ACTA ALIMENTARIA POLONICA
Vol. XIII (XXXVII), No. 1 1987

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PHYTASE ACTIVITY IN FERMENTATIVE MICROFLORA OF DOUGH

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Key words: Phytase, *Saccharomyces cerevisiae*, lactic acid bacteria, dough.

The research was intended to establish whether phytase is produced by the baker's yeast *Saccharomyces cerevisiae* and by the liquid preferment microflora: the yeasts *Saccharomyces minor* and lactic acid bacteria. It was found that phytase is present in *Saccharomyces cerevisiae* and *Saccharomyces minor*, and absent in lactic acid bacteria cells. The determined activity values suggest that the yeast phytase is probably of slight practical significance since it is capable of decomposing less than 5% of the total phytates in flour.

INTRODUCTION

Phytase, an enzyme hydrolyzing phytates occurs mainly in plant material alongside its substrate. The enzyme was also found to occur in the gastrointestinal tract of some animals, such as rats chicken, calves and sheep [1]. It is believed that it may also be produced by some microorganisms [4, 9, 10].

It is still unclear whether phytate hydrolysis during fermentation of bread leavens and dough is due exclusively to the phytase contained in flour. It may be that the enzyme is also produced by the fermentative microflora, thereby participating in the decomposition of phytic compounds. These issues have never really been investigated. There were only a few studies of phytates hydrolysis in the successive phases of rye and wheat dough preparation indicating, the possibility that this decomposition may also be connected with the action of fermentative microflora [2, 4, 8, 12].

The literature contains diverse views on the role of pressed yeasts added to the fermenting dough in this matter. According to Ranhotra et al. [8] the yeasts contain active phytase, since their addition intensifies phytate hydrolysis. However, some researchers do not link the effect of

yeats on the hydrolysis of phytic compounds with the presence of phytase in these microorganisms. For example, Tangkongchitr et al. [12] suggest that a yeast addition merely creates better conditions for phytate decomposition by changing the acidity of the medium.

It was thus decided to investigate whether phytase is produced by the fermentative microflora of bread leavens, i.e. by yeasts and lactic acid bacteria.

MATERIAL AND METHODS

The experiments were performed with five samples of baker's yeasts *Saccharomyces cerevisiae*:

— commercial pressed baker's yeast,

— commercial dry active baker's yeast,

— commercial pressed baker's yeast dried at 32°C in laboratory conditions,

— freeze-dried "Fleischmann's yeast active dry",

— freeze-dried "DCL active dried yeast" from Distillers Company (Yeast) Ltd.

The sample of commercial pressed yeast was also used to multiply the biomass of these yeasts in the laboratory (on suitably diluted beer wort, with intense aeration).

The yeasts *Saccharomyces minor* and lactic acid bacteria were isolated from the liquid preferment and multiplied on the Blickfeldt and the MRS medium, respectively, until there was enough biomass to perform the analytic determination [5].

The microorganism cultures, both of the yeasts and the bacteria, were maintained on two kinds of media, either with or without sodium phytate. The sodium phytate (sodium salt of inositol hexaphosphoric acid obtained from BDH Chemicals, Ltd) was applied in doses of 0.5, 0.8 and 1.0 g/100 cm^3 of the medium, corresponding to 100, 160 and 200 mg phytic P, respectively.

Phytase activity was determined by Peers' method [6] consisting in the colorimetric determination of the amount of inorganic phosphorus liberated over a given period by phytase from the sodium salt of inositol hexaphosphoric acid under conditions optimal for the enzyme's activity (55°C, pH 5.1).

RESULTS AND DISCUSSION

PHYTASE ACTIVITY IN *SACCHAROMYCES CEREVISIAE*

Table 1 contains results of the study of phytase activity in the various samples of commercial baker's yeasts. The only increment of inorganic P

Table 1. Phytase activity in various samples of the yeasts *Saccharomyces cerevisiae*

Sample	Inorganic P (mg/100 g of dry mass) liberated from sodium phytate after:		
	3 min	60 min	180 min
Commercial pressed baker's yeasts	0	0	383
Commercial dryactive baker's yeasts	545	545	545
Commercial pressed baker's yeasts dried in laboratory	397	397	397
Fleischmann's active dry yeast	131	131	131
DCL active dried yeast	132	132	132

during the three hours of determination was observed in the pressed fresh yeasts which indicates that they contain phytase. This confirms the results of earlier studies demonstrating the presence of active phytase in baker's yeasts. For example, Ranhotra et al. [8] found that an addition of yeasts to fermenting wheat dough doubled the amount of hydrolysed phytic acid, explaining this phenomenon by the presence of phytase in the yeasts. These authors determined the phytase activity in yeasts by the method used in this research and found it to be 135 units (mg inorganic P liberated during 1 h of sample incubation).

It is hard to say why, unlike in the case of fresh yeast, no increment of inorganic P was found during the three hours of incubation of the dried yeast samples. Perhaps the effects of phytase would have become apparent if the incubation period was extended beyond the three hours specified in Peers' method. However, such an experiment would have no practical significance since the fermentation of dough usually takes no more than three hours.

One should also comment the high values of inorganic P in the "blank" sample (the amount of inorganic P determined after 3 min of sample incubation, i.e. directly after the addition of the studied enzymatic extract to the sodium phytate solution) that were obtained for dried yeast. It is possible that during the production of lyophilized yeasts (DCL and Fleischmann) the freezing of the microorganisms could damage cell walls which in turn might lead to a passage of a part of the phosphates from the yeast cell to the water extract during the preparation of the enzymatic extract. As for the yeasts dried traditionally, the high inorganic P content determined in the "blank" sample could have been due to the following causes:

— the temperature during drying could have increased the activity of yeast cell enzymes, including those hydrolysing phosphorus compounds,

— drying could have reduced the permeability of cell membranes, a consequence of which would be easier leaching of phosphate ions during the preparation of the enzymatic extract.

Given the fact that the determinations of phytase activity in yeasts did not give positive results in all cases, it was assumed as possible that the enzyme is adaptive. Accordingly, this activity was studied in the presence of a substrate for phytase activity. To this end, yeasts were multiplied for 24 h on wort with 0, 100 and 200 mg phytic P additions per 100 cm^3 of wort. The activity of phytase in yeast samples thus prepared was determined by measuring the liberated inorganic P after 3 (blank sample), 60, 180 and 240 min.

The obtained results (Table 2) apparently indicate the presence of phytase in yeast cells, since about 600 mg increase of inorganic P content was found in the samples multiplied on wort with sodium phytate after 60 min of incubation. The return of the inorganic P content to the level observed in the "blank" sample after a further several hours of incubation could have been due to phytic acid rephosphorylation. According to Ranhorta [7], phytic acid is decomposed by the enzyme, and then, as the PO_4^{3-} ions and mioinositol accumulation progresses, there is a partial "rebuilding" of this acid.

Table 2. Phytase activity in *Saccharomyces cerevisiae* multiplied on beer wort with and without sodium phytate addition

Sample	Phytic P (mg/100 cm^3 of medium)	Inorganic P (mg/100 g of dry mass) liberated for sodium phytate after			
		3 min	60 min	180 min	240 min
Yeast	0	612	612	612	612
	100	650	1300	650	650
	200	575	1150	1150	575
Supernatant (wort after centrifugation of yeast)	0	62	62	81	62
	100	112	125	137	112
	200	125	131	150	125

The obtained results also indicate that the contribution of yeast phytase in the decomposition of phytate during dough fermentation is of no great consequence. Considering the small yeast addition to flour (about 3%) and the fact that dough fermentation takes place in conditions which are not optimal for the enzyme activity (pH, temperature, phytase inhibitors in the flour) it must be assumed that yeast phytase is not capable of decomposing more than 5% of the phytates in the flour (or, in absolute values, 10 mg phytic P/100 g of flour).

Also studied were supernants obtained by separating the yeasts from the wort by centrifugation with the view of determining whether phytase is an exoenzyme. The amount of inorganic P determined in supernatants was 3-20-fold lower than in the yeast extracts (Table 2). It was found that during three hours of sample incubation the inorganic P content

increased slightly, this being indicative of modest enzyme activity. The prolongation of incubation time to 4 h caused a drop of inorganic P content to the blank sample level (similarly as in the case of yeast); a possible cause of this was partial rephosphorylation of phytic acid.

ACTIVITY OF PHYTASE OF *SACCHAROMYCES MINOR* AND OF LACTIC ACID BACTERIA ISOLATED FROM LIQUID PREFERMENT

The yeasts isolated from liquid preferment were cultured in the Blickfeldt medium with a sodium phytate addition of 0.8/100 cm^3 of the medium (160 mg phytic P) and in the same medium without this addition. After a morphological characteristic the yeasts were identified as *Saccharomyces minor* [11]. The results of determinations of phytase activity in the isolated yeasts and in the supernatants left after centrifugation of the yeasts from the medium are presented in Table 3.

Table 3. Phytase activity in *Saccharomyces minor* isolated from liquid preferment

Sample	Phytic P (mg/100 cm^3 of medium)	Inorganic P (mg/100 g of dry mass) liberated from sodium phytate after:			
		3 min	60 min	180 min	240 min
Yeast	0	312	312	625	625
	160	312	312	625	625
Supernatant (wort after centrifugation of yeast)	0	6.5	6.5	6.5	9.5
	160	12.5	12.5	12.5	18.7

An increase in the amount of liberated inorganic P (by about 300 mg) was observed after 3 h of sample incubation, and this would indicate the presence of active phytase in both kinds of yeast samples multiplied with or without the sodium phytate in the medium. It is difficult to compare the obtained results with those of other authors since the available literature contains almost no data on this subject; the only pertiment work is that by Ranhotra et al. [8], already quoted, which deals with phytate activity in *Saccharomyces cerevisiae*. No data about the activity of phytase in yeasts from liquid preferments were found. The phytase in *Saccharomyces minor*, similarly as that of *Saccharomyces cerevisiae*, has only a slight effect on the hydrolysis of phytates in flour.

The study of supernatants obtained by centrifuging the yeast from the medium also revealed (after as much as 4 h of sample incubation) a slight increase of inorganic P, of about 3 mg and about 6 mg (Table 3). These small amounts appear to indicate that only minute amounts of phytase are liberated from the yeast cells.

The bacteria were cultured similarly as the yeasts, with or without 0.8 g of sodium phytate added to 100 cm^3 of the medium. The results are shown in Table 4.

Table 4. Phytase activity in lactic acid bacteria isolated from liquid preferment

Phytic P (mg/100 cm^3 of medium)	Inorganic P (mg/100 g of dry mass) liberated from sodium phytate after:			
	3 min	60 min	180 min	240 min
0	1875	1562	1875	1875
160	1875	1562	1875	1875

No increase of the inorganic P content was found during 4 h of determinations, and this would mean that there is no phytase in bacteria isolated from the liquid preferment. This is true of both kinds of culture, either with or without the sodium phytate addition. Noteworthy in Table 4 is the very high value for the blank sample. It seems that this value may be due to the composition of the medium used for culture [5], as well as to the duration of the cultivation (about five days) during which the organic phosphate compounds may have decomposed.

In conclusion, it is worth pointing out yet another fact, namely that although no phytase activity was observed in the lactic acid bacteria, one cannot preclude the possibility that this enzyme exists in the cells of these microorganisms. However, this phytase may have properties that are completely different from those of plant phytase. This is indicated by few reported research concerning the properties of phytase of microorganisms, e.g. of *Aspergillus niger* [10]. It is thus very likely that Peers' method, universally used to study phytase activity in cereal products and applied also in this work, ought to be modified for the study of phytase from fermentative microflora. It therefore seems advisable that a correct methodology be worked out in the future studies on the subject in hand.

CONCLUSIONS

1. The obtained results do not allow to state univocally whether phytase is produced by the yeast *Saccharomyces cerevisiae*: phytase was found only in samples of commercial pressed yeasts while studies of dried baker's yeasts and of yeasts multiplied in laboratory conditions on media either with or without an addition of sodium phytate indicate the absence of active phytase in the cells of these yeasts.

2. The studies of phytase activity in *Saccharomyces cerevisiae* and in lactic bacteria isolated from rye leaven indicate the presence of active phytase in yeast cells and the absence of this enzyme in lactic bacteria cells.

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Manuscript received: August, 1985
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BADANIA NAD AKTYWNOŚCIĄ FITAZY MIKROFLORY FERMENTACYJNEJ CIASTA

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Streszczenie

Przebadano mikroflorę fermentacyjną zaczynów piekarskich — drożdże *Saccharomyces cerevisiae* i *Saccharomyces minor* oraz kultury bakterii kwasu mlekowego pod kątem możliwości wytworzenia enzymu fitazy. Podczas badania różnych próbek drożdży *Saccharomyces cerevisiae* zaobserwowano przyrost ilości $P_{nieorg.}$, świadczący o obecności fitazy jedynie w przypadku drożdży handlowych prasowanych. Natomiast wyniki uzyskane podczas badania drożdży piekarskich suszonych wskazują na brak aktywnej fitazy w ich komórkach (tab. 1). W czasie badania drożdży *Saccharomyces cerevisiae* namnażanych na pożywce z dodatkiem fitynianu sodu będącego substratem dla działania fitazy, stwierdzono również obecność aktywnej fitazy (tab. 2). Zaobserwowano również przyrost ilości $P_{nieorg.}$ w trakcie badania drożdży *Saccharomyces minor* wyizolowanych z zakwasu żytniego, co wskazywałoby na istnienie aktywnej fitazy w tych drożdżach (tab. 3).

Uzyskane wyniki pozwalają przypuszczać, że fitaza drożdży nie ma większego znaczenia praktycznego, ponieważ w warunkach fermentacji ciasta enzym ten jest w stanie rozłożyć nie więcej niż 5% fitynianów zawartych w mące. Wyniki uzyskane w czasie badania aktywności fitazy w bakteriach mlekowych wyizolowanych z zakwasu żytniego wskazują natomiast na brak tego enzymu w komórkach tych drobnoustrojów (tab. 4).