platinum electrodes and a large 150 cc. centrifuge tube.

The procedure was only slightly varied from the ordinary Hlish Sandstedt method, the main variation being that the 150 cc. tube was substituted for the 250 cc. flask specified for the method. Immediately after flour had been dispersed in the buffer solution the two platinum electrodes were immersed in the suspension and connected to a 110 volt A.C. circuit. The electrodes were fixed in position by means of a cork

care being taken in their adjustment that the maximum possible quantity of suspension lay between them.

Irrespective of the time in which the suspension was subjected to an electric current the total time of digestion was kept constant, i.e., one hour, to conform with the demands of the Blish Sandstedt method. For instance, if the suspension was subject to the current for 15 minutes another 45 minutes ordinary digestion was given, similarly after 30 minutes exposure to the current another 30 minutes was allowed. The procedure was of course carried out with centrifuge tube containing the suspension always immersed in the thermostat.

Care was taken throughout the experiment that no settling out of the suspension took place, the tube being agitated every five minutes.

The results obtained are tabulated in Table V.

Table V.

The Effect of a 110 Volt Alternating Current on the Diastatic Activity of a Flour

Time of Exposure to Current in Mins.	Diastatic Activity Blish Sandstedt Units
0 (Control)	488
15	492
30	486
45	487

The variations in activity noted were obviously such as would be expected from experimental error. Quite definitely the electric current had had no effect on the activity of the diastase. Therefore the conclusion is evident that as a means of inactivating wheat flour diastase a 110 volt alternating current is useless.

(c) Inactivation with Copper Salts.

Ling and McLaren[33] demonstrated the inactivating effect on malt diastase of small quantities of copper salts. These results were verified by many workers. The work of Jacoby and Shemizer[34] has indicated

that this inactivating effect of copper does not extend to taka diastase. It was hoped therefore that the differential effect of copper might be applicable to the problem being dealt with. Thus copper could be used to inactivate the natural wheat diastase, taka diastase could then be added and the amyloclastic susceptibility of the starch measured.

The tentative method outlined, however, proved impractical in application. All accurate methods of determining diastatic activity are based on a measuring of reducing sugars produced in digestion. As the concentration of the reducing sugars is invariably gauged by the reducing action on either alkaline cupric salt solutions or some other solution of an oxidizing agent of definite concentration, the presence of any additional cupric salts would lead to inaccuracies. All experiments in which a rapid removal of the copper present was attempted proved ineffective. The addition of other reagents only introduced new complications and so the method was discarded.

II. INVESTIGATION OF THE VALIDITY OF THE ADDED DIASTASE METHOD AS A MEANS OF DETERMINING AMYLOCLASTIC SUSCEPTIBILITY.

Section I has dealt with the attempts to inactivate the diastase present in wheat flour. From the evidence presented it was obvious that no method had appeared as suitable for the purposes required in this work.

In the introduction an outline of what has been termed the added diastase method has been given, and the objections to this method, as presented by Malloch, have been indicated. The chief of these objections was the possibility of a supplementary effect of the two diastases, i.e., an inhibitory or stimulating effect of one diastase on the other was thought to occur. Should it therefore be possible to demonstrate that no such effect existed, the main objection to the added diastase method would be removed.

With the object of determining if the above mentioned effects did occur, the following series of experiments was undertaken.

(a) Preparation of Wheat Flour Diastase Extract.

The first step in the new series of investigations was the development of a method of preparing a diastatically active extract of wheat flour.

Thatcher and Koch[35] succeeded in obtaining such extracts from wheat flour by simple aqueous extraction. In their work they demonstrated that such extracts could be obtained using ice water, thus illustrating that diastase could be extracted uninjured by such treatment. Using this as a basis to work upon, the following procedure was evolved.

A quantity of flour was weighed out into a 250 cc. centrifuge bottle. Several glass beads were put in and for every five grams of flour 15 cc. of ice water were added. One or two cubes of ice were also added and the bottle was immediately corked and shaken up vigorously to prevent the flour doughing. The corked bottle was then placed in a mechanical shaker and shaken up for 60 minutes. After removing from the shaker another cube of ice was added and the suspension centrifuged. The supernatant liquid was decanted off and enough ice water added to it to bring the volume up to 26.60 cc. for every 5 grams of flour extracted.

Another solution was then prepared in which 3 cc. of glacial acetic acid and 4.1 grams of anhydrous sodium acetate were dissolved in 250 cc. of water. One part by volume of this solution was mixed with three parts by volume of the flour extract. This buffered the flour extract to a pH of 4.6 - 4.8. Every 36 cc. of the solution contained the extract of 5 grams of flour. The reason for making up to this volume will be discussed later.

The extract so prepared was now ready to test for activity. The tests were conducted much as the ordinary Blish Sandstedt Diastatic Activity tests determinations, the buffered extract, however, being substituted for the ordinary sodium acetate-acetic acid buffer solution, and corn starch

substituted for flour.

In order to make sure that the maltose estimated at the end of an hour's diastasis was due to the action of the extract on the starch used, and not to diastasis during extraction a sample of extract was inactivated and its sugar concentration determined at exactly the same time as the digestion of the test sample was started. The maltose produced after an hour's digestion was determined by the ordinary Blish Sandstedt procedure and the difference assumed to be due to the activity of the extract.

The figures in Table VI indicate the activity of extracts from two different flours.

Table VI.

Extract from	(1) Mg. Maltose per 46 cc. extract before digestion	(2) Mg. Maltose per 46 cc. extract after 1 hour's digestion with starch	(3) = (2) - (1) Mg. Maltose due to Action of Extract on Starch
1 Hard	48	100	52
Sprouted	61	178	117

As noted from column three a fairly active diastase solution was indicated by its action on the starch. Furthermore diastasis during extraction (column one) was moderately low. The extract so obtained was therefore considered suitable for the required purpose.

(b) Preliminary Experiment to Determine Possibility of Additive Effect of Taka Diastase and Wheat Diastase.

1. Taka Diastase - This diastase preparation was obtained from Parke-Davis. It is in powder form and specified as undiluted, 700%. The preparation is identical with that used by Geddes in his work mentioned in the introduction. As both Malloch and Geddes found that about .01 gms. per 5 cc. of substrate was a suitable proportion in their investigations, the same quantities were used in this work. The method used was to dissolve

a quantity of the diastase in the sodium acetate acetic acid solution so that .01 gms. were contained 10 cc. of solution.

It should be noted at this point that in preparing the wheat extract the dilution was so planned that 36 cc. of extract contained the diastase from 5 gms. of flour. Hence as 36 cc. of extract, the extract from 5 gms. of flour was added to 5 gms. of starch on approximation to conditions as they exist in ordinary flour digestions was achieved. Probably it should also be noted that the extract was at the proper buffer strength, pH 4.6 - 4.8. Therefore by simply adding 10 cc. of taka diastase solution, also buffered, or 10 cc. of plain buffer to the 36 cc. of diastase extract, 46 cc. in all of buffer had been added to the substrate and the specifications of the Blish Sandstedt method complied with.

The procedure was to place the wheat diastase extract, the take diastase, and the plain buffer solution, in the thermostat. At the same time three flasks each containing 5 gms. of starch and a spoonful of sand were also placed in the thermostat. When the temperature of the bath had been reached the following were added to the flasks.

Flask (1) 10 cc. of taka diastase and 36 cc. of plain buffer solution

Flask (2) 36 cc. of wheat diastase and 10 cc. of plain buffer solution

Flask (3) 36 cc. of wheat diastase and 10 cc. of taka diastase.

As nearly as possible the one hour's digestion was run concurrently on all three flasks. With the aid of an assistant it was found that the solutions could be added to all three flasks within two minutes, and as inactivation at the end of the digestion could be accomplished in less than a minute, no more than a 3 minute error occurred. Within the author's experience this error does not largely influence results of diastatic activity determination. The remainder of the procedure, i.e. inactivation autolyses, etc., were conducted according to the Blish Sandstedt method and need not be detailed here.

The experiment was repeated several times with the same starch. Tabulation of the data is given in Table VII.

Table VII

The Mutual Effect of Two Diastases During Simultaneous Digestion of Starch.

(1) Mgm. Maltose produced by Digestion with .01 gm. Taka Diastase	(2) Mgm. Maltose produced by Digestion with Wheat Diastase.	(3) (1) + (2)	(4) Mgm. Maltose produced by Digestion with Taka Diastase plus Wheat Diastase
100	98	198	204
103	146	249	255
107	137	244	238

Colum three in Table VII is merely the sums obtained by adding results in columns one and two. It will be observed that the effects of wheat diastase and taka diastase acting separately, when summed closely approximate to the effects of the two diastases when acting together on the same substrate. It is believed that such variations from a directly additive effect as are observed may be attributed to the unavoidable experimental error inherent in the method.

The above data may therefore be taken to indicate that for the particular starch and diastase used the diastatic activities of the two diastases are additive.

It must, however, be admitted that as the above experiments were performed using only starch from one wheat and diastase extract from one wheat the results cannot be considered as conclusive for all cases. Further work varying both the starch and the diastase extract were therefore undertaken.

(e) The Preparation of Starches.

Before proceeding further with the investigations it was necessary to prepare starches from the different flours. As these starches were also to be used later for purposes of comparison, it was thought advisable to prepare them in a manner that would ensure the least possible change in characteristics.

Rask and Alsberg[36] had developed a method of preparing starch from wheat flour, which provided an excellent basis to work on. The chief advantage of this method lies in the comparatively mild treatment to which the starch is subjected during isolation. Malloch's work has indicated, however, that even such mild treatment as is used in the preparation, changes the amyloclastic susceptibility of the starch. The ether wash especially was shown to bring about a lowering of starch resistance. Before proceeding with the preparation of the starches it was determined, therefore, to make a thorough investigation of the effect of the various reagents used on the starch.

Up to the end of the 1% sodium chloride wash, the procedure followed in these tests was identical with that of Rask and Alsberg. After repeated washing with distilled water had removed all traces of chloride, a sample of the starch at this stage was set aside to dry in a warm dry current of air. The remaining starch was then washed twice with 95% alcohol and a sample of this portion set aside to dry in the same manner as the previous sample. The starch remaining was washed twice with anhydrous ether and dried as the previous portions.

Amyloclastic susceptibilities of the three samples were run concurrently using .01 gms. of taka diastase as in previous tests.

Table VIII

Amyloclastic Susceptibilities of Starches subjected to Different Treatment during Extractions.

1% Sodium Chloride and Water	1% Sodium Chloride and Alcohol	1% Sodium Chloride Alcohol and Ether
68	72	85
60	61	72
82	84	97

Table VIII shows the variations in susceptibility due to each treatment of the starch. The variations within each column are due to the fact that a different solution of taka diastase was prepared for each set of determinations. It is of special interest that these variations in the

strength of the solution occurred in spite of the extreme care with which they were prepared. Consideration of this point will be given again in a later section. The figures given in the table scarcely need any comment. The alcohol wash had practically no effect on the starch resistance, whereas the effect of the ether was most pronounced.

The above work lead to two important conclusions.

(1) The starch resistance is lowered by ether wash and it is therefore necessary to omit this part of the process in preparing starches for amyloclastic susceptibility determinations.

(2) In spite of the extreme care in making the solution, the separate preparation of two taka diastase solutions with the same diastatic power is impossible. For comparative work on different starches therefore it is essential that a series be run using the same solution of the diastase throughout.

(d) The Materials

Having finally determined the procedure to be used in preparing the starches, a selection of flours was made from which the starches were to be extracted. These flours were selected with the view of obtaining a series of flours which would fairly well represent all the major variations in world wheats, that are generally encountered.

Table IX gives a list of the wheat flours chosen along with the moisture, protein, and ash content. The protein was obtained by the usual Kjeldahl method. The moisture content was given by the moisture driven off by heating at 130°C. for one hour in an air oven. The flours were ashed in a muffle furnace for 12 hours at 1000°.

Table IX

Flours from which Starches were Obtained.

	Moisture Content %	Protein %	Ash %
English	12.43	8.34	.37
No. One Hard	12.81	13.30	.45
Kanred	10.83	12.34	.40
Gavilla	13.51	8.86	.39
Bahia Blanca	11.99	12.37	.41
No. One Hard Sprouted	12.38	13.09	.42
Australian	12.36	8.00	.44
Durum	9.40	12.68	.51

The starches were prepared in the manner already indicated viz. by the Rask Alsberg method omitting the ether wash. After being allowed to dry for twenty-four hours in a stream of warm dry air they were placed in glass flasks and carefully sealed to prevent changes in moisture content. The intensive drying to which Rask and Alsberg subjected their preparations was omitted as no particular need for such an intensively dried starch existed.

Protein, moisture and ash determinations were also run on the prepared starches.

Table X

Starches prepared from Flours in Table IX.

	Moisture Content %	Protein %	Ash %
English	6.86	.32	.06
No. One Hard	7.13	.31	.09
Kanred	7.10	.36	.10
Gavilla	6.14	.35	.10
Bahia Blanca	6.36	.39	.04
No. One Hard Sprouted	6.40	.38	.07
Australian	6.60	.30	.05
Durum	7.11	.32	.08

The ash and moisture content were determined by the same method as that used with the flours. The protein was determined by Folins Micro Kjeldahl method.

(e) Investigation to Determine if Additive Effect of Diastases holds when Applied to Different Varieties of Starch and Diastases of Different Origin.

The starches having been prepared, the method evolved in section II (b) was applied to determine whether the additive effect applied, irrespective of the starch or natural wheat diastase used. Two series were run using the above listed starches. Ine one series wheat diastase extract from sprouted wheat was used and in the other series diastase extract was made from No. One Hard sound wheat. The extracts were not prepared till immediately before the series in which it was to be used. This was done as a precaution against both bacterial action on the diastase, and the production of unduly large quantities of maltose due to the action of the diastase on small quantities of starch, unavoidably remaining in the solution after extraction.

Only a slight modification of the procedure previously used was necessary. In the preliminary work the entire wheat diastase extract was placed in the thermostat to reach optimum temperature and the required quantities pipetted off as required. Modification of this procedure for a large series was necessary for several reasons.

It was necessary to run the samples with taka diastase, wheat diastase and taka diastase plus wheat diastase, concurrently if the true effect of the two diastase on each other was to be determined. This was naturally much simpler with one set of samples (three determinations) that it would be in a series in which eight different sets (twenty-four determinations). When the larger series was to be run, consideration had to be taken of the effect on the wheat diastase solution of remaining at optimum temperature and pH for some seven or eight hours. The disadvantage of such a condition lay not only in the difficulty of obtaining accurate results due to the large quantities of maltose produced, but also in the unpredictable effect of such quantities of maltose on the diastatic action of both the

wheat and taka diastase.

The difficulty was obviated by keeping the flask of diastase extract packed in ice throughout the series. Experiment showed that ten to fifteen minutes gave ample time for the 36 cc. of the extract to reach the temperature of the bath when placed in it, provided sufficient surface was exposed. Hence the procedure adopted was to draw off two 36 cc. quantities of wheat diastase every half hour placing each in a long narrow test tube (to ensure as great as possible surface exposure) in the thermostat. By this means it was possible to run the series of eight starches (3 simultaneous digestions for each starch) with the same wheat diastase extract, and without any fear of deterioration of the extract before the finish of the series.

Except for the modification described above the tests were run exactly as in the preliminary experiment, a set of three flasks being put in to digest every half hour.

The results of these series are given in Tables XI and XII.

Table XI

Tests to Determine if Additive Effect of Wheat and Taka Diastase holds for Different Starches.
Wheat Diastases from No. One Hard Wheat.

	(1) Maltose produced by Taka Diastase Digestion	(2) Maltose produced by Wheat Diastase Digestion	(3) (1) + (2)	(4) Maltose produced by Digestion with Wheat plus Taka Diastase
English No. One Hard	64	210	274	279
Kanred	110	230	340	340
Gavilla	56	200	256	260
Bahia Blanca	46	220	266	278
Sprouted	38	200	238	244
Australian	62	210	272	274
Durum	106	270	376	370

Table XII

Tests to Determine if Additive Effect of Wheat and Taka Diastase holds for Different Starches.
Wheat Diastase from Sprouted Wheat.

	(1) Maltose produced by Taka Diastase Digestion	(2) Maltose produced by Wheat Diastase Digestion	(3) (1) + (2)	(4) Maltose produced by Digestion with Wheat plus Taka Diastase
English	65	74	139	142
No. One Hard Kanred	112	52	164	160
Gavilla	60	58	118	124
Bahia Blanca	46	58	104	96
Sprouted	40	62	102	100
Australian	65	58	123	113
Durum	103	110	213	213

In both tables column (3) is, as indicated, obtained by merely adding the results given in columns (1) and (2). Comparison of columns (3) and (4) show that the agreement between the added individual effects and the effect of the two diastases, acting together on the same substrate, is quite close. Even if the differences noted are significant the effect of the two diastases is so close to additive as to make the added diastase method a fairly accurate means of estimating amyloclastic susceptibility in flour.

From the evidence produced it was concluded that the added diastase method gives a true measure of the relative amyloclastic susceptibilities of starch in flour.

III. RELATIVE AMYLOCLASTIC SUSCEPTIBILITIES OF DIFFERENT WHEAT STARCHES AND THE RELATIONSHIP BETWEEN AMYLOCLASTIC SUSCEPTIBILITY OF THE FREE STARCH AND STARCHES IN SITU IN FLOUR.

In the previous section it has been demonstrated that the additive diastase method is at least sufficiently accurate to permit of its use in estimating starch resistance in flour. This method was therefore used to determine the relative amyloclastic susceptibilities of the eight flours already listed.

In order to ensure that the results obtained might be strictly comparable a certain amount of preliminary work was necessary.

(a) Effect of Concentration of Substrate on Diastatic Production of Maltose.

Malloch investigated this question with surprising results. Despite the large excess of substrate considerable variation in reducing sugar was produced by varying the substrate concentration.

Malloch demonstrated this by using the same concentration of substrate for 8, 10 and 12 grams. Variations of as much as one hundred milligrams of maltose were produced by a change in concentration from 8 to 12 grams of starch.

As, however, Malloch's work only covered the above mentioned concentrations and all concentrations in this work were in the neighborhood of 5 gms. it was thought advisable to determine the effect of variations in the neighborhood of 5 gms.

The quantities of starch used were 4, 5, 6 and 7 gms. of starch (dry basis), the same quantity of taka diastase (.01 gms.) being used in each case. Table XIII shows the results obtained. In Fig. 2, these results are plotted and show an almost perfectly linear relationship. This is the same as the results obtained by Malloch for larger quantities of starch.

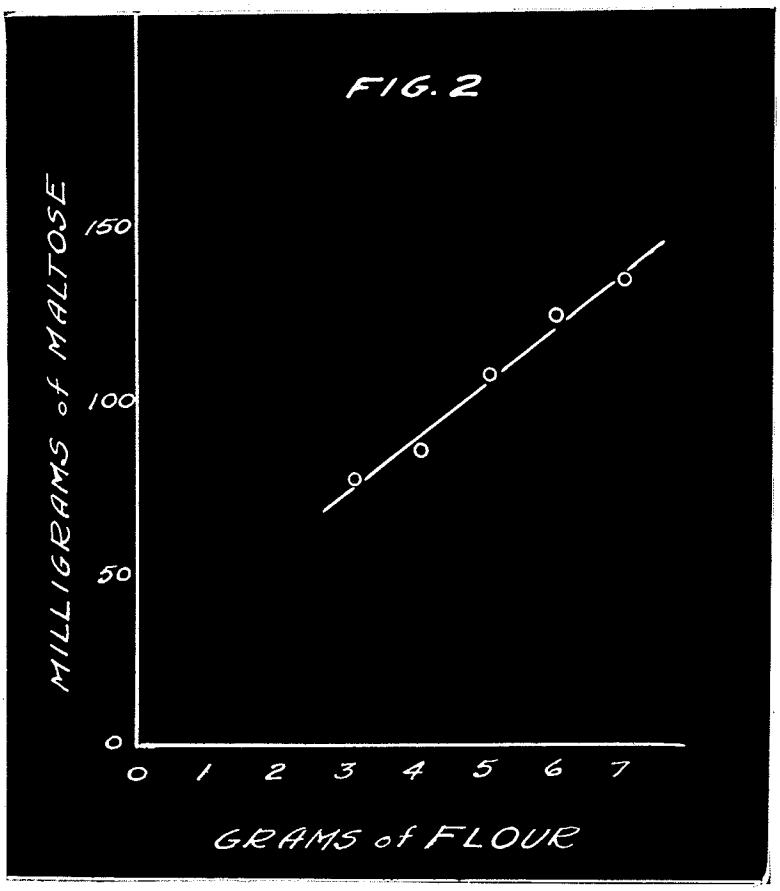
FIG. 2
MILLIGRAMS of MALTOSE
150
100
50
0
0 1 2 3 4 5 6 7
GRAMS of FLOUR

Table XIII

The Variation of Diastatic Activity with Concentration of Substrate.

Substrate Gms. of Starch	Diastases Mgms. of Maltose
3	78
4	83
5	106
6	122
7	132

It is evident from the above that if the amyloclastic susceptibility of a series of flour, as determined by the added diastase method, are to be comparable, the flours must be weighed out on a constant dry starch basis.

(b) Determination of Relative Amyloclastic Susceptibilities of Flours and Free Starches.

The impossibility of reproducing a diastase solution of exactly the same strength as any previously prepared one has been already mentioned. Further it was the author's experience throughout this work that diastase solutions rapidly deteriorate, such deterioration being noticeable within a period of twenty-four hours. Hence any series in which comparable results are required must be run using the same diastase solution throughout. In addition it is essential that there be not more than 12 to 15 hours between the first and last determination of a series.

In the following series a digestion was started every fifteen minutes so that in a series of 24 done in duplicate, there was no more than 12 hours between the first and last digestion.

The determinations were run in the usual manner with only one slight variation from the ordinary added diastase method. This was the weighing out of flours on the constant pure dry starch basis. Diastatic activities were run in the usual manner on the plain flour and on the flour plus diastase. In the same series amyloclastic susceptibilities of the free starches were also determined.

Table XIV

Amyloclastic Susceptibility of Wheat Flours

	(1) Maltose produced by Digestion of Flour with Taka Diastase	(2) Maltose produced by Digestion of Flour	(3) Amyloclastic Susceptibility of Flour (1) - (2)
English	330	120	110
No. One Hard	470	142	328
Kanred	750	223	527
Gavilla	405	125	280
Bahia Blanca	455	140	315
No. One Hard Sprouted	780	512	268
Australian	415	112	303
Durum	620	278	342

Table XIV shows the results of the series with the flours. The amyloclastic susceptibilities in column (3) are obtained by simply subtracting the results in column (2) from those of column (1).

As shown by previous investigators wide variations in susceptibility are noticeable. Of particular interest is the remarkably low susceptibility of the English in comparison to the others. Kanred represents the least resistant with a susceptibility of about 500%, that of the English. A rather puzzling state is represented by the No. One Hard sound and No. One Sprouted, a considerably higher susceptibility being indicated in the sound than in the sprouted. Wheter or not this is typical cannot of course be concluded from these results. Should, however, such a state be general, a possible explanation suggests itself. It has frequently been noticed that the starch granules nearest the are the most susceptible to amylases and during germination these granules are the first to be acted on by the amylase. Hence in flour from a sprouted wheat the most susceptible portion of the starch will have been disposed of. The net result would be that the unsprouted wheat would have a greater proportion of the more susceptible starch granules and hence a greater susceptibility. In passing it may be noted that the high susceptibility of durum is in agreement with the work of other investigators.

Table XV

Susceptibility of Starch in Situ and Free Starch

	(x) Susceptibility of Free Starch	(y) Susceptibility of Starch in Situ in Flour	$\frac{y-x}{y} \times 100$
English	86	110	22
No. One Hard	104	328	37
Kanred	166	527	68
Gavilla	72	280	74
Bahia Blanca	62	315	80
Sprouted	48	268	82
Australian	84	303	72
Durum	152	342	55

Column (x), Table XV, represents the relative susceptibility of the isolated starches. Column (y) is the same as column (3) in Table XIV. The most striking feature in this table is the great difference in all cases between the isolated starch and the starch in situ in the flour. In every case, it may be observed that isolating the starch has decreased its susceptibility. Furthermore the decrease is not proportional as shown by an examination of the percentage decrease in susceptibility in the third column. Decreases are observed to vary from 22% to 80%. No apparent relationship exists between the decrease in susceptibilities and the original or final susceptibility.

It is of course evident that the process of separation has caused this decrease in susceptibility but there is no means of determining whether it is due to the actual treatment given in the process or whether it is due to co-enzymes which have been washed out of the starch during its separation. On the basis of what this last set of data indicate there is small justification in judging the characteristics of starch as an ingredient of flour from starch as an isolated product. In consideration of the large amount of work that has been done on isolated starches in attempts to index flour quality by some starch characteristic, this latter conclusion appears of particular importance.

(c) Changes in Susceptibility due to the Treatment with 1% Sodium Chloride and Subsequent Washing with Water.

The surprising changes in susceptibilities of starches after isolation noted in the previous section, were such as to merit further investigation. The probability that such treatment was due to the treatment received by the starches during isolation has already been broached. Under that section dealing with changes during isolation the effect of alcohol treatment was dealt with and there can be little doubt that it was not the cause. The effect of the 1% sodium chloride was not investigated as no method has yet been indicated as to how such treatment could be avoided and a product with any degree of purity be obtained. The possibility, however, arrises that the greatest change did take place during the initial washing with distilled water and the subsequent treatment with the 1% sodium chloride.

It should be borne in mind that starch is a biochemical substance with largely colloidal properties, and it may well be that the initial treatment would cause the most radical change. The inherent difficulties of investigating such changes are at present insurmountable. It was thought, however, that by retreating the already isolated starch with water, sodium chloride, and again with distilled water, as in the original separation, certain of the discussed changes might again be brought about. No great hope was entertained that any great variations would be apparent, the maximum change probably having already taken place.

In this series of tests that follow only four starches were used no particular purpose being served by an investigation of all eight starches.

The starches were allowed to soak in cold water for two hours. Enough sodium chloride was then added to the suspension to make a one percent solution. After addition of the salt another half hour of soaking was allowed. The starches were then washed free of chloride as in the first separation and dried in the idental manner used in the original treatment.

Amyloclastic susceptibility determinations were then made on samples of the retreated and original preparations, the two sets being run in one series so as to be comparable.

Table XVI gives the results of the determinations.

Table XVI

Susceptibilities of Original Starch and Retreated Starch Preparations.

	Original Preparation	Retreated Preparation
English	93	90
Gavilla	77	76
Bahia Blanca	66	62
Durum	165	161

The data shows a very slight decrease in susceptibility in the retreated starch. It must be admitted, however, that the figures although consistently showing a decrease are not conclusive. Each result given is the result of triplicate determinations but such slight variations as are noted might still be the result of experimental error and cannot with certainty be stated as significant. The experimental results are inconclusive and do little to spread any new light on the problem.

IV. INVESTIGATION OF POSSIBLE RELATIONSHIP BETWEEN IODINE ABSORPTION AND AMYLOCLASTIC SUSCEPTIBILITY OF STARCH.

Allusion has already been made to the fact that determinations of amyloclastic susceptibilities of starches, to be of any value, must be done in series. The value of such work is greatly reduced by the inability of obtaining either a standard substrate or a standard diastase preparation. In short, an absolute unit for starch susceptibility is greatly needed. The problem has been given consideration by several investigators but to date advances in this direction have been negligible. If instead of such a unit some other starch characteristic, that paralleled susceptibility, coudd be discovered, the same purpose would be served.

In reviewing the different starch characteristics the starch iodine reaction being the most familiar received first attention. There were several considerations that made this property particularly worthy of investigation.

The heterogenity of the starch granule has been discussed in the introduction. The existence of α and β amylose and hemicellulose was outlined and mention was made of differences in properties. The chief differences in these fractions are their iodine reactions and their susceptibility to diastases. Their susceptibilities are in ascending order, hemicellulose, α amylose, and β amylose. The hemicellulose is as far as is known entirely impervious to diastase, the α amylose is attacked only slowly, the β amylose being the chief fraction affected by the diastase.

The reaction of iodine closely parallels the susceptibility of the different fractions. Clayson and Schryver[37] have shown that hemicellulose only gives a faint brown coloration. Hemicellulose is known to give a faint violet or no coloration, the chief factor in the reaction being the β amylose.

Reasoning from this it seemed probable that a measure of iodine absorption in the starch granule might also be a gauge of the β amylose content. As β amylose is the chief source of the maltose produced in diastases, then indirectly the iodine absorption might provide an index of amyloclastic susceptibility. An investigation was therefore projected in which the iodine absorption of the different starches was to be determined.

The first step in the investigation was the devising of a suitable method for measuring the absorption. Essentially the problem was that of affecting a complete separation of the starch iodide complex and the iodine solution. The most obvious method being filtration, it was the first to be attempted.

A quantity of starch was shaken up with iodine in potassium iodide solution and filtered under pressure through an alundite cup. The process was

extremely slow but the solution came through quite clear. However, the possibility existed that the accuracy might be affected by adsorption of some of the iodine from the filtered solution onto the alundite. Tests were made by simply filtering the clear iodine solution through a clean dry cup. A decrease in concentration of as much as 40% in a .001N iodine solution was observed. The method of separation by filtration was therefore discarded and the possibilities of centrifuging next considered.

In proceeding with the centrifuging tests, one gm. of starch was shaken up with 25 cc. of .001N iodine in potassium iodide solution in a 100 cc. centrifuge tube and the suspension was centrifuged for 30 minutes. On examining the centrifuged suspension it was found that the starch iodide had completely settled out leaving a clear supernatant liquid without the slightest trace of blue coloration.

The efficacy of centrifuging having been demonstrated, the next step was to determine the most suitable strength of iodine solution to be used. It had been observed in the separation experiments that when one gram of starch was used with 25 cc. of .001 N Iodine, all the iodine was completely withdrawn from solution. The experiment was therefore repeated using a .01N solution. This concentration was found suitable for the purpose, a measurable quantity of iodine being left in solution.

As the iodine absorption action of starch is probably colloidal and would therefore be influenced by slight differences of temperature of pH, it was thought advisable to keep these factors controlled. The pH was controlled by using a sodium acetate-acetic acid buffer (pH 4.7) instead of distilled water as solvent for the iodine potassium iodide solution. The reason for using this particular buffer was that as it was desired to a measure a property which might parallel amyloclastic susceptibility, it would be advisable to duplicate conditions under which amyloclastic susceptibility tests were made. For the same reason the temperature chosen at which the starch iodide and

iodine solution were allowed to reach equilibria was set at 86°F.

The final procedure was to weigh out a sample of starch or flour containing 1 gm. of starch dry basis. After the sample had reached the set temperature in the thermostat 25 cc. of the buffered iodine solution at the same temperature was added. The tube was then corked, shaken up for about five minutes to ensure complete dispersion of the starch and replaced in the thermostat. Every fifteen minutes the tube was shaken up to redisperse the starch. At the end of an hour the tube was finally removed from the thermostat and centrifuged for thirty minutes, the centrifuge being brought rapidly to a halt at the end of this time by means of the brake.

The estimation of iodine remaining in solution was immediately proceeded with. 10 cc. of the clear supernatant iodine solution was pipetted into a 100 cc. flask and 1 cc. of at least 2% soluble starch solution added. Another 15 cc. of distilled water were added and the solution then titrated with .001 N $Na_2S_2O_3$. The end point was not sharp but with very little practice it was found possible to obtain very close checks.

The following is a typical calculation of the iodine absorbed by the 1 gram of starch.

10 cc. of the supernatant iodine solution required 7.35 cc. of .001 $Na_2S_2O_3$.

∴ 10 cc. " " " " " contained

$$7.35 \times .001 \times \frac{126.93}{100} = .929 \text{ Mg. Iodine}$$

25 cc. " " " " " contained $2.5 \times .929 = 2.32$ Mg. Iodine

Hence starch absorbed = 31.75 - 2.32 = 29.43 mgms. of iodine.

The results given by the procedure were easily duplicated, using either one gm. of pure starch or a quantity of flour weighed out on the one gm. dry starch basis. The experiment was carried out on all 8 starches and the flours from which they were derived.

Table XVI

Iodine Absorptions of Flours and Starches.

(1) Flour Absorption Mgm.I per gm. Starch	(2) Starch Susceptibility	(3) Starch Absorption Mgm.I per gm. Starch	(4) Starch Susceptibility
30.45	110	29.43	86
30.53	328	30.00	104
30.89	527	29.33	166
30.55	280	29.90	72
30.99	315	29.81	62
30.10	268	29.31	48
30.75	303	30.20	84
30.96	342	30.32	152

Table XVI shows the iodine absorptions both for pure starches and flours. Columns (2) and (4) are the amyloclastic susceptibilities given in Table XV.

It is apparent at first glance that there is no relationship between amyloclastic susceptibility and iodine absorption. Compared with the large variations in amyloclastic susceptibilities the variations in iodine absorption are negligible. In view of the fact that the variations in absorptions observed appeared consistently and were definitely not due to experimental error it would seem that some significance might be attached to them. However exactly what they might signify is beyond the scope of this work.

It might be noted that in every case the iodine absorption was greater in the flour than in the starch. This might have been due to a certain amount of adsorption by the protein and no particular importance could be attached to it without a much more detailed investigation.

Although the very slight variations of iodine absorption and the lack of relationship between the iodine absorption on starch susceptibility were disappointing, the investigation was not entirely fruitless. The almost constant absorption, irrespective of the starch variety, noted in Table may have an important practical application. The possibility arrises that

phenomenum may be applied to the quantitative estimation of starch in mill offals. Should such an application prove feasible, one of the most frequent and annoying problems of mill control would be solved. Many methods of quantitative estimation of starch content are known, unfortunately however, these methods usually involve either expensive laborious processes or highly specialized and expensive apparatus. Consequently they are scarcely suited for the type of laboratory available in many mills. The method suggested by the results of this work would be both simple and inexpensive enought to conform with the requirements of the most sparsely equipped laboratory. Hence the development of a method for quantitative estimation of starch based on the starch iodine absorption is at least a possibility that might well merit further investigations

SUMMARY

The inactivating effect of ultra-violet light on wheat diastase was investigated. No inactivation of the dry flour could be detected. A definite inactivation of an aqueous suspension of the flour was noted. The inactivation was, however, too slow and diastases during inactivation too great to be applicable to amyloclastic susceptibility determinations. Similar results were obtained with a flour suspension in a buffer solution of pH 4.7.

On 110 volt alternating current applied for from 15 to 45 minutes to a flour in buffer solution suspension had no inactivating effect on the diastase.

Attempts were made to apply the inactivating effect of copper on wheat diastase to a solution of the problem. The reducing action of the copper on maltose produced in diastasis, introduced complications that necessitated the discarding of the project.

An examination of the Alsberg and Rask method of preparing starches showed that the alcohol treatment had only a negligible effect on the starch resistance. The ether treatment caused an appreciable lowering of starch resistance. The method, with the ether treatment omitted, was used to prepare starches from eight different wheat flours. These starches were used in the succeeding experiments.

An investigation was conducted into the possible supplementary of taka diastase and wheat diastase acting together on the same substrate. In the eight starches investigated and with the two wheat diastases used in conjunction with taka diastase the diastatic activities were very closely additive.

The results obtained were taken as justification for the use

of the added diastase method in determining the relative amyloclastic susceptibilities of the eight wheat flours mentioned.

Before proceeding with the amyloclastic susceptibility determinations, investigation was made of the effect of substrate concentration on diastases. A linear relationship between diastases and starch concentration (in the neighborhood of 5 gms.) was indicated.

Amyloclastic susceptibility determinations revealed wide variations in the wheat flours investigated, susceptibilities ranged from 110 in English wheat to 522 in Kanred. The susceptibilities were found to be much greater in flour than in the isolated starch. Furthermore the decrease in susceptibility after isolating the starch was not proportionate ranging from 22% in the English to 82% in the sprouted.

Tests were made to see if any proof could be obtained that this decrease was due to the initial treatment of the starch during its preparation. Only very slight indication was given that such was the case, the experiment in general being inconclusive.

A method was developed for the quantitative determination of iodine absorption by starch. Application of this method to the eight starches showed no relationship between their amyloclastic susceptibilities and iodine absorption. The absorption of iodine by the different starches varied only very slightly but the variations were too consistent to be due to experimental error. In every case the iodine absorption was greater with the flour than with the purestarch, the indication being that the increased absorption was due to adsorption by the protein. The absorptions ranged from 29.31 to 30.32 mgms. of iodine per gram of starch with the pure starch and from 30.10 to 30.99 mgms. of iodine per gram of starch with the flour.

Suggestion is made that the almost constant iodine absorption by the different starches may be applied to the development of a simple

inexpensive method of quantitative estimation of starch in mill offals.

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