Measuring Damaged Starch by Polarimetric Method[1]

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ABSTRACT

A method for estimating damaged starch quantitatively in flour has been developed. This method is based on polarimetric determination of starch in calcium chloride solution and on the fact that damaged starch is digested more easily by α-amylase than in undamaged starch. A sample of flour was subjected to α-amylase digestion under controlled conditions for at least 30 min. After the mixture was centrifuged, the residue was washed with alcohol and then dissolved in calcium chloride solution. The protein was precipitated by uranyl acetate and then filtered. The optical rotation of the clear filtrate was determined using a polarimeter. The total starch (including damaged starch granules) was determined by a similar procedure but without enzymatic digestion. The difference in the two readings represented starch damage. The polarimetric method gave higher values for damaged starch than the official AACC method. However, these values can be readily converted to the AACC values by use of the regression equation: % damaged starch, AACC methods =

$$\frac{\text{\% damaged starch, polarimeter method} + 2.69}{2.60}$$

The method appears equally applicable to soft or hard wheat flour.

Starch granules imbedded in the protein matrix of the endosperm (1) are easily damaged by pressure, shear, or strain such as that applied by grinding procedure (2,3). Starch damage generally affects the end-use performance of flour. Ponte et al. (4) showed that starch damage increased gassing power and water absorption, reduced tolerance to mixing, and generally was deleterious to bread quality. Schlesinger (5) reported that ball-milling of flour increased absorption, lowered loaf volume, and reduced baking scores. Lorenz and Johnson (6), working on the effect of the damaged starch on quality of continuous-mix bread, indicated that starch damage increased dough-mixing speed requirement but mixing tolerance decreased; also, bread grain became open and the texture harsh.

A number of methods have been used to measure starch damage in wheat flour. Medcalf and Gilles (7) and Williams and Fegol (8) used iodine absorption by soluble amylose. The susceptibility of damaged starch to enzymes has been used to determine damaged starch in flour (6,9-15).

We propose a new method, based on polarimetric estimation of starch in calcium chloride ($CaCl_2$) solution, for estimating quantitatively starch before and after its digestion with α-amylase. The difference represents the amount of damaged starch. In developing the method, we recognized that measuring total starch polarimetrically in high fiber fractions is not recommended (16) because of interference from non-starch polymeric carbohydrates in these materials.

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MATERIALS AND METHODS

A 100-g. sample of hard red winter (HRW) wheat flour was subjected to ball-milling at room temperature for 10 hr. using 50 g. of flour with 850 g. of ceramic balls (2.5 cm. diam.) in a 1,875-ml. porcelain jar. This ball-milled flour was added in varying percentage to sound HRW flour to provide samples of varying degrees of starch damage. A few samples of sound soft red winter and HRW were also included to establish a wide range of starch-damage samples. A fungal (*Aspergillus oryzae*, Calbiochem Co., catalogue no. 17155, grade B) α-amylase standardized at 5,000 SKB activity units per g. and a Kern full-circle polarimeter equipped with monochromatic sodium light were used.

The total starch of wheat flour was determined according to AACC method 76-20 (17,18). The damaged starch in flour as determined by the AACC (76-30A) method (17) was compared with that determined by polarimetric method.

Determining Damaged Starch by Polarimetric Method

The method is applicable to wheat flour or any component parts of the flours containing appreciable quantities of starch. A sensitive polarimeter capable of reproducing within ± 0.025° with 2-dm. tubes and a monochromatic sodium vapor lamp were used. Optical rotation was expressed in angular degrees.

Reagents

Acetic acid buffer. Dilute 4.1 g. anhydrous sodium acetate and 3.0 ml. acetic acid to 1 liter, pH 4.6 to 4.8.

Mercuric chloride solution. Dissolve 1.07 g. mercuric chloride in 950 ml. water, add 120 ml. ethanol, and mix.

Trichloracetic acid (TCA). Dissolve 50 g. TCA in 100 ml. water.

Starch dispersant. Dissolve 550 g. $CaCl_2$ dihydrate in 760 ml. of water and adjust to pH 2.2 to 2.5 with glacial acetic acid.

Uranyl acetate. Dissolve 10 g. uranyl acetate in 80 ml. water, add 20 ml. glacial acetic acid, and heat to 60°C.; then add 100 ml. of starch dispersant solution.

Enzyme solution. Disperse 2.463 g. fungal α-amylase (5,000 SKB units/g.) in 250 ml. acetate buffer, filter rapidly, and use within 2 hr.

Neutralizing solution. Dissolve 12.0 g. sodium carbonate in 100 ml. water.

Procedure. Flour samples (2.463 g.) were placed in 50-ml. centrifuge tubes. To the first tube, 25 ml. of α-amylase solution was added and starch digested for 30 min. at 30°C. After the enzyme was inhibited with 2 ml. of TCA, 2 ml. of sodium carbonate solution was added. The solid material was recovered by centrifugation and filtration. The flour in both tubes was treated with 20 ml. of mercuric chloride solution to inactivate traces of enzyme present and to remove soluble sugars, and then centrifuged. The mercuric chloride treatment was performed twice. The solids of each tube were transferred to 500-ml. beakers with 10 ml. of water and then 60 ml. of $CaCl_2$ solution added. The mixture was brought to full boil in 5 min., continued to boil for 15 min., with water level kept constant. The dispersed solids were cooled to room temperature, 10 ml. of uranyl acetate solution was added, and the contents were transferred with $CaCl_2$ solution to a 100-ml. Kohlrauch flask and made to volume. The contents were filtered and 20 to 50 ml. collected for reading of the optical rotation with a polarimeter.

Calculation. The total starch on a dry matter basis was calculated from the angular rotation (α) as follows:

$$\frac{\%\ \text{starch}}{(\text{dry basis})} = \frac{\alpha \times 100}{100 - \%\ \text{moisture}} \times 100.$$

% of damaged starch = % of total starch - % of undamaged starch

RESULTS AND DISCUSSION

Inactivating α-amylase

Several reagents (including mercuric chloride, cupric sulfate, uranyl acetate, sodium tungstate, and TCA) to inactivate α-amylase were investigated. In these experiments inactivating solutions were mixed with the flour, then 25 ml. of α-amylase of solution was added to digest the damaged starch. After standing for 30 min. at 30°C., starch was dispersed in $CaCl_2$ solution as described above and determined polarimetrically. The native flour starch had an optical rotation of 6.600° ± 0.0050°. If the α-amylase were not completely inactivated by the treatment, a lower optical rotation would be expected. All reagents partially inactivated the enzymes. At a concentration of 2.0 ml. of 50% TCA, the inactivation of the α-amylase was found to be complete.

Effect of α-Amylase Concentration

The effects of α-amylase concentration on rate of damaged and undamaged starch digestion, followed polarimetrically, are shown in Fig. 1. The degree of

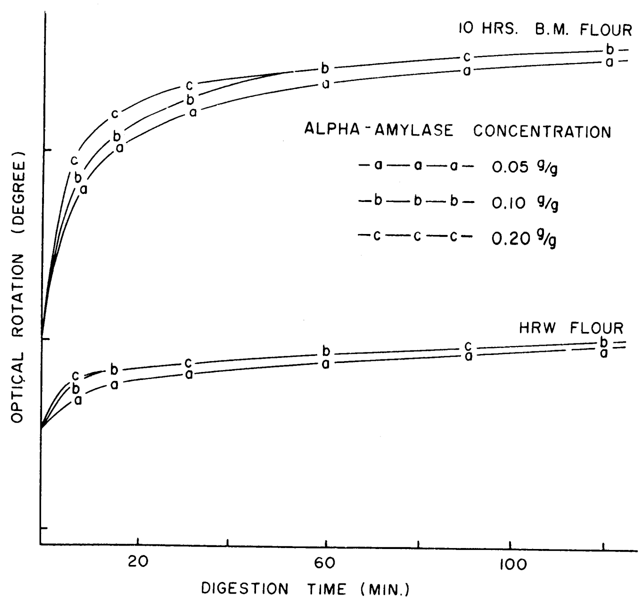

Fig. 1. Effect of α-amylase concentration on optical rotation of starch solution.

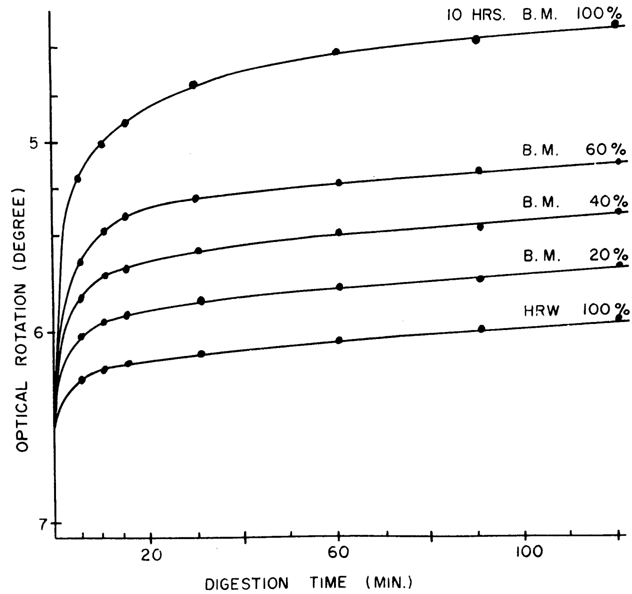

Fig. 2. Effect of amount of ball-milled flour on optical rotation of starch solution.

optical rotation was influenced drastically by the presence of damaged starch. These data showed that 0.1 g. of α-amylase per g. of flour provides adequate conversion of the damaged starch, if allowed to react for 30 min. It was difficult to filter the starch when 0.20 g. of α-amylase per g. of flour was used.

Effect of α-Amylase on Digestion Time

As shown in Fig. 2, optical rotation of starch solution decreased markedly after 15 min. of digesting of normal flour, but the rate of change was greatly reduced thereafter. It suggested that the damaged starch and part of the undamaged starch were digested during the first 15 min. and that the shape of the curve from 15 to 120 min. represented mainly digestion of the undamaged starch granules. Note that the optical rotation after 30 min. of digestion was proportional to the amount of damaged starch present.

The portion of the curve before 30 min. represents a combination of the rates of digestion of the damaged and the undamaged starch. The undamaged starch was assumed to be digested at the same rate after 30 min. as after 60 min. By subtracting the value of the undamaged starch after 30 min. digestion from the total value of the undamaged and damaged starch, the digestion of the damaged starch could be obtained and converted to the percentage of damaged starch in the flour. Because the digestibility of the undamaged starch of normal hard and soft wheat flour was almost equal, a single determination of optical rotation after 30 min. of digestion (with a correction factor of 0.05° for the digestion of the undamaged starch) could be a measure of the damaged starch in the flour.

$$\text{Optical rotation of damaged starch} = \text{optical rotation of total starch} - \left(\text{optical rotation of starch after 30 min. digestion}\right) + 0.05$$

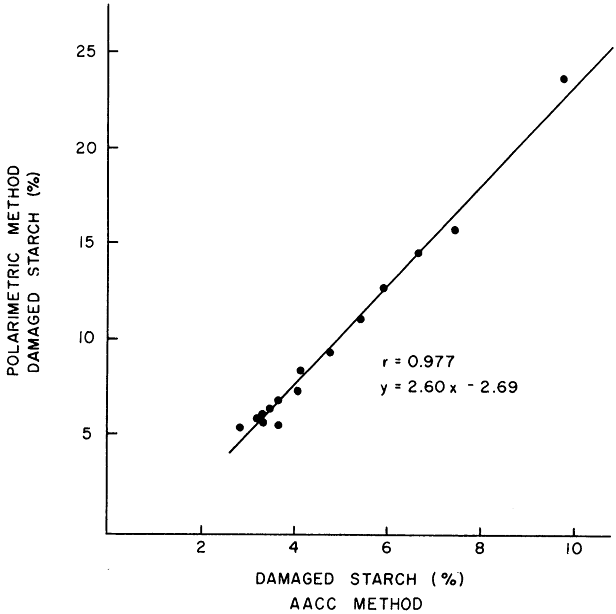

Fig. 3. Damaged starch measured by polarimetric and AACC methods.

Polarimetric Method Compared with AACC Method

Starch-damage values by the AACC method are compared with those by the polarimetric method in Fig. 3. These data represent HRW and soft red winter wheat flours having a wide range of starch damage. While the polarimetric method gave higher readings than did the AACC method, the correlation between the two methods was excellent.

SUMMARY

A method for quantitative estimation of damaged starch in flour was developed. The method is based on the polarimetric determination of starch in $CaCl_2$ solution and on the fact that damaged starch is digested enzymatically more readily than is undamaged starch. In the method starch is determined on a separate sample, with and without α-amylase digestion; the difference represents starch damage. Total starch and damaged starch of a flour can be estimated in a single test by the method. The damaged starch may be expressed as a percentage of the starch that is damaged or as a percentage of the flour that is damaged starch.

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