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The use of fungal enzymes for breadmaking purposes

by

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The Use of Fungal Enzymes for Breadmaking Purposes (*)

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Before discussing in more detail the use of fungal enzymes in bread making a few remarks should be made on the function of amylases in dough.

The action of beta amylase on starch is relatively simple and is characterized by the cleavage of terminal maltose units.

The prime function of alpha amylase is to liquefy and dextrinize starch, the net result being the starch becoming more available to attack by beta amylase and the formation of dextrins. The combined action of alpha- and beta amylase in dough results in a rapid saccharification which provides the fermentable sugar for the yeast. The action on starch, however, is limited since mainly injured granules, either damaged by mechanical force during the milling process or by gelatinization, are susceptible to attack by amylases.

In normal sound flour the amount of beta amylase far exceeds that of alpha amylase, but when the wheat is allowed to sprout there is a pronounced production of alpha amylase, whereas the increase in beta amylase is only small. Flours made of sprouted wheat are difficult to bake out properly due to the formation of excessive dextrins. The dough and the bread crumb are inclined to be sticky and there may be a sagging of the loaves. On the other hand, if a flour has a natural deficiency of alpha amylase, the saccharification is almost limited to that caused by beta amylase and gas production will tend to stop or at any rate be insufficient in the final stages. Obviously an adequate level of alpha amylase activity is essential to produce the desired starch degradation and to ensure sufficient gas production.

In order to correct the deficiency of this enzyme in flours, malt supplements have long been employed in baking. In the past decade, however, there has been considerable interest, especially in the U.S.A., in enzyme preparations from several moulds, in particular from certain strains of *Aspergillus oryzae*, which form, in addition to other enzymes, an amylase of the alpha type (3), (2), (4), (6). Various means of purification are employed in the preparation of these fungal enzymes and it is possible to make concentrates which show marked activity in one direction but very little in certain other directions, for instance with high amylolytic activity but with little effect on protein, or the reverse (7) (5).

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Some properties of the fungal alpha amylase will be mentioned briefly. The enzyme has starch degrading properties similar to cereal alpha amylase but is rather ineffective in reducing maximum viscosity of flour paste, as measured by the Brabender amylograph. This is due to lack of thermostability as a consequence of which the enzyme is already inactivated before much of the starch becomes gelatinized. The crystalline fungal alpha amylase, as obtained by fractional precipitation with ammonium sulfate, is stable in the cold between pH 4.7 and 7.8 and has its isoelectric point at pH about 4. Its activity does not depend on the presence of any ion, in contrast to crystalline malt alpha amylase, which is activated by calcium ions (1).

Coming to the experimental part, the objective of our investigations was to study the feasibility of using fungal enzyme preparations to improve the quality of Dutch white bread. Two types of enzyme concentrates were employed, *Diastase 33* and *Rhozyme-S*, both obtained from the U.S.A. The first exhibited high amylolytic activity with only very small proteolytic activity, the second showed the same high amylolytic activity as well as high proteolytic activity. Ten commercial flours were used, milled from a mixture of Dutch and foreign wheat (extraction about 75 %). In all baking tests a lean dough formula was employed, consisting of flour, water and 2 % salt and yeast, based on the weight of flour. Baking tests were performed under strictly defined conditions, according to the scheme given in table I.

TABLE I

Scheme of the baking test

mixing	10 minutes	
first proof	25 minutes	Dough temp. 28°C.
punching		
second proof	20 minutes	
scaling and rounding		
third proof	20 minutes	
moulding and panning		
pan (final) proof	± 60 - 75 minutes [1]	
baking	30 minutes	Baking temp. ± 250°C.

Dough consistency appeared to decrease in the presence of fungal enzymes, resulting in improved dough handling properties in the case of proper supplementation but in an undesirable slackening of the dough if excessive dosages were used, the effect of *Diastase*, however, being less than that of *Rhozyme*. The explanation is found in the fact that decrease in dough consistency under the influence of alpha amylase is limited by the quantity of susceptible starch, whereas the change in dough consistency under the influence of proteolytic enzymes is proportional to the

[1] **The duration of the pan proof is determined by the time required to produce a given amount of carbon dioxide.**

concentration of the proteinase present and is not limited by the nature of the gluten substrate.

Representative data from the baking tests with various concentrations of *Diastase* and *Rhozyme* are presented in the tables II and III. Attention should be drawn to the fact that very small amounts of enzyme supplements exert a beneficial effect on bread properties as shown by the baking score in the last column. In the case of flour A marked improvement was only obtained by the addition of *Diastase*; with flour B only through *Rhozyme* supplementation. Flour C responded nearly equally well to both *Diastase* and *Rhozyme* supplements, but there was some evidence of too much proleolysis at the higher dosages of *Rhozyme*. In most cases the colour of the crust was slightly improved.

With one out of the ten flours examined detrimental effects were observed through the use of either *Diastase or Rhozyme*. These findings, however, were not surprising since this flour was characterized by a high amylase activity.

TABLE II

Effect of fungal enzyme supplements on baking results

	enzyme supplement		loaf volume	external	crumb	baking
	type	amount mg/1000 g. flour	control = 100 %	appearance (0-12)	characteristics (0-15)	score [1]
Flour A	control	—	100	9	9	40
	Diastase	15	103.5	10	9	48
	Diastase	50	107.5	10	10	57
	Diastase	100	104	10	10	50
Flour B	control	—	100	10	9	42
	Rhozyme	30	105.5	10	10	56
	Rhozyme	50	106.5	10	11	59
	Rhozyme	100	107.5	10	11	62

TABLE III

Effect of fungal enzyme supplements on baking results

	enzyme supplement		loaf volume	external	crumb	baking
	type	amount mg/1000 g. flour	control = 100 %	appearance (0-12)	characteristics (0-15)	score [1]
Flour C	control	—	100	10	8	72
	Diastase	50	105.5	11	9	87
	Diastase	100	105	11	9	87
	Diastase	200	103.5	11	9	82
	Rhozyme	50	106	11	9	91
	Rhozyme	100	101	11	9	82
	Rhozyme	200	99.5	11	8	75

[1] Baking score : $<$ 15 bad; 15 - 30 poor; 30 - 47 nearly sufficient; 47 - 67 sufficient; 67 - 93 good; $>$ 93 excellent

Figure 1 illustrates the effect on loaf volume and crumb characteristics. The upper part shows loaves, baked without and with the addition of small amounts of *Diastase;* the lower part is a cross-section picture of these loaves. A marked improvement in loaf volume and crumb characteristics is easily observed in the case of loaf number two, but with loaf number three the upper safe limit of supplementation has already slightly been exceeded, which is shown by the less desirable break at the side of the loaf and by a less

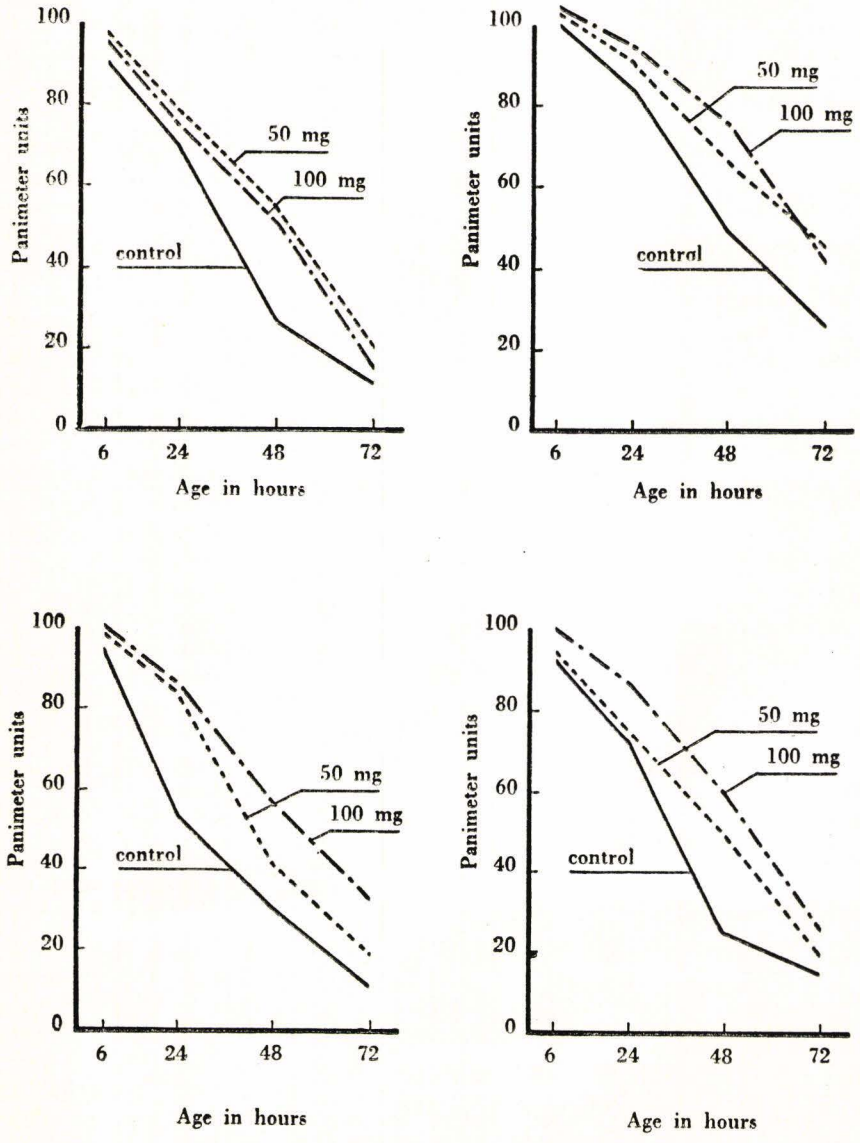

Fig. 4. Effect of *Diastase* supplementation (above) and of *Rhozyme* supplementation (bottom) on crumb compressibility.

uniform grain of the crumb. Figure 2 illustrates similar effects, obtained by the addition of small amounts of *Rhozyme*.

Crumb compressibility was determined after different storage times with the panimeter, shown in figure 3. A standardized piece of bread crumb is subjected to a fixed load and the compression is automatically recorded on a moving strip of paper. The results are expressed in arbitrary units, a higher value indicating better crumb softness. In figure 4 representative schematic diagrams are given of the fall in compressibility with time and the influence of enzyme supplements on the crumb softness of loaves baked from different flours. These diagrams clearly illustrate the beneficial effects, which may be obtained by fungal enzyme supplementation. Even in those cases where the softness of the control was very good, slight improvements have been observed.

Summarizing, the results of baking tests and compressibility measurements indicate that the fungal preparations, if correctly used at a suitable level, may improve the quality of Dutch white bread to a considerable extent.

In order to get a better understanding of the effect of fungal enzyme supplementation on baking results, the influence on maltose value, gassing power and maximum flour paste viscosity was determined. Employing the same levels of enzyme concentrates as in baking, the maltose value was raised only very slightly, whereas gas production, measured over a period of six hours, was increased considerably. The influence of *Diastase* appeared to be less than that of *Rhozyme*. A typical example of this effect is given in figure 5, obtained by plotting the increase in carbon dioxide per unit of time at intervals from one to six hours. It should be noted that the increase in the rate of gas production is small up to about two hours, becoming more significant from the third hour on. The major effect is apparently not produced until several hours have passed. Furthermore, the rate of gas production of the control dough starts to diminish at a point, where the increase in gas producing capacity of the treated doughs is still maintained. In view of the relatively short fermentation time usually employed in the Netherlands, it is obvious that this beneficial effect is only partly utilised. Nevertheless it may be assumed that the increased gas production, which becomes even more pronounced under the action of heat during the first half of the baking process, contributes to better oven spring and hence to the emprovement in loaf volume.

It was further found that the increase in maltose figure, brought about by the addition of the enzyme concentrates is not a reliable measure of the concomitant improvement in gas production.

Viscosity measurements with the Brabender amylograph showed that the same small amounts of *Diastase* or *Rhozyme* hardly affected maximum flour paste viscosity, because of their rather low inactivation temperature. Treatment with fungal enzymes therefore permits the formation of sugars without any appreciable decrease in the viscosity of the gelatinized starch. The formation of dextrins at the elevated temperatures during the baking process will be held to a minimum and the choice of fungal alpha amylase level might consequently not be as critical as that of malt alpha amylase, which is characterized by a relatively high inactivation temperature.

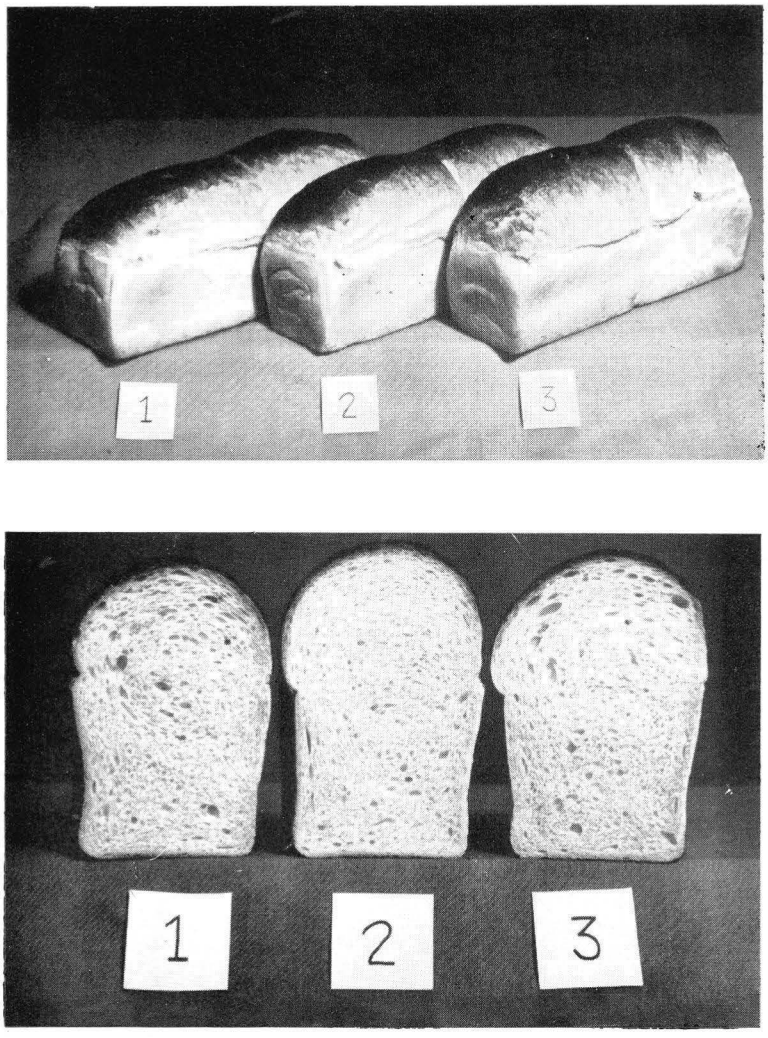

Fig. 1. Effect of *Diastase* supplementation on loaf properties and crumb structure. 1 = control; 2 = + 100 mg. *Diastase*/1000 g. flour; 3 = + 200 mg. *Diastase*/1000 g. flour

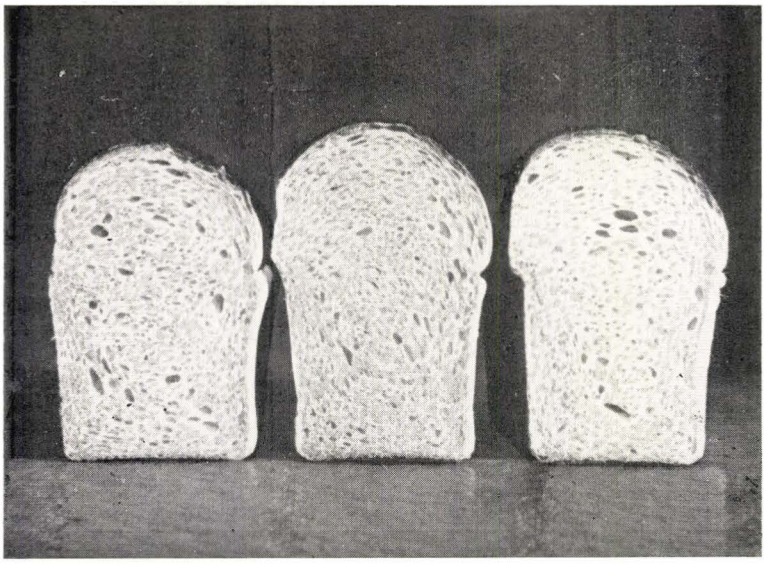

Fig. 2. Effect of *Rhozyme* supplementation on crumb structure.
Control + 50 mg. *Rhozyme*/ 1.000 g flour + 100 mg. *Rhozyme*/ 1.000 g flour

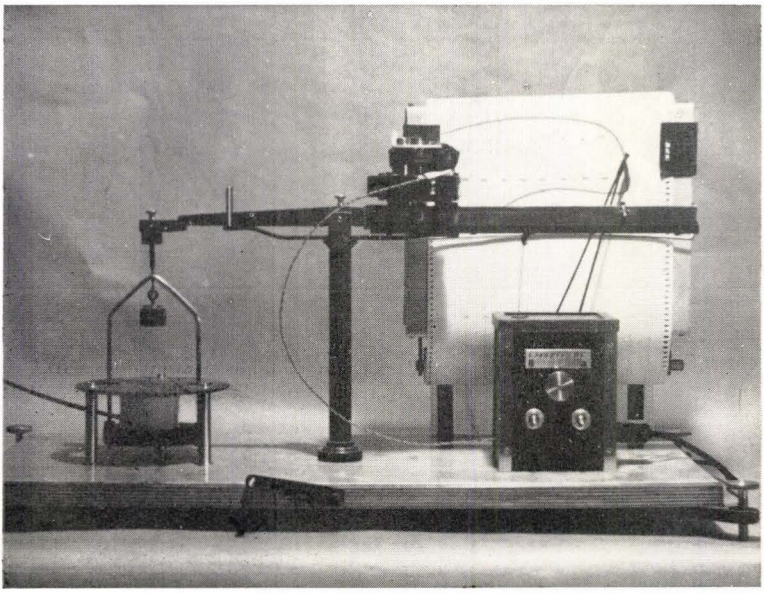

Fig. 3. Panimeter.

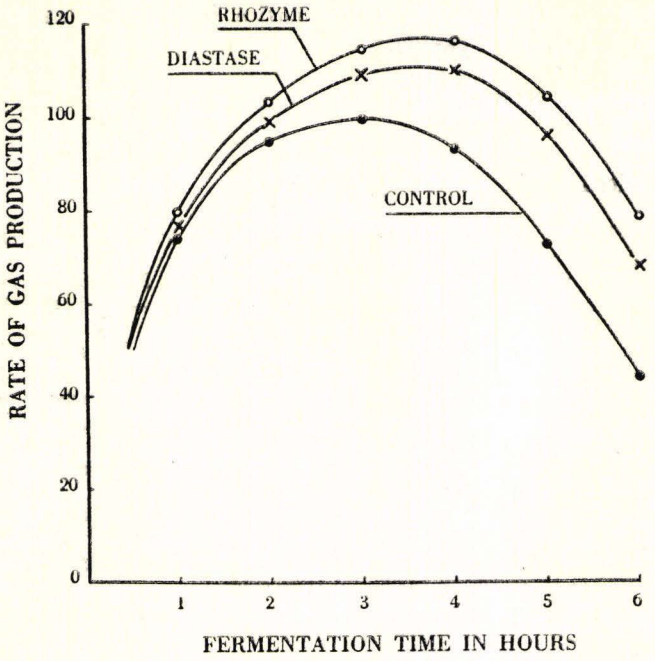

Fig. 5. **Effect of fungal enzyme supplementation on rate of gas production.**

On the whole these findings, which confirm the observations of other investigators, seem to indicate that the influence of *Diastase* and *Rhozyme* on baking results is only partly paralleled by the influence on gassing power. It is not quite impossible that increased softness and keeping quality may partly be accounted for by mild degradation of the starch under the action of the fungal amylases. The proteolytic activity of *Rhozyme* may possibly cause an increase in availability of starch and a liberation of bound beta amylase, resulting in an acceleration of starch hydrolysis and finally in increased gas production. It is realized, however, that other factors, not determined by the three methods employed, influence the response of a flour to both types of fungal enzymes, such as the alpha-amylase content of the flour, the nature of the starch granules, the quantity of susceptible starch, the properties of the gluten proteins and the influence of the proteolytic enzymes on bound or so-called latent amylases.

Some remarks will finally be made as to the means of controlling fungal enzyme supplementation and to the use of these enzymes in the flour industry. Since maximum paste viscosity is not correlated with maltose value and gassing power determination, and these methods often yield divergent results, the baking test up to now still remains the final criterion of proper supplementation.

Although much has been learned about the action of these enzymes, additional fundamental information is required to control their action in breadmaking. However, the advantage of a product, carefully standardized

as to both amylolytic and proteolytic activity and with the further advantage that generally very small additions will suffice, promise well for the future use of fungal enzymes in baking.

(Wageningen, September 1952.)

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