A STUDY OF

DIASTATIC ACTIVITY AND AMYLOCLASTIC SUSCEPTIBILITY OF STARCH IN WHEAT FLOUR

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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 Leoberg[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 Leoberg'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.