A POLARIMETRIC STUDY OF FLOUR DIASTATIC VALUE[1]

A. H. Woodhead[2] and Catherine A. Wyatt[2]

ABSTRACT

The diastatic values of flour samples measured polarimetrically differ in a regular and notably simple manner from results obtained by chemical reduction methods of analysis. The reducing substances present in bran and germ were not recorded by the polarimeter, and the results by the latter method are therefore suggested as affording a more convenient measure of the main products of flour autolysis.

There are several well-known procedures for estimation of flour diastatic values, the one currently used being that of Blish and Sandstedt (1), which depends upon a chemical reduction principle. Molin (5) has described a refractometric method to determine the total products of starch hydrolysis as an estimation of germination damage in wheat, by autolysis at 62°C. Munz and Bailey (7) extended this work

[1] Manuscript received June 17, 1959.
[2] Unga Limited, Nairobi Mills, Nairobi, Kenya.

by including in their refractometric studies the effects of temperature and of various buffer solutions, and the incremental effects of added malt preparations.

Kent-Jones and Amos (3) used the polarimeter in studying the dextrinogenic activity of oat flour, using autolysis at 62°C. and basing their calculations upon the large specific optical rotatory power for dextrins which they assumed to be +200°. Though the method was not applied to wheat flour, they claimed that it sufficed to put a number of oat flour samples in the correct order of alpha-amylase activity as indicated by the method of Hills and Bailey (2).

The experimental work described in this paper concerns the estimation of what are commonly referred to as "maltose values" by autolysis of flour suspensions at 30°C. in a weak buffer solution, the result being determined by polarimetric measurements on the clear extract obtained by a technique developed by the present authors. The accurate measurement of optical rotation depends upon the use of perfectly clear solutions; hence it was necessary to develop clarifying agents of high efficiency. These were found to act conveniently as diastasis inhibitors, thereby making the procedure experimentally stable and reliable.

Theory

The simplified expression for specific optical rotation at 20°C. referred to sodium (D) light is:

$$[a]_D^{20} = 100R/C.1$$

where R is the measured angular rotation in degrees;

C is the concentration of substance in 100 ml. solvent; and

1 is the length of the tube used, in decimeters.

This expression is applicable to dilute solutions having unit density.

The high specific rotation for maltose (+ 138°) suggests that a reasonably large rotation could be anticipated at a maltose concentration of the order of 1.5% using a 2-dm. tube. By using 25-g. flour samples and a total of 69 ml. of liquid diluents, including clarifying and inhibiting reagents, it is readily seen that R = percent maltose, numerically. This assumes, for the purpose of experimental design only, that maltose is the sole product of diastasis.

It is known that flour contains small amounts of natural sugars (4,6,8,9) which have been characterized. It was expected that the effect of these sugars would be small but would be superimposed upon the final results, assuming them to have a definite resultant optical rotation. It was decided to compare the polarimetric results with those

obtained by the Blish-Sandstedt procedure on the same samples.

Materials and Methods

Materials. The flour samples used included straight-run bakers' and family white flours of 75% extraction or lower, whole-meal, brown or "standard" flour of 85% extraction, biscuit and mill stream flours of very low diastatic value, and medium wheatmeals known in East Africa as "Atta" and made for the local Indian community. These provided a wide range of diastatic activity.

The reagents used were:

Acetic acid/sodium acetate buffer, pH 4.6 – 4.8, 0.05M;

Potassium ferrocyanide solution, 15% w/v;

Zinc acetate, 15% w/v, in 50% v/v acetic acid.

The equipment used included a simple polarimeter fitted for sodium (D) light; a centrifuge accepting 100-ml. tubes; flat-bottomed, wide-necked flasks of 250-ml. capacity; a thermostatically controlled water bath operating at $30°C. \pm 0.1°C.$; and fine filters either of paper (Whatman No. 42, 12.5-cm.) or sintered-glass Büchner funnels of No. 4 porosity and 60-ml. capacity.

Method of Analysis. A storage flask containing sufficient buffer solution was kept in the 30°C. bath. Flour samples of 25 g. ± 0.01 g. were weighed on paper and transferred to the flat-bottomed flasks, together with about 10 g. of coarse sand. The flour and sand were mixed by rotation of the flasks, and then conditioned in the bath for 15 minutes. The flasks were then removed; 55 ml. of buffer solution at 30°C. were quickly added to each; the mixture was homogenized by a vigorous swirling motion and replaced in the bath. The samples were incubated for 1 hour, the pH being 5.0. After 1 hour the flasks were removed, and 7 ml. of the potassium ferrocyanide solution were added to each and mixed by shaking; the flasks then were placed in a water bath at room temperature. To each were added 7 ml. of the zinc acetate/acetic acid solution, with thorough shaking. The pH fell to 4.0. The mixtures were then centrifuged for 15 minutes at 2,500 r.p.m. at a mean radius of 15 cm.

The resulting supernatant liquid was filtered without loss through Whatman No. 42 (12.5-cm. diameter) papers or through the sintered-glass funnels under moderate suction. The whole of the liquid was filtered by one of these methods and the perfectly clear filtrate was polarized in a 2-dm. tube using sodium (D) light. Results reproducible to $\pm 0.2°$ were afforded by this procedure. Mutarotation was not observed, and measurements may therefore be made immediately or at convenience. Solutions in fact had prolonged stability and could

be checked either immediately or on the following day by the same observer or by an independent one.

Results and Discussion

A comparison of the polarimetric values with those obtained by the Blish-Sandstedt procedure is shown on Fig. 1. Linear relationships were
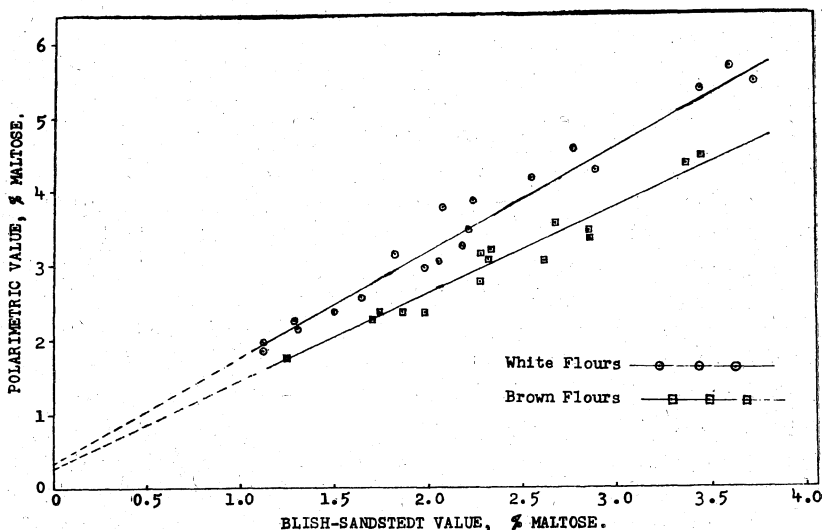

Fig. 1. Relationship between polarimetric and Blish-Sandstedt diastatic values. Circles indicate white flours; squares indicate brown flours.

found, the regression equation being slightly but positively different for white flours as compared with that for the brown flours. The extrapolated curves almost converge on the ordinate axis representing the polarimetric values. The polarimetric values are always higher than the chemical values, both being expressed as a percentage of maltose in the flour. The results show that polarimetric value equals:

For white flours .... (B-S Value) 1.438 + 0.33 $r = 0.98$
For brown flours .... (B-S Value) 1.19 + 0.23 $r = 0.97$

The correlation coefficients are highly significant. The constants 0.33 and 0.23 respectively indicate points of zero diastasis; i.e., they are the polarimetric equivalents of natural nonreducing sugars initially present in the two types of flour. At the dilution used, they correspond to approximately 0.8% sucrose or its optical rotatory equivalent of a mixture of nonreducing sugars. Detailed independent

work on these natural sugars by Williams and Bevenue (9), by Koch, Geddes, and Smith (4), and by Montgomery and Smith (6) supports this result. Their collective findings may be summed up in the statement that wheat flour contains 0.8–1.1% of nonreducing sugars, and only 0.1% reducing sugars.

The regular divergence between the chemical and the optical assays of the products of diastasis for both types of flour is somewhat larger than one would expect. The reducing value of maltose has been carefully evaluated in the work of Blish and Sandstedt, while the specific optical rotation of pure maltose is an accepted physical constant. It is likely that much if not all of this divergence is due to the presence of dextrin or dextrin-maltose complexes formed by autolysis. These are known to have a higher specific optical rotation than pure maltose, according to Kent-Jones and Amos.

On the other hand, bran extracts have a very high chemical reducing value and practically no optical activity — points which we have checked experimentally. These features of bran account for the small but apparently significant difference in the regression equations for the brown and white flours. It is suggested that the polarimetric scale is more convenient than the chemical reduction values given by the Blish-Sandstedt procedure, since the reducing substances other than maltose which are present in bran and germ are not measured by the polarimeter, the latter recording only the optically active products of autolysis, which are chiefly maltose and the malto-dextrins.

Literature Cited

1. BLISH, M. J., and SANDSTEDT, R. M. An improved method for the estimation of flour diastatic value. Cereal Chem. 10: 189–202 (1933).
2. HILLS, C. H., and BAILEY, C. H. The nature of the increase in amylase activity of germinating barley. Cereal Chem. 15: 273–281 (1938).
3. KENT-JONES, D. W., and AMOS, A. J. Modern cereal chemistry (5th ed.), p. 609. Northern Pub. Co., Ltd.: Liverpool (1957).
4. KOCH, R. B., GEDDES, W. F., and SMITH, F. The carbohydrates of Gramineae. I. The sugars of the flour of wheat (*Triticum vulgare*). Cereal Chem. 28: 424–430 (1951).
5. MOLIN, G. Determination of sprout damage in wheat and rye by means of the dipping refractometer. Cereal Chem. 11: 153–159 (1934).
6. MONTGOMERY, R., and SMITH, F. The carbohydrates of the Gramineae. IV. Identification of the sugars of the flour of wheat (*Triticum vulgare*). Cereal Chem. 31: 490–494 (1954).
7. MUNZ, E., and BAILEY, C. H. Refractometric methods of determining diastatic activity of flour. Cereal Chem. 14: 85–96 (1937).
8. PERLIN, A. S. Isolation and composition of the soluble pentosans of wheat flours. Cereal Chem. 28: 370–381 (1951).
9. WILLIAMS, K. T., and BEVENUE, A. The chromatographic examination of sugars in wheat flour. Cereal Chem. 28: 416–423 (1951).