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Effect of Ultrasonic Power on the Activity of Barley's Alpha-amylase from Post-sowing Treate of Seeds

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Abstract: In the present study, the effects of ultrasound as emerging technology were investigated on the alpha-amylase activity of barley seeds after sowing along with the normal watering of seeds. The seeds of barley after steeping were exposed to ultrasonic irradiation in vibration amplitude controller setting 20, 60 and 100% of nominal power for times (5, 10 and 15 min) at 20 KHz and 30°C. For determining the effects of these parameters on enzyme the Fuwa method assay based on the decreased staining value of blue starch-iodine complexes employed for measurement an activity. The results of these assays were analysed by Qualitek4 software using the Taguchi statistical method to evaluate the factor's effects on enzyme activity. It has been recorded; an increase in enzyme activity at the lower power densities followed a decrease in activity at the higher power densities employed.

Key words: Ultrasonic power % alpha-amylase activity % post-sowing and Taguchi statistical method

INTRODUCTION

Alpha-Amylase (1,4-a-D-glucano-hydrolase, endoamylase) hydrolyzes starch, glycogen and related polysaccharides by randomly cleaving the internal a-1, 4-glucosidic linkages. It is widely distributed in nature, i.e. in the higher plants, animals and microbes however, the activity of these enzymes is high in germinating cereals. Cereal alpha-amylases play a very important role in the starch metabolism in developing as well as germinating cereals [1] because of the commercial importance of diastatic power of malt in brew producing as it is popularly known is very important for assessing the activity of starch-degrading enzymes, many efforts have been taken for increasing the alpha-amylase activity in the germination process of barley seed. diastatic power or in another word the alpha-amylase activity increasing has been attempted in the past by genetic recombination [2] or through the use of natural/artificial chemicals such as gebberllin [3]/ethylene [4] or combination of this materials by KBr for treatment of barley in order to the release of amylase from barley aleourn layer. None of these known methods, however, have been found to significantly improve the activity of alpha-amylase without adversely affecting the quality of the resultant malt from the treated seeds. In addition the chemicals methods have the disadvantages that final malt product contains residues due to the treatment. Therefore these methods are problematic for use as general-purpose technique and it would be highly desirable to find a method for increasing the alpha-amylase activity. This risk has been made with developing the physical methods such as ionizing radiation [5] or water restriction stress [6] and etcetera. even through the effects of ultrasound have been studied in over hundreds of seeds type [7-14] to our knowledge, it's effect on the activity of alpha-amylase of barley grain, has not been investigated, therefore this research was focused on the post sowing treatment of the barley seeds by using the different energy of ultrasonic waves.

MATERIALS AND METHODS

Chemicals: All chemicals with high analytical grade including iodine, KI (potassium iodide), KH_2PO_4 (monobasic phosphate), $K_2HPO_4 \cdot 3H O_2$ (dibasic phosphate) and soluble starch from potato (S-2630) which used for alpha-amylase assay were obtained from Sigma-Aldrich, Fluka and Merk companies.

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Raw materials: Karon in kavir barley varieties with moisture content of 9% and an average content of protein 11.5% was used in all experiments. To prevent absorption of moisture it was stored in a dry place at 20°C until malting. Also it must be mentioned, that for removal of dormancy, samples were stored at room temperature (25-37C°) for 3 months after harvest.

Apparatus: The sets of Gerhardt Kjeldatherm The sets of Gerhardt Kjeldatherm and Gerhardt Vapodest 30 instrument were used for determination of the protein amount in barley seeds.

Ultrasonic irradiation was given by means of UP 200 H ultrasonic processor horn type(20 kHz, maximum wave amplitude of 210 µm and maximum nominal power 460W) equipped with a radial Sonotrode S3 (3mm diameter) designed by Dr. Hielscher GmbH (Treptow, Germany).

Experiments design: In this study 4 important effective parameters namely, ultrasound power, the time of ultrasonic irradiation, temperature and frequency were selected. Since in our design problem the operating temperature and frequency were fixed, only 2 variables were remained for the design of experiments. Taguchi method at our disposition was employed to design experiments condition and evaluate the factor's effect on stimulation and activity of enzyme. L9 orthogonal array is used to design of experiments. 9 experiments, repeated for 3 times, considering the 2 parameters as defined above in the 20 KHz frequency were done for barley seeds. Qualitek4 analysis using the ANOVA approach was employed for finding the average effects of individual parameters on enzymatic process condition as shown in the Fig. 3 and 4 according to software output.

Experimental

Sonication of sample and malting stage: Barley seeds were micromalted manually in laboratory scale according to the following procedure: samples after stepping at 16-17C° for 6 h in the incubator chamber, were air-rested for 8h.This process was done 3 times periodically to reach a moisture content of 45%. At the end of steeping, when the rootlets were noted at the edge of grain the sonication process was started.The ultrasonication experiments were carried out at 20 kHz on the ultrasonic generator. The tip of the horn was immersed about 9 mm into the solution to be processed. all experiments were performed on samples (10 g barley seeds) dispersed in 80 ml of tap water in direct sonication at ultrasonic intensity of 20, 60 and 100% power setting of device with additional agitation or shaking that was employed, to avoid standing waves or the formation of solid free regions for the uniform distribution of the ultrasonic waves. The ultrasonic energy was pulsed using a duty cycle control in order to reduce the formation of free radicals. The cycle was set on 50% in all experiments. The solution was processed at constant temperature of 30C° with the sonication horn for 5, 10 and 15 min. (by circulating water continuously constant temperature was attained during experimentation.) after soaking or sonification, the grains placed on water-saturated filter paper in a Petri-dish and the subsequent germination phase followed 96 h with keeping the 45% moisture content (water was added to filter paper every four hours to maintain saturation). Then the samples were kilned in the drying oven in gradually ramping temperature from 17 to 55C° over 20 h, from 55 to 65C° over 20 h, from 65 to 75C° over 6 h and finally from 75 to 82C° over 4 h. The drying process was stopped with reaching the moisture content of samples to 4%. Afterwards with removing the rootlets, the samples were milled and the malted flour was prepared for the next stages of the experiments. Control was treated similarly with the exception the elimination of the sonication stage. All the experiments in this study were performed in triplicate.

Extraction of enzymes from malt: In this research commonly 50 mM Na-phosphate buffer with pH = 8 was used as the best extraction media. This buffer enhances the release of more enzyme rather than another media such as the mixture of NaCl in water owing to the fact that either the high pH or added phosphate ions (higher concentration), as pointed out by Osman [15]. Approximately, 0.75 g malt flour was weighed in duplicate into centrifuge tubes and 4 mL extraction media was added with mixing. Extraction was performed for 30 min at 30C° with regular vortexing for 5 s at 5 min intervals and was terminated by centrifugation for 10 min at 2826g.

Determination of alpha-amylase activity based on decrease in starch/iodine colour intensity: Starch forms a deep blue complex with iodine and with progressive hydrolysis of the starch, it changes to red brown. Several procedures have been described for the quantitative determination of amylase based on this property. This method determines the dextrinising activity of alpha-amylase in terms of decrease in the iodine colour reaction. The dextrinising activity of alpha-amylases employs soluble starch as substrate and after terminating the reaction with dilute Hcl, iodine solution is added. The

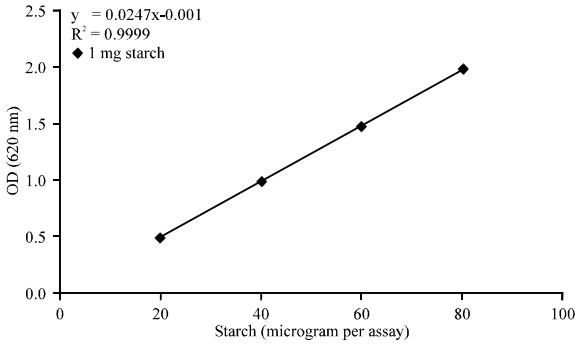

Fig. 1: Standard curve for starch-iodine assay

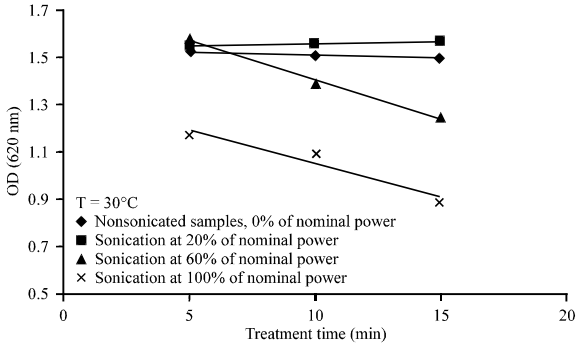

Fig. 2: Optical density versus time, barley sonication after steeping at 30C°

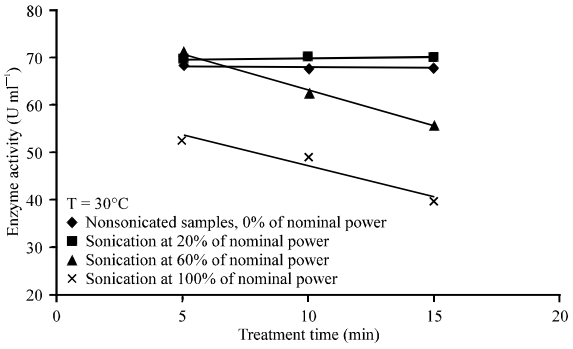

Fig. 3: Alpha-amylase activity versus time, barley sonication after steeping at 30C°

decrease in absorbance at 620 nm is then measured against a substrate control. One percent decline in absorbance is considered as one unit of enzyme [16].

Enzyme assay: starch-iodine assay according the Fuwa method was carried out as follows: assay reactions were initiated by adding 0.5 ml of starch solution (20 mg mL^{-1} in 0.1 M phosphate buffer pH = 7) and 0.5 ml of enzyme in 0.1 M phosphate buffer at pH 8.0 to reaction tube and incubated at 37EC for 30 min. The reaction is then terminated by adding 1ml of 1N HCL. Following reaction termination, the mixture then diluted to nearly 9 ml with H2O, followed by the addition of 1ml of iodine reagent (0.2% iodine and 2% potassium iodide). Finally, the volume is adjusted to 10 ml with distillated water and the amount of color development is determined by measuring the absorbance at 620 nm [17].

The enzyme activity was calculated according to the following equation [17]:

$$U/ml = \frac{(OD_{620}control - OD_{620}sample)}{OD_{620}mg\ starch \times t \times V}$$

Where, OD_{620}control is the absorbance obtained from the starch without the addition of enzyme, OD_{620}sample is the absorbance for the starch digested with enzyme, OD_{620}/mg strach is the absorbance for 1 mg of starch as derived from the standard curve in Fig. 1, t is the assay incubation time and V is the volume of the enzyme used in the assay.

RESULT AND DISCUSSION

The effect of acoustic power and irradiation time on barley's alpha-amylase activity: In order to investigate the effect of ultrasonic power, the post sowing seeds in water were sonicated at different acoustic powers ranging from 20 to 100% setting from total power of device at 30C°. Data shown in Fig. 2 and 3 indicate the optical density and corresponding alpha-amylase activity versus time. Concerning the Fig. 2 and 3 the following results have been obtained: the enzyme activity increased with increasing the irradiation time at 20% of power setting. Typically the activity increased from 69.678(U mlG^{-1}) for 5min to 70.128(U mlG^{-1}) for 15min. the enhancement on alpha-amylase activity given by the use of ultrasound at 20% of nominal power is attributed to the cavitation bubbles that induce a physiological or biochemical changes in the post-sowing seeds which cause a speeding up of metabolic process within the barley seed so that upon exposure of the seed to malting conditions, more enzyme realesed. when applied power was increased, enzyme activity could generally be improved due to the effect of ultrasonic cavitation.the same trend was resulted at 60% which the enzyme activity increased up to the first 5 min, but then it has surprisingly decreased considerably with increasing the irradiation time and employment power (for example enzyme activity at 60% of power setting in 5min was 71.52 U mlG^{-1} but it decreased to 40.008 U mlG^{-1} in sonication for 15 min at 100% of power setting). This

Table 1: Analysis of variance (ANOVA) showing the effect of ultrasonic (P) and the variable of time as significance of the main effects

Number	Factors	DOF	Sums of squares	Variance	F-ratio	Pure sum	Percent
1	P	2.00	1.041	0.520	116.502	1.032	79.353
2	t	2.00	0.161	0.080	18.051	0.152	11.714
Other/error		22.00	0.098	0.004			8.933
Total	26	1.301					100.000%

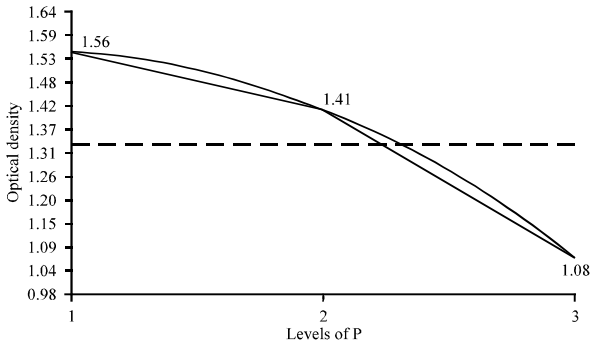

Fig. 4: Average effect of ultrasonic power by Taguchi method using qualitek 4 software

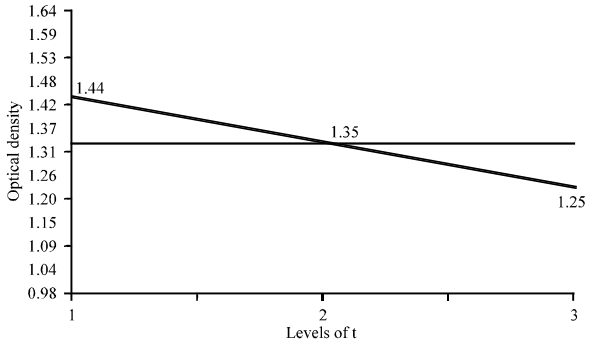

Fig. 5: Average effect of exposure time by Taguchi method using qualitek4 software

could be reasonably explained by the increased production of free radicals from the ultrasound dissociation of water. In the presence of these high energy spices, modification of the molecular structure of enzyme could take place. Furthermore during cavitation, microbubbles form at various nucleation sites in the fluid and grow during the rarefaction phase of the sound wave. Then, in the compression phase, the bubbles implode and collapsing bubbles release a violent shock wave that propagates through the medium. Cavitation is associated only with power ultrasound and is used to explain the performance enhancing effects of ultrasound in biological systems. Cavitation causes intense local heating with temperature rising to 4000C° and pressure in a collapsing cavitation bubble can reach 1000 atm. Local temperature in the vicinity of a forming or collapsing bubble can change extremely rapidly [18]. Cavitation, often accompanied by emission of light, can break apart relatively robust small molecules and bioactive macromolecules and thus activity does not remain for long.

Qualitek4 statistical analyses: Table 1 shows the detailed analysis of variance results of experiments conducted under Taguchi method. In this table the contribution of each factor quantitatively was determined by using ANOVA approach. Also Fig. 4 and 5 depict the main effects of ultrasonic power, temperature and exposure time respectively. By the term “main effects”, the average of obtained results (as an optical density), in which each factor is at a given level, is meant. As it is shown in these figures the average effects all of these parameters on enzyme activity are negative and the maximum effects of these parameters were in the third level of them. Also the results of the ANOVA (Table 1) reveal that acoustic power which reached 79.353%, made the major contribution to overall performance.

It should be mentioned, in these curves the longitudinal axis is a level of selected parameters such as p = ultrasonic power, T = temperature and t = the time of exposure to ultrasonic irradiation along with conventional treatment and transverse axis is optical density as a criteria of the process yield in statistical analysis.

CONCLUSION

The following are the main conclusions drawn from the study:

Most probable mechanism for ultrasonic enhancement of alpha-amylase activity at low power density may be due to the mobilization of storage materials such as sugars, proteins by activation or de novo synthesis of alpha-amylase in treated seeds.

Alpha-amylase activity is shown to be either stimulated or inhibited, depending on the conditions of ultrasound exposure. Since when malting barley is irradiated higher than 20%, activation is inhibited. From the practical view the use of higher power of ultrasound for the treatment of post-sowing barley

seeds is harmful. An explanation for this is that the larger the amplitude of ultrasound wave traveling through a mass medium, the more violently the bubbles collapse.

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REFERENCES

1. Muralikrishna, G. and M. Nirmala, 2005. Cereal alpha-amylases-an overview. Carbo. Poly., 60: 163-173.
2. Tull, D., B.A. Phillipson, B. Kramhùft, S. Knudsen, O. Olsen and B. Svensson, 2003. Enhanced amylolytic activity in germinating barley through synthesis of a bacterial alpha-amylase. J. Cereal Sci., 37: 71-80.
3. Taiz, L. and J.E. Starks, 1977. Gibberellic Acid enhancement of DNA turnover in barley aleurone cells. Plant Physiol, 60: 182-189.
4. Eastwell, K.C. and M.S. Spencer, 1982. Modes of ethylene action in the release of amylase from barley aleurone layers. Plant Physiol., 67: 563-567.
5. Ress, P., I. Kiss, G. Miltenyi, I. Petro, J. Farkas, P. Biacs, I. Kozma and I. Debreczeny, 1987. Process for controlling the germination of malting barley. Patent No. 4, 670, 279.
6. Scott, L., 2003. Treatment of germinating malting grain, patent application publication, Pub. No. US2003/0148012A1.
7. Povey, M.J.W. and T.J. Mason, 1998. Ultrasound in food processing. Blackie Academic and Professional, International Thomson Publishing I T P, London SE1 8HN, UK, pp: 115-125.
8. Gordon, A.G., 1963. The use of ultrasound in agriculture. Ultrason, 1 (2): 70-77.
9. Toma, M., M. Vinatoru, L. Paniwnyk and T.J. Mason, 2001. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction, ultrason. sonoch, 8: 137-142.
10. Shors, J.D., D.R. Soll, K.J. Daniels and D.P. Gibson, 1999. Method for enhancing germination, United state application publication, patent No. 5, 950, 362.
11. Shimomura, S., 1990. The effects of ultrasonic irradiation on sprouting radish seed, Ultrasonics Symposium, in the Proceedings, IEEE, 3: 1665-1667.
12. Aladjadjiyan, A., 2002. Increasing carrot seeds (*Daucus carota* L.), cv. Nantes, viability through ultrasound treatment. Bulg. J. Agric. Sci., 8: 469-472.
13. Hebling, S.A. and W.R. da Silva, 1995. Effects of low intensity ultrasound on the germination of corn seeds (*Zea mays* L.) under different water availabilities. Sci. Agric. (Piracicaba, Braz.), 52 (3): 514-520.
14. Weinberger, P. and M. Measures, 1968. The effect of two audible sound frequencies on the germination and growth of a spring and winter wheat. Can. J. Bot., 46 (9): 1151-1158.
15. Osman, A.M., 2002. The advantages of using natural substrate-based methods in assessing the roles and synergistic and competitive intractions of barley malt starch-degrading enzymes. J. Inst. Brew., 108 (2): 204-214.
16. Gupta, R., P. Gigras, H. Mohapatra, G.V. Kumar and B. Chauhan, 2003. Microbial a-amylases: A biotechnological perspective, Process Biochem., 38: 1599-/1616.
17. Xiao, Z., R. Storms and A. Tsang, 2006. A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. Analyt. Biochem., 351: 146-148.
18. Suslick, K.S., 1990. Sonochemistry. Sci., 247: 1439-1445.