THE INFLUENCE OF SOYA FLOUR IN BREAD DOUGHS

IV. Alpha-Amylase of Soya[1]

E. MITCHELL LEARMONTH[2] and J. C. WOOD[2]

ABSTRACT

The existence in raw soya of a starch-liquefying enzyme as demonstrated by the amylograph is confirmed. The enzyme is chiefly, if not wholly, a true alpha-amylase. The activity of soya is only of the order of one-hundredth of that of an ordinary wheat malt and it diminishes rapidly with rising temperature and with falling pH, in contrast with that of wheat malt. These results contradict the findings of some other workers and lead to the conclusion that the alpha-amylase activity of raw soya is not significant in commercial breadmaking.

For many years soya has been recognized as a rich source of beta-amylase, but it has been a matter of controversy whether or not it contained more than traces of alpha-amylase. Orestano (8) reported that soya contains only one amylase – beta-amylase. Newton and Naylor (6) found only traces of alpha-amylase in soya beans, both before and after germination. Although their soya amylase had the ability to re-

[1] Manuscript received August 21, 1958.
[2] British Soya Products Limited, Puckeridge, Herts., England.

duce the viscosity of starch paste, they attributed this to beta-amylase. Teller (13) showed that there were two amylases in soya, but Laufer, Tauber, and Davis (3) found only very slight alpha-amylase activity. The work of Peat, Whelan, and Pirt (10) showed the presence of a small amount of alpha-amylase, but they made no attempt to measure it. Ofelt, Smith, and Mills (7), using the amylograph, claimed that soya could be considered an active source of alpha-amylase when compared with a pure alpha-amylase preparation. Learmonth (4) had earlier attempted to measure the alpha activity of several varieties of raw soya beans by the method of Sandstedt, Kneen, and Blish (11) and Stone's modification (12) of it. The latter method is expected to measure traces of alpha activity in supposedly pure preparations of beta-amylase. In no case did he obtain evidence of more than a dubious trace of alpha activity in soya.

The work described here was undertaken to resolve this discrepancy, especially with reference to the use of raw, enzyme-active soya in commercial breadmaking. In the United Kingdom and in Europe, enzyme-active soya is widely used, as a commercial bread improver, in quantities of approximately 1% of the wheat flour in a dough. Ofelt *et al.* (7) had reported so high an alpha-amylase activity for soya (5% raw soya flour equivalent to 12 SKB units of alpha-amylase) as to lead to the supposition on the one hand that it could be used as an alpha-amylase supplement instead of malt or fungal amylase preparations, or on the other, that it could produce deleterious results in doughs that did not require such a supplement. Although their work was confined to soya supplements at the rate of 5% of the wheat starch, their findings were so much at variance with all earlier work and with general bakery experience in the United Kingdom as to require confirmation or disproof.

Their experiments with the amylograph have therefore been repeated and the activity of the soya has been rigorously compared, by the more exact quantitative methods of Sandstedt, Kneen, and Blish (11) and of Peat, Thomas, and Whelan (9) with that of an ordinary commercial wheat malt whose activity (23.0 SKB units) was measured at the same time. To complete the picture, the response of the soya activity to pH and temperature has also been examined in comparison with the wheat malt, and the effect of soya in breadmaking from this point of view is discussed in detail.

Materials and Methods

The soya used was prepared by grinding whole raw beans to a fineness such that less than 2% was retained by a 60 British Standard

sieve. Defatted soya was prepared by Soxhlet extraction of this flour for 4 hours with petroleum ether (b.p. range 30°–40° C.).

Commercial raw wheat starch and a commercial wheat malt were used.

Measurement of Alpha-Amylase Activity. (1) *Amylograph.* The amylograph experiments were carried out exactly as described by Ofelt *et al.* (7). To demonstrate the thermolability of the starch paste-liquefying factor, the following experiment was done:

One gram soya flour was extracted with 100 ml. water at 30°C. for 60 minutes, with shaking every 5 minutes. The extract was centrifuged at 4,500 r.p.m. for 5 minutes and supernatant decanted off. Forty-five milliliters of supernatant (equivalent to 0.45 g. soya flour) were taken for the amylograph test, the volume of water added to the starch being reduced from 405 to 360 ml. A further 45 ml. of supernatant were heated at 62°C. for 15 minutes and tested in the same way.

(2) *Sandstedt-Kneen-Blish Method (11).* The effect of sodium phosphate/citric acid buffers, pH 5.3 and pH 6.6, in a final concentration of 0.01*M* was investigated, together with the effect of raising the temperature of incubation. The micro method of Kneen, Sandstedt, and Hollenbeck (2) was also investigated, but not very thoroughly, for Stone's (12) modification of that method had not given very satisfactory results in earlier work (4). However, a 1% w/v wheat malt flour extract was diluted 1:100 and compared with a 1% w/v soya flour extract.

(3) *Sodium Starch Glycollate Method.* The method of Peat, Thomas, and Whelan (9) was modified as follows: the beta-amylase solution was prepared by extracting 2 g. soya flour with 20 ml. distilled water for 60 minutes at 35°C., with shaking every 5 minutes. The extract was centrifuged and the supernatant removed. Five milliliters of supernatant plus 7.5 ml. 0.2*M* sodium acetate buffer, pH 4.8, well mixed and kept at 62°C. for 15 minutes, cooled immediately to 20°C. and centrifuged. Supernatant used as source of beta-amylase. There was no alpha-amylase activity in this beta-amylase as measured by the Sodium Starch Glycollate Methods I and II (see below).

The substrate was prepared by mixing 200 mg. sodium starch glycollate (British Drug Houses Ltd., Poole, Dorset, England) with 23 ml. water and 1.5 ml. beta-amylase solution (excess) and 9 ml. sodium phosphate/citric acid buffer, pH 5.3 (prepared according to Ofelt *et al.*, 7) or 9 ml. 0.2*M* sodium acetate buffer, pH 6.6.

The substrate was incubated at whatever temperature the digestion was to be done until the Absorption Value (A.V.) fell to a constant level. The A.V. was measured at 680 mμ in an EEL Portable Colorim-

eter (Evans Electroselenium Limited, Harlow, Essex, England), using 1 ml. test solution, 4 ml. 0.022% w/v iodine in 0.2% w/v potassium iodide, and 1 drop of 6*N* sulfuric acid, made up to 50 ml. The A. V. usually fell by 3 to 5% of the original value.

Preparation of alpha-amylase extracts: 1 g. of the soya flour or wheat malt flour was extracted for 60 minutes at 30°C. with 100 ml. water, with shaking every 5 minutes. The extract was centrifuged at 4,500 r.p.m. for 5 minutes and the residue discarded. The malt extract was diluted before use and the soya extract used as such.

The digests were prepared thus:

Method I: One milliliter alpha-amylase extract to be tested was mixed with 4 ml. substrate and 6 ml. water; 1 ml. removed immediately for A.V. determination. The remainder of the digest was incubated at 35°C. for 5 hours; then 1 ml. was removed for A.V. determination. The percentage fall in A.V. was taken as a measure of the alpha-amylase activity. A.V. was measured as described above, but the final volume was 30 ml. instead of 50 ml.

Method II: Five milliliters test solution with 4 ml. substrate and 2 ml. water were incubated for 60 minutes at the required temperature; A.V. measured immediately before and after incubation as in Method I.

Results and Discussion

The Amylograph. The liquefying effect of unheated soya flour on starch paste, as shown by Ofelt *et al.* (7) with the amylograph, is qualitatively supported by the graphs in Fig. 1. The thermolability of the starch-liquefying factor is also shown. Ofelt *et al.* used soya at the rate of 5% of the wheat starch, a much larger proportion than is customarily used in breadmaking in the United Kingdom. Figure 2 shows the effect of 0.9% addition of enzyme-active full-fat soya – the product customarily used as a bread ingredient in approximately the concentration at which it is used. The liquefying effect is appreciably reduced at this concentration. Figure 2 also shows that the factor is extractable with water and is destroyed by heating the extract for 15 minutes at 62°C. (Fig. 2, b and c). Commercial debittered soya flour was without liquefying power (Fig. 2, d, and Fig. 1, d and e).

It is by no means certain that this starch-liquefying activity of soya is attributable entirely to alpha-amylase. The Z-enzyme of Peat, Thomas, and Whelan (9) may also have some liquefying effect. Z-enzyme would not be measured by the starch glycollate method, however (see below), and its effect, if any, on the amylograph results is considered negligible.

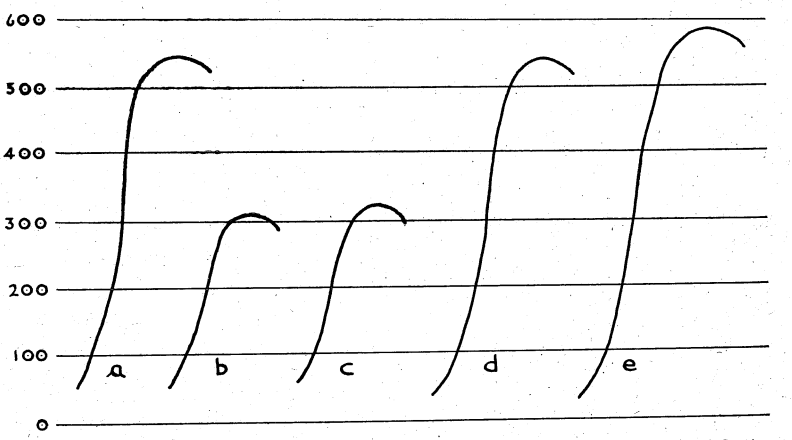

Fig. 1. Amylograph results: a, wheat starch; b, wheat starch + 3.1 g. (6.2%) raw soya flour (assuming 20% fat equivalent to 2.5 g. (5.0%) defatted raw soya flour); c, wheat starch + 2.5 g. (5.0%) defatted soya flour; d, wheat starch + 3.1 g. (6.2%) full-fat soya flour (heat-treated); e, wheat start + 2.5 g. (5.0%) defatted soya flour (heat-treated).

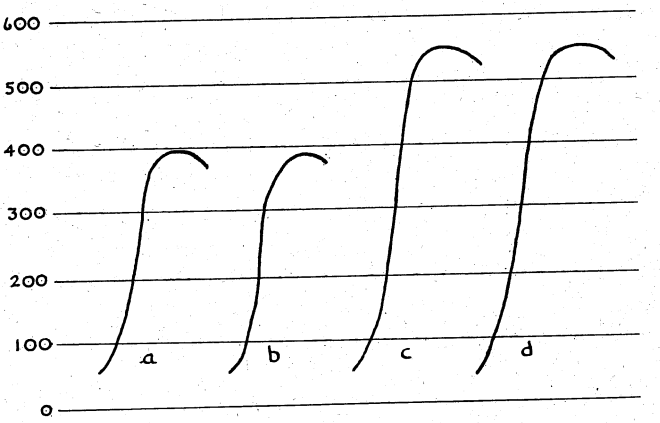

Fig. 2. Amylograph results: a, wheat starch + 0.45 g. (0.9%) raw soya flour; b, wheat starch + 45 ml. 1% aqueous soya flour extract; c, wheat starch + 0.45 ml. 1% aqueous soya flour extract (heated at 62°C. for 15 minutes); d, wheat starch + 0.45 g. (0.9%) soya flour (heat-treated).

Ofelt *et al.* (7) assessed the supposed alpha-amylase activity of their soya flours by comparison with a purified alpha-amylase preparation of known activity, and on this basis arrived at a figure of approximately 12 SKB units for the activity of 2.5 g. of their defatted soya. The evidence for the "known activity" of the purified alpha-amylase does not appear in their paper.

In the United Kingdom the customary source of alpha-amylase for

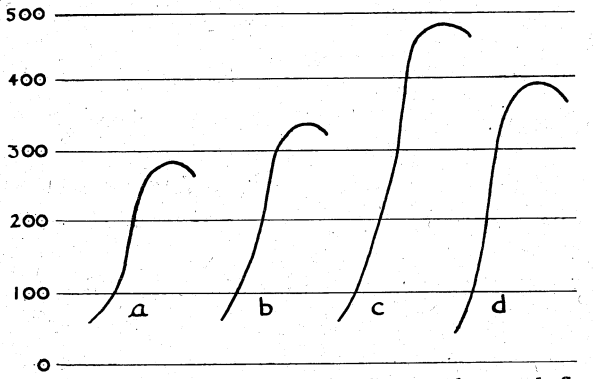

Fig. 3. Amylograph results: a, wheat starch + 20 mg. wheat malt flour; b, wheat starch + 10 mg. wheat malt flour; c, wheat starch + 5 mg. wheat malt flour; d, wheat starch + 450 mg. soya flour.

breadmaking is malt flour, or an active malt extract. Comparison was therefore made of the liquefying power of soya with that of a wheat malt of known Lintner value, and the alpha-activity of the malt flour was also determined by the SKB method. The amylograms are shown in Fig. 3, from which it is clear that 450 mg. of soya were equivalent to somewhere between 5 and 10 mg. of malt flour. Evidently the soya had a liquefying power about one-sixtieth of that of the malt flour. In bakery terms, 2.5 lb. of soya per sack (280 lb.) of wheat flour had the same effect as about 0.75 oz. of malt flour per sack. So small an addition of malt would be ridiculous in bakery and milling practice.

Iodine Staining Methods. (1) *Sandstedt-Kneen-Blish Method (11).* The influence of pH on the alpha-amylase activity of the malt flour, as measured by the method of Sandstedt, Kneen, and Blish (11), is brought out in Table I. It is clear that the alpha-activity of this particular malt (23.0 SKB units) was not exceptionally high, though it had a Lintner value of 83°. Sandstedt, Kneen, and Blish (11) quote figures for the alpha-activity of malts ranging from 39.0 to 17.5 in SKB units. That the alpha-activity of soya, as indicated by the amylograph, was so much

TABLE I

ALPHA-AMYLASE ACTIVITY OF A SAMPLE OF WHEAT MALT FLOUR AND ITS VARIATION WITH TYPE OF BUFFER, pH, AND TEMPERATURE (SKB METHOD)

BUFFER	pH	ACTIVITY	TEMPERATURE
			°C
Sodium acetate/acetic acid	4.6	23.0	30
Sodium acetate/acetic acid	6.6	12.6	30
Sodium phosphate/citric acid	5.3	22.0	30
Sodium phosphate/citric acid	6.6	12.0	30
Sodium phosphate/citric acid	5.3	36.9	45

below this not-very-active malt is a further indication of the negligible influence of soya as a source of alpha-amylase (or starch-liquefying enzyme).

In fact, the activity of soya proved to be too small to be measured by the SKB method, as had been found earlier (4), and attention turned to the micro method of Kneen, Sandstedt, and Hollenbeck (2). The results obtained by this method are given in Table II. Sandstedt

TABLE II

ALPHA-AMYLASE ACTIVITY (KNEEN, SANDSTEDT, and HOLLENBECK MICRO METHOD) OF SOYA AND WHEAT MALT IN DIFFERENT BUFFERS. TEMPERATURE 30°C THROUGHOUT

SOURCE	BUFFER	pH	ALPHA-AMYLASE UNITS
1% w/v Aqueous soya flour extract	Sodium acetate/acetic acid	4.6	0.081
1% w/v Aqueous soya flour extract	Sodium phosphate/citric acid	5.3	0.115
1% w/v Aqueous soya flour extract	Sodium phosphate/citric acid	6.6	0.148
1% w/v Aqueous wheat malt flour extract diluted 1:100	Sodium acetate/acetic acid	4.6	0.100

has already pointed out that the unit of measurement obtained by the micro method is not the same as the SKB unit of the earlier paper. In terms of the micro-method units, it is clear from Table II that the soya has only one one-hundred-twenty-fifth of the activity of the malt at pH 4.6.

An interesting contrast appears in the influence of pH on the alpha-activity of soya and of malt. That of soya increases by nearly 50% as the pH rises from 4.6 to 6.6 (Table II), whereas that of malt falls about the same amount over the same pH range (Table I). This comparison is fairly drawn from the figures obtained, since both gross and micro methods measure the same end point in the same reaction. Hollenbeck and Blish (1) observed a similar trend with wheat malt.

(2) Sodium Starch Glycollate Method. The most convenient method of studying alpha-amylase proved to be the Sodium Starch Glycollate Method of Peat, Thomas, and Whelan (9), modified as described above. Though the method is open to criticism on the ground that the substrate is an artifact foreign to bread dough, it offers a convenient means of comparing the alpha-activities of similar sources of the enzyme and is especially useful for measuring the very low activities found in soya.

Table III compares the activities of seven different samples of raw soya beans in terms of the percentage fall in Absorption Value of the digest after incubation, as described in the first modification (Method I; see "Materials and Methods" section) of the method developed by

TABLE III

ALPHA-AMYLASE ACTIVITY OF SOYA BEANS OF DIFFERENT GEOGRAPHICAL ORIGIN (SODIUM STARCH GLYCOLLATE–METHOD I)

SAMPLE	PERCENT FALL: ABSORPTION VALUE	SAMPLE	PERCENT FALL: ABSORPTION VALUE
	%		%
A	22.6	E	11.3
B	17.7	F	9.7
C	17.7	G	4.8
D	14.5	H	4.8

Peat, Thomas, and Whelan (9). Sample A was the material used in the amylograph tests and in the micro method of Kneen, Sandstedt, and Hollenbeck (2) (see above). It was clearly the most active of the seven, and the other samples would have had even less effect on the viscosity of starch. All were commercial samples of different geographical origin. The fivefold variation in activity is of no significance in breadmaking, since the most active sample has so small a potency in comparison with malt.

The activity of the wheat malt as measured by this method is shown in Fig. 4, where the dilution of a 1% extract is plotted against the percentage fall in absorption value. The fall in pH from 6.6 to 5.3 is accompanied by a *rise* in alpha-amylase activity of 33%. The effect of an identical pH change on the alpha-activity as measured by SKB method can be seen (Table I) to be an increase of 45%. With soya, on the other hand (Table IV), a fall in pH from 6.6 to 5.3 (the range over

TABLE IV

SOYA FLOUR ALPHA-AMYLASE ACTIVITY. EFFECT OF CHANGE OF BUFFER AND PH (SODIUM STARCH GLYCOLLATE–METHOD I. TEMPERATURE 35°C. THROUGHOUT)

BUFFER	PH	PERCENT FALL: ABSORPTION VALUE
		%
Sodium acetate/acetic acid	6.6	30.5
Sodium phosphate/citric acid	5.3	21.0
Sodium phosphate/citric acid	6.6	31.2

which the pH of a bread dough is likely to fall from the time of mixing to the beginning of baking) is accompanied by a *fall* in alpha-activity of 31%, as indicated by the Glycollate Method, compared with a fall of 22% over the same pH range in the micro method of Kneen, Sandstedt, and Hollenbeck (2) (Table II). The discrepancy between the two methods in this response to pH is not of much significance in view of the very low activities of all soya varieties tested. It may be due to inherent inaccuracy in the micro method.

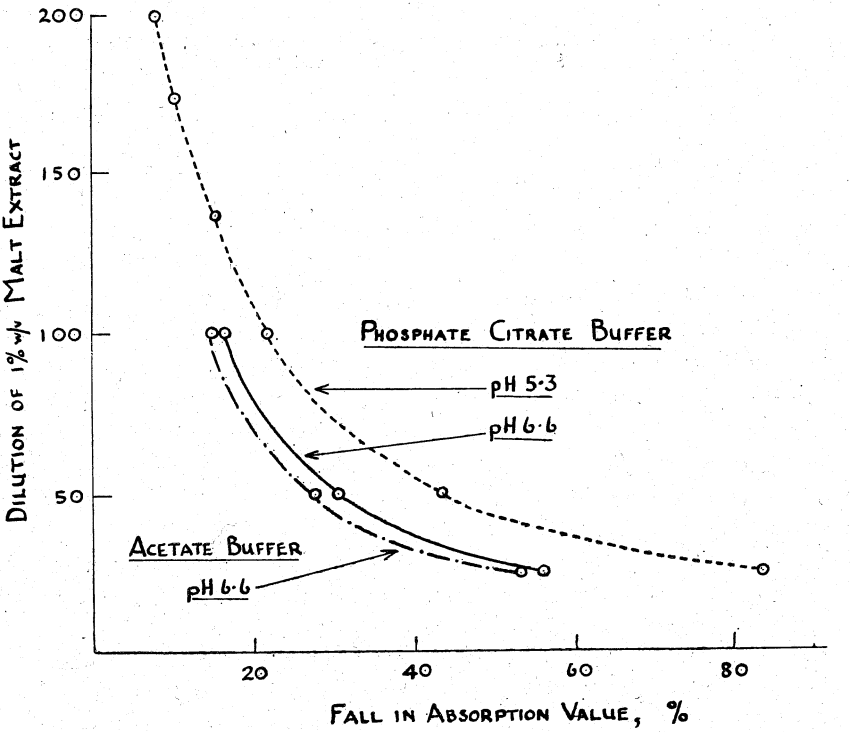

Fig. 4. Alpha-amylase activity of wheat malt extract at various dilutions and different pH values (Sodium Starch Glycollate–Method I).

The most active soya sample, A, giving a fall in absorption value of 22.6%, has an activity only one-ninetieth of that of the malt, and the other samples range downwards to negligible values. This method, therefore, gives results in general agreement with those of the micro method of Kneen, Sandstedt, and Hollenbeck and with the amylograph, so far as this can be used for quantitative assessment.

Effect of Temperature. Sodium Starch Glycollate Method was used to study the response to temperature changes of the alpha-amylase from both soya and malt. The long incubation time adopted in the first modification of the original method gave anomalously low results for malt as the temperature rose, and it seemed probable that some loss of activity was occurring during the incubation owing, perhaps, to digestion by the proteolytic enzymes in malt. The second modification (Method II; see "Materials and Methods" section) overcame this difficulty and enabled comparable measurements at five different temperatures to be made of the malt extract at five different dilutions and of the soya (sample A) at a single concentration (1%). These results

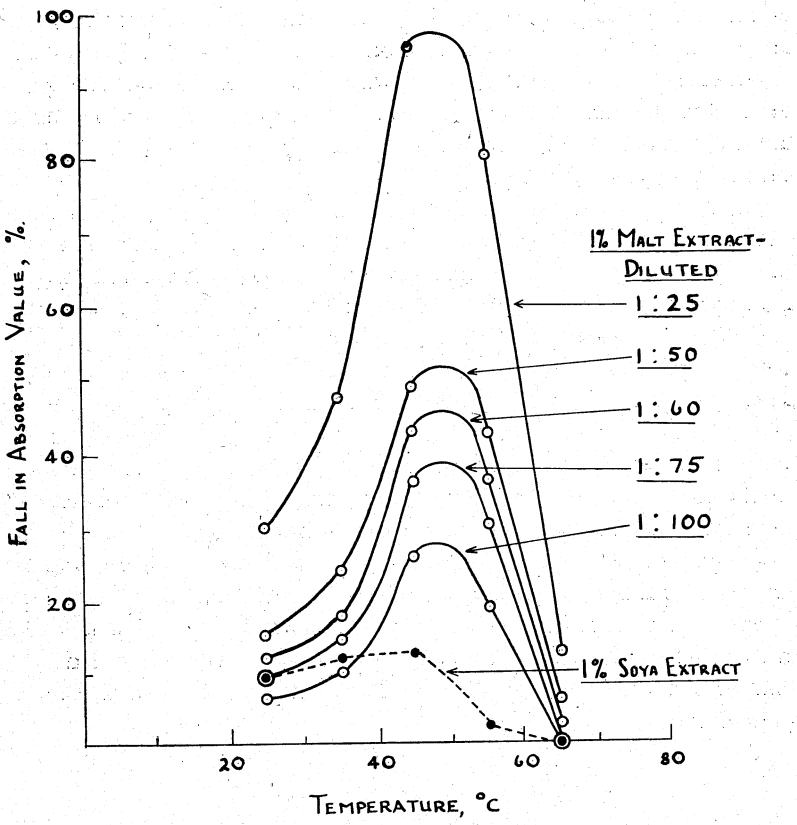

Fig. 5. Effect of temperature on wheat malt flour and soya flour alpha-amylase activities (Sodium Starch Glycollate–Method II).

are grouped in Fig. 5.

The soya alpha-amylase showed little increase with rise in temperature from 25° to 45°C., and beyond this point it fell rapidly to a scarcely measurable activity at 55°C. and complete extinction at 65°C. The 1% malt extract, on the other hand, even when diluted 100 times, showed a sharp increase in activity with rise in temperature, having a peak about 50°C. Thereafter the activity declined, but at 55°C. was still greater than that of soya at any temperature. More concentrated extracts of malt had proportionately greater activity. They also had markedly greater resistance to temperature; the 1:25 dilution of 1% malt extract at 65°C. was still as active as the 1% soya extract at its peak value.

This variation in resistance to temperature with concentration raises interesting questions about the true thermal death point of en-

zymes in the dough during baking. It is difficult to assess the true water-enzyme ratio in a dough, for some of the added water may not be "available." The "availability" of water in a bread dough is a very uncertain matter and becomes more obscure when baking begins. It is therefore difficult to interpret these data in terms of fermentation or baking behavior of doughs.

It is well known that the stability at high temperatures of most, if not all, enzymes is greatly increased as available moisture is reduced. Clearly the stability of alpha-activity of a malt supplement added to a dough at the rate of 0.5 to 1% of the wheat flour (and therefore of the order of 1 to 2% of the total water present) is likely to be appreciably higher than that found for the dilute extracts used in these experiments. The gap between the minute activity of soya and the useful value of malt at fermentation temperatures can only widen as the temperature rises. It is abundantly clear, however, that soya, having a negligible alpha-amylase activity at fermentation temperature, can have little or no effect on starch breakdown from this cause at this stage; and since its activity rises so little with temperature, and is destroyed so quickly at temperatures above 45°C., it cannot, in the quantities in which it is used (about 1% of the wheat flour) contribute anything appreciable to the total alpha-amylase activity of the dough in comparison with malt, or indeed, the wheat flour itself when the bread goes to the oven.

Conclusion

The existence of a starch-liquefying enzyme in raw soya has been confirmed and it has been shown, even in the most potent sample, to be quite negligible in comparison with a sample of malt of normal activity. It is clear that the small liquefying effect of soya is attributable chiefly, if not wholly, to a true alpha-amylase, since Z-enzyme is not measured by the Sodium Starch Glycollate Method, and the ratio of soya activity to malt activity is of the same order when measured by both methods.

The small activity of soya diminishes as pH falls during fermentation and vanishes rapidly as temperature rises. In sum, it cannot have a significant effect on starch breakdown during either fermentation or baking of bread.

The use of soya in breadmaking is not likely, therefore, to contribute on the one hand to difficulties associated with excessive alpha-amylase activity, for example in sprouted wheats, or, on the other hand, to the useful alpha-amylase of malt or fungal enzyme supplements where these are required. In view of its antiproteolytic activity

(4) when prepared from selected beans (5), it does offer a useful natural means of controlling the undesirable proteolysis sometimes produced by malt.

Acknowledgments

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