The Effects Of Ultrasound On The Activity Of Alpha-Amylase During Barley Germination

M. Yaldagard[1*], S.A. Mortazavi[2] and F. Tabatabaie[2]

Abstract

In this study, the effects of ultrasound as emerging technology on the activity of alpha-amylase after germination were investigated. All experiments were carried out at 20KHz on the ultrasonic generator by considering the three effective factors, temperature (30, 50 and 70 C°) and time (5,10 and 15 min) in different intensities (20, 60 and 100% of total power of device(460w)).For determining the effects of these parameters the enzymatic activates measured by DNS method due to determination of the reducing sugar equivalents released. The results of these assays were analyzed by qualitek4 software using Taguchi statistical method to evaluate the factor's effects on the enzyme activity. Consequently the results of assays showed that the activity of this enzyme was reduced after germination after thermosonication by comparing to the blank.

Key words: ultrasound, alpha-amylase activity, germinated barley and Taguchi method.

[1]Department of Chemical Engineering, Ferdowsi University of Mashhad-Iran
[2]Department of Food Science and Technology, Ferdowsi University of Mashhad-Iran

Introduction

Amylases(E.C:3.2.1.0) are a class of hydrolases widely distributed in the higher plants,animals and microbes.They can specifically cleave the O-glycosidic bonds in starch.These enzymes have a great significance with extensive biotechnological applications in food, brew, textile and paper industries.Industrial applications generally require amylases with a high activity profile. For this purpose many efforts to increase the alpha-amylase activity in the process of barley germination have been done[1,2].some of these efforts are based on the endosperm modification and alourn protoplast to create suitable condition to do anabolic reactions in amylase synthesis sites to increase the amylolytic enzymes activity [3].In the case of enzymes activity several reports have been recorded an increase in activity in the presence of ultrasound for free enzymes in vitro.Unexpectedly, at low acoustic power, some enzymes are not deactivated, whether supported on porous silica gel or free[a-amylase,glucoamylase][4].The application of power ultrasound in enzyme inactivation has been explored in recent years.The inactivation effect of ultrasound is attributable mainly to a phenomenon called cavitation.Cavitation refers to the formation, growth, and implosion of cavities in a liquid when ultrasound propagates travel through it. Extreme physical phenomena (1000K & 500MPa)at micro-scale take place when the bubbles collapse and these phenomena are considered to be the cause of enzyme inactivation. Reports that the use of ultrasound may enhance the activity of certain enzymes have led us to examine the feasibility of ultrasound-induced enhanced enzyme activity[4-7].

Materials and methods

Soluble starch, Sodium potassium tartarate tetrahydrate,3,5-dinitrosalicylic acid, Sodium phosphate,Maltose and Maleic acid obtained from Sigma-Aldrich, company.Karon in kavir barley varieties with moisture content of 9% and an

* Corresponding author:Fax:+985118787430,Tel:+985118795618, E-mail:m_yaldagard@yahoo.com

average content of protein 11.5% was used in all experiments.Ultrasonic irradiation was given by means of UP200H horne type equipped with a radial Sonotrode S3.

Experiment design

3 important and effective parameters, time, ultrasound intensity and temperature were selected.Taguchi method was employed to design experiments condition and evaluate the factor's effect on the activity of enzyme.L9orthogonal array is used to design of experiments. The results were shown in figures 4to 6.

Sonication of the samples

The ultrasonication experiments were carried out at 20 kHz on an ultrasonic generator.All experiments were performed on samples indirect sonication at ultrasonic intensity of 20, 60 and 100% of 460W.The solution was processed at three temperatures of 30, 50 and 70 C° with the sonication for 5, 10 and 15 min.

Malting stage

Barley seeds were micro malted manually in laboratory scale[8].

Extraction of enzymes from malt

Extraction of enzyme from malt performed by means of NaH2PO4[8].

Alpha-Amylase Assay

Enzymatic activity measured according to method of reducing sugars[8].

Results and discussion

At 30 C° when the temperature is not high enough to cause a decrease in α-amylase activity, α-amylase inactivation in an ultrasound treatment at 30 C° is due to sonication itself(fig1).Effects of ultrasound on enzymes are often ascribed to several mechanical and sonochemical processes induced by cavitation. The micro jets of liquid generated by the asymmetrical collapse of cavitation bubbles, the shear stress in a sonicating liquid, and the microstreaming caused by stable oscillating bubbles might mechanically damage the integrity of the barley α-amylase protein structure and causes loss in enzyme activity.Much faster inactivation in combination of ultrasound and temperature obtained at 50 and 70 C° (fig2,3)compared to the thermal treatment and sonication at 30 C°.The activity amount obtained from thermosonication at any observed temperature was much smaller than those for thermal and sonication inactivation.

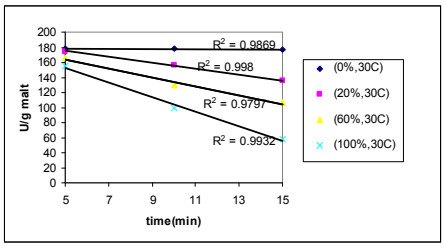

Fig1: Alpha- amylase activity vs. time, barley sonication after germination at 30 C°

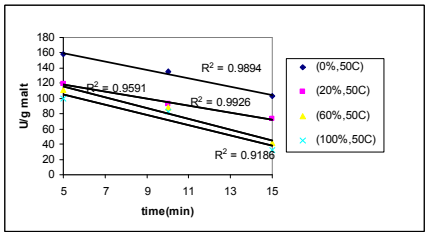

Fig2: Alpha- amylase activity vs. time, barley sonication after germination at 50 C°

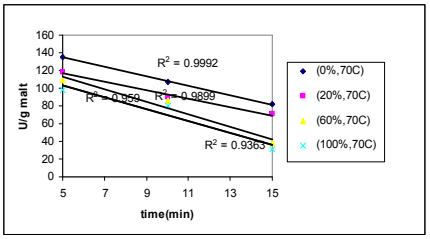

Fig3: Alpha -amylase activity vs. time, barley sonication after germination at 70 C^{o}

Fig4: Average effect of intensity by tagochi method using qualitik4 software

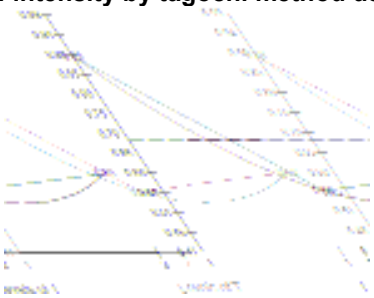

Fig5: Average effect of temperature by tagochi method using qualitik4 software

Fig6: Average effect of time by tagochi method using qualitik4 software

Conclusions

Ultrasound treatment effectively increased the barley a-amylase inactivation compared to a thermal treatment at the same temperature. When sonication was combined with a heat treatment at temperatures high enough to cause thermal inactivation, grater inactivation was observed. At first the aim of this study was enhancing the a-amylase activity via ultrasonic irradiation in germination stage but as were shown by results we noticed that ultrasound has a destruction effect on this enzyme and cause inactivation of this enzyme.

Acknowledgements

The laboratory of emerging technology,department of science and food technology, Ferdowsi university of Mashhad and Khorasan cereals organization are gratefully acknowledged for the equipmental and experimental support.The authors also would like to thank from research office,department of chemical engineering for partial financial support.

References

1.Taiz. L. and Starks. J. E. 1977. Gibberellic Acid Enhancement of DNA Turnover in Barley Aleurone Cells, Plant Physiol, 60:182-189.

2. Eastwell. K. C. and Spencer. M. S. 1982. Modes of Ethylene Action in the Release of Amylase from Barley Aleurone Layers', Plant Physiol.,67:563-567.

3.Tull .D., Phillipson. B. A., Kramhùft. B., Knudsen. S., Olsen. O. and Svensson . B.,2003. Enhanced Amylolytic Activity in Germinating Barley through Synthesis of a Bacterial Alpha-amylase, J. Cereal Science ,37:71-80.

4.Schmidt, P.,Rosenfeld, E.,Millner,R.,and Schellenberger,A.1987.Effects of Ultrasound on the Catalytic Activity of Matrix-Bound Glucoamylase, Ultrason, 25:295-299.

5. Barton. S., Bullock C. and Weir. D. 1996. The Effects of Ultrasound on The Activities of Some Glycosidase Enzymes of Industrial Importance, Enzyme and Microb.Technol. 18:190-194.

6. Czerner,R.,Millner, R., Roenfeld,E., Schellenberger,A., and Schmidt,P. 1987. Theoritical and Experimental Studies on the Influence of Ultrasound on Immobilized Enzymes . Biotechnol Bioengin.,30:928-935.

7. Ishimori,Y.,Karube,I., and Suzuki, S. 1981.Acceleration of Immobilized Alpha-chymotrypsin Activity with Ultrasonic Irradiation. J.Mol Catal.,12:253-259.

8.Osman, A.M(2002),The Advantages of Using Natural Substrate-Based Methods in Assessing the Roles and Synergistic and Competitive Interactions of Barley Malt Starch-Degrading Enzymes,J.Inst.Brew.108(2):204–214.