

The effects of ultrasound on the stimulation barley seed and alpha-amylase enzyme activity

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Alpha amylase is one of the important enzymes found in barley grain which is activated in the malting stages. This enzyme, because of its stability against heat and specific ability in starch liquefying and formation it to simple sugars is very important in industry & medicine. Different methods including adding growth hormones and or genetic altering to increase the activity of this enzyme during the germination stages have been reported. In this study, the effects of ultrasound as emerging technology on germination stimulation, amount of activity before steeping grains in the dry state, by using on the ultrasonic generator in 20KHz operating frequency by considering the three effective factors, temperature and time in different intensity was investigated. About determining these effects the Enzymatic activates measured by DNS method due to determination of the reducing sugar equivalents released. The results of this assays analyzed by Qualitek4 software using Taguchi method to evaluate the factor's effects on enzyme extraction yield and enzyme activity. Consequently the results of this analysis were increasing activity of this enzyme as result of the increasing germination rate in the sonicated of dry grains comparing to the blank.

Keywords: Ultrasound, enzyme activity, stimulation, alpha-amylase, Taguchi method

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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 [alpha-amylase, glucoamylase] [4]. In recent years, extensive efforts have applied sonication under dry conditions witch may be carried out up to several months before actual sowing. Examples of the use of this process include ultrasonic treatment leading to a three fold enhancement in sunflower seed germination in soil and a ten-day reduction in the ripening time of tomatoes or shorter period to form a gel as a result in a faster release of starch during subsequent cooking in a sonicated rice grain in water[5].

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 average

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

Experiment design

2 important effective parameters, time, ultrasound intensity at constant temperature were selected. Taguchi method was employed to design experiments condition and evaluate the factor's effect on stimulation and activity of enzyme (fig 2,3). L9 orthogonal array is used to design of experiments.

Sonication of the samples

The ultrasonication experiments were carried out at 20 kHz on a 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 30 °C with the sonication for 5, 10 and 15 min.

Malting stage

Barley seeds were micro malted manually in laboratory scale [6].

Extraction of enzymes from malt

Extraction of enzyme from malt performed by means of NaH₂PO₄ [6].

Alpha-Amylase Assay

Enzymatic activity measured according to method of reducing sugar

RESULT AND DISCUSSION

Possible explanation for increased alpha-amylase activity according to results as shown in figures 1 to 3 and table 1 could be as follows: When cavitation occurs in a liquid near a solid surface cavity collapse generates high-speed jets of liquid. These jets hit the surface with tremendous force. This process can cause severe damage at the point of impact and fragment the seed shell and additional porosity of barley grains. Therefore as result of these actions, the resistance of the seeds shell against the water diffusion decrease and sonicated tissue absorb an extra volume of water so that the absorbed water are given to embryo as easily and freely. In consequence at constant interval time the activity and extractive value of alpha-amylase from the grains that subjected to sonication was much greater, compared by unsonicated seeds.

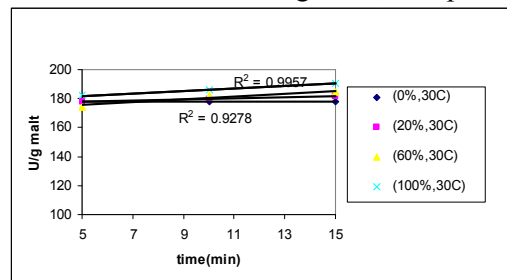


Fig1: Alpha- amylase activity vs. time, barley sonication before steeping at 30 °C

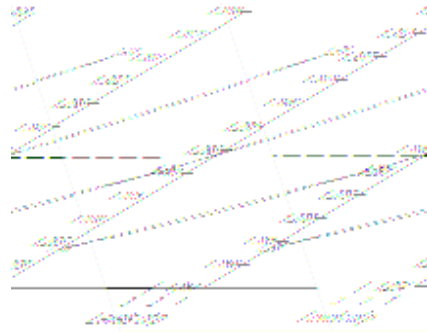


Fig2: Average effect of ultrasound intensity by tagochi method using qualitik4 software

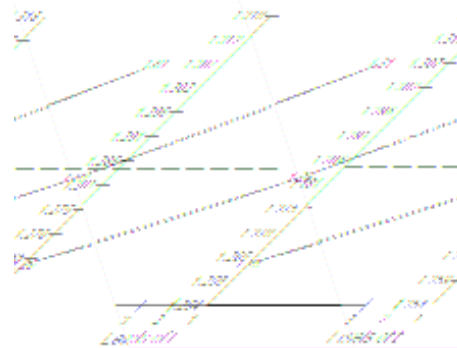


Fig3: Average effect of time by tagochi method using qualitik4 software

Table1: Analysis of variance (ANOVA)

Number	Factors	DOF	Sums of Squares	Variance	F-Ratio	Pure Sum	Percent
1	P	2	0.009	0.004	101.468	0.009	
48.456							
2	t	2	0.008	0.004	94.868	0.008	
45.273							
Other/Error		22	0.000	0.000			
6.271							
Total		26	0.019				
100.000%							

CONCLUSIONS

Ultrasound waves used to treating the barley seeds before soaking and this could be one of the explanations for their better and faster germination after sonication. This behavior is due to shell fragmentation so more water retention capacity in dry grains. The enhancement on the germination process caused by ultrasound is attributed to the facilitating in hydration process as result of enhancement on the mass transfer due to the intensification of diffusion phenomena.

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.

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