Relation of Starch Damage and Related Characteristics to Kernel Hardness in Australian Wheat Varieties

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ABSTRACT

A simple test, the particle size index (PSI) test, is described. Its principle depends on the breakdown of wheat kernels under a standard grinding procedure. The test gives a consistent measure of kernel hardness. Over a very wide range of wheat varieties, grown under different environmental conditions in Australia, kernel hardness as measured by this test has been shown to be closely related to the damaged-starch content of flour milled from the wheat by a standardized milling procedure, and to be associated with characteristics of water absorption, diastatic activity, and gassing power. Regression formulas are presented for computation of these factors from PSI data. The PSI test may also be used to assess the degree of alpha-amylase activity of a flour before milling. Evidence is presented to show that the starch of hard wheat flours is apparently more susceptible to attack by diastatic enzymes than is starch from soft wheat flours. Furthermore, under Australian conditions there appears to be little or no relation between the distribution of starch granules of different sizes within and between varieties, with regard to damage incurred during milling.

Since the classic work of Jones on the mechanical damage incurred by starch during flour milling (1), more attention has been paid to this aspect of cereal chemistry. The damaged-starch content of flours has been shown to exert both adverse and beneficial effects on the flour. For example, a series of studies initiated by Bird indicated that the presence of excessive amounts of damaged starch could lead to erroneous interpretations of results from the wheat-meal fermentation time test, the sedimentation test, and many physical dough parameters (2,3). On the other hand, Greer and Stewart (4) pointed out the contribution of damaged starch to flour water absorption, and this phenomenon has come to be exploited by flour millers, who have contrived to increase the water absorption of flour by milling techniques modified so as to result in a higher incidence of damaged starch. Several chemical methods are now available for estimating damaged starch in flours, all of which must be considered to be superior to the staining techniques preferred by earlier workers.

Preliminary studies on the association between kernel texture and diastatic activity revealed a highly significant relation between these two parameters (5). Hence, it was considered likely that an estimation of the damaged-starch content could be made on a basis of the precise determination of kernel texture by means of the particle size index (PSI) system. Such a procedure would furnish information as to the damaged-starch content likely to be encountered in a flour before milling, and would also enable a preliminary estimation of the water absorption of the final flour to be made. It was considered that such a technique would be of assistance in the blending of wheats prior to milling.

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Two projects were conceived. The first was a classification on the basis of kernel texture of the wheat varieties commercially grown in Australia, as well as others utilized by wheat breeders as parental material. The validity of this classification was to be tested under different environmental conditions with an appropriate range of varieties. The second project was aimed at assessing the value of the particle size index in the prediction of starch damage and the associated characteristics of water absorption, diastatic activity, and gassing power in flour.

Previous workers have shown the importance of heredity (6,7), protein content (7), and environment (6–10) on diastatic activity in Australian wheat. Although these studies contributed valuable information on the range and variability of diastatic activity, none of these workers investigated the characteristics of kernel texture as such; moreover, the lack of replication prevented any assessment of the variability of the parameters measured within a location.

MATERIALS AND METHODS

For the purpose of the investigations described herein twelve varieties were selected, the PSI of which covered the entire range described by Symes (11). These varieties were grown in standardized trials consisting of four randomized blocks on thirteen locations throughout the Australian Wheat Belt during 1961–63. Locations ranged from East Central Queensland down to Tasmania and across to Western Australia, and previous accumulation of seed enabled all sowings to be made from the one original source of seed supply. In addition, seed of over 120 wheat varieties, cross-breds and pure species, was accumulated during 1960 and 1961 and sown in replicated rod-row trials at Wagga Wagga, New South Wales, during 1962 and 1963.

Kernel texture was determined by the method described by Symes (12). Grain (10 g.) was ground in a Labconco mill set at its finest setting, and the meal sieved for 10 min. on a 200-mesh wire sieve, the percentage of throughs being recorded as the PSI. Moisture content of all samples at testing varied between 9.3 and 10.6%, and periodic interpolation of hard and soft control samples confirmed that this moisture range did not affect the results obtained. Flour was milled from 1-kg. samples of wheat by means of a Buhler automatic mill, the flours being stored at –12°C. after milling. Damaged starch was determined in all flours by the procedures described by Sandstedt and Mattern (13), Sullivan *et al.* (14), and Farrand (15). Starch for estimation of starch granule size distribution was obtained by exhaustive washing of dough balls made from 10 g. of flour. The starch was then suspended in distilled water in a tall cylinder and maintained in complete suspension by means of a stream of air. Samples were withdrawn from midway down the cylinder, placed immediately on a glass slide, and stained with a 0.5% solution of iodine in 50% glycerol. Duplicate estimations of starch granule size distribution were made. It was established by experiment that no swelling of granules took place over a period of 18 hr. when slides were prepared by this method. Diastatic activity and gassing power were determined as prescribed in *Cereal Laboratory Methods* (7th ed.).

RESULTS AND DISCUSSION

Application of the PSI test to over 120 wheat varieties revealed a complete spectrum of kernel texture in Australian wheat, ranging from a PSI of 5% (very hard) to 40% (very soft). The twelve varieties included in the series of trials conducted throughout the wheat belt showed that the range of kernel texture represented by them covered virtually the whole range revealed in the larger collection. Daily measurements of maximum and minimum temperatures and rainfall at each location indicated the conditions under which trials were grown. The PSI classification of the twelve varieties was maintained at each location with only minor variations in relative positions of varieties at certain locations. The results of this series of experiments will be presented more fully in a later publication.

Relation between Kernel Texture and Damaged-Starch Content

The damaged-starch content of flours milled from the wheat varieties included in both series of experiments showed that, as expected, the highest levels of damaged starch were associated with the harder wheats, and vice-versa. Multiple regression analysis confirmed that by far the majority of the variance in damaged-starch content was accounted for by differences in kernel texture. In general, classification of varieties on the basis of damaged-starch content as determined by either of the chemical methods was less precise than classification based on the PSI (Table I).

TABLE I

Ratio of Variance in Twelve Wheat Varieties of Different Kernel Hardness

Classification	Reference	Variance (D.F.): Due to Varieties (12)	Variance (D.F.): Due to Environment (32)	Ratio: Varieties / Environment
Particle Size Index		795.03	2.10*	397.13
Starch damage by Farrand method	15	243.67	22.47**	10.84
Starch-damage index, Sullivan *et al.*	14	207.61	3.47**	59.83
Sandstedt and Mattern procedure	13	239.67	3.53**	67.90

The simple correlations of PSI with damaged-starch content are of the same order of magnitude for all three chemical methods employed in this series for the determination of damaged starch (Table II).

Evidence was obtained which suggested that the relationships would improve if secondary factors were included in a multiple correlation. Protein

TABLE II

Relation between Particle Size Index and Damaged-Starch Content, Diastatic Activity, and Gassing Power

	r
Damaged starch (Farrand), %	—0.908
Damaged starch (Sandstedt *et al.*), %	—0.899
Starch-damage index (Sullivan *et al.*)	—0.919
Log diastatic activity (mg./10 g. flour)	—0.992
Log gassing power (mm. Hg 6th hr.)	—0.956

content and the differential susceptibility to diastatic attack displayed by different wheat varieties are two factors which were considered likely to be significant in this respect.

A multiple regression analysis was carried out relating PSI to damaged-starch content as determined by the Sandstedt-Mattern procedure, but incorporating protein content as a second variable. The relation between PSI and damaged-starch content was improved slightly from r = —0.899 to r= —0.914, which suggested that protein content had relatively little influence on the relationship. The reason for this is worthy of additional comment. It was found that individual varieties, when tested statistically by this procedure, varied in their response to fluctuations of protein content. The PSI of some hard wheats decreased as protein content increased, whereas that of others increased. A similar situation existed with soft wheats. This finding is inconsistent with the widely held view that all soft wheats tend to become more vitreous at higher protein contents.

In the Sandstedt-Mattern procedure, the difference between the "damaged-starch content" after 2-hr. autolysis and that at zero time gives a measure of the susceptibility of starches to diastatic attack by the combined alpha- and beta-amylases (Fig. 1). This difference is referred to for the sake of convenience as the "susceptibility factor" and was found to be significantly related to the PSI (D.F. = 171, r=—0.671**). A second multiple regression analysis was carried out relating PSI to the Sandstedt-Mattern procedure, including the "susceptibility factor" as a second variable. This procedure improved the relation between PSI and damaged-starch content from r= —0.908 to r= —0.988, showing that the susceptibility factor exerts a very

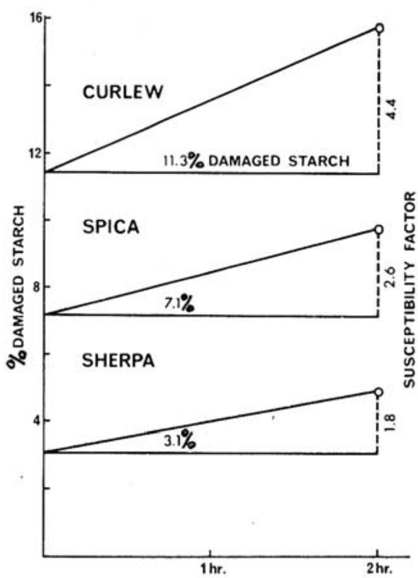

Fig. 1. Illustrating the "susceptibility factor" for three flours of different damaged starch content.

material effect upon the correlation. One implication here is that not only do hard wheats yield flour which contains a higher proportion of damaged starch, but that the starch itself is more susceptible to diastatic attack even in the undamaged state. No relation could be demonstrated between either the damaged-starch content, the susceptibility factor, or PSI and any feature of starch granule size distribution, either between or within varieties. The number of small granules (less than 7 μ in diameter) was, however, significantly correlated with kernel size, ($r = 0.608^{**}$, D.F. = 50).

Relation between Kernel Texture and Gassing Power, Diastatic Activity, and Water Absorption

Kernel texture was shown to be logarithmically related to gassing power as well as diastatic activity (Table II). Neither kernel texture nor damaged-starch content is closely related to diastatic activity and gassing power when alpha-amylase activity rises to significant levels. Two types of relationship appear to exist: one between kernel texture and damaged-starch content, the second between diastatic activity and gassing power, all four factors being interrelated in sound flour.

Since the relation between kernel texture and damaged-starch content is not affected to any great extent by variations in grain protein content, it is unlikely that the nature of the association between kernel texture (PSI) and flour water absorption will be very markedly affected. In fact, kernel texture gave a rather closer prediction of flour water absorption than any measurement of damaged-starch content when protein content was omitted from the regression equation (Table III).

TABLE III
RELATION OF PARTICLE SIZE INDEX AND DAMAGED-STARCH CONTENT TO FLOUR WATER ABSORPTION

	r	Regression	Root Mean Square[a]
Particle Size Index	—0.957	$y = 68.69 - 0.312x$	1.206
Starch damage (Farrand)	—0.904	$y = 58.4 + 0.1852x$	1.376
Starch damage (Sandstedt *et al.*)	—0.894	$y = 55.6 + 0.99x$	1.442
Starch damage index	—0.897	$y = 56.9 + 0.0351x$	1.442

[a] Root mean square of the deviation of predicted water absorption from observed flour water absorption.

It was considered that a useful estimation of the probable water absorption of flour milled from a given sample of wheat could be made from a knowledge of the PSI of the grain before milling. Current methods for estimating water absorption are based on chemical or physicochemical principles. The methods outlined by Farrand (15) and Greer and Stewart (4) are based on the relative content of starch, damaged starch, and protein and may be classified as chemical methods. Physicochemical methods include the interpretation of farinograph and alveograph data as well as that of other instruments. In practice, however, it frequently becomes necessary in individual laboratories and bakeries to "adjust" these series of data to comply with local requirements. The water absorption figures used in compiling Table II were the actual amounts of water used to bake loaves from the respective flours by the standard AACC procedure.

Influence of Alpha-Amylase

The presence of appreciable alpha-amylase activity in flour seriously affects any computation of the water absorption made from a knowledge of either damaged-starch content or kernel texture. The damaged starch present which normally contributes materially to the water-absorption capacity of a flour is rapidly liquefied in the presence of appreciable amounts of alpha-amylase. During our studies on the relation of kernel texture to diastatic activity several samples of flour were encountered, the diastatic activity of which was considerably higher than predicted from the regression equation described in an earlier paper (5). These samples were assayed by the Hagberg test and were found to contain abnormally high amounts of alpha-amylase. It seemed likely, on the basis of these tests, that herein may lie a method for the estimation of alpha-amylase activity, based on the difference between observed flour diastatic activity and that predicted from the PSI test. The first consideration was for the development of a method of accurately predicting actual flour diastatic activity from the original grain before milling, regardless of the possibility of high alpha-amylase content. After a series of preliminary investigations the following procedure was found to be admirably suited to this purpose.

With the residual throughs from the PSI test, 400-mg. samples are equilibrated at 30°C. with 9.2 ml. of acetate buffer (pH 4.6 as for the Blish-Sandstedt test) for 1 hr., with periodic agitation. The tube contents are clarified by means of 0.4 ml. each of 3.58*N* sulfuric acid and 12% sodium tungstate, and then centrifuged. Aliquots of 1 ml. are transferred to 6 × 1-in. boiling tubes with 2 ml. of saturated picric acid and 2 ml. of 20% sodium carbonate solution. The tubes are immersed in a boiling-water bath for 30 min. and cooled; contents are accurately diluted to 50 ml. before measurement of color intensity at 490 mμ. It is recommended that the saturated picric acid solution be maintained at 30°C. to obtain a satisfactory degree of constancy in the blank determinations. Any suitable sensitive method for measuring reducing sugars may of course be substituted for the picric acid test, which was first applied to plant material by Thomas *et al.* (16). A regression equation was computed on the basis of the results obtained from more than 100 samples of wheat. Subsamples were analyzed by the PSI test, the throughs being retained for the studies on diastatic activity prediction described herein. Samples (1 kg.) of the same wheat were then milled to flour in the Buhler mill, and diastatic activity was determined on the flour by the Blish-Sandstedt procedure. The relation between predicted and observed diastatic activity ($N = 112$) was $r = +0.994$, quite satisfactory for the preparation of a regression equation.

The diastatic activity of a flour depends on its damaged-starch content, and on both alpha- and beta-amylase activity. Since beta-amylase is virtually a constant factor, the predicted diastatic activity is dependent mainly on the damaged-starch content of the flour. The difference between observed and predicted diastatic activity of the PSI throughs may be considered to be the result of alpha-amylase activity. Since the absolute levels of diastatic activity vary from hard to soft wheats, the difference between observed and predicted

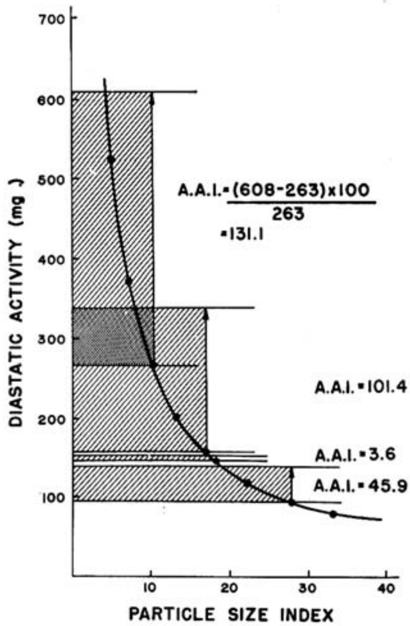

Fig. 2. Method of computation of alpha-amylase index (AAI) for flour samples.

$$AAI = \frac{\text{(observed diastatic activity — predicted diastatic activity)}}{\text{predicted diastatic activity}} \times 100$$

diastatic activity has to be related to the predicted diastatic activity for the purpose of estimating alpha-amylase activity (Fig. 2).

Twenty-seven samples of wheat and the flour milled from them were tested by PSI and diastatic activity tests, the latter being determined on both "sieved" and "milled" flours. The difference between observed and predicted diastatic activity expressed as a percentage of the predicted diastatic

TABLE IV

RELATION BETWEEN KERNEL TEXTURE (PSI), OBSERVED AND CALCULATED DIASTATIC ACTIVITIES, ALPHA-AMYLASE INDEX, AND HAGBERG NUMBER

PSI	Observed Flour Diastatic Activity	Calculated Diastatic Activity[a]	AAI	Hagberg No.	PSI	Observed Flour Diastatic Activity[a]	Calculated Diastatic Activity	AAI	Hagberg No.
	mg.	*mg.*				*mg.*	*mg.*		
10.7	616	256	140.6	58.0	19.3	284	141	101.4	28.0
10.4	608	263	131.1	56.5	15.3	344	179	92.2	26.0
13.2	418	207	101.9	36.0	11.0	292	249	17.3	11.8
17.6	337	155	117.4	42.0	12.7	218	215	1.4	9.5
25.1	194	109	78.0	39.5	33.1	83	82	0.1	9.5

[a] Predicted from formula log Y = 3.441 — 1.004 log x, where X = PSI of grain and Y = diastatic activity of flour.

activity is referred to as the alpha-amylase index (AAI). Alpha-amylase activity was measured in the flour by the Hagberg test (17). The relation between AAI and the Hagberg figure ($r = +0.902^{**}$) was sufficiently close to give a very satisfactory indication as to whether the alpha-amylase level of the flour was above the level normally encountered. Table IV illustrates some typical data from this experiment.

On the basis of the results obtained, an AAI up to 30 indicates that the alpha-amylase level is low. Figures of 40 and upward are obtained from flours having higher alpha-amylase activities.

The influence of high alpha-amylase depends to a large extent on the nature of the flour. Flours with low damaged-starch content may actually benefit as a result of the higher enzyme activity, whereas the water absorption of flours of highly damaged starch content is usually lowered. Even the influence of alpha-amylase on flours with highly damaged starch depends on the purpose for which the flour is to be used and the baking procedures employed. For example, the Chorleywood process appears to be more tolerant of high levels of alpha-amylase than—say—the AACC remix baking test.

CONCLUSIONS

The relations between wheat kernel hardness and several chemical properties of the flour milled from the wheat by a standardized milling procedure are described. Kernel hardness as measured by the particle size index (PSI) method has been shown to be closely related to damaged-starch content as measured by three chemical methods. It is also related to diastatic activity, water absorption, and gassing power. Regression equations are presented for the prediction of these parameters from the particle size index. The regression of damaged-starch content on PSI is improved when the susceptibility of the starch to enzyme attack is included in a multiple regression equation, although grain protein content exerts a less significant influence. No relation could be found between starch granule size and kernel texture, damaged starch, diastatic activity, gassing power, or water absorption. A significant positive correlation was, however, revealed between starch granule size and kernel size.

The regression equation by which flour diastatic activity can be computed (5) takes no account of the possible presence of excessively high levels of alpha-amylase. A method is presented for the prediction, from a small sample of grain, of the true diastatic activity of flour milled by a standard milling procedure. From the difference between observed true diastatic activity and diastatic activity as predicted from the PSI figure, an estimation can be made of the extent to which alpha-amylase is present. The figure obtained, referred to as the alpha-amylase index, is shown to be closely related to such figures as the Hagberg penetrometer figure.

The relations demonstrated between kernel texture and the factors enumerated above assume greater significance in the light of a recent contribution by Symes (12), who has reported that the difference in kernel texture between several series of hard and soft wheats is apparently controlled by a single major gene, the expression of the minor modifying genes differing with different parental types. It thus becomes conceivable by means of the back-crossing technique to introduce kernel hardness (and all of the factors associated with it) to wheat varieties possessing attractive agronomic and quality characteristics, but which by reason of undesirable characteristics associated with the soft kernel are unsuitable for milling.

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