THE DETERMINATION OF STARCH DAMAGE OF FLOUR[1]

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ABSTRACT

A quantitative method for the determination of "starch damage index" is outlined. The method is based on the fact that damaged starch granules are much more susceptible to the action of beta-amylase than undamaged granules. The "starch damage index" is the difference in maltose value between flour inactivated by trichloroacetic acid in butanol and the inactivated flour plus an excess of beta-amylase. The method is not affected by the usual malting and maturing treatments.

Starch damage index of flours is dependent mainly on the type of wheat milled and the kind and severity of grinding. Tempering time, except when very short, has no significant effect on starch damage index. Absorption increases as starch damage increases; both are positively correlated with particle size and specific surface only when the same type of grinding action is employed.

It has been generally recognized that starch granules can be damaged and made more susceptible to enzyme attack by grinding, and that grinding practices vary significantly from mill to mill. Excessive

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[1] Manuscript received June 17, 1961. Contribution from the Research Laboratories of Russell Miller-King Midas Mills, Minneapolis, Minnesota.

amounts of starch damage can harm the machining characteristics of dough and impair loaf volume and crumb resilience. Starch damage of flour has assumed an even greater importance with the increasing number of continuous-bread-system installations where higher dough temperatures are employed. Until very recently, there was no quantitative measurement of starch damage available and, consequently, the effect of this variable has not had the study it deserves. Sandstedt and Mattern (5) recently have reviewed the literature and proposed a quantitative method. The procedure in use in this laboratory is based on some of the same general principles, and it was thought that it might prove useful to other laboratories.

Our objective was the development of a method for determining starch damage that could be adapted to control work and would provide a sufficiently useful guide for studying and predicting the effect of starch damage on certain baking characteristics of flour. Sandstedt and Mattern (5) correctly pointed out that all such methods are empirical, since the complete differentiation of damaged and undamaged starch granules is virtually impossible. There is a continuous spectrum of degrees of damage. Basic assumptions of the method are that the trichloroacetic acid-butanol treatment inactivates the amylases of the flour and that the ratio of amylose to amylopectin is relatively constant in wheat flours.

Practical observations have indicated that, when other variables are kept constant, the starch damage index (SDI) correlates well with crumb strength as observed in loaves from laboratory baking tests and from actual plant production.

Materials and Methods

Beta-Amylase. Wallerstein, standardized at 2,000° Lintner without further purification.

The enzyme was kept in the refrigerator and brought to room temperature before being used. In routine determinations, 80 mg. of the enzyme were weighed into glass flasks or plastic bottles and stored in the refrigerator. The flasks or bottles were allowed to warm to room temperature prior to use.

Flours. A random selection of different flours from various mills and wheat mixes was checked for starch damage index, including winter and spring short patent flours and bakery patent grades. Patent, first clear, and second clear flours from two different spring wheat mixes and from an amber durum wheat mix were among the samples. Coarse middlings were ground on rolls and in an impact mill to increase the amount of damaged starch. Several of the flours had been milled especially for bakeries using continuous systems. Analytical data on most of these flours are included in Table I or, in a few separate experiments, in the other graphs or tables.

TABLE I
ANALYSES AND STARCH DAMAGE INDEX OF FLOURS

SAMPLE	ASH[a]	PROTEIN[a]	MALTOSE VALUE,[a] INACTIVATED FLOUR	MALTOSE VALUE,[a] INACTIVATED FLOUR + 80 MG. BETA-AMYLASE	STARCH DAMAGE INDEX[a]
	%	%	*mg/10 g*	*mg/10 g*	
North Dakota spring					
Patent	0.39	12.40	21	214	193
First clear	0.69	16.00	22	158	136
Second clear	1.72	17.40	41	222	181
North Dakota-Montana spring					
Patent	0.40	12.20	17	188	171
First clear	0.70	15.70	20	149	129
Second clear	1.60	17.00	35	208	173
North Dakota spring					
Patent	0.39	14.10	20	202	182
North Dakota-Montana winter					
Patent	0.39	12.10	20	213	193
Straight	0.49	13.50	20	191	171
Spring-winter					
Bakers patent	0.43	12.70	24	192	168
Durum flours					
Fancy patent	0.64	13.40	28	281	253
Patent	0.70	13.60	29	347	318
First clear	1.27	15.50	34	250	216
Second clear	1.54	17.60	33	224	191

[a] At 14.0% moisture.

Inactivation of Enzymes. The procedure was as follows: The solution used to inactivate the enzymes of the flour was prepared by placing 30 g. of trichloroacetic acid, A.R., in a volumetric flask and making up to 1 l. with 90% (v/v) butanol. The ratio of butanol to water is critical; hence care must be taken in preparing this solution. Forty grams of flour were placed in a 300-ml. Erlenmeyer flask and 150 ml. of the inactivation solution added. The flask containing the flour and solution was then stoppered and shaken until all the particles were in suspension. The suspension was allowed to stand for 30 minutes, during which period the flask was shaken two or three times. After 30 minutes, the contents were filtered through a No. 560 S&S fluted filter paper. The flour on the filter paper was washed twice with 50-ml. portions of 100% butanol. The flask was rinsed with the first

50-ml. portion of butanol. After the two butanol rinses, the flour was rinsed twice with 50-ml. portions of ethyl ether. After the solvent had ceased to drip, the flour on the filter paper was transferred to a 1000-ml. beaker and the remaining butanol and ether evaporated on a steam bath. During the drying period, the flour was stirred occasionally with a glass rod. The flour was dried until an easily handled powder resulted. Any remaining lumps were broken up and the sample was thoroughly blended. A trace of butanol did not affect the results. Most of the inactivated samples produced values between 16 and 30 maltose units.

Maltose. Maltose was determined by the standard ferricyanide method (1) using 5 g. of inactivated flour and 5 g. of the same flour plus 80 mg. of beta-amylase. In a few cases where the starch was damaged drastically, 2.5 g. of flour and 40 mg. of beta-amylase were used. The difference in milligrams of maltose per 10 g. of flour between the two determinations represents the starch damage index (SDI).

Measurement of Particle Size. Particle size was measured by the technique of Whitby (7,8) using the Mines Safety Appliance Particle Size Analyzer. Mass median diameter (MMD) and degree of dispersion were read from the cumulative particle size distribution curve.

Results

Amount of Beta-Amylase. First it was necessary to determine how much beta-amylase was needed. Three flours, a soft wheat, high-ratio cake flour (0.29% ash, 7.7% protein), a hard-spring-wheat, patent flour (0.40% ash, 14.0% protein), and a hard wheat, impact-ground cake flour (0.36% ash, 7.7% protein), were inactivated according to the above procedure and each flour was treated with increments of beta-amylase from 5 to 320 mg. Results are shown in Fig. 1.

Other flours from various wheat mixes and of different extractions gave similar results on the addition of successive levels of beta-amylase. The derivative of the curves was relatively constant at 80 mg. of beta-amylase and above; consequently, this amount was selected for routine determinations. There was a continued, though less rapid, rise in the curve on increments of beta-amylase over 80 mg. This might have been due to a trace of alpha-amylase with the beta-amylase, the product being standardized to have not more than 0.05 alpha-amylase units (SKB units) per gram[2] or, more probably, to the action of beta-amylase on undamaged starch granules.

Completeness of Inactivation: Beta-Amylase. Experiment 1. One proof

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[2] Private communication from the manufacturer.

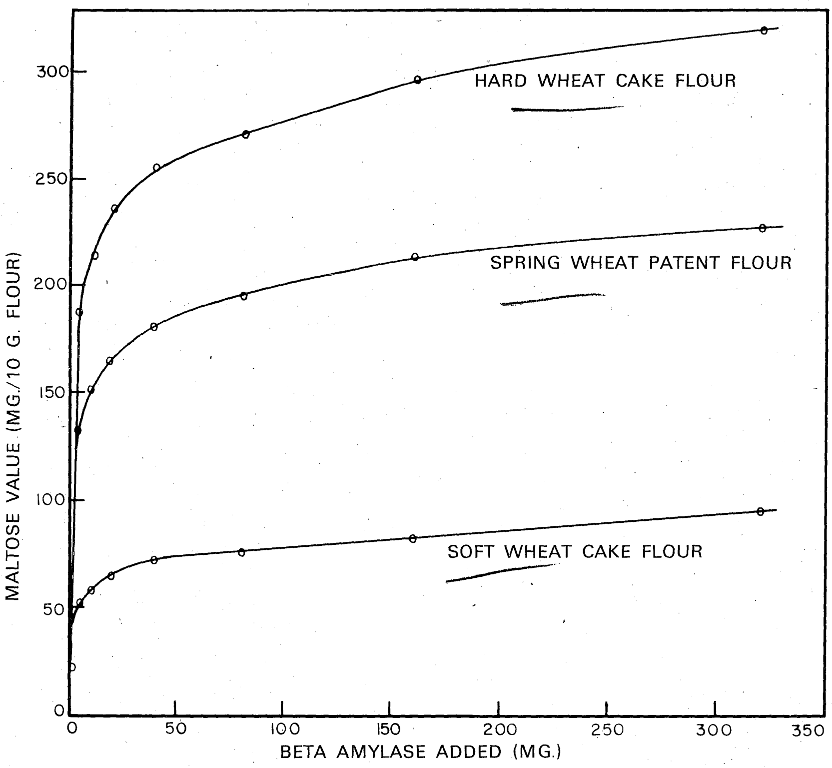

Fig. 1. Effect of increments of beta-amylase on inactivated flours.

of complete inactivation is the independence of maltose value and digestion time. A spring wheat, patent flour (0.39% ash, 12.4% protein) was inactivated in the manner described and the maltose value found on a 1-hour digestion period was 25 units and, on a 2-hour digestion, 21. The longer time produced no additional maltose, thus indicating complete inhibition of enzyme activity.

Experiment 2. Another proof of inactivation is the inhibition of exogenous beta-amylase. A definite amount of beta-amylase (0.25%) was added to the flour used in Experiment 1, before inactivation. The original sample on a 1-hour digestion period gave a maltose value of 25 and the same sample plus beta-amylase a 20 maltose value, thus showing complete inactivation of the enzyme.

Completeness of Inactivation: Alpha-Amylase. A high-protein, spring wheat flour (0.39% ash, 14.1% protein) was inactivated by the method outlined and another sample of the same flour to which 0.25% alpha-amylase (Wallerstein) had been added was also inactivated.

To each sample 80 mg. of beta-amylase were added and the maltose determined on both samples. The control sample gave a 195 maltose value and the sample to which alpha-amylase had been added showed a 196 maltose value, thus proving the inactivation of alpha-amylase was complete.

Time of Digestion. The effect of digestion time on the starch damage index of the three flours described above is shown in Fig. 2. The rate of change, shown in maltose units, decreased significantly after 1 hour; therefore, a 1-hour digestion period was chosen. A constant amount of beta-amylase (80 mg.) was added to an inactivated sample to determine each point on the curve.

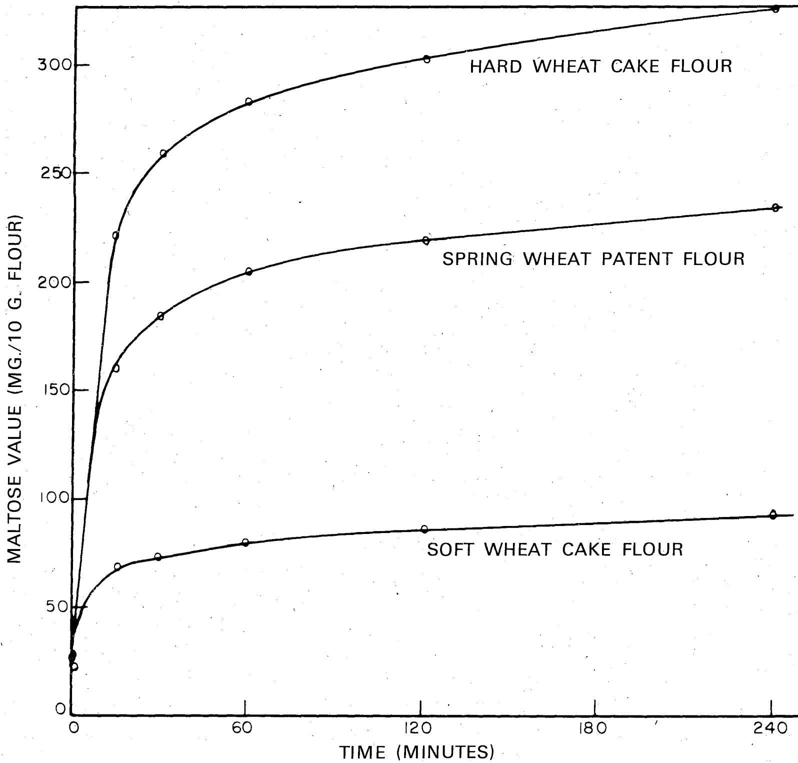

Fig. 2. The relation of time of digestion to the maltose value of inactivated flour to which 80 mg. of beta-amylase were added.

Roll Versus Pin Mill Grinding. Hard wheat middlings (0.38% ash, 11.5% protein) were used in this experiment. One sample of the middlings was ground on rolls several times, using a very close setting, and another sample was ground once in an Alpine 160-Z Pin Mill operating at 17,000 r.p.m. The inactivated, roll-ground sample gave a 27 maltose value and, with 80 mg. of beta-amylase, a 286 maltose value, giving a starch damage index of 259. The impact-ground, inactivated sample gave a 24 maltose value and, with 80 mg. of beta-amylase, a 235 maltose value, giving a starch damage index of 211. The particle size distribution of the two ground flours is shown in Fig. 3. The roll-ground and impact-ground samples had specific surfaces of 2,100 cm^2/cm^3 and 3,150 cm^2/cm^3, respectively.

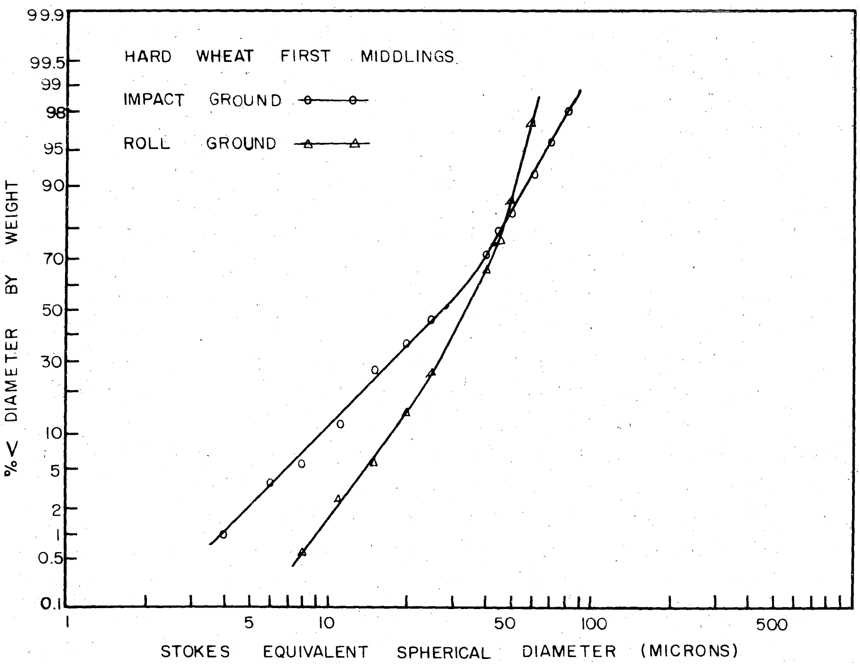

Fig. 3. Particle size distribution of roll- and impact-ground first middlings.

In spite of the finer granulation of the impact-ground sample, the starch damage index was considerably less than that of the roll-ground flour. This subject has been discussed by Sullivan, Engebretson, and Anderson (6). Normally, roll grinding or a shearing action on the starch granule causes considerably more damage than pin mill grinding to the same granulation. Figure 3 shows also that, in pin mill grinding, there is usually a greater dispersion in particle size than in roll grinding. The coarsest particle found in a roll-ground sample is no larger than the spacing of the rolls, whereas the coarsest particle in a pin-milled sample can be as large as the coarsest particle of the starting material or the spacing between the pins, whichever is smaller.

Usually, the spacing of the pins in a pin mill is much larger than the spacing between rolls.

With conventional milling, closer-than-normal roll settings produce stocks with finer granulation, greater specific surface and starch damage, and higher absorption. There is no significant correlation between particle size or specific surface and absorption when more than one type of grinding is employed. Thus, in Fig. 3, the sample ground in a pin mill, with 3,150 cm^2/cm^3 specific surface and an MMD of 27μ, showed 61.7% farinograph absorption at 14.0% moisture whereas the same middlings ground on rolls had a specific surface of 2,100 cm^2/cm^3, and MMD of 35 μ, but an absorption of 64.4%.

The above data and other work done in this laboratory indicate that starch damage and absorption are related independently of the type of grinding, but that the relationship between granulation and starch damage or absorption is dependent on the type of grinding used. The relationship between any of these variables is also dependent on the type of stock used for grinding.

Variation of Starch Damage Index with Grade. Short patent, first clear and second clear flours from two different spring wheat mixes were compared for starch damage index. Data are listed in Table I. The first clear flours showed less starch damage (SDI 136 and 129) than the short patent flours (SDI 193 and 171); the second clear flours (SDI 181 and 173) showed about the same as the respective patent flours. The second clear and durum flours gave a higher maltose value on the inactivated flours than the other samples. The reason for the relatively low SDI figures for the first clear flours is attributed to the large amount of break flour they contained. This flour is removed as the throughs of some of the break sifters and has not been ground on reduction rolls.

Effect of Flour Treatment on Starch Damage Index. Since most flours routinely tested are treated with various combinations of malt flour, potassium bromate, benzoyl peroxide, and chlorine dioxide, the influence of representative flour treatments on starch damage was measured. A winter wheat, patent flour (0.40% ash, 11.5% protein) with no treatment was compared with the same flour treated with 0.5% wheat malt, with 0.5% malt and 30 p.p.m. bromate, with 0.5% malt and 0.25% benzoyl peroxide, and with 0.5% malt and 0.5 g. chlorine dioxide per hundredweight. The results are shown in Table II.

No significant effect of flour treatment on starch damage index was demonstrated, as would be expected.

Replication. A spring wheat, patent flour (0.40% ash, 12.1% protein) was used to check the reproducibility of the method. Twelve

TABLE II

EFFECT OF TREATMENT ON STARCH DAMAGE INDEX[a]

TREATMENT	STARCH DAMAGE INDEX[b] (SDI)
None — control	135
1.5% Wheat malt	133
1.5% Wheat malt + 0.25 g. per cwt. benzoyl peroxide	129
1.5% Wheat malt + 30 p.p.m. potassium bromate	135
1.5% Wheat malt + 0.5 g. per cwt. chlorine dioxide	134

[a] Flour used was a hard-winter-wheat patent (0.41% ash, 11.5% protein).

[b] At 14.0% moisture.

samples were inactivated at different times. Twelve determinations were run on twelve different days. The standard error was 4.3 SDI units.

Effect of Tempering Time on Starch Damage Index. It might be expected that tempering time would have some effect on the starch damage index, since the mellowness of the endosperm influences grinding. Patent flours from a large mill where the wheat mix had a 24-hour and a 100-hour temper to 15.5% moisture showed no significant difference in starch damage index. The same wheat was milled on an experimental Allis mill, using three different tempering times: 3-, 24-, and 100-hour tempers to 15.5% moisture. The results are shown in Table III.

TABLE III

EFFECT OF TEMPERING TIME ON STARCH DAMAGE INDEX[a]

FLOUR SAMPLE	TEMPERING TIME	ASH[b]	PROTEIN[b]	STARCH DAMAGE INDEX[b]
	hours	%	%	
Patent, large mill	24	0.38	12.10	133
Patent, large mill	100	0.38	12.00	139
Patent, experimental mill	3	0.39	12.80	117
Patent, experimental mill	24	0.39	12.60	110
Patent, experimental mill	100	0.39	12.60	107

[a] Wheat used was a spring-winter wheat blend tempered to 15.5% moisture.

[b] At 14.0% moisture.

It appears from this and other work that, providing tempering time and conditions are adequate for good milling practice, little difference in starch damage index will be encountered. Too short a tempering time or insufficient temper from other causes will give a higher starch damage index.

Effect of Wheat Types on Starch Damage Index. Since wheats vary markedly in kernel hardness and friability of endosperm, wheats tempered and milled as far as possible in the same manner and with the same roll settings were compared for starch damage. There were a few instances, as with one sample of amber durum, where the extraction rate and percent patent were below the average. Table IV illustrates the results.

TABLE IV
EFFECT OF WHEAT TYPE ON STARCH DAMAGE INDEX[a]

WHEAT	EXTRACTION[b]	PATENT[c]	MOISTURE	ASH[d]	PROTEIN[d]	STARCH DAMAGE INDEX[d]
	%	%	%	%	%	
North Dakota spring, Selkirk	68.9	95.0	14.5	0.37	14.00	116
Southern Montana spring	67.7	93.6	14.3	0.42	11.50	156
Northern Montana spring	65.1	91.8	14.4	0.41	14.80	97
Montana winter, Cheyenne	67.8	94.2	14.3	0.35	12.50	111
Texas-Oklahoma winter	68.3	93.6	14.4	0.38	12.20	98
Nebraska winter	68.3	94.8	14.3	0.39	12.00	129
Kansas winter	68.3	94.5	14.6	0.41	12.00	103
Illinois soft	67.1	93.7	14.5	0.35	9.70	50
Amber durum	64.7	88.7	14.4	0.53	14.20	192
Amber durum	68.5	94.8	14.4	0.54	14.30	205

[a] All samples were tempered to 15.0% moisture for 20 hours at room temperature and milled to a long patent on an Allis Experimental Mill with the same roll settings.

[b] (Dry flour weight/dry wheat weight) x 100.

[c] Based on 75% extraction of clean sample weight as-is.

[d] At 14.0% moisture.

The soft wheat flour showed much less starch damage than any of the other wheats; less grinding is necessary. Flours from durum wheats showed very high damage, as might be expected from the hardness and vitreousness of the durum kernel. Flours from bread wheats, both winter and spring, varied between these limits. Thus, the wheat mix, as well as the severity and kind of grinding, affects the amount of starch damage.

Additive Effect of Starch Damage. As shown in Table V, a soft wheat, high-ratio cake flour (0.29% ash, 7.7% protein) showed a starch damage index of 51 whereas a hard wheat, impact-ground flour (0.36% ash, 7.7% protein) gave a starch damage index of 247. The two samples showed approximately the same particle size distribution. Various blends of these two flours were made to see if starch damage results

TABLE V
ADDITIVE EFFECT OF STARCH DAMAGE

FLOUR	ASH[a]	PROTEIN[a]	STARCH DAMAGE INDEX[a], AS FOUND	STARCH DAMAGE INDEX[a], CALCULATED
	%	%		
100% Soft wheat cake	0.29	7.70	51	. . .
100% Hard wheat cake	0.36	7.70	247	. . .
75% Soft — 25% hard	0.31	7.70	95	100
50% Soft — 50% hard	0.32	7.70	143	149
25% Soft — 75% hard	0.34	7.70	191	198

[a] At 14.0% moisture.

were additive in the blends. Table V gives the data showing that this is the case.

Discussion

Starch damage is known to affect such important baking properties as absorption, malt response, rate of fermentation, and crumb strength. Many observations on the effect of starch damage on these and other factors have been more or less qualitative because of lack of a more definitive method for measuring starch damage. It has been long recognized that finer grinding and greater surface area increase absorption and amylase activity. As Halton (2) has noted, both factors are interrelated and dependent on time of fermentation and, if a significant increase in water absorption is to be effected by an increase in starch damage, the amylase activity of the flour must be kept as low as possible consistent with satisfactory performance. Crumb strength and resilience are important in the appearance, texture, and commercial handling of bread. Moreover, with the advent of continuous systems of bread production where the dough comes from the developer at around 100° F., more attention than ever has been focused on starch damage and its relation to the physical properties of starch, such as swelling and gelatinization, and the effect of these properties on dough structure.

Microscopic observations, the Hampel method (3), and the more recent procedure proposed by Sandstedt and Mattern (5) are all useful in evaluating starch damage. The method proposed here lends itself more to routine control. Results have checked well with flour performance in our laboratory and in bakeries. This method, like other methods proposed, is empirical and depends on the greater susceptibility to beta-amylase of damaged starch compared to normal starch. The degree of susceptibility to beta-amylase of various kinds of starch damage is not known. The incomplete degradation of amylose by beta-amylase is generally attributed to retrogradation of amylose. Complete conversion to maltose can be attained by adding, drop by drop, amylose in a highly dispersed state to an excess of enzyme. Under such conditions, the formation of an enzyme-substrate complex is more likely than the association of amylose particles and a consequent enzyme-resistant substrate.

Moreover, there is a more rapid initial action of beta-amylase on amylopectin than on amylose, and hence the number and length of branch chains affect the maltose produced.

A number of techniques were tried in this laboratory in an endeavor to inactivate flour as completely as possible with the minimum difficulty. In our original method, the procedure used was a modification of that proposed by Lee and Geddes (4) where the flour was inactivated by heating and stirring with absolute ethanol. The inactivated flour showed maltose values over double those obtained by the present procedure and, although reasonably good duplication could be achieved, the original high values indicated lack of complete inactivation, with the consequent greater probability of variability. Several means of inactivation were tried and the best was a solution of trichloroacetic acid in butanol, as outlined. Proof of the completeness of the inactivation is given in the experimental section.

As Fig. 1 shows, the starch damage index (reported as maltose value, mg. per 10 g. flour) increases rapidly with the first addition of beta-amylase to the inactivated flours. The rate of reaction changes markedly at around 40 mg. One flour in particular (hard wheat cake flour) that shows a high starch damage is unusual, but is included to show the continued increase in maltose production on increasing the levels of beta-amylase in excess of that required for action on damaged starch. With normal flours and under the experimental conditions outlined, there is a definite leveling off in maltose formation as the amount of enzyme is increased from 70 mg. There is, however, a slight but definite increase of maltose with increasing amounts of enzyme with all the flours tested. This may be due to the action of beta-amylase on the undamaged starch granules or to the action of the traces of alpha-amylase present in the enzyme employed. Two samples of beta-amylase, one repurified to contain half the alpha-amylase of the enzyme routinely employed (0.047 SKB units), gave the same reaction rate.

There are two main factors influencing starch damage: the first and probably the most important is the wheat, and the second, the kind and severity of grinding. Table IV shows the results of the experimental milling of a wide variety of wheats where the flow and roll settings were kept as constant as possible. The hardest wheat, amber durum, showed the highest starch damage and the soft red winter, for which the least power was required for grinding, the lowest. Hard red winter and spring wheats varied widely between these two extremes. Thus, on the 1960 crop, the southern Montana spring wheat showed one and a half times the damage of northern Montana spring wheat. The Oklahoma-Texas winter wheats thus far examined have given a low starch damage.

The wide ranges of tempering conditions commonly employed in milling seem to have little effect on the starch damage index.

The second factor influencing starch damage is the type and amount of grinding. Rolls, or any shearing action, apparently cause more starch damage than pin mill grinding to the same granulation. Other factors, such as wheat stock, being the same, starch damage is positively correlated with absorption but not always with granulation or specific surface.

It is interesting to speculate if, perhaps, many properties of wheat, including starch damage potential, might be due to amyloses of different chain lengths or different degrees of branching.

A fuller knowledge of the structure of the starch granule of the endosperm of wheat would provide the basis for many physical and chemical factors affecting flour performance.

Acknowledgment

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