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A new method and system for real time fermentation process monitoring

OPTICAL ANALYSYS REGARDING STARCH HYDROLYSYS REACTION IN REAL TIME - kinetic studies in real time Internal Report

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OPTICAL ANALYSYS REGARDING STARCH HYDROLYSYS REACTION IN REAL TIME

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Abstract: *This paper presents the influence of the some exogenous enzymes on the starch hydrolysis reaction using laser interferometry techniques. The principle of this method consists in determination of refractive index changes of starch - amylase biochemical system using a laser interferometer. The fringes of interference modification in time are acquired in real time by a C.C.D. camera. The image processing and analysis and also the drawing of the graphics are realised by using a computer program made by the authors.*

1. Introduction

The starch hydrolysis with amylases is a very important reaction in biotechnology [1]. The amylases which catalysed starch hydrolysis, so called α- and β-amylase (E.E.C. 2.1.1.1. and E.E.C. 2.1.1.2) attack the α-1, 4-glycosidic bonds from amylopectine and amylose, and form maltose as major hydrolysis product. The classic methods for amylase activity determination do not allow the study of rate of hydrolysis reaction in real time [2].

To determine the rate of the amylase activity, the authors had created and realised a device which function principle is based on the determination of the refractive index variations at the starch hydrolysis with amylase. At each hydrolysis step it takes place a modification of the refractive index, variation that is determined in real time.

The system realised is one complete integrating. So, using a Michelson interferometer is determined the modification in real time of the interference fringes due to the refractive index variations of the biochemical starch-amylase system, modification that is acquired and processed in real time by a computer which had attached a CCD camera [3].

The Michelson interferometer used provides interference fringes, which are formed on a screen made by a white sheet of paper, and so, the visual sensor that is located on the opposite side of the screen, acquires the image in optimum conditions. On the screen appear successive images with interference fringes (Heidegger rings).

The solution refractive index is changing in time, in the same way that the hydrolysis reaction occurs, so on the screen appear new interference maxims that correspond to the different hydrolysis steps.

The CCD visual sensor acquires the image formed on the screen and sends it to the Matrox IP 8 data acquisition board. The CCD sensor used had a density of 10000 receptors/mm^2 uniform distribute, and the total number of the receptor is 640x480, that determine a high resolution of the system.

The program realised and elaborated in C++ language offers the possibility of acquiring and processing images, processing which consists in the determination of the number of changes in the interference fringes [4, 5, and 6].

In figure 1 is presented the principle scheme of the conceptual and realised device.

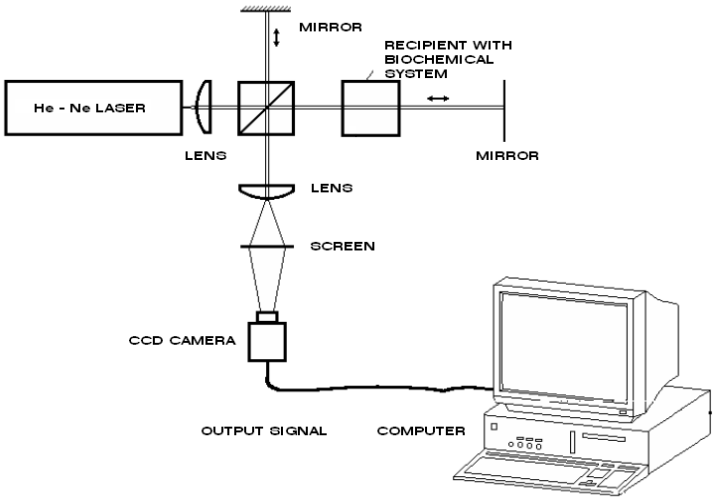
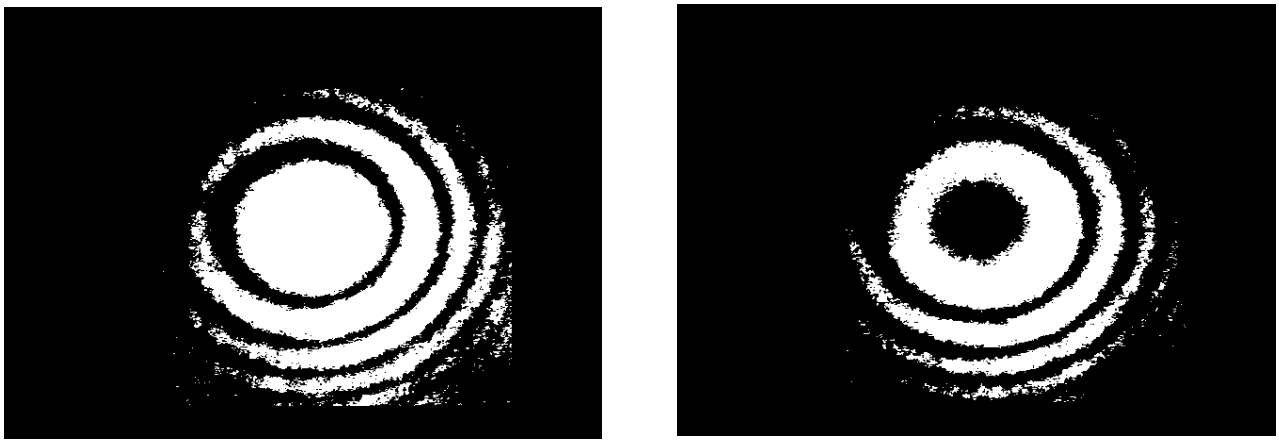


Figure 1 a. The principle scheme of the device



a) minimum of interference; *b) maximum of interference*

Figure 1b. Imagines acquired by CCD

2. Experimental

***Reagents*:**

1. Soluble starch supplied by Merck, Darmstadt was used in 1% concentration in aqua's solution.

2. 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 for 10 minutes. The supernatant obtain was dilute 10 times in distillate water. The extract represents the endogenous amylase.

3. Exogenous enzyme preparations containing alpha-amylase, xylanase, cellulase and fungal and bacterial protease:

- AF1 - fungal α-amylase, SKB 1800 isolated from selected strains of Aspergillum oryzae.
- AF2 - fungal α-amylase, SKB 40000, isolated from selected strains of Aspergillum oryzae,
- AF3 - SKB 10000 fungal α-amylase isolated from selected cultures of Aspergillus oryzae;
- AF4 - fungal and bacterial α-amylase SKB 2000AF5 - fungal α-amylase SKB 2000C - fungal cellulase derived from Trichoderma reesei strains;
- AX - α-amylase and xylanase isolated from fungal sources SKB 2500 Aspergillus niger and Aspergillum oryzae;
- AP1 - fungal α-amylase and protease isolated from Aspergillum oryzae;
- P2 - papain extracted from Carica Papaya.
- P3 - bacterial protease from Bacillus subtilis enzyme with activity influenced by starch.
- C- Cellulase extracted from Trichoderma reesei strains

Interferometer analysis:

It was studied the influence of the presence of some exoenzymes on the starch hydrolysis reaction with endo-amylases using laser interferometry techniques following the mode work presented in table no. 1

In the hydrolysis system soluble starch - endogene amylase from wheat flour is inserted after the work mode presented in table 1, diferent exoenzymes and measure the rate of hydrolysis for each sample.

At the same time, is performed and a control containing no exoenzymes, and which is determining the rate of hydrolysis, under the same conditions.

Table 1. Lab techniques

	Control	Sample
Starch (ml)	0,6	0,6
Endogen enzymes (ml)	0,6	0,3
Exogenous enzyme* (ml)	-	0,3
DW (ml)	0,8	0,5

In the device cuvette are inserted starch solution, distilled water and simultaneously two types of enzymes: endoamylase extract of wheat flour and the fungal exoenzymes. The changes of the interference fringes are monitored and plotted on the computer screen.

3. Results and discussions

The graphics (numbers of changes vs. time) were realised by using a computer program made by the authors and are shown in the following figures and represent the kinetic enzymes.

Figure 1. Control

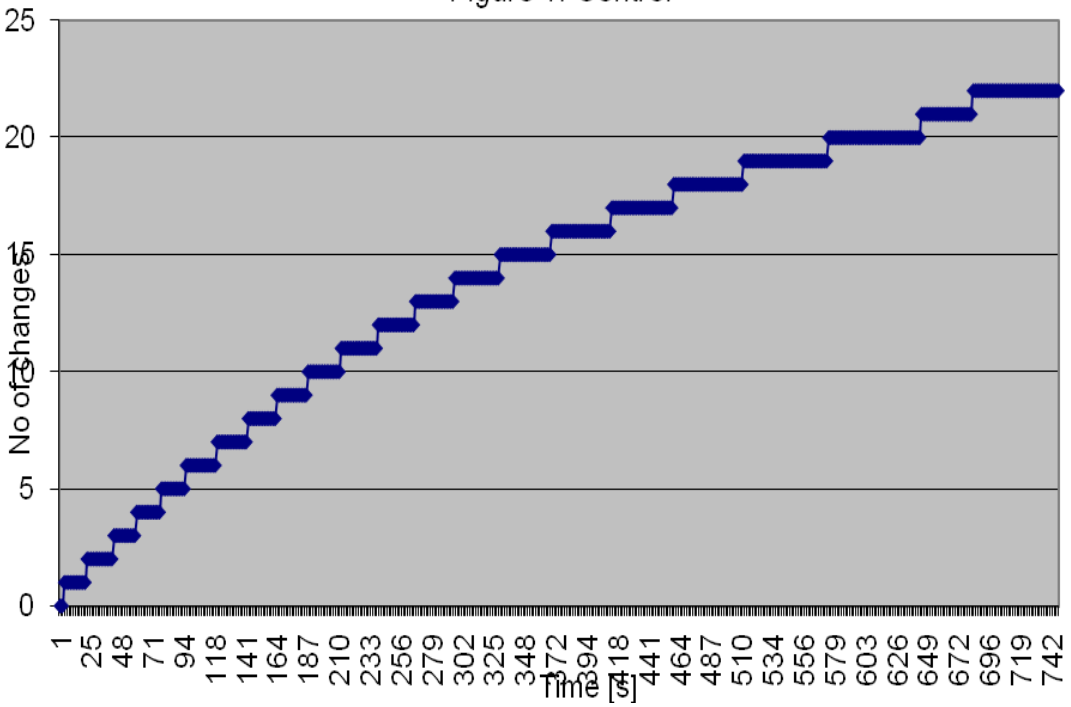


Figure 2. Influence AF1

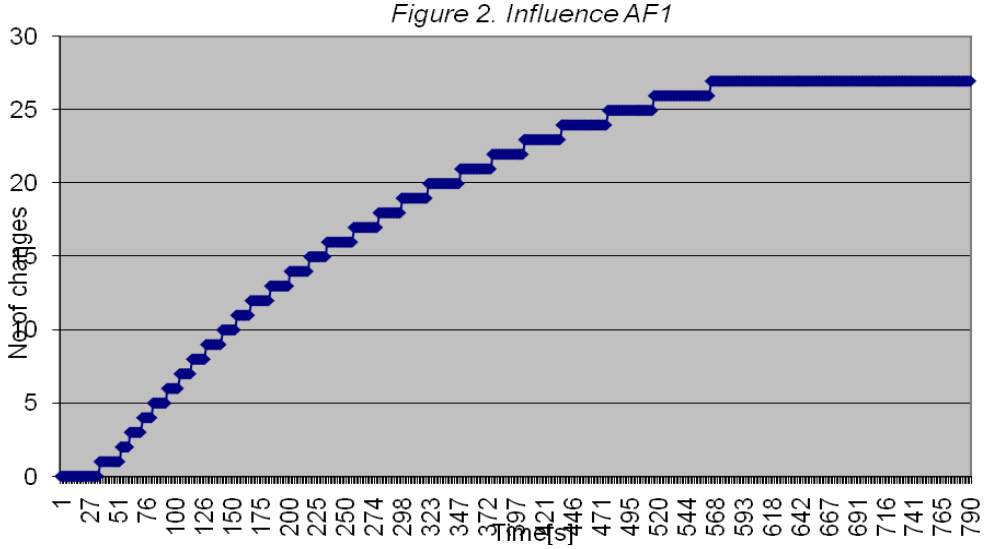


Figure 3. Influence AP1

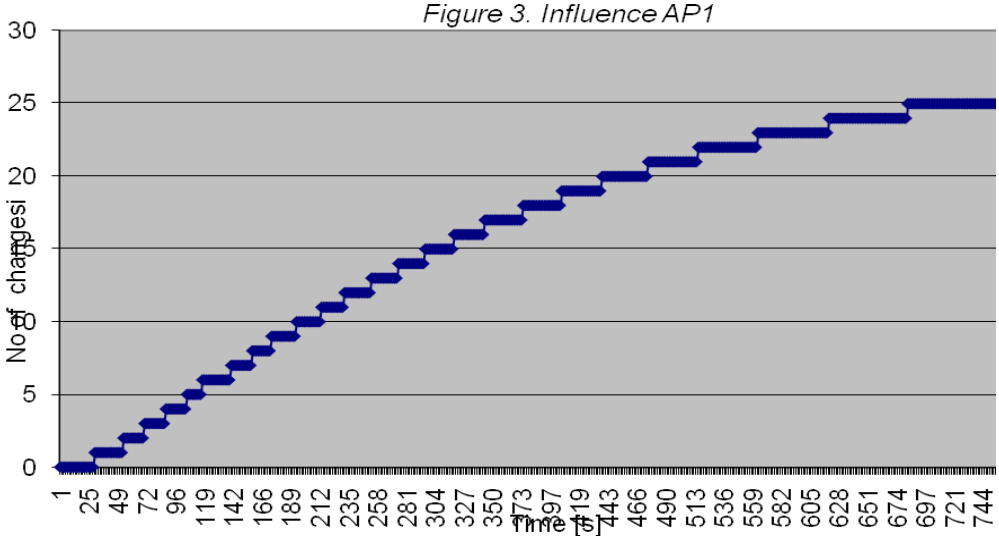


Figure 4. Influence AX

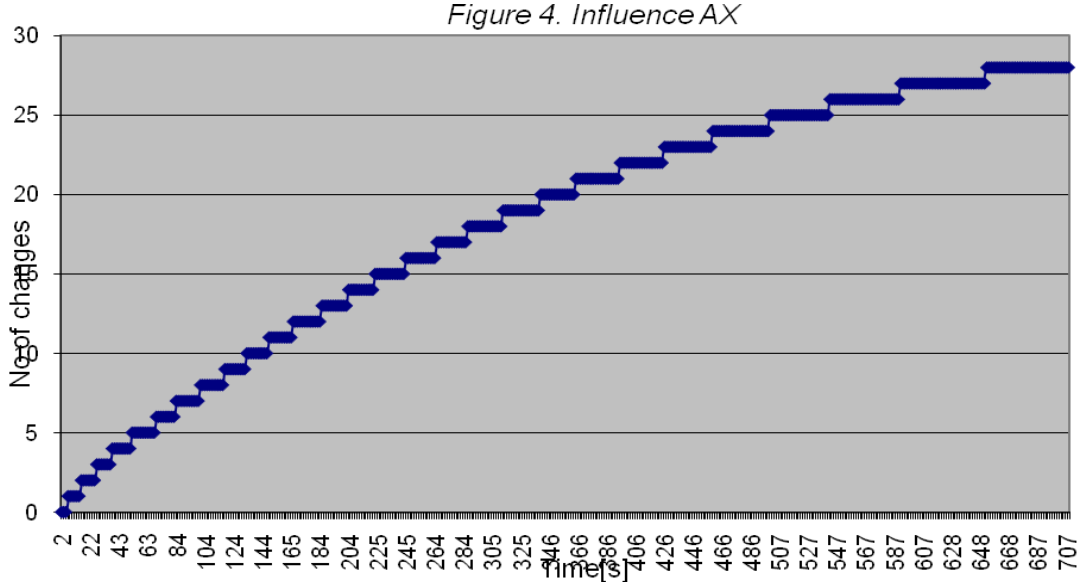


Figure 5. Influence P2

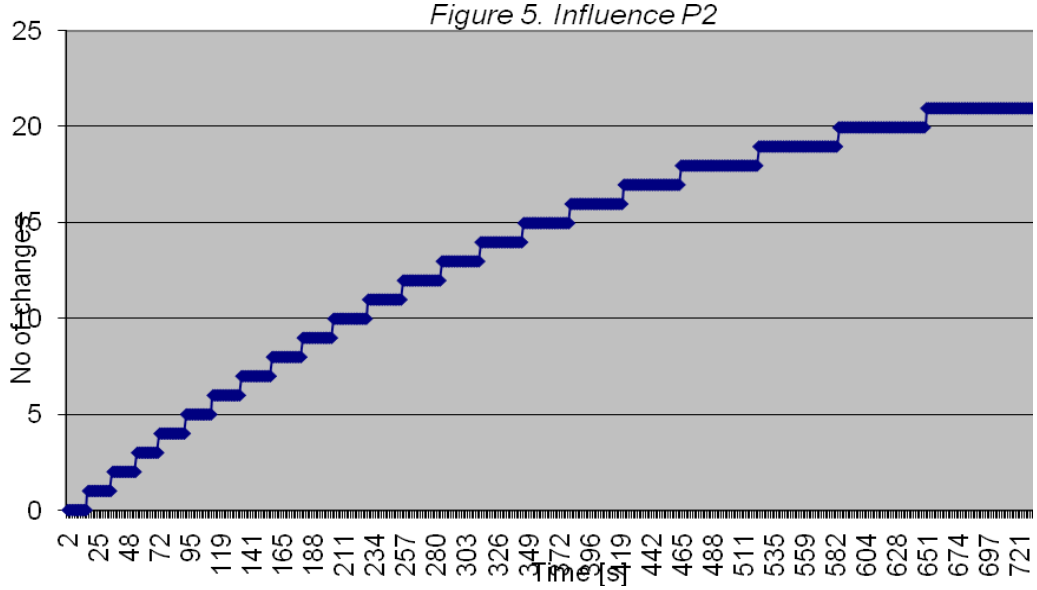


Figure 6. Influenta P3

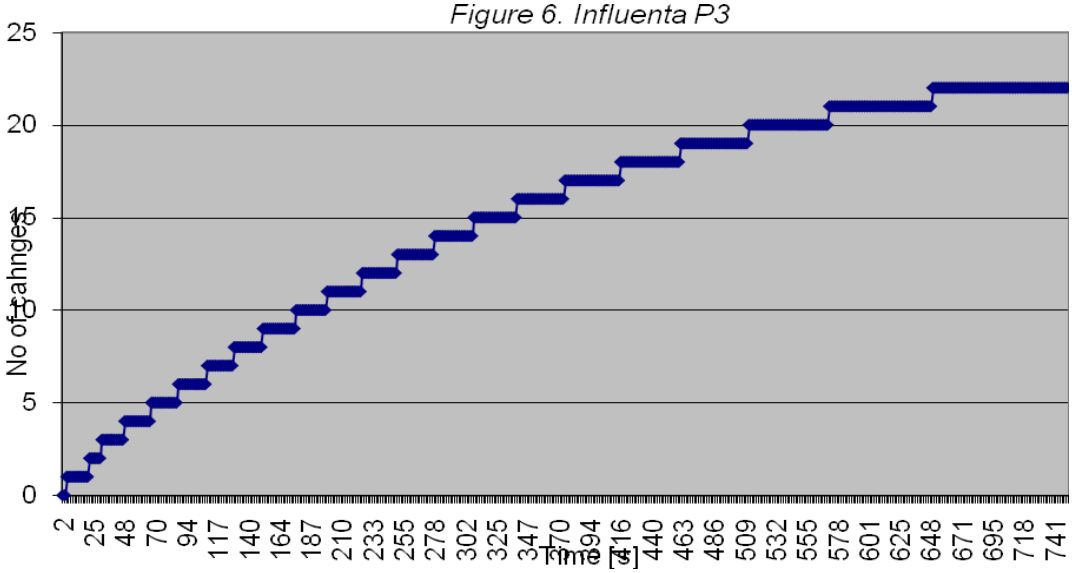


Figure 7. Influence of cellulase

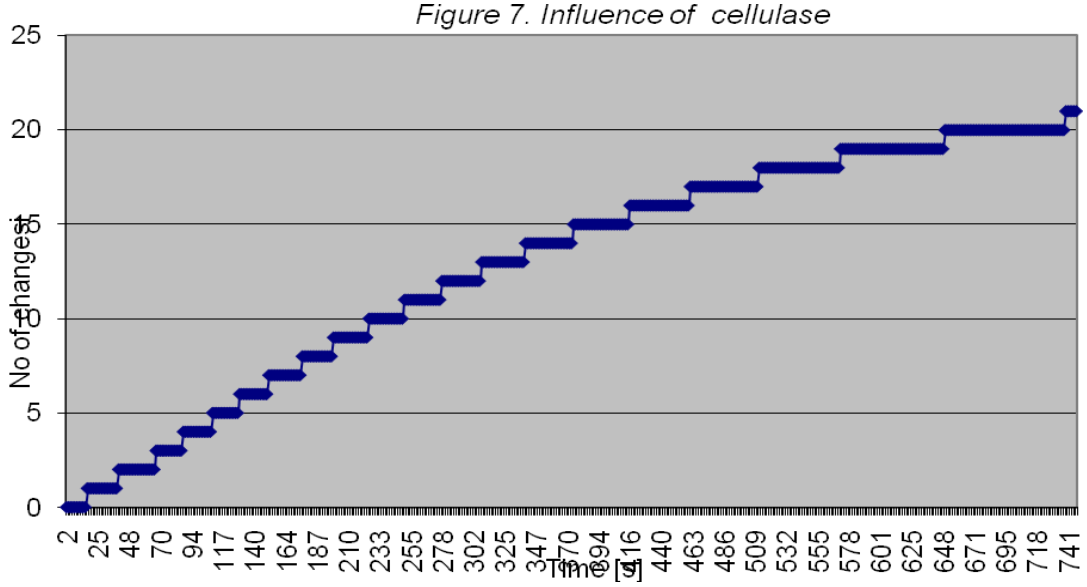


Figure 8. Influence AF2

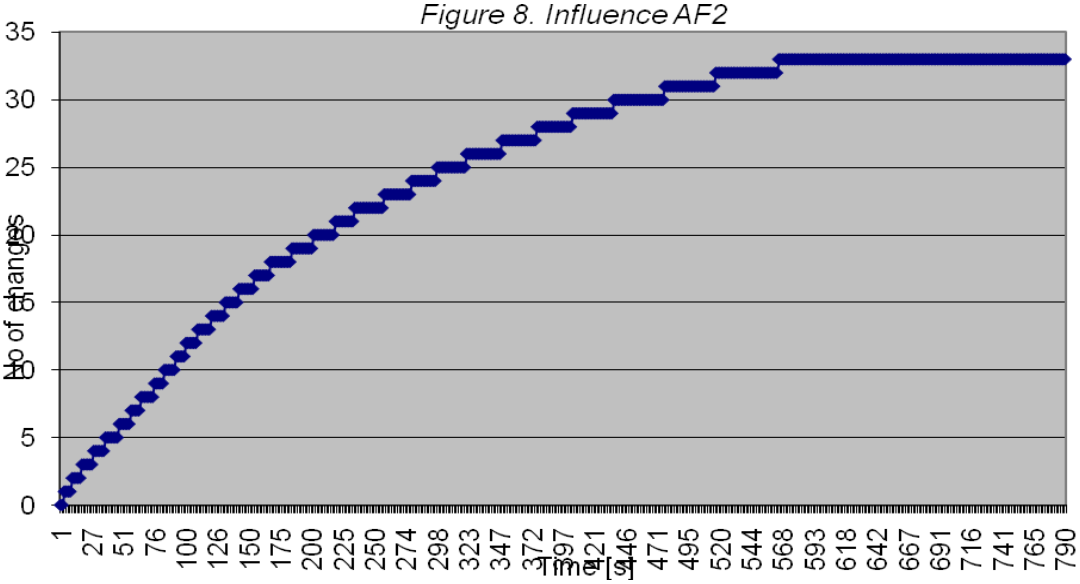


Figure 9. Influence AF3

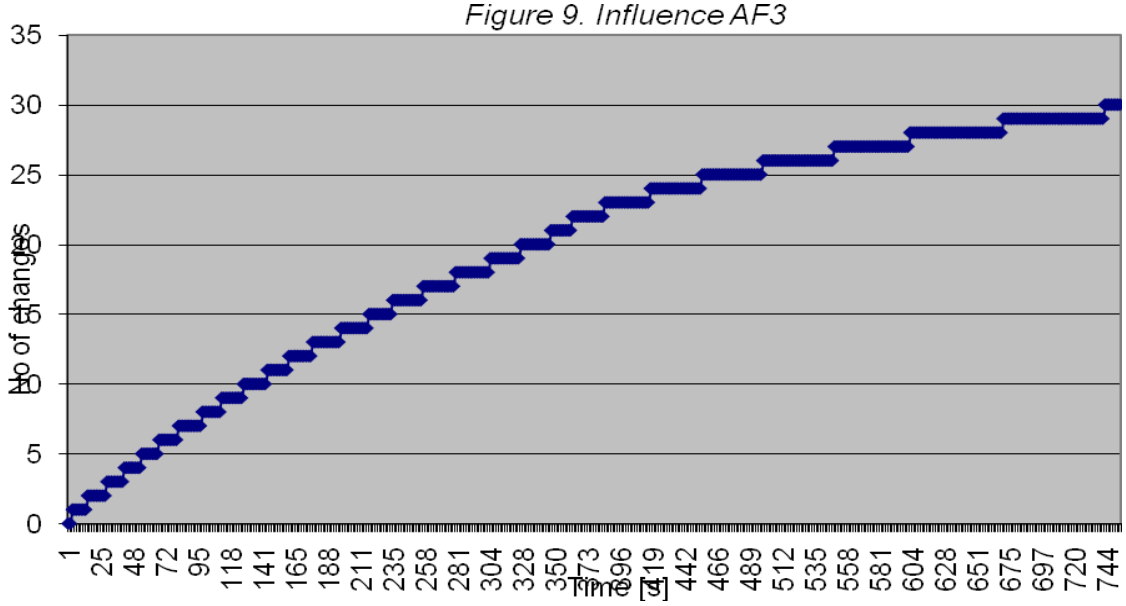


Figure 10. Influence AF4

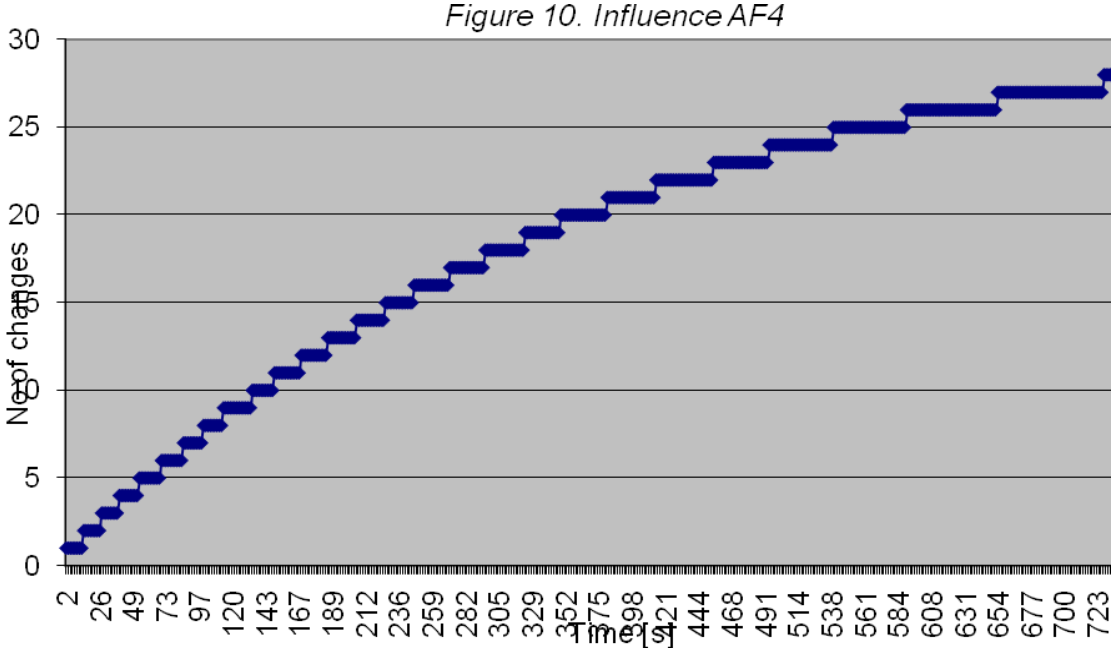


Figure 11. Influence AF5

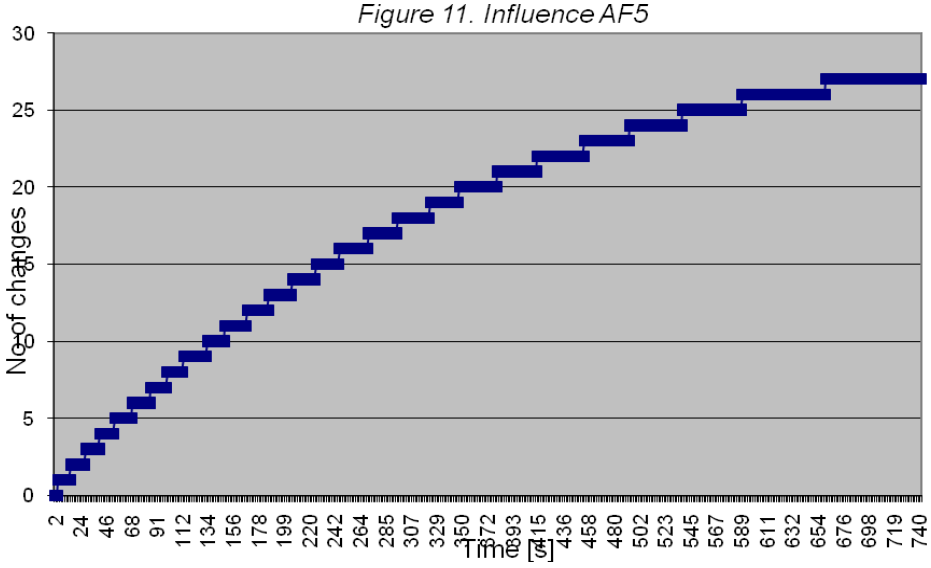
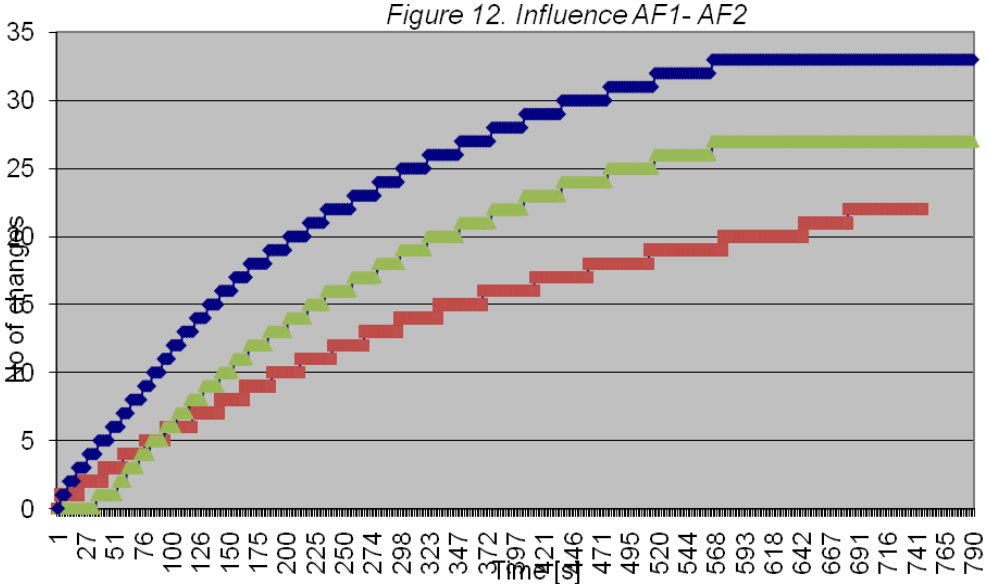


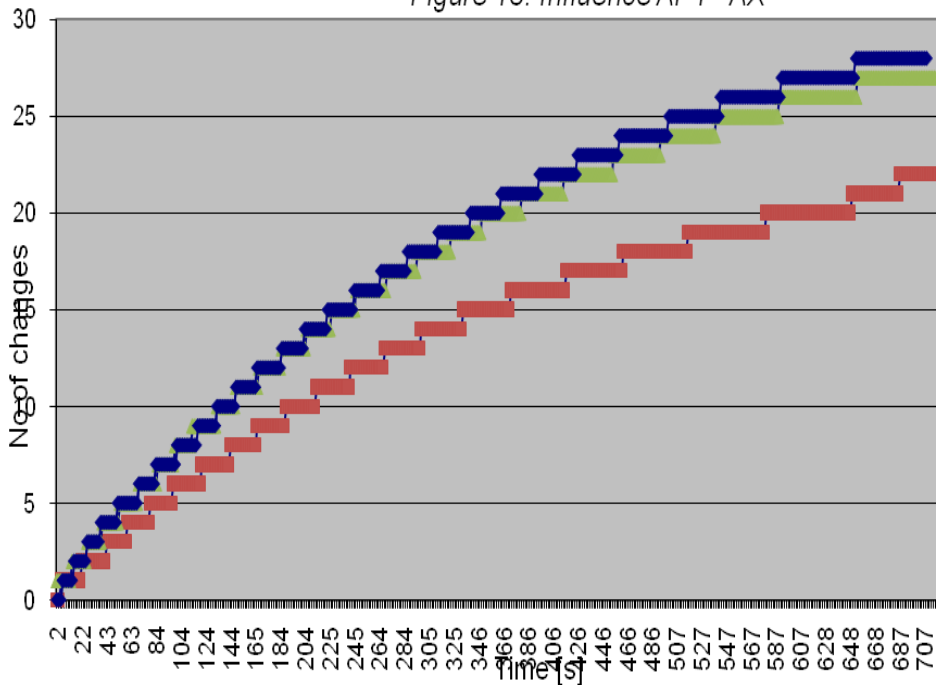
Figure 12. Influence AF1- AF2



Legend:

Control
AF1
AF2

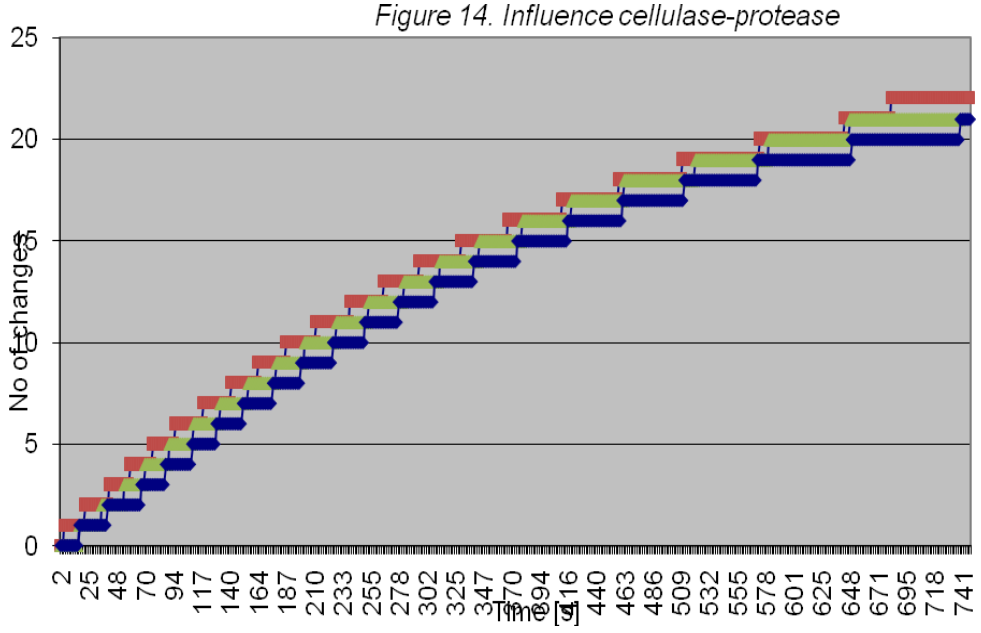
Figure 13. Influence AF4 - AX



Legend:

Control
AF4
AX

Figure 14. Influence cellulase-protease



Legend:

Control

C

P

Exoenzymes influence the rate of starch hydrolysis reaction with endoamylase.

From the experimental results, it is noted that the presence of alpha-amylase from the fungal sources in the starch hydrolysis reaction with endoamylase, determined an increase in the rate of hydrolysis proportional to their amylolytical capacity.

Thus, in addition to the hydrolysis of soluble starch with endoenzymes, of an exogenous fungal amylase, this will change the rate of hydrolysis, depending on its amylolytic capacity:

• in the addition of fungal α- amylase AF1 (SUB 1800) the hydrolysis takes 575 seconds to achieve the 27 steps of hydrolysis;

• the addition of α- amylase AF2 (SKB 40000) determines a duration of 568 seconds in order to obtain 33 stages of hydrolysis (reaction rate is higher);

• the addition of α- amylase AF3 (10,000 SKB) determines the hydrolysis of starch in 730 seconds and achieves 30 steps of hydrolysis;

• the addition of enzyme AF4 (2000 SKB) determines a period of 680 seconds for hydrolysis which was carried out in 22 hydrolysis stages.

4. Conclusions

Exoenzymes influence on the rate of starch hydrolysis reaction with endoamylase.

By comparison with control containing no exoenzyme M (hydrolysis taking place in 741 seconds with 22 stages of hydrolysis), it is noted that in all the cases studied for fungal amylases, the hydrolysis time decreases. It was increased the steps of hydrolysis and it was reduced the duration of the hydrolysis.

The use of the mixtures for different types of exoenzymes namely amylase - xylanase mixture (AX), fungal protease and amylase (AP), determined the increases of the hydrolysis rate by increasing the number of the hydrolysis steps (28 to AX and 25 for AP compared with 22 steps to control M) and reduced hydrolysis duration (650 seconds AX and 675 seconds for AP, compared with 741 seconds for control).

Proteases from different sources (Carica papaya and Bacillus subtilis) influence endogenous amylase action on soluble starch, approximately equal, resulting in a decrease in the duration of hydrolysis of 741 seconds to 655 seconds for P2 and 650 seconds for P3, the number of changes hydrolysis system being unmodified. (22 steps in all cases M, P2, and P3).

The exogenous fungal cellullase (C) slightly decrease the hydrolysis duration from 741 seconds to 680 seconds, but does not alter the number of hydrolysis links (22 steps as control).

This means that these exogenous enzymes do not interference in the starch hydrolysis reaction but their presence determined some sterical impedimences.

The use of laser interferometry techniques to determine the rate of hydrolysis of the starch, the original analysis is a method that can be generalized for any chemical reaction in which the refractive index varies in real time.

Determination of the rate of reaction using laser interferometry techniques can be applied to the study of any system in which a chemical refractive index varies over time

Laser interferometric techniques can be used to determine the rate of hydrolysis of starch and hydrolysis of the exact duration at any field of the food industry, the use of the reaction;

• the method of determining the rate of reaction using laser interferometry technique is extremely sensitive, being able to view the hydrolysis of very low concentrations of reactants (ppm);

• the method of determining the rate of reaction with laser interferometry technique requires small amounts of reagents and short-term analysis;

• the method of determining the rate of reaction with laser interferometry techniques can be used for industrial analysis and is easy to use

• the computer program developed practical results can be represented graphically, making it easy to interpret;

• knowledge of the factors that influence the activity of amylase from wheat flour, may be the reaction of hydrolysis of the starch in the desired direction, activation or inhibition of amylase by appropriate variation of these factors.

This method has particular advantages in particular in the steps of hydrolysis study in real-time fast determination of the extent and duration of hydrolysis of starch and requires small amounts of reagents. It is a very sensitive, convenient and very fast, which is suitable for both analysis and industrial research laboratories

The hydrolysis kinetics study demonstrates that exogenous enzymes affect endogenous amylase activity.

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