THE INFLUENCE OF SOME EXO-ENZYMES ON THE HYDROLYSIS REACTION OF STARCH WITH WHEAT AMYLASE

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Abstract

The influence of some exo-enzymes (xilanase, protease, and papain) was studied on the hydrolysis reaction of starch with amylase isolated from wheat flour, using the colorimetric determination of amylase activity with DNS reagent, modified by the authors. The exo-enzymes studied, acted as enzymatic effectors, xilanase being activator and protease and papain being amylase inhibitors.

Key words: exo-enzymes, wheat amylase, starch hydrolysis

INTRODUCTION

This paper presents the influence of some exo-enzymes (xilanase, protease and papain) usually used as bread improvers (Reed G., et.al.1993).

The amylase activity can be measured following the decrease of the viscosity of a starch solution, the decrease of the turbidity of a starch suspension, the decrease of the intensity of a starch-iodine reaction and the increase of the reducing groups in the reaction medium. The last method is in agreement with the EC-IUB demands (Grog N, et. al, 1992).

The amylase activity is measured using a colorimetric method with DNS V.Grog,et.al.1985), modified by the authors in order to ensure the appropriate conditions for the hydrolyse of starch in baking industry;

- The hydrolyse reaction had place at pH 5.5 (the optimum pH for wheat amylase);
- As enzymatic activator we used a solution of $CaCl_2$ (Ca ions are activators for the wheat amylase) in appropriate concentration 0,01M (McGregor A.,et.al.1993);
- The hydrolyse reaction occurred at 30ºC, in accordance with that of the dough fermentation in baking industry.

The starch is hydrolysed on the catalytic action of amylase to fragments, which can be determined with 3, 5-dinitrosalicylic acid, due to their semiacetalic reducer groups. The formed nitroaminosalycilic acid concentration is corresponded to the enzymatic activity of amylase.

MATERIALS AND METHODS

Reagents

Soluble starch supplied by Merck, Darmstadt was used in 1% concentration in acetate buffer solution at pH 5, 5 which contain $CaCl_2$ 0,1M (Kennedy J, et.al.1988).

The DNS reagent (Merck, Darmstadt) was obtained from 1g 3, 5-dinitrosalcylic acid dissolved in 20 ml NaOH 2N, adding 30g double tartrat of natrium and potassium and completed with distillate water to obtain 100ml solution.

It was used an enzymatic extract of alpha and beta amylase from wheat flour prepared by extracting 10 g wheat flour in 100 ml distillate water for 30 minute using a magnetic stirring, than centrifuging at 6000 rpm and the supernatant obtain was dilute 10 times in distillate water (Cărăban A. et.al.2008)

Maltose was used as standard solution in the concentration as 100 μg/ml.

As exoenzymes were used fungic xillanase from *Aspergillus niger* (X), protease from *Carica Papaya,* (P1), protease from *Aspergillus oryzae* (P2) and bacteria protease from *Bacillus subtilis,* (P3) supplied by Worthington Biochemical Corporation, USA.

Colorimetric analysis

The reaction mixture formed by soluble starch solution, the amylase extract and the analysed factors (exoenzymes) was incubated for 5 minutes at 30ºC for the enzymatic hydrolyse reaction and then the reaction was stopped with DNS reagent and by boiling the reaction medium for 5 minutes. After cooling, the mixture was colorimetered at 546 nm related to distillate water (Cărăban A. et.al.2006).

To determine the reducing sugars existing in the reaction medium at initial moment, for all samples were made controls, identically with the tests, except that in the controls there was no enzyme.

To transform the optical densities read for the tests and controls in moles maltose it was made a standard curve in concordance with the data from table 1.

Table 1

Standard curve

Maltose (ml)	0,1	0,3	0,5	0,7	1	-
Distillate water (ml)	0,9	0,7	0,5	0,3	-	1
DNS reagent (ml)	1	1	1	1	1	1
Boiling 5 minutes						
Distillate water (ml)	8	8	8	8	8	8
Extinction	0,150	0,305	0,455	0,605	0,765	0,800

The experiment results were calculated as follow: the optical densities for the controls were subtracted from the test extinction and the results were expressed in μmoles maltose enzymatic delivered/1ml/1min.

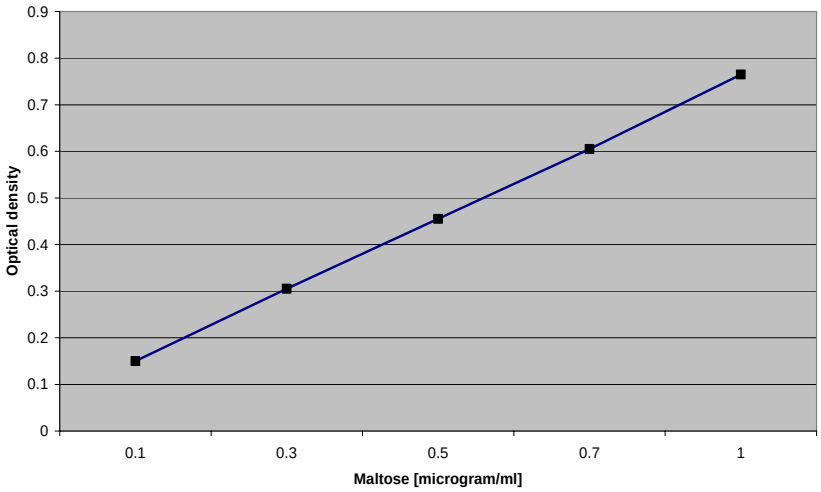

Fig.1. The standard curve

The experiments were made as shown in table 2.

Table 2

Lab techniques

	Control	**Sample**
Starch (ml)	0,5	0,5
Distillate water (ml)	0,3	0,3
Exoenzymes analysed	0,1	0,1
Amylase extract (ml)	-	0,1
Incubation 5 minutes		
DNS reagent (ml)	1	1
Boiling 5 minutes		
Distillate water (ml)	8	8

RESULTS AND DISCUSSION

The influence of the exoenzymes on the hydrolysis reaction is presented in table 3 and in the figure 2.

Table 3

The influence of the exoenzymes on the hydrolysis reaction of starch with amylase

No.crt.	**Exo-enzymes**	**Control extinction**	**Sample extinction**
1.	Xilanase (X)	0,450	0,510
2.	Protease Carica papaya (P1)	0,450	0,385
3.	Protease Aspergillus oryzae (P2)	0,450	0,405
4.	Protease Bacillus subtilis (P3)	0,450	0,360

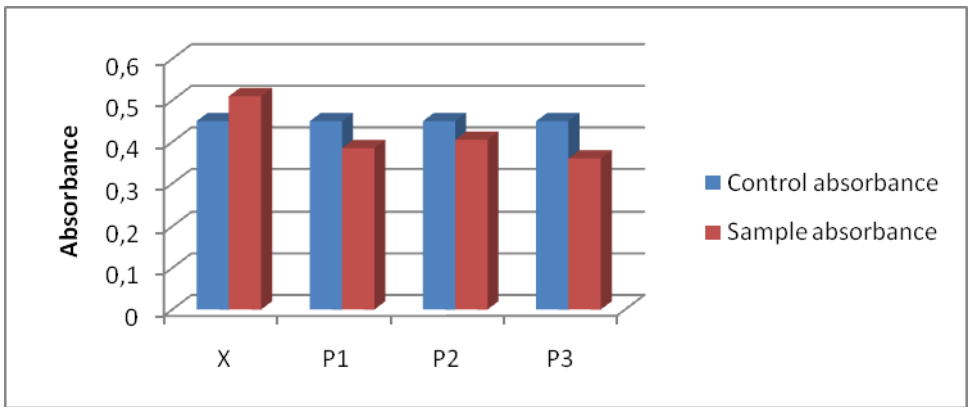

Fig.2 The influence of exo-enzymes studied over the hydrolysis reaction

CONCLUSIONS

The substances used as food additives influenced the reaction of starch hydrolysis with wheat amylase, some of them acting as activators or inhibitors for wheat amylase.

Xillanase acted as wheat amylase activator increased the rate of the starch hydrolysis reaction. All the proteases studied acted as amylase inhibitors and had a slightly effect in inhibition the wheat amylase activity.

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