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Effects of Amylases and Metals on the Pasting Properties of Wheat Flour, Determined by the Amylograph and by Hagberg's Falling-Number Method

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ABSTRACT

Sound and sprout-damaged wheat flours, flour from steamed enzyme-inactivated wheat, and flour with superior pasting strength have been examined by the falling-number (FN) technique and by the amylograph. These two techniques have been used to study the effects of various additions of silver, copper, and mercury salts, Versene, iodate, N-ethylmaleimide, and a sulfhydryl compound to flour. The effects of alpha-amylase may be decreased by many of the reagents, but there is also diminution of some desirable pasting characteristics. Silver has an especially complex effect. Alpha-amylase of flour is clearly not a sulfhydryl-dependent enzyme. The FN method is much less sensitive than is the amylograph to low levels of alpha-amylase because of more rapid heating.

Wheat flour is one of the more economical thickening agents in the manufacture of soups and other canned foods; it has additional advantages of texture and flavor. However, not all flours have this thickening power in sufficient degree, and it has been usual to select flours on the basis of the amylograph value. A low amylograph maximum is assumed to be indicative of sprout damage – that is, excessive alpha-amylase activity. This demerit can be overcome by preliminary treatment of wheat or flour with steam to inactivate the enzyme, according to the Hutchinson (1) or other (2) processes.

It is well known that alpha-amylase can be destroyed by poisoning with various metal ions such as silver, mercury, and copper, and that its activity can be reduced by removal of calcium ions (3).

The Hagberg falling-number (FN) method has been adopted as a standard technique in many countries where sprout damage is widespread, as a simple method of measuring the effect of alpha-amylase in flour.

It is the purpose of this paper to show in more detail than hitherto the effects of various metals on the FN of flour, to show that alpha-amylase activity is not sulfhydryl-dependent, and to compare the amylograph and FN techniques for assessing flours of low amylase activity.

MATERIALS AND METHODS

A single bulk of wheat of the variety Aotea, representative of the poorer-baking grades of the New Zealand harvest of January-March 1967, has been termed "normal." Part of this wheat was steam-inactivated by passage of low-pressure steam (15 p.s.i.) through the wheat, spread in a thin layer on a sieve and continuously stirred. Under these conditions, 6 min. of steaming was optimal. The steamed wheat was immediately cooled and dried back to 15% moisture. Both "normal" and "steamed" wheat were milled with a Brabender Quadrumat Junior mill. The normal wheat flour had amylogram maximum 105 B.U., protein 9.0%, damaged starch 6.1%, ash 0.46%.

Two commercial flours of extreme properties have also been used in the main part of the work. That called "sprouted" was inadvertently produced from New Zealand sprouted wheat in 1962. It had amylogram maximum 10 B.U., Farrand alpha-amylase 127 units, protein 10.6%, damaged starch 9.0%, ash 0.54%. The other commercial flour, called "supersound," was Australian, produced in 1963. It had amylogram maximum 835 B.U., protein 12.1%, damaged starch 8.8%. Both commercial flours had been stored in a deep-freeze.

A bulk of Aotea wheat of the 1967 harvest, somewhat sprouted, was used in the original and in the steamed state, Quadrumat-milled, only for the amylograms represented in Fig. 2 under "Results and Discussion."

A bulk of Aotea wheat representing the average quality of the 1967 harvest was used in the original and in the steamed state, Quadrumat-milled, only for the experiment of Table I.

Reagents were dissolved in distilled water used to prepare flour slurries. Concentrations are expressed relative to flour, and all flour weights are as-is basis. The flours used have all approximated to 14% moisture content and no attempt has been made to work to a precise dry weight. Damaged-starch analyses are by Donelson and Yamazaki's method (4), and alpha-amylase analyses are according to Farrand (5).

Amylograph

There has been little agreement so far on a standard method for the amylogram. The AACC Approved Method (22-10)[1] suggests 65 g. of unmalted wheat flour plus 460 ml. buffer. Past workers have used a range of quantities and concentrations from 43 to 100 g. flour with 400 to 470 ml. of water or buffer. They have used sensitivities of 250, 350, and 700 cm.g. per 1,000 chart units, but in most cases have continued to call the chart units B.U. There is thus some confusion of meaning in comparison of results.

In the present work 60 g. flour has been suspended by shaking in 440 ml. distilled water and poured into the machine bowl, with 10 sec. of drainage allowed. The thermostat drive was started from the 35°C. setting and the machine set to run for 50 min., rising to 97°C., then holding that temperature. Bowl speed 75 r.p.m., 700 cm.g. spring capsule, and thermometer drive 1.5°C. per min. were used. The whole operation was carried out in a room thermostated at 80°F. The unpasted viscosity, between 40° and 60°C., has been subtracted from the maximum viscosity reading. Results, measured in chart units, are called Brabender Units; i.e., 1,000 B.U. equals 700 cm.g.

[1]American Association of Cereal Chemists. AACC Approved Methods (formerly Cereal Laboratory Methods, 7th ed.). The Association: St. Paul, Minn. (1962).

Falling Number

Determinations were made substantially according to AACC Approved Method 56-81[2] except that 5 g. flour was used (5:25:60 method) unless otherwise noted.

No correction of the water bath for varying barometric pressure has been made, all points of an experimental series being completed within 1 day. No reflux condenser was used on the water bath, since this has in our experience led to variable results. Tubes were used randomly; the results shown are independent of tube-to-tube variation.

Falling time (Table I) is FN with the 60 sec. of stirring time deducted.

In contrast to the amylase methods of clinical science, it is not necessary to protect FN or amylograph determinations from salivary contamination, because salivary amylase is destroyed at a temperature lower than the commencement of starch gelatinization.

RESULTS AND DISCUSSION

Inactivation by Silver

Various concentrations of silver nitrate have been included in the FN method applied to various flours, with results shown in Fig. 1.

The unusual nature of the results with silver obviously called for confirmation, and a similar experiment was carried out with the amylograph, with results shown in Fig. 2. Maxima and minima typical of Fig. 1 have been observed repeatedly with a variety of flours. The data of Fuller et al. (6) for silver nitrate additions of about 1 to 60 μeq. per g. are also in accord with the observations of Fig. 1.

Hutchinson (7,8) used silver to poison alpha-amylase, as a check on the completeness of steam-treatment. He used a fixed concentration of silver nitrate, corresponding to 3 μeq. silver per g. of flour. The same fixed concentration of silver nitrate was used by Yasunaga et al. (9) as an enzyme poison. It is clearly evident from Fig. 1 that 3 μeq. per g. is insufficient silver to completely inactivate amylase in a sprouted flour assayed by FN. Nor does this dosage produce complete improvement of sprouted flour assayed by amylograph, though the apparent improvement is greater by this technique. Much more serious, however, is the reduction of FN brought about by this dosage of silver applied to "supersound" flour, as seen in Fig. 1.

If silver is to be used as Hutchinson suggested, to check the completeness of an inactivation process, then it is evident that dosage of the order of 100 μeq. per g. is required. But there is an obvious trend (Fig. 1) of different flours to give about the same FN at this silver concentration.

Quite apart from the inhibiting effect on amylase activity, the addition of silver ion to the FN determinations produced the curious series of maxima and minima. All flours showed a maximum (Fig. 1) in the vicinity of 35 μeq. per g., this maximum being greatest for the supersound flour. Another maximum occurred at 5 μeq. per g. only for the sprouted and for the steamed flour (Fig. 1). There is a particular contrast to be seen here between the normal and steamed versions of the same wheat. In the course of these FN determinations, silver ion is being reduced to metallic silver, and we must conclude that the inflections represent titrations of

[2]See footnote 1. (Editor's Note: Method 56-81 has been revised and is now 56-81A.)

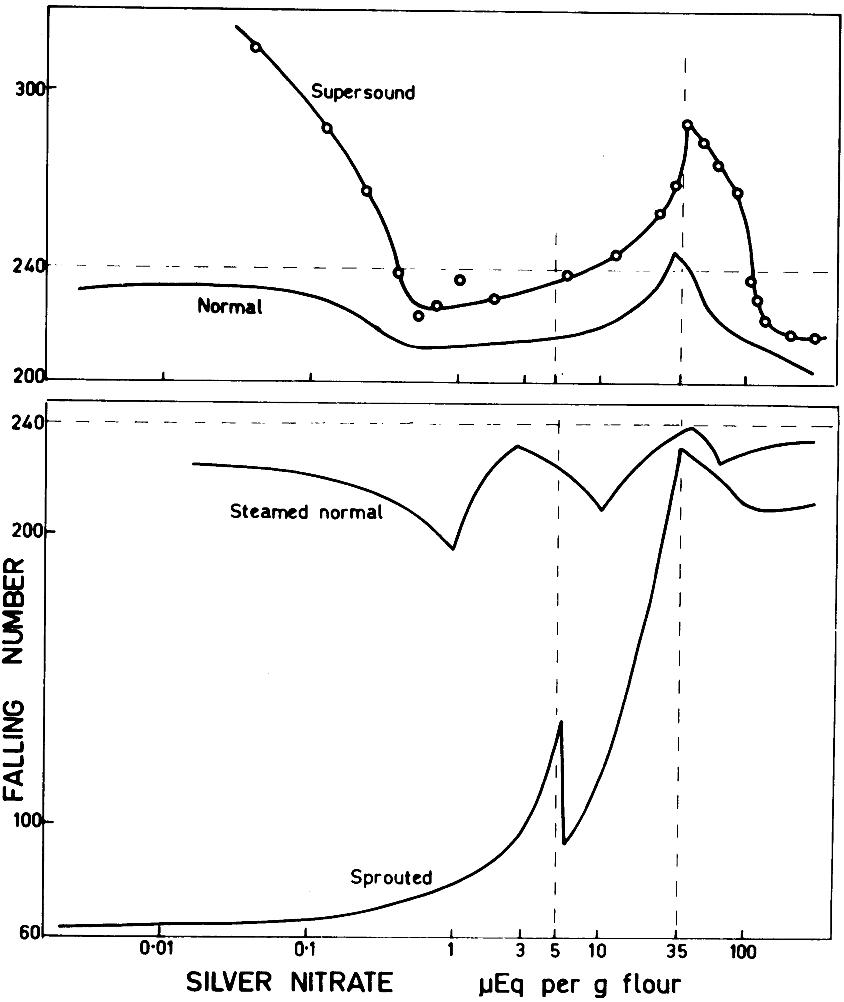

Fig. 1. Variation of FN with concentration of silver nitrate, for "normal," "supersound," "steamed," and "sprouted" flours. Concentration is a logarithmic scale. Experimental points are shown for the "supersound" curve, but are omitted from all other curves. Coordinates shown dashed are to facilitate comparison with other figures.

particular groupings in the flour. Possibly the 5 μeq. per g. inflection represents something which is only accessible in the starch granule damaged by steaming or alpha-amylase attack. None of the silver inflection points correspond to the SH or SS group content of the flour.

The two maxima have been confirmed by the amylograph technique applied to a sprouted wheat flour and to the corresponding steamed wheat flour (Fig. 2). The maxima appear here as steps in the curves. It is noteworthy that silver at low concentration is a more effective amylase inhibitor in the amylograph than in the FN method.

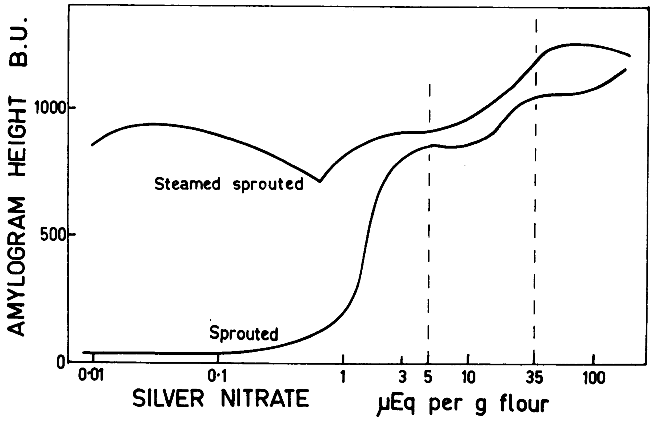

Fig. 2. Variation of amylogram height with concentration of silver nitrate, for two flours from the same sprouted wheat, one of them milled after steaming.

Inactivation by Copper and Mercury

Copper ion has been stated by Mercier and Colas (10) to be an inhibitor of both alpha- and beta-amylases. Ford (11) in 1904 showed that copper was toxic to the activity of malt diastase on starch. Sherman and Wayman (12) in 1921 also demonstrated the toxicity of copper to all kinds of amylases at a concentration of 4.2 μM per g. starch. Figure 3 shows the relatively low efficiency of copper sulfate as an amylase inhibitor in sprouted wheat. Copper produced a drastic reduction in FN when applied to the supersound flour, as with silver. Figure 3 also shows a small modifying effect of copper at high concentrations on the FN of normal flour.

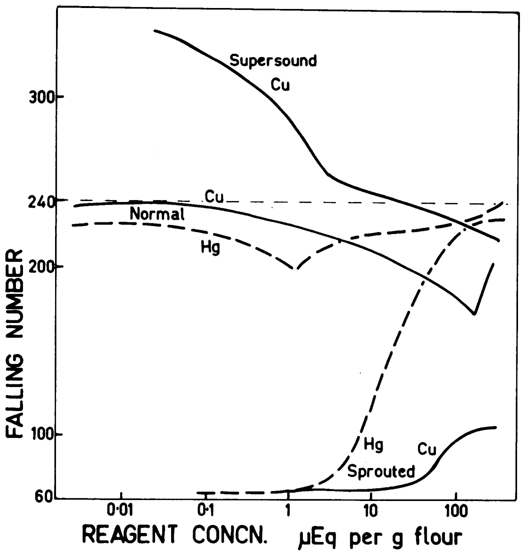

Fig. 3. Variation of FN with concentrations of copper sulfate and mercuric chloride.

A similar small modifying effect was shown by mercuric chloride, but at much lower concentration. The extreme sensitivity of amylases to mercuric chloride was demonstrated by Ford (11), who showed 50% loss of malt saccharogenic activity at mercuric chloride concentration of only 0.0037 μM per g. starch. Comparison of the curves of Figs. 1 and 3 shows mercuric chloride to be not quite as effective as silver nitrate at very low concentrations, but the curves are entirely coincident between 6 and 20 μeq. per g. Möttönen (13) has used high concentrations of mercuric chloride, ranging from 286 μeq. per g. to 7.15 meq. per g., to inactivate alpha-amylase in the FN test.

Addition of Versene (ethylenediamine tetraacetic acid, disodium salt) (Fig. 4) had little effect on sound wheat or on steamed wheat, except for a small inflection at high concentration. It was rather better than copper at inhibiting the effects of sprouting, presumably complexing calcium required by amylase as cofactor. Versene had a small effect of lowering the FN of supersound flour, but copper and silver lowered it much more.

Sulfhydryl Status

Because of the inhibitory effect of metals, it was possible that alpha-amylase might be a SH-functional enzyme, so two reagents that would inactivate SH groups were tried. The effects of potassium iodate and of N-ethylmaleimide (NEMI) are shown in Fig. 5. The converse situation, the addition of extra sulfhydryl as mercaptoethylamine to normal flour, gave a curve very similar to the copper sulfate curve for normal flour of Fig. 3. Mercaptoethylamine had no effect on sprouted flour.

Although alpha-amylase is commonly stated not to be an SH-dependent enzyme, it is said to be inhibited by oxidants (10,14). These experiments offered an

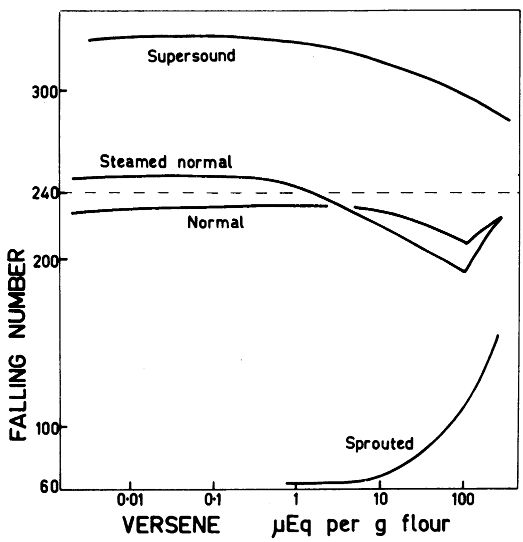

Fig. 4. Variation of FN with concentration of Versene (ethylenediamine tetraacetic acid, disodium salt).

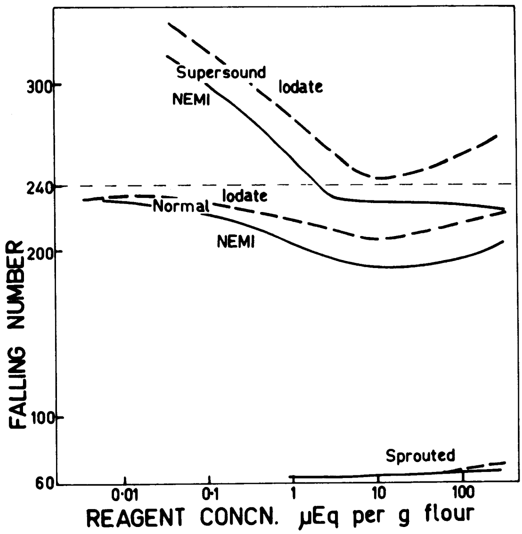

Fig. 5. Variation of FN with concentrations of potassium iodate and N-ethylmaleimide (NEMI).

opportunity to confirm such statements. Certainly the specific SH-complexing reagent NEMI had negligible inhibiting effect in doses up to 200 μeq. per g., nor did it have any marked effect on sound flour. It did, however, have a pronounced degrading effect on the FN of the supersound flour. The oxidant, potassium iodate, had a similar degrading effect on the supersound flour and, like NEMI, had little effect on either the sound or the sprouted flours. The converse, attempted potentiation of amylase effect by addition of extra SH in the form of mercaptoethylamine, was without significant effect. Since all sound flours contain a small amount of alpha-amylase activity (15,16), we can firmly conclude that SH groups are not involved in this enzyme activity. However, inhibition of alpha-amylase did not occur with doses of potassium iodate up to 200 μeq. per g., this finding being at variance with the literature statement about oxidants in general. More work on the oxidant inhibition of alpha-amylase is required, since inhibition of activity by the ascorbic-dehydroascorbic acid system has been described (17).

Comparison of Amylograph and Falling Number

In the present work it has been found, in agreement with Hutchinson's observations (18), that although the amylogram maximum is raised by the steaming treatment, the FN may not be. These two methods seem to be measuring separate features of pasting properties. The two techniques differ in several obvious properties: notably, rate of heating, concentration, and rates of shear during mixing and during sensing of viscosity. These differences were systematically tested (Table I) over a pair of flours, from steamed and unsteamed wheat, that differed in amylogram height but not in FN. The conclusion was quite clear, that it is the rate of heating that is of prime importance. From published data the contrast is quite

TABLE I. COMPARISON OF TWO FLOURS BY AMYLOGRAPH AND FALLING-NUMBER METHODS WITH INTERCHANGE OF THE PRINCIPAL EXPERIMENTAL VARIABLES

	Concentration %	Heating Time min.	Speed r.p.m.	Viscosity: Normal Flour B.U.	Viscosity: Steamed B.U.	Viscosity Ratio, S/N
Amylogram maximum						
Normal technique	12	27	75	50	670	13
Decreased shear rate	12	27	30	25	280	11
Increased shear rate	12	27	145	130	1,030	8
Increased concentration	16.7	27	75	270	2,400	9
Increased heating rate	12	8	75	490	1,100	2
				sec.	sec.	
Falling time						
Normal technique	16.7	1		93	104	1
Decreased concentration	12	1		2.0	2.0	1
Decreased heating rate	16.7	27		0.5	6.7	13

clear also; Perten (19) states that it takes about 30 sec. to pass the critical temperature range from 55° to 80°C. in the course of a 5-g. FN determination. Möttönen (20) showed temperature curves for the 7-g. FN determination and concluded that 85 sec. was required to pass the critical range. The amylograph takes about 17 min. to pass the same range. By comparison, Perten suggests times of 40 to 140 sec. for bread crumb to pass the critical range. The practical result of this difference in heating rates is that the FN method is less sensitive, or insensitive, to low levels of alpha-amylase, the region in which the amylograph is most sensitive, so that a flour with a very low level of alpha-amylase activity is capable of having its amylogram value raised by amylase destruction, though the FN cannot be raised further.

CONCLUSIONS

Our program of work on wheat starch and amylases explores possibilities for commercial exploitation of sprouted wheat. It is patently obvious that the metals considered here could not be tolerated for food use, nor could they be considered for many technological thickening applications. It is doubtful if silver and mercury are even of use analytically as a check on the completeness of other inactivation treatments.

That alpha-amylase of wheat flour is clearly not a sulfhydryl-dependent enzyme removes another possible attack on the enzyme.

The inadequacy of the FN for assessing the alpha-amylase activity of normally sound flours has been demonstrated.

It is evident that a thickening property can be found which is superior to that of the average sound flour. This "superpasting" is easily destroyed by several of the reagents used. A superpasting flour may be defined as one having a FN (5:25:60 method) in excess of 250 sec. The generation of superpasting will be explored in a future paper.

Acknowledgments

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