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The effect of ultrasound in combination with thermal treatment on the germinated barley's alpha-amylase activity

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Abstract−The effects of ultrasound as emerging technology along with thermal treatment were investigated on the activity of barley's alpha-amylase after germination. All experiments were carried out at 20 kHz on an ultrasonic generator by considering the three effective factors, temperature (30, 50 and 70 °C) and ultrasonic intensities (20, 60 and 100% setting from total power of device (460 W)) in different time intervals (5, 10 and 15 min). For determining the effects of these parameters, the enzymatic activity was assayed by measuring the reducing sugars released as a result of the alpha-amylase action on soluble starch using 3,5-dinitrosalicylate regent (DNS). The results of these assays were analyzed by Qualitek4 software by using the 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 from germinated barley was reduced after thermosonication by comparing to the blank.

Key words: Ultrasound, Alpha-amylase Activity, Germinated Barley, Taguchi Statistical Method

INTRODUCTION

The application of ultrasound to biotechnological processes has recently attracted the attention of some research groups [1,2]. In biotechnological processes, the ultrasonication method is widely used for laboratory scale and it does not require sophisticated equipment or extensive technical training. The structure and function of biological molecules can be changed by ultrasound irradiation. The most common interaction mechanisms which are involved in this case are either heat or chemical effects and acoustically induced cavitational activity. In addition, inactivation of biomolecules by ultrasonication can also be caused by mechanical effects, i.e., shear stress developed by eddies arising from shock waves [3]. It is well known that the ultrasonic waves have the potential to influence the enzymes. The effects of ultrasonic power employed on enzymes can be divided basically into (1) aiding in biological reactions, (2) causing a decrease in the activity of many enzymes *in vitro*, and (3) in some cases increasing the activity of free enzymes instead of their deactivation. Many studies have documented enhanced rates of enzyme-catalyzed reaction by using ultrasound. For example, there are some reports that demonstrate the potential role of the ultrasound whether in enhancing the rate of the enzyme-catalyzed hydrolysis of starch and sucrose by means of alpha-amylase [4,5] or improvement in the rate of hydrolysis of milk lactose under sonication [6]. In the case of enzyme activity there have been numerous reports that ultrasound decreases enzyme activity [7-18], but only a limited number of reports have 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 (EC 3.2.1.1), glucoamylase (EC 3.2.1.3)] [19]. Different researchers reported that the activity of free enzymes increased under mild ultrasound irradiation, and in some cases an increase in rate at lower intensities followed by a decrease in rate at higher intensities was recorded, most notably for the activity of alpha-chymotrypsin on casein [19]. It has been reported that high intensities of ultrasound cause a decrease in the activity of many enzymes *in vitro*, and this is probably attributed to the change in the structure and function of biological molecules. The low intensity of ultrasound in some cases can increase the activity of free enzymes instead of their deactivation. Therefore, the intensity level of ultrasound plays a major part in the activity or inactivity of many enzymes. It also seems that the enzymes, which are the key of the biochemical reaction, increase their activity with a good proportioning of ultrasound. Generally, ultrasonication in combination with other treatment(s) is more effective in enhancing the inactivation efficacy. The efficacy of combining heat with power ultrasound (thermosonication) was explored by some authors who found that the enzyme inactivation of thermosonication was greater than the sum of the inactivating effects of heat and ultrasound when acting independently [14]. It was also reported that the combination of heat and ultrasound is much more efficient with respect to treatment time and energy consumption compared to either treatment individually [14]. The inactivation of peroxidase has been found to be improved with thermosonication as a result of these benefits [15]. The concept of a combination treatment has been further explored by introducing elevated static pressure in an ultrasound treatment chamber in a process called manothermosonication (MTS). MTS has proved to be an efficient procedure to inactivate not only heat-sensitive enzymes such as peroxidase, lipoxygenase and polyphenoloxidase [7], but also heat-resistant enzymes such as lipase, protease [12] and the thermostable fraction of pectinmethylesterase from tomato [13] or fruit and vegetable juices [14]. In order to analyze the mechanisms of the ultrasonic enzyme inactivation, there are several assumptions that can be considered. Ultrasound exerts its effects mainly through a phenomenon called cavitation. Cavitation

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is the formation, growth and, sometimes, the implosion of microbubbles created in a liquid when ultrasound waves propagate through it. The collapse of the bubbles leads to energy accumulation in hot spots where temperatures of above 1,000 K and pressures of approximately 500 MPa have been measured [3]. This phenomenon can cause enzyme inactivation through three mechanisms, which can act alone or combined. The first one is purely thermal, due to the enormous temperatures achieved during cavitation. The second one is due to free radicals generated by water sonolysis [7], and the third one is due to the mechanical forces (shear forces) created by microstreaming and shock waves [3]. The influence of the dissolved gas on the enzyme inactivation can also be explained by the formation of free radicals by cavitation.

Reports, and the intriguing possibility that the use of ultrasound may enhance the activity of certain enzymes [19-22], have led us to examine the feasibility of ultrasound-induced enhanced alpha-amylase activity. Amylases (E.C: 3.2.1.0) are a class of hydrolases widely distributed in nature, i.e., in the higher plants, animals, and microorganisms (bacteria, Fungi origin). They can specifically cleave the O-glycosidic bonds in starch, a principal storage polysaccharide present in seeds of various plants and other related oligo and polysaccharides. These enzymes have a great significance with extensive biotechnological applications in food, brew, textile and paper industries. Alpha-amylase, one of the most valuable enzymes is important in the metabolism of maltose and maltodextrins. In addition to being used as an additive in detergents, it can also be used for the removal of starch sizing from textiles, the liquefaction of starch, and the proper formation of dextrin in baking. Alpha-amylase is a common enzyme added to fruit juices for its dextrinizing (liquefying) action on starch and other macromolecules to facilitate their filtration. Starch depolymerization by amylases is the basis for several industrial processes such as the preparation of glucose syrups and brewing. Cereal alpha-amylases play a very important role in the starch metabolism in developing as well as germinating cereals [23]. Industrial applications generally require amylases with a high activity profile. For this purpose much effort to increase the alpha-amylase activity in the germination process of barley has been undertaken. Various articles have reported the increase of alpha-amylase activity in biologic, genetic molecular and biochemical literature. All of these reports are based on the genetic altering (recombination techniques) or endosperm modification and aleurone protoplast to mobilization of endosperm nutrients and create suitable conditions to do anabolic reactions in amylase synthesis sites to increase the amylolytic enzymes activity. In this regard aleurone protoplasts of barley were transfected to express synthetic genes encoding cytosolic and secreted forms of the alkalophilic Bacillus alpha-amylase, alkBA. The alpha-amylase activity in the cytosol of transfected protoplasts was increased 4-fold compared to the controls [24]. Also, the action of botanical gebberlic acid growth hormones [25] and ethylene [26,27] was investigated in the release of amylase from barley aleurone layer. The aleurone cells of barley secrete substantial quantities of protein in response to GA3 [28]. GA increases the synthesis of poly (A)RNA, as well as the level of translatable alpha-amylase messenger RNA. However, the massive cell walls of the aleurone layer pose a formidable barrier to the release of protein into the surrounding medium. Extensive degradation of barley aleurone cell walls in response to GA3 was detected [29,30] Taiz and Starks have found that the hormone simultaneously stimulates both DNA synthesis and DNA degradation in aleurone cells, resulting in enhanced rates of DNA turnover and finally increase alpha-amylase release [25]. A similar result was recorded by Eastwell and Spencer as a result of ethylene treatment. They found that ethylene promotes the release of amylase from isolated aleurone layers by enhancing the production of the cell wall-degrading enzyme. Ethylene affects the production of only those enzymes that require GA3 for their synthesis [26].

Because of the commercial importance of diastatic power of malt in brewing and industrial application of alpha-amylase, in this work, the attention was focused on studying the effects of ultrasound in combination with conventional heat treatments on the germinated barley's alpha-amylase activity. As far as we know, no reference about the effects of ultrasound on the barley's alpha-amylase activity has been found in the literature.

MATERIALS AND METHOD

1. Chemicals and Regent

The chemicals include soluble starch obtained from potato, $(C_6H_{10}O_5)_n$ (S-2630), sodium potassium tartarate tetrahydrate, KOCOCH(OH)CH(OH)COONa·$4H_2O$ (S-2377), 3,5-Dinitrosalicylic acid, $(O_2N)_2C_6H_2$-2-(OH)CO_2H (D-0550), sodium phosphate monobasic anhydrous, NaH_2PO_4 (S-0751), maltose monohydrate, $C_{12}H_{22}O_{11}\cdot H_2O$ (M-5885), sodium metabisulfite $Na_2S_2O_5$ (71928), Na-phosphate NaH_2PO_4 (S0751) and maleic acid (disodium salt) $C_4H_2Na_2O_4\cdot xH_2O$ (M-9009). All of these materials with high analytical grade were obtained from Sigma-Aldrich and Fluka Companies and used in alpha-amylase assay.

3,5-dinitrosalicylic acid solution is used for measuring the reducing sugar. For preparation of this solution first 10 g of 3,5-dinitrosalcilic acid and 10 g of NaOH were dissolved in almost 600 ml distilled water. Then 192 g of tartarat sodium potassium with constant mixing was added to the solution. Afterward by adding 2 g of melted phenol and 0.5 g of metabisulfit, the total volume of the solution was adjusted to 1,000 ml with distilled water.

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 (25-37 °C) for 3 months after harvest.

2. Equipment

The sets of Gerhardt Kjeldatherm and Gerhardt Vapodest 30 instruments were used for determining the amount of protein in the barley seeds.

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

3. Experiments Design

In this study 3 important and effective parameters, i.e., temperature, time and ultrasound intensity in three levels, were selected for experimental design. Due to the sensitivity of working with enzymes and their behavior, to close monitoring and evaluate exact control-

ling of parameter's effects, 27 experiments each repeated for 3 times were done in the 20 kHz frequency for germinated barley. And the results are shown in figures 3 to 5 in order to analyze the results, 9 data×3 that were selected among the 27 experiments×3 were given to software according to the prediction of the Taguchi technique in design experiments condition (L9 orthogonal array), and Qualitek4 analysis using the ANOVA approach was employed for finding the average effects of individual parameters on enzymatic process condition as shown in the Figs. 6 to 8 according to software output. Finally, with determination of the optimum condition by software, conformation experiments were done with 90 and 95% confidence interval.

4. Malting Stage

Barley seeds were micro-malted manually in laboratory scale according to the following procedure: samples after steeping at 16-17 ºC for 6 h in the incubator chamber, were air-rested for 8 h. This process was done 3 times periodically to reach a moisture content of 45%. The subsequent germination phase followed 96 h with keeping the 45% moisture content by watering the samples every four hours. Then the samples were kilned in a drying oven with gradually ramping temperature from 17 to 55 ºC over 20 h, from 55 to 65 ºC over 20 h, from 65 to 75 ºC over 6 h, and finally from 75 to 82 ºC over 4 h. The drying process was stopped when the moisture content of samples reached 4%. Afterwards with removing the rootlets, the samples were milled, and the malted flour was prepared for the next stages of the experiments [31].

5. Sonication of the Samples

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) dispersed in 80 ml of tap water in direct sonication (probe system) at ultrasonic intensity of 20, 60 and 100% power setting of device. Ultrasonic irradiation was employed with additional agitation or shaking to avoid standing waves or the formation of solid free regions for the uniform distribution of the waves. The ultrasonic energy was pulsed by using a Duty Cycle Control. The cycle was set on 50% in all experiments. The solution was processed at three temperatures--30, 50 and 70 ºC--with the sonication horn for 5, 10 and 15 min. The temperature of water circulating in the water batch was set and the temperature inside the solution was intermittently checked so that the temperature of the solution remained constant during the experiments.

6. 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 enzymes rather than other medias such as the mixture of NaCl in water owing to either the high pH or added phosphate ions (higher concentration). The procedure of the extraction of enzymes from malt was performed exactly according to the method that has been presented and used by Osman [31].

7. Alpha-amylase Assay Using 3, 5-Dinitrosalicylic Acid (DNSA) According to Method of Reducing Sugars

This method determines the increase in reducing sugars as a result of amylase action on starch. The major defect in this assay is a slow loss in produced color and destruction of glucose by constituents of the DNSA reagent. To overcome these limitations, a modified method was developed for the estimation of reducing sugars. Sodium Sulphate was added in order to prevent the oxidation of the reagent [32]. The reagent is composed of dinitrosalicylic acid, potassium sodium tartrate tetrahydrate (Rochelle salt), phenol, sodium metabisulfite (or sodium disulfite), and sodium hydroxide. During the reaction, color development is stabilized by rochelle salt and enhanced by phenol, and sulfite protects the reagent and reducing sugar from oxidation [33,34].

8. Enzyme Assay

The progress of substrate hydrolysis was measured by determination of the reducing sugar equivalents released by using the modified 3,5-dinitrosalicylic acid (DNS) assay following a 10 min fixed-time incubation period with enzyme according to the method used for alpha-amylase assay by Osman [31] with this difference that the optical densities of samples, controls and the standard were read at 540 nm after cooling to room temperature and making up the contents of solution to 10 ml with distilled water. Reducing equivalents were calculated from the calibration graph obtained by using absorbance data for standard solutions of maltose reacted with DNS as described [31]. All enzyme assays were performed in duplicate.

One unit of alpha-amylase activity was defined as the quantity of enzyme that released the amount of reducing sugars per minute equivalent to one micromole of maltose, under the above defined assay conditions.

The enzyme activity was calculated according to the following formula [31].

$$\text{U/g malt} = \left(\frac{OD_t}{OD_s}\right) \times \left(\frac{\mu g_{realised\ maltose} \times EV \times DF}{t \times V \times m \times Mw_{maltose}}\right)$$

It has been established that 4 mL used to extract 0.75 g malt flour yielded on average 2.95 mL maltose.

RESULTS AND DISCUSSION

1. Thermal Inactivation of Barley's Alpha-amylase

In order to investigate the effect of thermal treatment on barley's alpha-amylase activity, the experiments were performed at temperatures ranging from 30 to 70 ºC, at the different times. The results are shown in Fig. 1. As can be seen from this figure, with increasing the temperature, the enzyme activity decreases. Based on the knowledge of the heat treatment feature, it seems it is because a large temperature gradient may lead to thermal inactivation of the enzyme or pyrolysis of bonds in the protein. Moreover, it is of in-

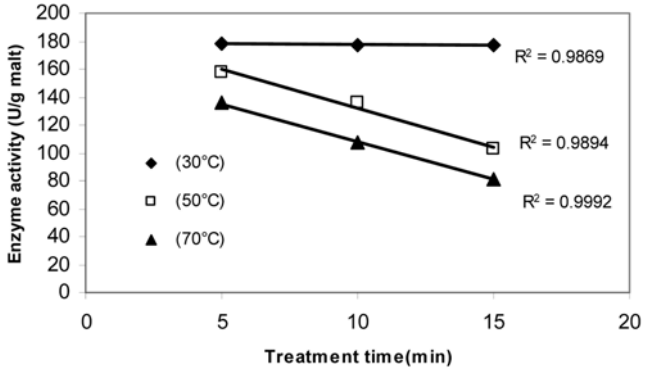

Fig. 1. Thermal inactivation of alpha-amylase.

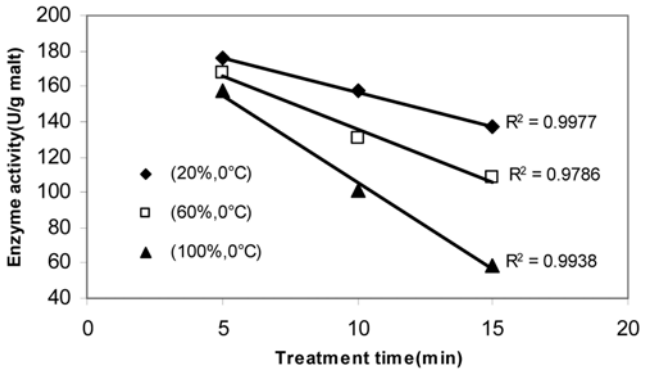

Fig. 2. Alpha-amylase activity versus time, barley sonication after germination at 0 °C.

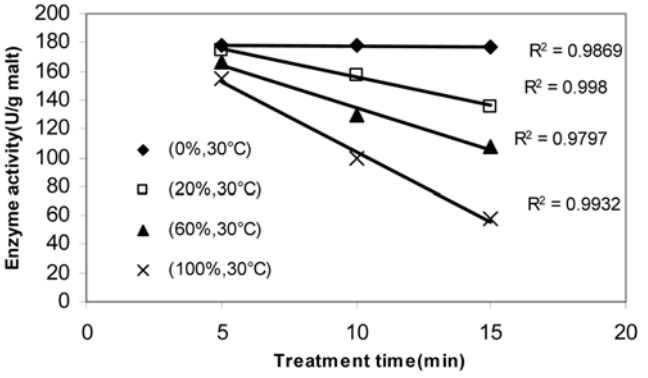

Fig. 3. Alpha-amylase activity versus time, barley sonication after germination at 30 °C.

terest to discuss the effect of thermal treatment on the activity of alpha-amylase at 30 °C. From Fig. 1, it is clearly seen that at 30 °C enzyme inactivation caused by heating is negligible as indicated ranging from 177.904 to 177.192 (U/g malt). This can be explained by summarizing the work of several research groups on barley's alpha-amylase activity. For instance, Muralikrishna and Nirmala noticed that the temperature optima of cereal amylases are between 40 and 55 °C, and above this temperature range most of the cereal amylases undergo inactivation [23]. Therefore, at 30 °C no evident deactivations of alpha-amylase were detected.

2. The Effect of Acoustic Power on Barley's Alpha-amylase Activity

In order to investigate the effect of ultrasonic intensity, the enzyme solutions were sonicated at different acoustic powers ranging from 20 to 100% setting from total power of device at 0 °C (the beaker of solution was put in a mixture of ice and water). Data shown in Fig. 2 indicates the enzyme activity versus time. As can be seen from this figure, the enzyme activity decreased with increasing the acoustic powers. Typically, the activity decreased from 136.870 (U/g malt) for 20% to 58.885 (U/g malt) for a cavitation intensity of 100% power setting at the end of the 15 min processing time. 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 enzyme protein structure and cause loss in enzyme activity. Another mechanism that enzymes inactivated during the sonication is due to the modification or damage of enzymes' molecular structure. Free radicals are species that possess an unpaired electron with highly reactive activity, which can alter the charge distribution on the protein surface, damaging enzyme active site geometries, thus leading to the loss of enzyme substrate affinity. So in this regard, the reason for barley's alpha-amylase inactivation lies in the free radicals and shear force (shock wave), which caused the modification or damage of alpha-amylase molecular structure. Obviously, at high cavitation intensity levels there will be more damage to the barley's alpha-amylase structure, resulting in a higher inactivation no matter what mechanisms might be involved.

It must be mentioned that the examination of the experiments at 0 °C was done merely for studying the effect of ultrasound alone on barley's alpha-amylase activity. Therefore, the obtained data from the experiments with sonication at this temperature were not used in the statistical analysis. This assumption was considered because of the possible solving difficulty encountered when using the asymmetric matrix.

3. Inactivation of Barley's Alpha-amylase by Ultrasonication at 30 °C

The efficacy of ultrasonication on barley's alpha-amylase inactivation was investigated at 30 °C and cavitation levels between 20% and 100% power setting of the device. Resulting activation and the corresponding regression coefficients (R^2) are shown in Fig. 3. It is obvious that increasing cavitation intensity and the time of exposure to ultrasound increases inactivation. The activity decreased from 177.904 (U/g malt) for 0% to 57.590 (U/g malt) for a cavitation intensity of 100% in the time intervals of 5 to 15 min, respectively. To examine the effect of thermosonication on barley's alpha-amylase, the inactivation at 30 °C due to sonication is compared to inactivation due to heat treatment at the same temperature. Comparing thermal inactivation (corresponding to 0% in figures) with sonication tests, one can see that sonication substantially decreases barley's alpha-amylase activity at 30 °C. Since 177.904 (U/g malt) is for thermal treatment at 30 °C, in the time frame of the inactivation tests, i.e., <15 min, the inactivation caused by heating at 30 °C is negligible. Therefore, at 30 °C when the temperature is not high enough to cause a decrease in barley's alpha-amylase activity, barley's alpha-amylase inactivation in an ultrasound treatment at 30 °C is due to sonication itself. This result can most likely be explained by the inactivation mechanism at low temperature (0 °C) as described above.

4. Inactivation of Barley's Alpha-amylase by Ultrasonication at 50 and 70 °C

The impact of various power settings and treatment times on the inactivation of barley's alpha-amylase at 50 °C and 70 °C is presented in Figs. 4 and 5. From both of these figures much greater inactivation can be observed compared to the thermal treatment and sonication at 30 °C. The activity amount obtained from thermosonication at any observed temperature was much smaller than that for thermal and sonication inactivation. However, the experimental results clearly indicate that the deactivating efficiency of ultrasound becomes low with increasing the temperature close to 70 °C. Inactivation by the combined action of heat and ultrasound can also be examined with the ratios of the obtained activity in sonication over

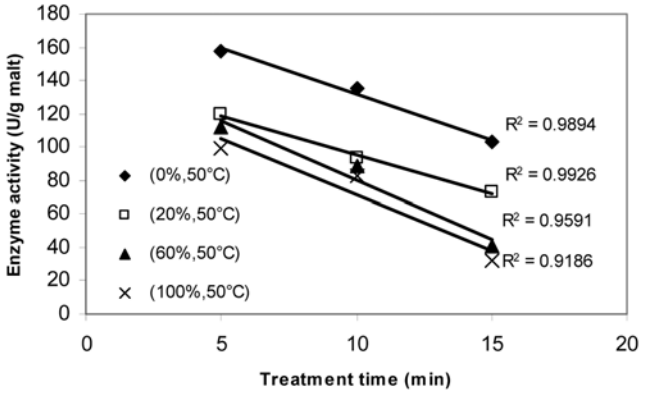

Fig. 4. Alpha-amylase activity versus time, barley sonication after germination at 50 °C.

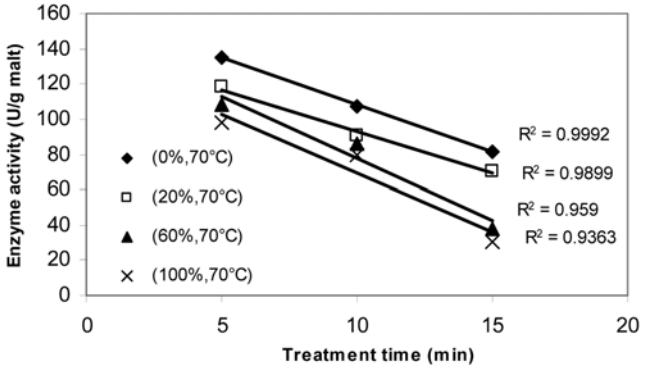

Fig. 5. Alpha-amylase activity versus time, barley sonication after germination at 70 °C.

thermosonication at the same cavitation level. For instance, the enzyme activity for sonication (100% of power setting) at 30 °C was 57.59 (U/g malt). When the temperature was increased to 50 °C to introduce heat-induced inactivation, the activity was reduced to 31.980 (U/g malt) at the same cavitation level. This decrease is several times higher than when temperature was increased from 50 °C to 70 °C (proportional with enzyme activity 30.530 U/g malt) at the end of 15 min processing time. From a practical point of view, this means that in order to improve the efficiency of the thermosonication treatment, the use of very high temperatures may not be very useful. A possible explanation for this could be the following: It seems that the increase in inactivation in thermosonication of barley's alpha-amylase is more pronounced at 50 °C. The well-known fact that thermosonication effects diminish with temperature contributes without doubt to this effect. This is mainly due to the higher vapor pressure of water at elevated temperatures. Higher vapor pressures serve a cushion effect during the implosion of cavitating bubbles, making this phenomenon less intense [35]. Another reason to this case could be related to the synergistic effect between the heat treatment and the ultrasound treatment at the same temperature. Several mechanisms have been suggested to explain the synergistic effect of combined ultrasound along with other treatments on enzyme inactivation. One mechanism is the free radicals so produced by decomposition of water inside the oscillating bubbles could be scavenged by some amino acid residues of the enzymes participating in structure stability, substrate binding, or catalytic functions. These mechanisms are responsible for the synergistic effect observed in thermosonication alpha-amylase inactivation [7]. It is worth noting that both Figs. 4 and 5 are roughly parallel and show the same trend with temperature. It implies that the sonochemical and mechanical effects of ultrasound are dominant over the whole cavitation level and time interval examined, although its efficiency decreases with the rise of temperature.

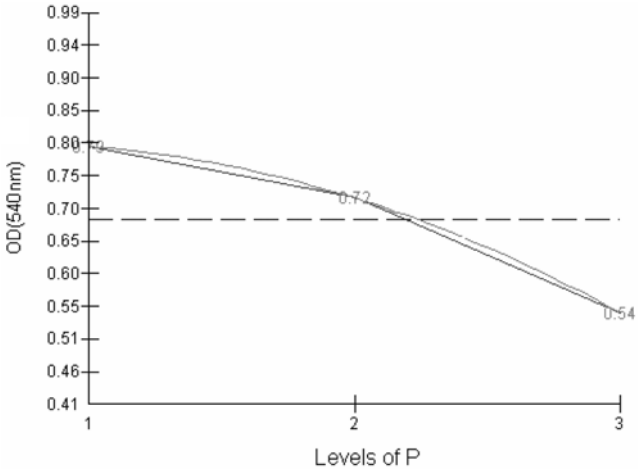

Fig. 6. Average effect of ultrasonic intensity by Taguchi method using qualitek4 software.

It is important to note that similar results in the present study but minor differences in the behavior of enzyme at elevated temperatures a long with sonication have been reported with respect to bacillus alpha-amylase more recently by Kadkhodaee and Povey [18]. How-

Table 1. Analysis of variance (ANOVA) showing the effect of ultrasonic power (P) and other variables (t, T) as significance of the main effects

Number	Factors	DOF	Sums of squares	Variance	F-Ratio	Pure sum	Percent
1	P[a]	2	0.289	0.144	172.633	0.287	17.049
2	t[b]	2	0.798	0.399	476.174	0.796	47.201
3	T[c]	2	0.583	0.291	347.884	0.581	34.457
Other/Error		20	0.016	0.000			1.293
Total		26	1.688				100.000%

[a]Ultrasonic Power
[b]Irritation Time along with Thermal Treatment
[c]Temperature

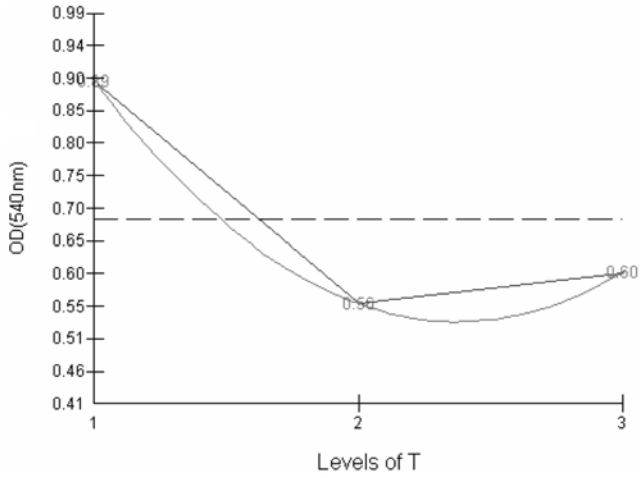

Fig. 7. Average effect of temperature by Taguchi method using qualitek4 software.

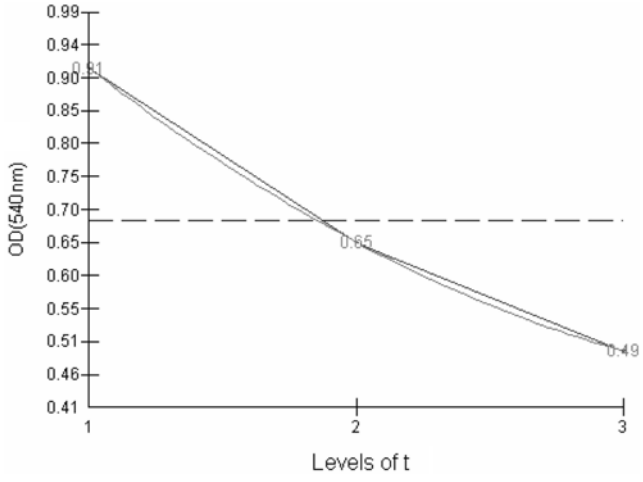

Fig. 8. Average effect of time by Taguchi method using qualitek4 software.

ever, these differences may correspond to differences in the origin of enzyme or perhaps due to differences in the ultrasonic generator performance (frequency, power), the size and geometry of the sonotrode and the characteristics (viscosity, surface tension, nature and concentration of dissolved gas, etc.) and the volume of suspension submitted to the treatment.

5. Qualitek4 Statistical Analysis of Barley's Alpha-amylase Thermosonication

Table 1 shows the detailed analysis of variance results of experiments conducted under the Taguchi method. In this table the contribution of each factor quantitatively was determined by using an ANOVA approach. Also, Figs. 6 to 8 depict the main effects of ultrasonic power, temperature and exposure time, respectively. By the term "main effects," the average of the 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 of all of these parameters on enzyme activity are negative and the maximum effects of these parameters were in the third level of them.

Based on the data listed in Table 1 there is no doubt the alpha-amylase was inactivated during the ultrasound irradiation. Both ultrasound power and irradiation time obviously affect the alpha-amylase biological activity. The results of the ANOVA (Table 1) reveal that time of exposure to ultrasonic irradiation along with thermal treatment, which reached 47.201%, made a major contribution to the overall performance.

It should be mentioned that in these curves the longitudinal axis is the level of selected parameters such as P=ultrasonic power, T= temperature and t=the time of exposure to ultrasonic irradiation along with thermal treatment and transverse axis is an optimal density in accordance with the activity of enzyme as a criterion of the process yield in statistical analysis.

CONCLUSIONS

Ultrasound treatment effectively increased the barley's alpha-amylase inactivation compared to a thermal treatment at the 30 °C. When sonication was combined with a heat treatment at temperatures high enough to cause thermal inactivation, greater inactivation was observed. Although as the temperature increased much further, the efficiency of ultrasound decreased, but the overall inactivation rate still remained higher than the thermal inactivation rate. Also, from the results presented above it can be concluded that the activity of alpha-amylase significantly decreased with extending irradiation time.

At first, the aim of this study was to enhance the barley's alpha-amylase activity via ultrasonic irradiation in the germination stage of the malting process, but as it is shown in the results section, we noticed that our results not only are not in agreement with our primary idea, but also are totally in disagreement with it. As a matter of fact, ultrasound has a destructive effect on barley's alpha-amylase and causes inactivation of this enzyme.

The results of this research can be profitable in case the inactivation of this enzyme is a main goal. For instance, the inactivation of the alpha-amylase after liquefying is a necessity in fruit juice applications.

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NOMENCLATURE

OD_t : optical density of test sample at 540 nm
OD_s : optical density of the maltose standard
EV : extraction volume [ml]
DF : dilution factor
t : incubation time [min]
V : used volume of diluted enzyme extract [ml]
m : weight of malt flour [g]

Mw : molecular weight of maltose

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