THE ESTIMATION OF DAMAGED STARCH USING POLARIMETER

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INTRODUCTION

The properties of damaged starch differ from intact starch in many respects such as viscosity, water absorption, solubility, gas production, susceptibility to staining with iodine and certain dyes and digestibility by amylases. It generally affects the end-use performance of flour. In bread-making, the damaged starch is, to a large extent, responsible for gas production and water absorption. Excessive levels of damaged starch may lead to a very dark color in the crust of bread, a lower loaf volume, and an undesirable, coarse-waxy texture in the final crumb.

A number of methods have been used to measure starch damage in wheat flour. The methods are based on rheological changes in the dough, the selective staining of damaged starch with certain dyes, or the preferential enzymic digestion of damaged starch. These methods are time-consuming or lack precision. The enzymic procedure is the most widely used at present.

REVIEW OF LITERATURE

Description of Intact Wheat Starch

Starch is the major carbohydrate in wheat flour. It is found in the endosperm and constitutes between 48 and 62% of weight of the kernel at 14% moisture content. The starch content of flour ranges from 65 to 71% at 14% moisture, depending on the type of wheat and flour grade (1). In general, the starch content of flour is inversely related to protein content. It is found that the starch is higher in flours from soft wheat than in those from hard wheat (2).

The size of the mature wheat starch granules are from 2μ to 35μ in diameter, with a maximum of about 50μ. The small granules, with a diameter up to 10μ, are spherical while the large granules are lenticular (3). In a study by Bice et al. (4), starch granules of three samples of mature wheat starches ranged in size as follow:

Less than 14μ	60 to 75%
11μ to 22μ	16 to 24%
Greater than 22μ	9 to 16%

As shown by the electron microscope, the surface of wheat starch granules is smooth, without extensive wrinkles, pits, or crevices (5). Sound starch granules show a characteristic birefringence pattern when viewed under the microscope in polarized light, which demonstrates the crystallinity of the starch granules (18). In wheat starch granules those shells of more intense crystallinity appear to start at the center of the granule. This is referred to as the hilum of the granule (10). The mechanism of the formation of the granule which leads to this characteristic is appositional growth; starting at the hilum, concentric shells of higher crystallinity alternate with shells

of relatively low crystallinity (11, 12). The areas of low crystallinity are considered generally amorphous in character with a preponderance of linear (amylose) molecules while the crystalline areas are considered to have a preponderance of branched (amylopectin) molecules interspersed with linear molecules (12). Natural wheat starch consists of two fractions; the straight chain type known as amylose and a branched chain type known as amylopectin. Both are polymers of D-glucose. Wheat starch is usually considered to contain about 20-25% amylose and about 75-80% amylopectin (8, 9). Amylose consists of unbranched chains of D-glycopyranose joined by alpha-D(1-4) linkage while amylopectin consists mainly of D-glucopyranose joined linearly but frequently joined by alpha-D(1-6) linkages to other D-glucopyranose units (6).

Woodrall and Weber (13) observed that the first changes on heating of wheat starch in water occurred at 55° C. The largest granules swelled slightly and lost their birefringence. In 5% suspension, birefringence completely disappeared at 60° C. from granules derived from hard wheat, and at 65° C. from granules from soft wheat. The temperature range at which these changes occur depends on the concentration of starch. With increasing concentration this loss of birefringence occurs at higher temperatures. At 50% concentration birefringence disappeared from the starch of hard wheat at 75° C., and from that of soft wheat at 85° C. (14).

Retrogradation is a partial crystallization of starch molecules which have been freed from one another during gelatinization (15). Retrograted starch becomes less available to the action of amylases; pastes become increasingly opaque; the ability to form a colored complex with iodine diminishes and part of the starch may precipitate (14). Linear starch molecules retrograde much more rapidly than branched molecules, since they can more readily

form crystalline regions. The retrogradation of linear fration of wheat starch is approximately 87% complete in 24 hours at 25° C. (16). The retrogradation of starch can be reversed by heat, but complete reversal requires heating in an autoclave at 125° C. (14).

Description of Damaged Wheat Starch

Wheat starch granules in nature are embedded in the protein matrix of the endosperm (19). During the milling operation, the starch granules are subjected to many passes through rolls, both smooth and corrugated. It is obvious that the starch granules have many opportunities to be mechanically damaged.

Williams (18), in a study of the nature of mechanically damaged starch, indicated that there were four types of mechanically damaged starch in wheat flour. He termed the radial cracked starch as type A, the chipped or split starch as type B, the abrased starch the type C and the squashed or flattened starch as type D. Jones (25) stated that two factors were at work creating mechanical damage: the surface factor involving shearing and scraping of the granule surface and internal factor involving crushing or partial flattening of larger fragments as the endosperm passed through the reducing rolls. Miller (9) indicated that increased grinding pressures caused various types of physical damage to starch cells, which increased the susceptibility to amylase action. It was found that too little conditioning moisture resulted in higher starch damage, while too much moisture tended to cause excessive flaking which in turn resulted in lower flour yield (18). Pelshenke and Hampel (29) showed that soft wheat starch was less affected by milling than hard wheat starch. Hardness of wheat is very closely related to starch damage. Williams (18),

after the study of a series of 12 wheat varieties grown over a wide range of environmental conditions in Australia, stated that the damaged starch increased with the increase of the kernel hardness of wheat. Wilson et al. (28) reported that a small increase in starch damage resulted from the air classification of flour.

The damaged starch granules stain pink in aqueous congo red, whereas the undamaged granules are unstained; and the use of polarized light shows that injured starch has lost birefringence (20). Osman (21) stated that fully damaged as well as partially damaged wheat starch granules swelled considerably in cold water and the swollen areas were readily attacked by amylases. Sedimentation valves are increased with high starch damage (10). Maltose value test shows that high levels of damaged starch flour gave high maltose values.

Lorenz (24) by use of the farinograph demonstrated that water absorption of a dough increased in proportion to the increase of damaged starch. Farrand (23) showed a close agreement with estimates based on the assumption that, per unit weight, damaged starch absorbed 1 unit of water, undamaged starch absorbed 1/3 unit, and protein absorbed 2 units of water. Ponte et al. (22) showed that starch damage increased gassing power, water absorption, reduced farinograph tolerance to mixing and was generally deleterious to loaf volume and bread quality. Schlesinger (26), after working with ball-milled hard winter wheat flours, reported that ball-milling increased farinograph absorption, lowered loaf volume and reduced baking scores.

Enzymatic Degradation of Wheat Starch by Amylases

Amylases, the enzymes that catalyze the hydrolysis of starch, have been divided into two classes known as alpha- and beta-amylases. The existence of

alpha- and beta-amylases was reported by Kuhn in 1924 and further substantiated by Ohlesson, who fractionated malt diastase into two components, alpha- and beta-amylases in 1930 (30). In 1949 Schwimmer and Balls (31) described the isolation and crystallization of alpha-amylase from germinated barley. The beta-amylase of barley malt was crystallized by Fischer and his co-workers in 1950 and that of wheat malt in 1953 (30, 32).

Alpha-amylase randomly attacks alpha-1-4 glucosidic bonds of amylose and gives a mixture of glucose and maltose, whereas beta-amylase attacks the alpha-1-4 glucosidic bonds of amylose, commencing at the nonreducing end, and gives pure maltose. Amylopectin, when treated with alpha-amylase, undergoes random rupture of alpha-1-4 glucosidic bonds and yields a mixture of branched and unbranched oligosaccharides in which alpha-1-6 glucosidic bonds are abundantly present. When amylopectin is hydrolyzed by the action of beta-amylase, successive maltose units are liberated. This process continues until a branched point is approached (6). Both alpha- and beta-amylases have no capacity to hydrolyze the alpha-1-6 glucosidic bonds of amylopectin that they here encounter, and all reaction stops (6, 34).

Alpha-amylase from various source shows different action patterns. The alpha-amylase of fungal origin hydrolyzes amylose mainly to glucose, maltotriose, maltotetraose and maltopentose. That of salivary origin produces maltose, maltotriose and maltotetraose. Bacterial alpha-amylase yields mainly maltose, maltotriose and maltohexose (35). Sorghum and barley alpha-amylases produce maltohexaose, maltoheptaose and maltooctaose (35, 36).

Many investigations have been made to find the relationship between enzymatic attack and starch damage. Brown and Heron (43), as early as 1879, indicated that the starch granules with mechanical injury were found to lower

the resistance to diastatic attack. Sandstedt et al. (37) showed that the undamaged starch granules of wheat flour were not attacked by beta-amylase, whereas the damaged fraction was readily available to this enzyme. Sandstedt and Mattern (38) also stated damaged starch was readily and rapidly digested by the amylases, whereas the native undamaged starch was markedly more resistant to digestion.

Methods of Determination of Damaged Wheat Starch

Water suspensions of damaged wheat starch differ from intact starch in many respects such as viscosity, water absorption, solubility, susceptibility to staining with iodine and digestibility by amylases etc. Some of these properties are used as a basis for determination of damaged starch in wheat flour.

Metcalf and Gilles (39) used the phenomenon of iodine absorption to measure the damaged starch. Their method is based on the principle that the damaged starch granules absorb iodine at a faster rate than do the undamaged ones. Williams and Fegol (41) developed a rapid colorimetric test for measuring damaged starch in flour. It is based on the observation that the amylose present in the mechanically damaged starch granules is extracted more rapidly by a strong solution of sodium sulfate containing 15% formamide and 0.2% sulfosalicylic acid than undamaged starch.

The susceptibility of damaged starch to enzymatic attack is one of its most characteristic properties. Many enzymatic methods have been developed for determining damaged starch in flour. It is postulated that the initial rapid rate of enzymatic digestion is due to damaged starch and then is followed by a slow rate of enzymatic digestion on the undamaged starch. The conventional

enzymatic methods fall into two groups, according to whether mainly alpha- or beta-amylase is used.

The Sandstedt and Mattern method (7) is based on the property that damaged starch is easier digested than undamaged starch by the malt amylase. Duplicate one gram samples of flour, to which have been added a buffer and 160 SKB units of malted wheat α-amylase are incubated at 30° C. The percent of maltose based on the flour is determined after one hour and two hours respectively. The percent of maltose produced the second hour is used to correct for the digestion of the undamaged starch. The percentage of damaged starch is obtained by extrapolation back to zero time. The slope of the curve is an indication of the susceptibility of the undamaged starch to digestion.

Sullivan et al. (40) developed a method for determination of damaged starch by starch damage index. The endogenous enzyme of flour, which that they thought might affect the maltose value, was inactivated by treating the flour with a solution of trichloroacetic acid in butanol. To a five gram the sample of flour 80 mg. of beta-amylase was added and digested for one hour at 30° C. in a water bath. The maltose value was measured by the alkaline ferricyanide reduction method. The starch damage index was the difference in maltose value between the flour inactivated by trichloroacetic acid in butanol and the inactivated flour plus and excess of beta-amylase.

The method of Greer and Stewart (17, 42), takes into account the effect of the endogenous enzymes in flour. It is similar in the principle to that of Sullivan et al. (40). The enzymic activity of the flour is destroyed by refluxing it with 82.5% (v/v) alcohol for 10 minutes. The air-dried inactivated flour and an excess of beta-amylase are then incubated at 30° C. for four hours. Reducing sugars are determined by the ferricyanide reduction method.

The damaged starch is calculated by multiplying the milligrams of maltose produced from one gram of flour with 100/371. The factor of 371 is based on the finding that 1 gram of wheat starch with more than 90% damaged starch produces 371 mg of maltose.

The Farrand method (23) does not consider the effect of endogenous enzymes in flour. A five gram of flour sample is subjected to enzymatic hydrolysis by excess of alpha-amylase from a fresh extract of malt flour. Digestion at 30° C. in a buffer solution of pH 4.6 is carried out with shaking every 15 minutes for one hour. Reducing sugar is measured by ferricyanide method and multiplied by 5 to obtain maltose figure. The damaged starch is calculated by the formula below:

$$\text{Damaged starch} = (\text{Maltose figure} - 3.5) \times 6$$

A rapid method for the determination of damaged starch in soft wheat flour was developed by Donelson and Yamazaki (33). The method is similar to that developed by Sandstedt and Mattern (7). Both use alpha-amylase and do not have endogenous enzyme inactivation treatment. The digestion time of this method is reduced to 15 minutes at 30° C. One gram of flour sample at 14% M.B. is used and 0.10 gram of Rhozyme 33 (Fungal amylase with 5,000 SKB units per gram) is added. The digestion is carried out without shaking. Maltose value is determined by ferricyanide reduction method and can be converted to damaged starch by multiplying the empirical factor 1.64 after correction for the blank.

A new method based on polarimetric estimation of starch in calcium chloride solution is proposed for the quantitative estimation of starch damage in flour. This method involves the estimation of starch before and after its digestion with alpha-amylase; the difference represents the amount of damaged starch.

MATERIALS AND METHODS

The flour used for this investigation was hard red winter (HRW) class of the 1970 crop. It was identified and analyzed as follows:

Class	HRW
Code	70-338
Variety	65A1682
Protein	13.3%
Ash	0.43%
Starch	75.4%
Sample size	10 lb

Creation of the starch damage by ball milling ---- 100 gram of this flour sample was subjected to ball-milling at room temperature for exactly 10 hours. The milling was performed on 50 gram of the flour and 850 grams of ceramic balls with diameter 2.5 cm. in a 1875 ml. porcelain jar.

The alpha-amylase used in the experiments was of fungal origin from Aspergillus oryzae standardized at 5,000 SKB activity units per gram. This was purchased from Cabiochem Co., Los Angeles, Calif. 90054, catalogue number 17155 grade B. The alpha-amylase was stored in the refrigerator and brought to room temperature before being used.

Polarimeter

The polarimeter used in this work is a Kern full-circle polarimeter (Plate 1), which employs the sodium light to measure the optical rotation. It is a polarimeter with tripartite field of vision, full-circle scale, micrometric adjustment and variable penumbral angle.

The polarimeter can be employed for measuring rotations up to $+180^{\circ}$.

Plate 1

This requires a full-circle scale of rather large diameter, which has the advantage over the semi-circles, or even smaller segments of other instruments, of offering greater protection against dazzling. The graduate circle is divided into half-degrees and, in combination with the vernier, enables readings to be made to 0.05° and estimation to 0.01°.

Determination of Total Starch by Polarimetric Method (25, 27)

A 2.463 gram of wheat flour sample was weighed into a 50 ml. round-bottomed centrifuge tube and washed twice with 40 ml. of 15% ethanol. The ethanol was then removed from sample by the combination of centrifugation and filtration. The filter paper and residue were transferred to a 400 ml. beaker and 10 ml. of water was added. The residue was macerated with a stirring rod. Sixty ml. of calcium chloride solution was added to the beaker and the liquid level on the beaker was marked. The beaker was placed on a hot plate and brought to boil in about 5 minutes. The solution was stirred occasionally, water added as needed to maintain liquid level substantially constant and kept boiling vigorously for 15 minutes. The beaker and contents were cooled to room temperature in a water bath. Ten ml. of uranyl acetate solution was added to the beaker and mixed thoroughly. The solution was allowed to stand for 2 minutes, then transferred to a 100-ml. Kohlrausch flask and diluted to volume with calcium chloride solution. The contents were filtered. The optical rotation of the clear filtrate was determined using a polarimeter. The percentage of starch in flour can be calculated by the formula below.

$$\% \text{ of starch (dry basis)} = \frac{\alpha \times 1000}{100 - \% \text{ of sample moisture}}$$

Where α was observed optical rotation

Determination of Damaged Starch by AACC Method (27, AACC 76-30A)

One gram of flour sample was weighed into 125 ml. Erlenmeyer-flask. Forty-five ml. of enzyme-buffer solution containing 100 mg of alpha-amylase was added and mixed thoroughly. The mixture was incubated in a thermostatically controlled water bath at 30° C. for exactly 15 minutes. At the end of the 15 minutes 3 ml. of 3.68 N sulfuric acid and 2.0 ml. of sodium tungstate solution were added and mixed well. The solution was allowed to stand for 2 minutes and then filtered. A 5-ml. aliquot of filtrate was transferred to a 25 x 200 mm pyrex test tube and 10 ml. of 0.1 N alkaline ferricyanide reagent added, and the test tube was immersed in vigorously boiling water bath for exactly 20 minutes. The test tube and contents were cooled rapidly. Twenty-five ml. of acetic acid-salt solution and 1 ml. of soluble starch potassium iodine indicator were added. The contents were mixed thoroughly and titrated with 0.1 N sodium thiosulfate solution. The ml. of 0.1 N alkaline ferricyanide reduced by the liberated reducing sugar was calculated to mg maltose equivalent. The amount of damaged starch could be calculated by multiplying the mg maltose equivalent by a factor 1.65 (27).

Determination of Damaged Starch by Polarimetric Method
(specification for the proposed method)

Definition -------This method determines the percentage of starch granules in flour or starch preparations, which is susceptible to hydrolysis by alpha-amylase.

Scope ----------The method is applicable to wheat flour, corn flour or any of its component parts consisting appreciable quantities of starch.

Special apparatus-Use a sensitive polarimeter or saccharimeter capable of

reproducing to within ± 0.01 circular degree, together with 1 and 2 dm observation tubes and a source of monochromatic light such as a sodium vapor lamp or equivalent.

Reagents ---------

(1) Acetate buffer: Dilute 4.1 gram of anhydrous sodium acetate and 3.0 ml of glacial acetic acid to one liter of distilled water. pH 4.6 - 4.8.

(2) Alcohol solvent: Dissolve 1.07 gram of mercuric chloride (HgCl) in 950 ml of water. Then add 120 ml. of 95% ethyl alcohol and mix thoroughly.

(3) Trichloroacetic acid solution: Dilute 50 gram of trichloroacetic acid to 100 ml with distilled water.

(4) Calcium chloride solution: Dissolve 550 gram of reagent grade calcium chloride dihydrate ($Cacl_2 \cdot 2HO$) in 760 ml. of water and adjust gravity to 33 Baume at 60° F. (specific gravity = 1.30). Adjust the pH to 2.2 to 2.5 by the addition of glacial acetic acid.

(5) Uranyl acetate solution: Dissolve 10 gram uranyl acetate dihydrate ($UO_2(C_2H_3O_2)_2 \cdot 2H_2O$) in 80 ml. of distilled water and 20 ml. of glacial acetic acid heated to not over 60° C. and add 100 ml. of 33° Baume calcium chloride solution.

(6) Alpha-amylase solution: Dissolve 2.463 gram of fungal alpha-amylase, which contains 5,000 SKB units per gram, in 250 ml. of acetate buffer. Filter rapidly through a coarse filter paper (Whatman No. 4). The solution should be used within two hours.

(7) Sodium carbonate solution: Dilute 12.40 gram of Sodium Carbonate (Na_2Co_3) to 100 ml. with distilled water.

Procedure --------

(1) Weigh accurately a 2.463 gram of flour sample into a 50-ml. round-bottomed centrifuge tube. Set aside for assaying the total starch. Weigh another sample as before for hydrolysis with alpha-amylase to assay the undamaged starch.

(2) Bring alpha-amylase solution (reagent 6) and the second sample to 30° C in a water bath. Add 25 ml. of the alpha-amylase solution to the sample. Obtain a uniform suspension by use of a stirring rod. Incubate for 30 minutes from the time of adding reagent. At the end of 30 minutes add 2 ml. of TCA (reagent 3) and mix the solution thoroughly. Add immediately 2 ml. of sodium carbonate solution (reagent 7).

(3) Remove the liquid from sample by a combination of centrifugation and filtration (gooch crucible no. 4 and whatman no. 542). Then treat the first sample weighed and the second sample (alpha-amylase hydrolyzed) as follows:

(4) Add 20 ml. of alcohol solvent (reagent 2). Mascerate the sample with a stirring rod until dispersed thoroughly. Remove the alcohol solvent from sample with a combination of centrifugation and filtration as procedure 3. Add another 20 ml. of alcohol solvent and wash residue again.

(5) Add 10 ml. of water. Mascerate and disperse the residue with a stirring rod. Transfer filter paper from gooch crucible and residue from centrifuge tube to a 500-ml. beaker. Add

60 ml. of calcium chloride solution (reagent 4) and mark liquid level on beaker.

(6) Place the beaker on a hot plate and bring to boil in about 5 minutes while stirring. Continue boiling for 15 minutes, adding water as needed to maintain liquid level substantially constant. Cool to room temperature in a water bath.

(7) Add 10 ml. of uranyl acetate solution (reagent 5), mix thoroughly and let stand for 2 minutes. Transfer the contents quantitatively to a 100-ml. Kohlrausch flask. Rinse beaker and dilute to the volume with calcium chloride solution (reagent 4).

(8) Filter through folded filter paper (Whatman No. 12). Discard first 20 ml. of filtrate and collect 30 - 50 ml. for polarization. Polarize in a 2-dm tube, using sodium light.

Calculation ------The damaged starch in starch:

$$\% \text{ of damaged starch} = \frac{\% \text{ of Total starch} - \% \text{ of undamaged starch}}{\% \text{ of Total starch}}$$

The damaged starch in flour:

% of damaged starch = % of Total starch - % of undamaged starch

RESULTS AND DISCUSSION

The purpose of the present work is to develop a new method for the determination of damaged starch in flour. It is based on the property of optical activity of starch solutions is proportional to the starch content, and the damaged starch is much easier digested by amylases than undamaged starch. This method involves the determination of starch before and after its digestion with alpha-amylase. The difference represents the amount of damaged starch.

Optical rotation and starch concentration.

Theoretical consideration: By definition, the observed optical rotation of starch in solution is related to its concentration in the following manner:

$$[\alpha]_D^{20} = \frac{\alpha}{L \times C} \qquad (A)$$

Where $[\alpha]_D^{20}$ is the specific optical rotation of starch solution at 20° C. when D line of the sodium spectrum is used.

α is the observed optical rotation of starch solution.

L is the length of the polarimeter cell in dm.

C is the concentration of starch solution in gram per ml.

From the equation (A) it is evident that the observed optical rotation of a starch solution is directly proportional to its concentration of the solution. Equation (A) could be rewritten as follows:

$$C\ (g/ml) = \frac{\alpha}{L \times [\alpha]_D^{20}}$$

Hence the percentage of starch could be calculated by the following equation:

$$\% \text{ of starch} = \frac{\alpha \times \text{dilution factor}}{L \times [\alpha]_D^{20}} \Big/ \text{sample weight} \times 100$$

Normally, the flour sample contains a considerable amount of moisture. The equation should be corrected as follows:

$$\text{\% of starch (dry basis)} = \frac{a \times \text{dilution factor}}{L \times [a]_D^{20}} \Big/ \frac{\text{sample weight}}{\frac{100}{100 - \text{\% sample moisture}}} \times 100$$

In a preparation of dilution factor equal to 100 and a sample cell length of 2 dm., the equation would be expressed as follows:

$$\text{\% of starch (dry basis)} = \frac{a \times 100}{2 \text{ dm} \times [a]_D^{20}} \Big/ \frac{\text{sample weight}}{\frac{100}{100 - \text{\% sample moisture}}} \times 100$$

The specific optical rotation of pure wheat starch is 203°.

$$\text{\% of starch (dry basis)} = \frac{a \times 100}{2 \times 203} \Big/ \frac{\text{sample weight}}{\frac{100}{100 - \text{\% of sample moisture}}} \times 100$$

If a sample weight of 2.46 gram (including moisture) is used the equation should be simplified as follows:

$$\text{\% of starch (dry basis)} = \frac{a \times 100}{2 \times 203} \Big/ \frac{2.46}{\frac{100}{100 - \text{\% of sample moisture}}} \times 100$$

or

$$\text{\% of starch (dry basis)} = \frac{a \times 1000}{100 - \text{\% of sample moisture}} \times 100$$

Inactivation of alpha-amylase.

Several reagents to inactivate the alpha-amylase were used. Inactivating reagent was added to a 2.463 gram of flour followed by the addition of 25 ml. of alpha-amylase (0.2463 gram). The mixture was incubated at 30° C. in the water bath for 30 minutes. The starch content of the mixture was determined

by the polarimetric method. A optical rotation of 6.60°was measured with a 2.463 gram of flour without alpha-amylase treatment. A decrease in optical rotation with the alpha-amylase treatment indicated the incompleted inactivation.

Inactivation by various amounts of mercuric chloride, cupric sulfate and uranyl acetate were examined. The trival experiment shown the incomplete inactivation even with a relative high amount of these reagents. Sodium tungstate also proved inadequate, and a milky solution resulted which interfered with polarimetric measurement.

The effect of trichloroacetic acid on alpha-amylase was determined by measuring the optical rotation. The result is shown on Table 7 and plotted on Figure 6. Since the optical rotation of 2.463 gram of flour was 6.60° the addition of 2.0 ml. of 50% TCA to alpha-amylase treated flour showed complete inactivation.

With less TCA, a significant decrease of optical rotation showed incomplete inactivation. The decrease of optical rotation with a higher TCA was believed due to the hydrolysis of starch at low pH. A 2.0 ml. of 50% TCA was used in the experiment to inactivate alpha-amylase.

Alpha-amylase concentration.

As was to be anticipated, variations of enzyme concentration caused variation in rate of digestion of starches (both damaged and undamaged). It could easily be observed from the change of the slope of the curves. Normal HRW flour and the same flour after 10 hours ball-milling were used. Observed optical rotation of starch in flour after treatment of different amount of alpha-amylase was examined. Both normal HRW flour and ball-milled flour were treated with 0.20g/g (grams of alpha-amylase per gram of flour), 0.10g/g and 0.05g/g of alpha-amylase. The results are shown in Tables 1 and 2 and

plotted in Figure 1.

Hydrolysis with 0.20g/g and 0.10g/g of alpha-amylase showed no significant difference after most of damaged starch was digested, although the initial rate of hydrolysis of higher level was fast. The rate was limited at the 0.05g/g level and proceeded more slowly than the other two levels. The 0.20g/g alpha-amylase treatment was abandoned because of the difficult filtration of the hydrolyzed mixture. The 0.10g/g alpha-amylase was chosen and used throughout this experiment.

Alpha-amylase digestion time.

The effect of digestion time on starch was investigated using the normal HRW flour and the mixture of the normal HRW and the 10 hour ball-milled flour. The series of the mixtures contained 0, 20, 40, 60, 100% of the ball-milled flour. A constant amount of alpha-amylase (0.10 gram of alpha-amylase per gram of flour) was added to the flour sample. The comparison of the optical rotation at various digestion times is shown in Table 3 and plotted in Figure 2.

The rate of change, shown in degree of observed optical rotation, decreased significantly after 15 minutes digestion for the normal HRW flour. This indicated that the whole damaged starch and some undamaged starch were digested during the first 15 minutes and that the portion of the curves, from 15 to 120 minutes, represented the rate of digestion of the normal undamaged starch. In the mixture the curves were essentially parallel and approximately the same distance apart for each 20% increment of ball-milled starch after 30 minutes of alpha-amylase digestion.

For precision, a 30 minute digestion period was chosen for normal wheat flour in this procedure. Obviously the 30 minute digestion period was not enough for the 100% ball-milled flour, as the digestion of damaged starch was

not completed in 30 minutes. This could easily be solved by increasing the digestion time from 30 minutes to 60 minutes or more.

Calculation for the optical rotation of damaged starch.

The portion of the curve (Figure 7) before the 30 minutes point represented a combination of the rate of digestion of the damaged and the undamaged starch, which was shown in degree of observed optical rotation. The rate of digestion of the undamaged starch during the first 30 minutes was assumed to be the same as the rate from 30 minutes to 60 minutes. If the digestion rate of the undamaged starch during the first 30 minutes was subtracted from that of the undamaged and damaged starch, the digestion rate of the damaged could be obtained and converted to the percentage of damaged starch in the flour.

Since the digestibility of the undamaged starch of normal hard and soft wheat flour was almost equal, a single determination of optical rotation after 30 minutes digestion with a correction factor 0.05 for the digestion rate of the undamaged starch could be a measure of the damaged starch in the flour. Some special wheat flours, whose undamaged starch might have unusual digestibility to alpha-amylase, need two digestion periods, one for 30 minutes and the other for 60 minutes. This was also preferable for the precise determination of damaged starch. The detailed calculation formulas are shown below:

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

Or

$$\begin{pmatrix}\text{Optical rotation}\\ \text{of damaged starch}\end{pmatrix} = \begin{pmatrix}\text{Optical rotation}\\ \text{of total starch}\end{pmatrix} - \left[\begin{matrix}\text{Optical rotation}\\ \text{of starch after}\\ \text{30 min. digestion}\end{matrix} + \left(\begin{matrix}\text{Optical rotation}\\ \text{of starch after}\\ \text{30 min. digestion}\end{matrix} - \begin{matrix}\text{Optical rotation}\\ \text{of starch after}\\ \text{60 min. digestion}\end{matrix}\right)\right]$$

Or

$$\text{Optical rotation of damaged starch} = \text{Optical rotation of total starch} - 2 \times \left(\text{Optical rotation of starch after 30 min. digestion} \right) + \text{Optical rotation of starch after 60 min. digestion}$$

Comparison of polarimetric method with AACC method.

The damaged starch of soft wheat flour and hard wheat flour was determined by both AACC recommended method and polarimetric method. The result is shown in Table 5. The damaged starch content measured by polarimetric method was found higher than that measured by AACC method.

A plot of percentage of the damaged starch of 14 different wheat varieties measured by polarimetric method versus that measured by AACC method is shown in Figure 4. It was found that there was a linear relationship between the percentage of damaged starch measured by two methods. The correlation coefficient between those two methods was $r = +0.97$. The relation between two methods could be expressed by linear regression equation $y = 2.60x - 2.69$. Where x was the percentage of damaged starch measured by AACC method and y was the percentage of damaged starch measured by polarimetric method.

Some critical factors which might affect the accuracy of the results.

1) The pH value of the calcium chloride solution --- the pH value of calcium chloride should be controlled between 2.2 to 2.5. At pH value below 2.0 the optical rotation activity was depressed. At pH value higher than 4 the filtrate of the starch solution was hazy and cloudy (34).
2) Polarization temperature --- The starch filtrate is transferred to a 2 dm. polarimeter tube. The temperature of the tube and the contents

should be maintained within a range of 20° C. to 25° C. The higher polarization temperature would cause a decrease in optical rotation value, while the lower polarization temperature would cause an increase in optical rotation value. A comparison of different polarization temperatures was made using normal HRW flour. The data is summarized in Table 4 and plotted in Figure 3.

3) Starch filtrate storage --- The starch filtrate should be stored not below 18° C. The filtrate becomes hazy and cloudy at lower temperature.

TABLE 1

Hydrolysis of Various Amount of Alpha-Amylase

on Normal Hard Red Winter Flour

Sample Number	Observed optical rotation* (degree) at various alpha-amylase concentration			Alpha-amylase digestion time (min.)
	0.05g/g	0.10g/g	0.20g/g	
1	6.60	6.60	6.60	0
2	6.30	6.25	6.23	5
3	6.25	6.20	6.20	10
4	6.23	6.18	6.18	15
5	6.18	6.15	6.15	30
6	6.13	6.10	6.10	60
7	6.10	6.05	6.05	90
8	6.05	6.00	6.00	120

*Polarization temperature at 22° C.

TABLE 2

Hydrolysis By Various Amount of Alpha-amylase

on 10-hour Ball-milled HRW Flour

Sample Number	Observed optical rotation* (degree) at various alpha-amylase concentration			Alpha-amylase digestion time (min.)
	0.05g/g	0.10g/g	0.20g/g	
1	6.58	6.60	6.58	0
2	6.25	6.15	6.05	5
3	5.10	5.00	4.93	10
4	5.00	4.90	4.80	15
5	4.80	4.70	4.68	30
6	4.68	4.58	4.58	60
7	4.60	4.50	4.50	90
8	4.55	4.45	4.43	120

*Polarization temperature at 22° C.

TABLE 3

Hydrolysis of Alpha-amylase on HRW Flour Mixed with Different Percentage of 10-hr Ball-milled Flour

Sample Number	Observed optical rotation* at different percentage of 10 hours ball-milled flour					Alpha-amylase** digestion time (min.)
	0%	20%	40%	60%	100%	
1	6.60	6.60	6.58	6.60	6.60	0
2	6.25	6.00	5.80	5.60	6.15	5
3	6.20	5.95	5.70	5.50	5.00	10
4	6.18	5.93	5.65	5.40	4.90	15
5	6.15	5.85	5.58	5.30	4.70	30
6	6.10	5.80	5.53	5.25	4.58	60
7	6.05	5.75	5.50	5.20	4.50	90
8	6.00	5.70	5.43	5.13	4.45	120

* Polarization temperature at 22° C.

**Alpha-amylase concentration 0.10g/g.

TABLE 4

Effect of Polarization Temperature on Optical Rotation

Sample Number	Observed optical rotation (degree) at various polarization temperature			Alpha-amylase* digestion time (min.)
	10° C	22° C	50° C	
1	6.65	6.60	6.50	0
2	6.30	6.25	6.15	5
3	6.25	6.20	6.10	10
4	6.20	6.18	6.08	15
5	6.18	6.15	6.05	30
6	6.13	6.10	6.00	60
7	6.10	6.05	5.95	90
8	6.05	6.00	5.90	120

*Alpha-amylase concentration 0.10g/g

TABLE 5

Comparison of AACC Method and Polarimetric Method

Sample Number	Polarimetric Method		AACC Method
	Damaged starch in starch %	Damaged starch in flour %	Damaged starch in flour %
1	8.09	6.22	3.6
2	7.29	5.76	3.3
3	8.15	6.35	3.6
4	6.97	5.56	3.1
5	8.16	6.33	3.4
6	7.58	5.72	3.1
7	6.43	5.04	2.8
8	9.09	6.86	4.1
9	12.12	9.14	4.7
10	14.39	10.86	5.4
11	16.29	12.29	5.9
12	18.94	14.29	6.6
13	20.46	15.43	7.4
14	31.06	23.43	9.7

TABLE 6

Observed Optical Rotation of the Mixture of Various Percentage of 10-hr Ball-milled Flour

Sample Number	Percentage of Ball-milled flour	Observed optical rotation
1	0%	6.15
2	10%	6.00
3	20%	5.85
4	30%	5.70
5	40%	5.58
6	50%	5.40
7	60%	5.30
8	100%	4.70

*Polarization temperature at 22° C. Alpha-amylase concentration 0.10g/g. Digestion time 30 minutes.

TABLE 7

Effect of TCA Concentration on Observed Optical Rotation

	Amount of 50% TCA Added (per ml.)				
	0.5	1.0	1.5	2.0	4.0
Observed Optical Rotation	6.18	6.55	6.60	6.60	6.57
pH of the Solution	4.3	3.5	2.1	1.5	1.0

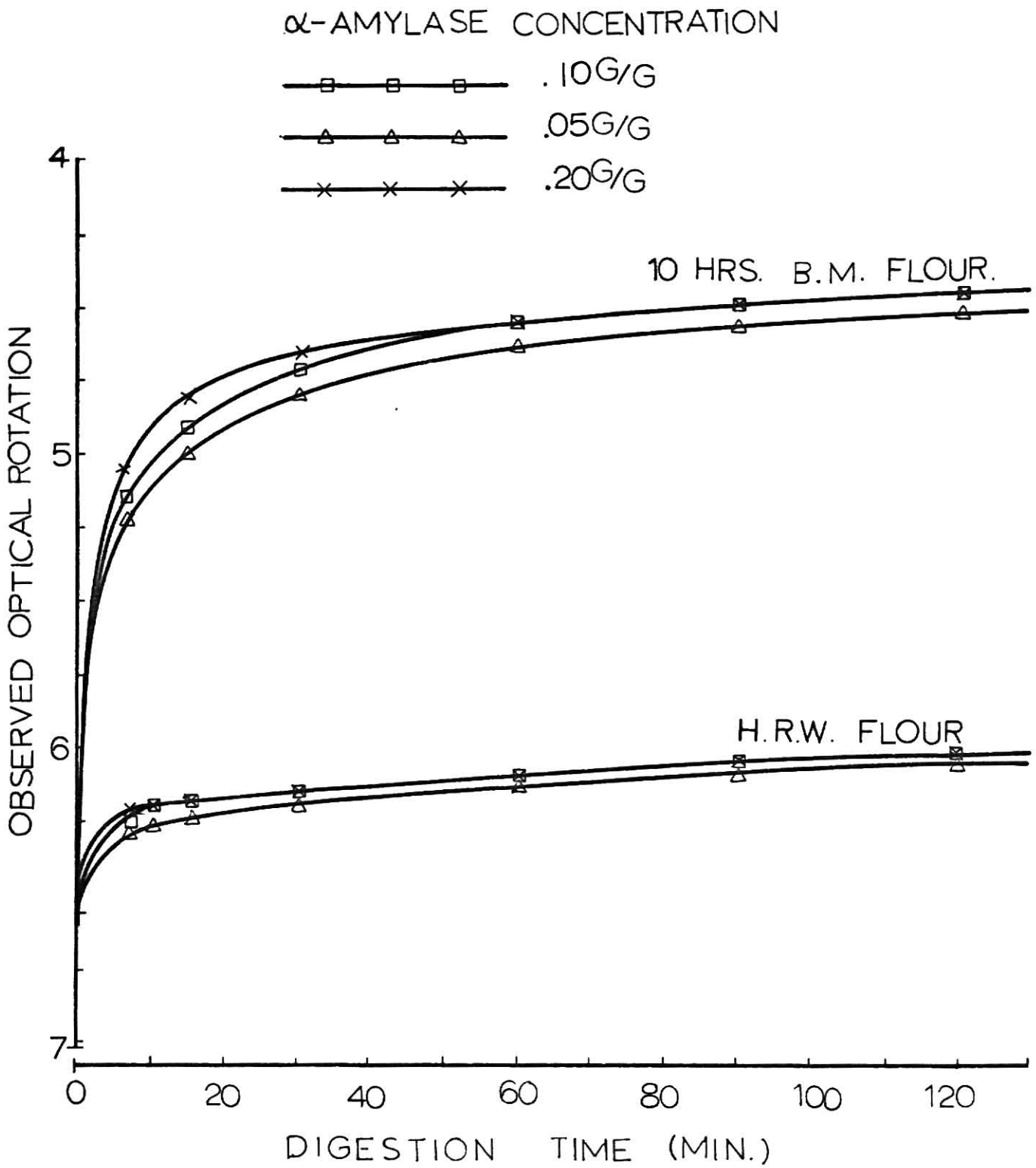
Explanation of Figure 1

Hydrolysis of various amount of alpha-amylase on normal Hard Red Winter flour and 10-hour ball-milled HRW flour:

Data were expressed in grams of alpha-amylase per gram of flour.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

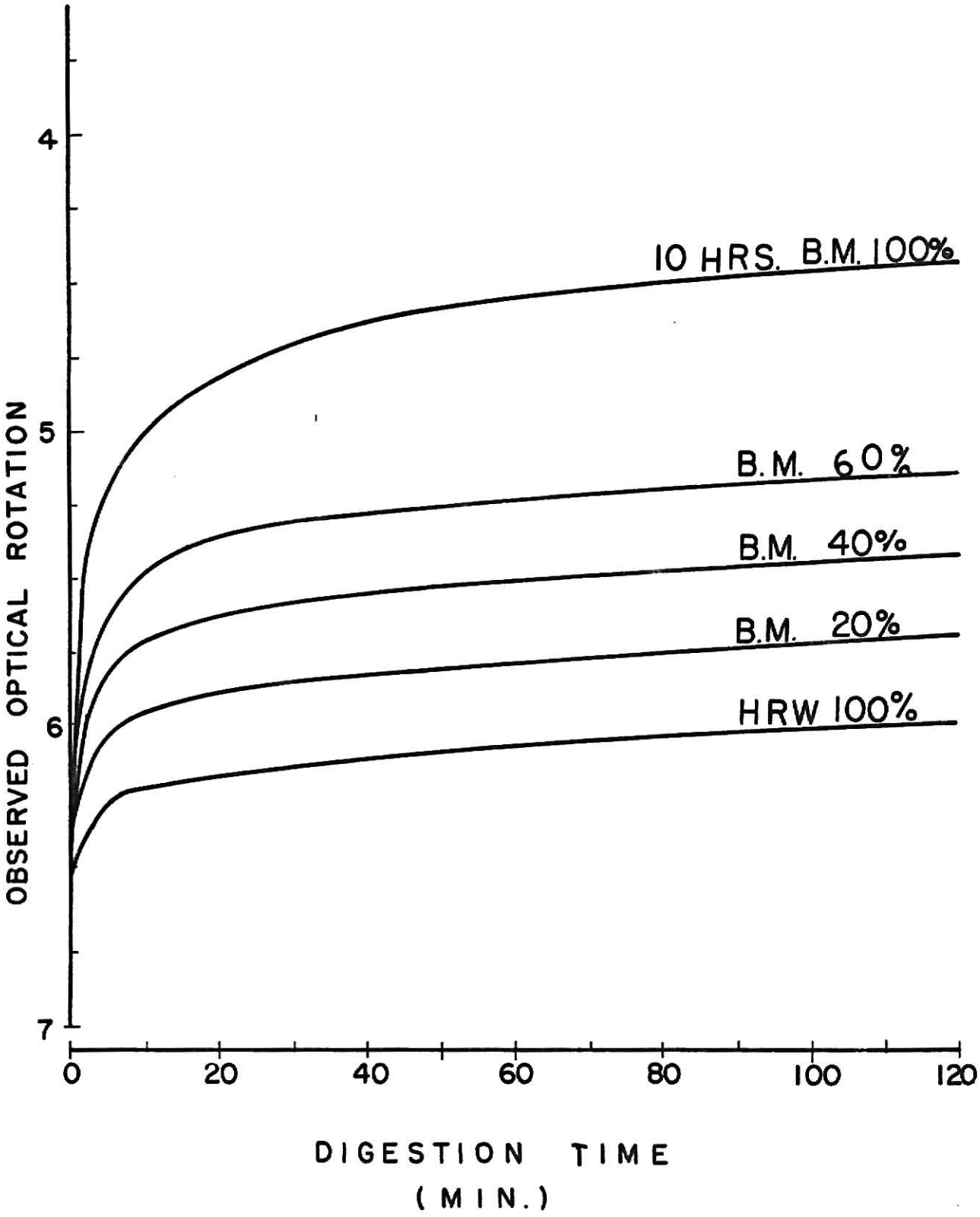
THIS IS AS RECEIVED FROM CUSTOMER.


α-AMYLASE CONCENTRATION
.10G/G
.05G/G
.20G/G
10 HRS. B.M. FLOUR.
H.R.W. FLOUR
OBSERVED OPTICAL ROTATION
4
5
6
7
0
20
40
60
80
100
120
DIGESTION TIME (MIN.)

Explanation of Figure 2

Hydrolysis of alpha-amylase on HRW flour mixed with different percentages of 10-hour ball-milled flour:

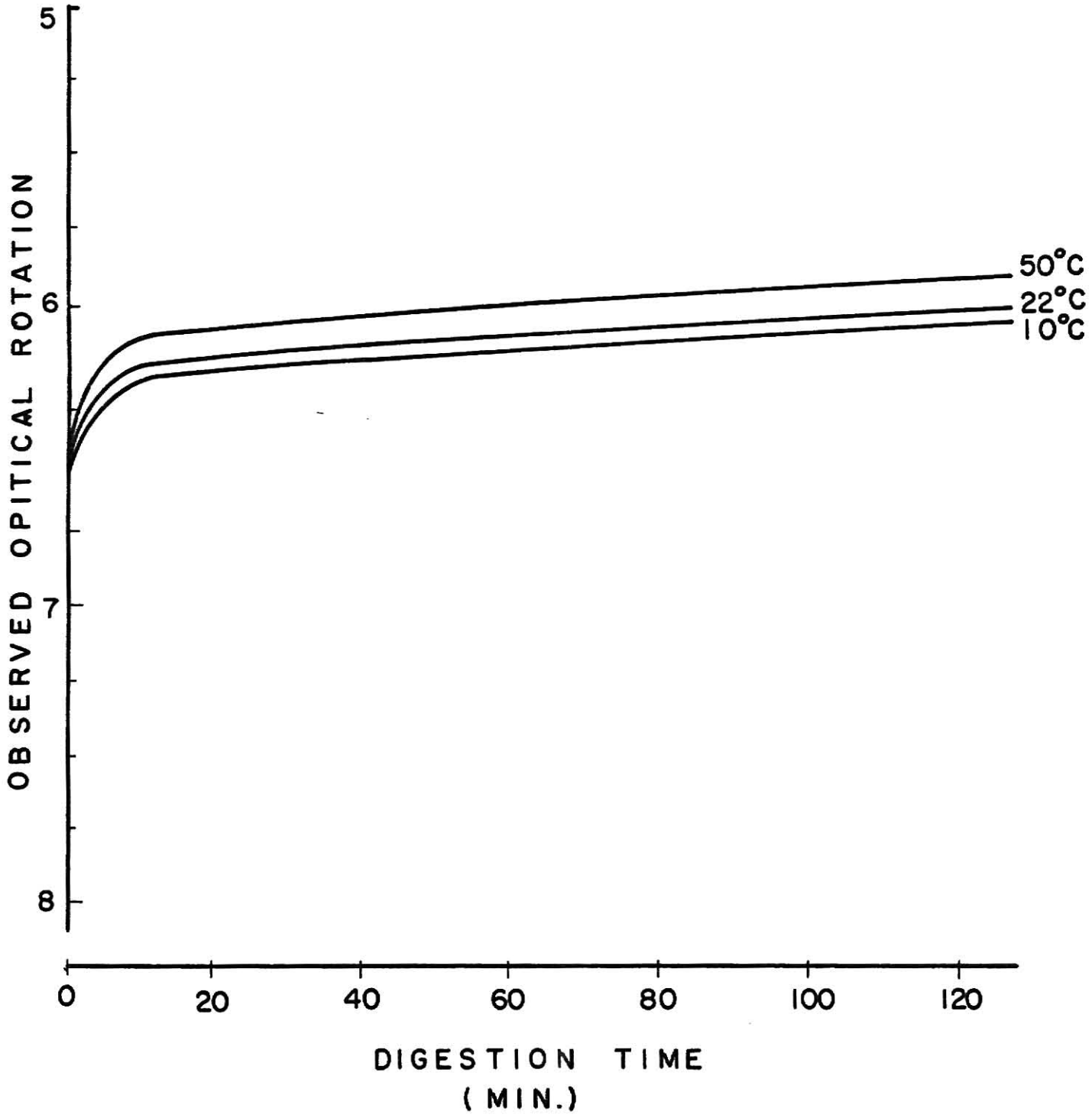
The samples were treated with alpha-amylase concentration 0.10g/g for 30 minutes digestion.


4
5
6
7
OBSERVED OPTICAL ROTATION
10 HRS. B.M. 100%
B.M. 60%
B.M. 40%
B.M. 20%
HRW 100%
0
20
40
60
80
100
120
DIGESTION TIME
(MIN.)

Explanation of Figure 3

Effect of polarization temperature on optical rotation:

The samples were treated with alpha-amylase concentreation 0.10g/g for 30 minutes digestion.


5
6
7
8
OBSERVED OPITICAL ROTATION
50°C
22°C
10°C
0
20
40
60
80
100
120
DIGESTION TIME
(MIN.)

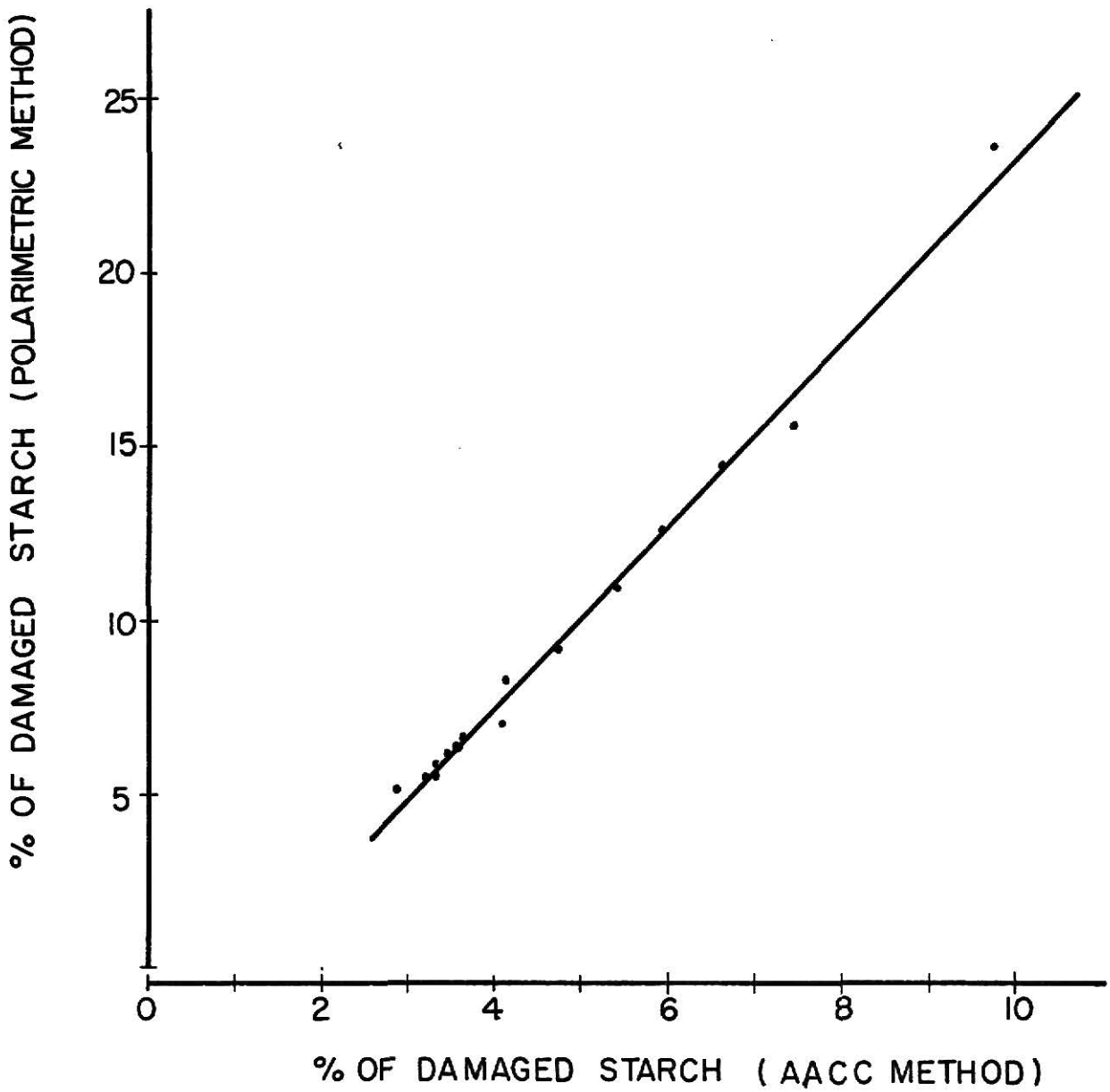
Explanation of Figure 4

Comparison of AACC method and polarimetric method:

Results by the AACC method are plotted on the X-axis and results of the polarimetric method are plotted on the Y-axis.

Correlation coefficient $r = +0.97$

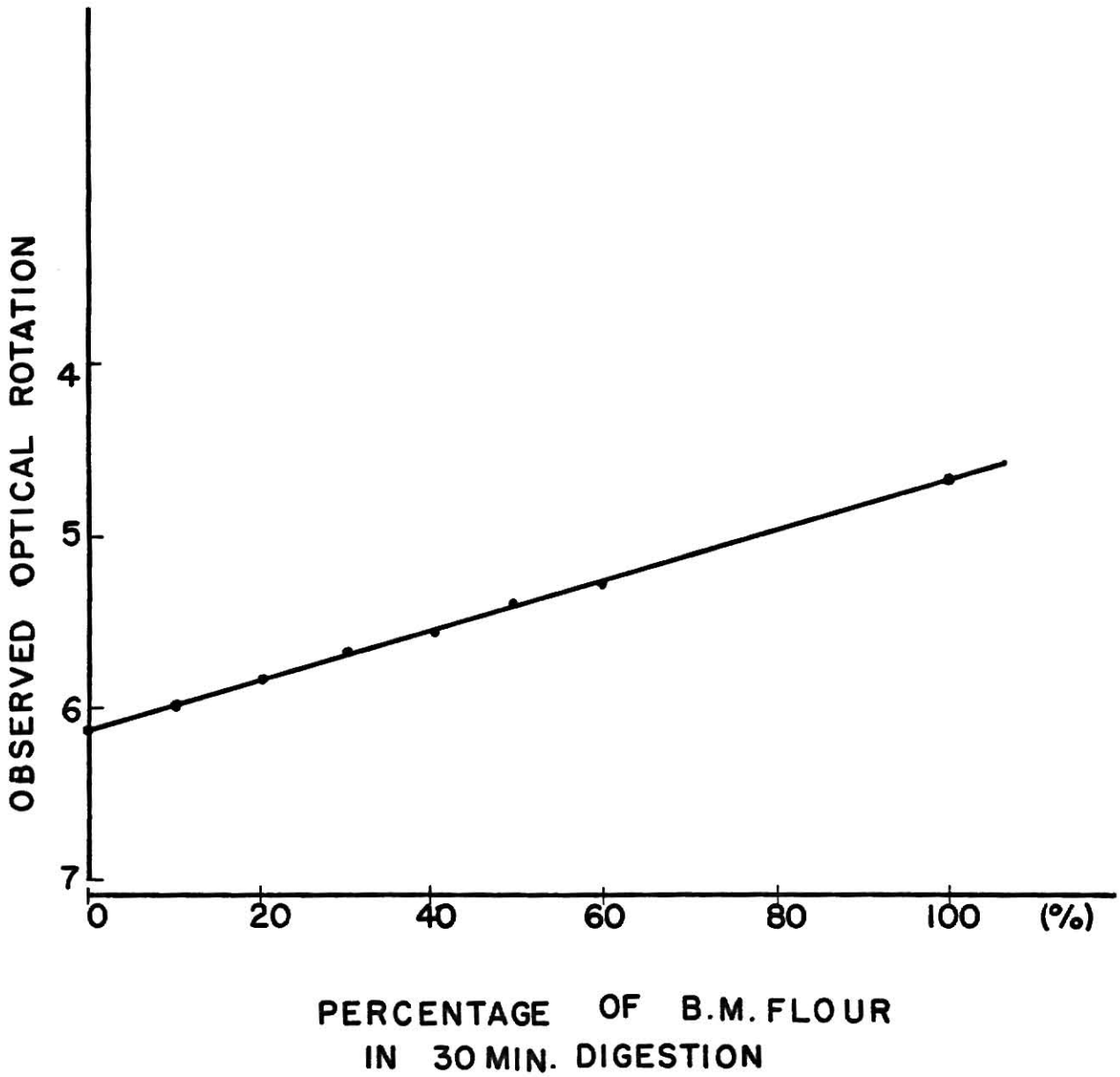
Linear regression equation $y = 2.60x - 2.69$


% OF DAMAGED STARCH (POLARIMETRIC METHOD)
25
20
15
10
5
0
2
4
6
8
10
% OF DAMAGED STARCH (AACC METHOD)

Explanation of Figure 5

Hydrolysis of different percentages of ball-milled flour by alpha-amylase:

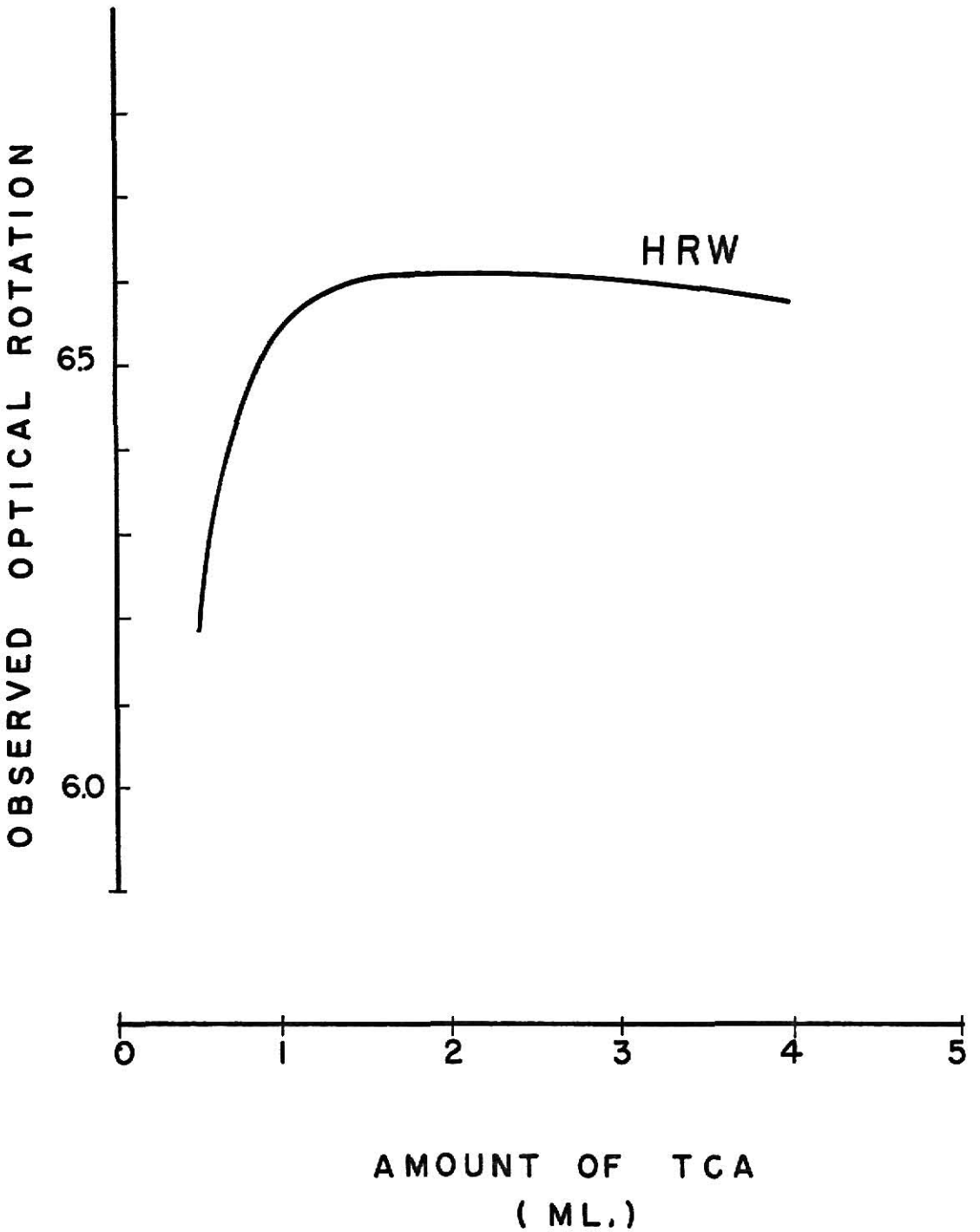
The samples were treated with alpha-amylase concentration of 0.10g/g for 30 minute digestion.


OBSERVED OPTICAL ROTATION
4
5
6
7
0
20
40
60
80
100
(%)
PERCENTAGE OF B.M. FLOUR
IN 30 MIN. DIGESTION

Explanation of Figure 6

Effect of TCA concentration on observed optical rotation:

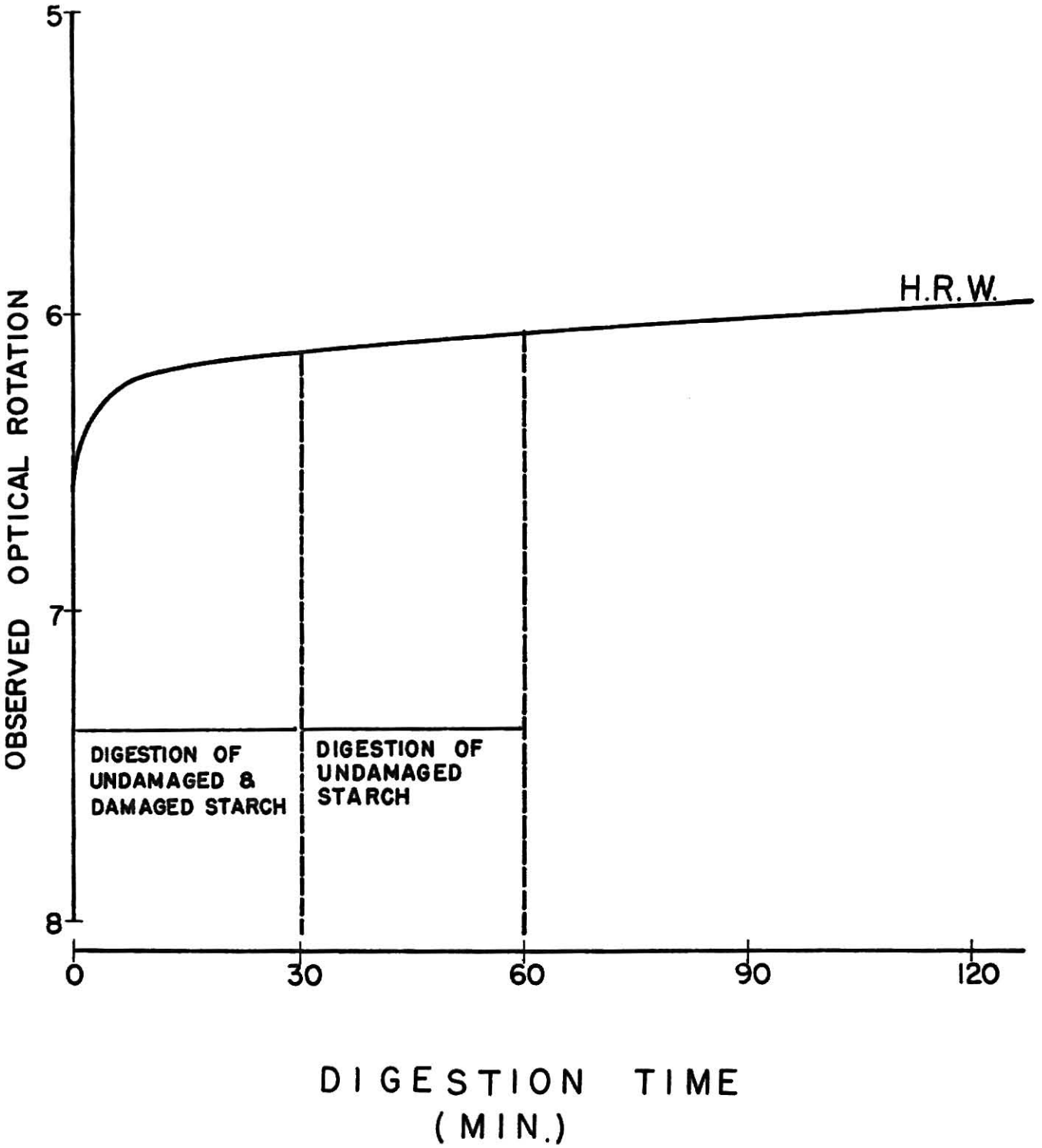
The concentration of TCA solution was 50%.


OBSERVED OPTICAL ROTATION
6.5
6.0
HRW
0
1
2
3
4
5
AMOUNT OF TCA
(ML.)

Explanation of Figure 7

Hydrolysis of undamaged starch granules and damaged starch granules by alpha-amylase:

The sample flour was Hard Red Winter.


5
6
7
8
OBSERVED OPTICAL ROTATION
H.R.W.
DIGESTION OF UNDAMAGED & DAMAGED STARCH
DIGESTION OF UNDAMAGED STARCH
0
30
60
90
120
DIGESTION TIME (MIN.)

SUMMARY

(1) A method for quantitative estimation of damaged starch in flour has been developed, based on the polarimetric determination of starch in calcium chloride solution and the fact that damaged starch is much easier to be digested than undamaged starch. The method involves the determination of starch before and after its digestion with alpha-amylase. The difference represents the estimation percent of starch damage.

(2) The method can give the estimations of both the total starch and damaged starch of the flour in a single test.

(3) The damaged starch may be reported as percent of the starch that is damaged (Farrand method) or percent of the flour that is damaged starch (Yamazaki and AACC method).

(4) The digestion of undamaged starch is appreciable. This method takes into consideration the low rate of alpha-amylase digestion of intact starch granules, and therefore makes the results more accurate.

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THE ESTIMATION OF DAMAGED STARCH USING POLARIMETER

by

BIN-YEA CHIANG

B.S., Taiwan Chung-Hsing University
Taichung, Taiwan, China, 1966

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1971

A new method based on polarimetric estimation of starch in calcium chloride solution is developed for the quantitative estimation of starch damaged in wheat flour. This method involves the estimation of starch before and after its digestion with alpha-amylase.

The sample of flour is subjected to alpha-amylase digestion under controlled conditions for a definite period of time. The mixture is centrifuged. The residue is washed with ethanol, and then dissolved in calcium chloride solution. The protein is precipitated by uranyl acetate. The solution is filtered. The optical rotation of the clear filtrate is determined by a polarimeter.

The total starch (including damaged starch granules) is determined by the similar procedure without enzymatic digestion. The difference represents the amount of damaged starch.