Application of Ultrasonic Waves as a Priming Technique for Accelerating and Enhancing the Germination of Barley Seed: Optimization of Method by the Taguchi Approach

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ABSTRACT

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In this study the effects of different intensities and exposure time of ultrasound on barley seed have been investigated to determine the optimal conditions for accelerating germination. For optimization, the Taguchi approach was used. The germination rates and/or yield of the treated sample seed were compared with those of the untreated seed germinated under normal conditions. The seeds were treated with ultrasonic energy (input power 20-90% of 460W) and were exposed to three time periods ranging from 5 to 15 min. It was found that germination of the treated barley seed was increased about 1.042–1.065 times compared with that of the untreated seed. The ultrasonic treatment proved to be efficient in reducing the germination period by 30-45%. It was concluded that priming of seeds with ultrasound was effective in improving water uptake and germination. The data presented in this paper suggest that the increase in final germination percentage, together with the reduced germination period in treated seeds, may be due to the action of ultrasound and that it led to an improved hydration process with concurrent shell fragmentation.

Key words: Barley seed, enhancing germination, optimization, Taguchi technique, ultrasonic priming technique.

INTRODUCTION

Barley is one of the major industrial seeds of monocotyledonous grains, in the malting industry. The barley grain has been of interest to plant biologists for many years, because of its importance for agricultural (germination of cereal grains) and industrial (the brewing industry) usage. The malt grain is used inter alia for the production of beers, lagers, barley wines, malt extract (powders and syrups) and diastase. Determining the germination capacity is an essential component in many aspects of the growing, marketing and utilization of crops. It is particularly important in the cereal grain industry, where the quality of the commodity may be seriously affected by precious germination or, in the case of barley, where the ability of the grain to rapidly and uniformly germinate is an essential component of malting quality. Generally speaking the malting process is designed to provide an acceptable product for the brewer, distiller or food processor in an efficient manner. Conventionally, this industrially very important grain is not always satisfactory in respect to its rate and/or yield of germination. Therefore improved yield and/or rate of barley seed germination would be valuable in the area of commercial brewing and agriculture. In addition, shortening the germination period may have significant commercial benefits to the brewing industry and may also affect yield as ungerminated seeds are prone to bacterial and fungal damage, due to environmental conditions.

Many seed priming treatments have been used to accelerate germination and seedling in most of the crops under normal and stress conditions[6]. Pre-sowing hydration treatments (priming) include non-controlled water uptake systems (methods in which water is freely available and not restricted by the environment) and controlled systems (methods that regulate seed moisture content preventing the completion of germination). There are several indications that many physiological mechanisms are involved in seed priming such as the repair of the age related cellular and subcellular damage that can accumulate during seed development[7,9] and an advancement of metabolic events of imbibition that prepare the radicle protrusion[11]. Some morphological changes also occur in the primed seeds. These are helpful in the later growth of the embryo (e.g., a portion of the seed endosperm is hydrolyzed during priming and permits faster embryo growth[9]). Several techniques are used for controlled water uptake of barley grain including priming with an additive solution such as chemicals[2,18], plant hormones, combination of plant hormones with natural or artificial inhibitors[5,30] or by controlled hydration with water[16,23]. Among these materials the best known is gibberellic acid (GA). Gibberellic acid is the botanic hormone, which regulates germination. Malt production of high quality is time consuming and expensive. In addition, chemical methods have disadvantages if the malt contains residues due to the treatment. In order to avoid these unfavorable results, developing other methods

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to increase the germination rate and germination capacity of barley seeds or enhancement of the ability of seeds to imbibe water are important.

Recent progress in the field of seed biology has been aided by molecular approaches (DNA recombination) utilizing mutant and transgenic seeds. In the case of barley seeds, the methods for stably transforming barley have been described by some research groups[22,33]. The metabolism of the physiological or biochemical changes of barley seeds can also be influenced by physical methods such as ionizing radiation[21] prior to the malting process, subjecting the slurry of water and seeds to gas pressure (carbon dioxide) or subjecting a bulk of the seeds to a vacuum for a selected period of time[8]. The ionization method, according to work by Ress et al.[21], is successfully applicable for malting barley grains within certain ranges, but there is a problem associated with this method. It is that the radiation treatment must be carried out in the starting stage of the processing; suitable germination of the treated barley should start within a week of irradiation, as the inductive effect of irradiation treatment tends to decline during storage. In respect to using either the pressure or vacuum system separately, although greater moisture content was obtained, it was noted a portion of the seeds treated by carbon dioxide suffered substantial bran damage and in the case of latter method, as soon as the vacuum was obtained, the seeds were observed to violently outgas. Considering the great importance of precious germination in malt quality, use of traditional methods (additive) or reported physical methods are a less acceptable approach economically and ultrasonication as a pretreatment may be a good technique to be tried along with normal steeping of barley. Ultrasound as a novel physical method acts as an alternative stress on cells or tissues. The most common interaction mechanisms which are involved in this case are acoustically induced cavitational activity which causes heat and chemical effects. In addition to these, acceleration of the rate of influx or uptake of a substance into a seed by ultrasonication can also be caused by mechanical effects, i.e. shear stress developed by eddies arising from shock waves. These effects arise principally from the phenomenon known as cavitation. Cavitation refers to the formation, growth and violent collapse of microbubbles in a sonication liquid due to pressure fluctuations[27]. The collapse of the bubbles leads to energy accumulation in hot spots and temperatures of above 1000 K and pressures of approximately 500 MPa have been measured[28]. Power ultrasound produces its effect via cavitation bubbles. When power ultrasound is applied to a liquid in sufficient intensity, the liquid is alternately compressed and expanded, forming bubbles. When power ultrasound is applied to a mixture of particles and liquid and the bubbles collapse near a solid surface, a high-speed jet of liquid is driven into the particles and this jet can deposit enormous energy densities at the site of impact, which can scour surfaces and damage cellular material[29].

Ultrasound has been used in conjunction with seeds for many purposes. In biotechnology processes and the food industry, ultrasonically stimulated seed germination and increasing the percentage of germination offers the possibility of increased productivity for large scale farm crops and for more general horticulture. Ultrasonic irradiation of the seeds of wheat[32], carrots[3], temperate Cymbidium species[10], corn[15,26], rice, tomatoes[1], and radishes[24], as well as sunflowers[20], in the dry-air state when carried out up to several months before actual sowing, led to the ripening of plants of grain and vegetable crops by 5–10 days sooner than control ones. There was a 10–30 day rise in their yield, an increase in the resistance of grain crops to falling and diseases, and a shorter period to form a gel as a result of a faster release of starch during subsequent cooking in sonicated rice grains in water[19]. Detailed discussions on the results of these investigations can be found in various articles[14] and books[20]. Reports on the intriguing possibility that the use of ultrasound may enhance the stimulation and germination of some seeds led us to examine the feasibility of ultrasound-induced effective germination of barley seeds and increasing the rate of germination.

Despite the fact that sonication has been applied on an extensive range of seed types[3,4,24-26,32], as yet, based our knowledge there is a no scientific literature about the application of ultrasonic waves for stimulating the germination of barley seed and the level of germination. The present investigation therefore, was designed with the objective to accelerate germination and to enhance the percentage of germination of barley seeds through an ultrasonic wave priming technique. For this purpose, the day of germination and the record of the numbers of seeds germinated were kept as a means of determining rate and/or yield of the process.

MATERIALS AND METHODS

Seed materials

Barley seeds (*Hordeum vulgare*, variety of karon in kavir), with moisture content of 9% (dry weight basis) and an average protein content of 11.5%, were used in all experiments. To prevent absorption of moisture, they were stored in a dry place at 20°C until required.

Instrumentation

Gerhardt Kjeldatherm and Gerhardt Vapodest 30 instruments were used for determination of protein in barley seeds.

Ultrasonic irradiation was performed by means of a UP 200 H ultrasonic processor horn type (20 kHz, maximum wave amplitude of 210 μm, and maximum nominal power of 460W) equipped with a radial Sonotrode S3 (3 mm diameter, maximum immersion length of 90 mm) designed by Dr. Hielscher GmbH (Treptow, Germany).

Experimental design

The Taguchi method is one of the most well known and widely applied robust design methods. The Taguchi approach enables a comprehensive understanding of the individual and combined effects of various design parameters to be obtained from a minimum number of simulation or experimental trials. The aim of the Taguchi design method is to establish the parameter settings that render the product quality robust to unavoidable variations in external noise. ANOVA is a standard statistical technique to interpret the experimental results and is used ex-

tensively to identify the performance of the group of parameters under investigation. The purpose of ANOVA is to investigate the parameters, whose combination to total variation is significant. If a design parameter is found to be significant, it implies that this parameter plays a fundamental role in determining the optimal solution of the design problem. The term interaction is used when the change in operational level of one factor influences the performance of other factors.

In this study, four important effective parameters were selected, namely, ultrasonic power, the time of ultrasonic irradiation, temperature, and frequency. Since the operating temperature and frequency were fixed in this design problem, only 2 variables remained for the design of experiments. Two factors with three levels were selected as shown in Table I. The factors and levels were used to design an orthogonal array L9 for experimentation. The layout of the trial condition is shown in Table II. (the letter "L" refers to Latin square, while the number "9" is the replication number of the trials). The nine experiments with introduced parameters at 30ºC and 20 KHz were conducted three times to ensure the reliability of experimental data for a standard analysis. The software, Qualitek-4 (Version 4.80.4), which is designed for Taguchi experiments, was used for the optimization, analysis of results, and determination of the main effect or average effects of individual parameters on process conditions and interactions between factors. The overall evaluation criteria (mean germination time) were calculated using software and depended on the specified quality characteristics. In this case, the mean germination time was chosen as a yield of process. Since the goal is to maximize the yield, "smaller is better" was performed as the optimization criteria.

Seed treatments

The ultrasonication experiments were carried out at 20 kHz on the ultrasonic generator. The tip of the horn was immersed into about 9 mm of solution to be processed. All experiments were performed on samples (10 g barley seeds) dispersed in 80 mL of tap water with direct sonication (probe system) at a power input of 20, 60, and 100% of 460 W, with additional agitation or shaking. This was employed to avoid standing waves or the formation of solid free regions for a 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 a constant temperature of 30°C with the sonication horn for 5, 10, and 15 min. The temperature of water circulating in the water bath was set and the temperature inside the beaker (100 mL laboratory glass beaker) was checked intermittently, so that the temperature of the solution remained constant during the experiments.

Table I. Control factors.

Number	Factors	Level 1	Level 2	Level 3
1	P[a] (%)	20	60	100
2	t[b] (min)	5	10	15

[a] Ultrasonic power.
[b] Irradiation time.

Imbibition test

First, the efficacy and optimal condition of ultrasonic waves for overcoming physical dormancy in this seed lot was studied. Of particular interest was the effect of sonication on the seeds' ability to rapidly imbibe water. For this purpose, the imbibition test with the treated seed samples, with 3 replications per treatment, was accomplished by placing seed samples in distilled water, stirring vigorously for 60 s to disperse the seeds, and allowing the seeds to remain undisturbed for the appropriate duration of steeping. Samples were allowed to air-dry for 10 h after soaking before weighing (initial weight). Samples were then placed in a 17°C incubator for imbibition for 36 and 44 h. After completion of imbibition, seeds were surface dried on paper towels and reweighed (final weight). The percentage increase in seed weight due to imbibition was calculated as follows:

$$Percentage\ increase\% = \left(\frac{\left(final\ weight - initial\ weight \right)}{initial\ weight} \right) \times 100$$

Germination test

Germination experiments were conducted with germination at 30°C in 100 mm Petri dishes (30 seeds per dish, 3 replicates) on a layer of filter paper moistened with distilled water. All sonicated seeds were surface sterilized with 2% (v/v) hydrogen peroxide followed by distilled water. After steeping at 16-17°C for 6 h in the incubator chamber, samples were air-rested for 8 h. This process was repeated 3 times. A treated seed was considered germinated when rootlets were noted at the edge of the grain. Water was added to the petri dish as needed. Germination counts were made daily for 7 days. Total numbers of seed germinated/emerged were counted and the percentage was calculated. Germination rate, the average number of days required for seeds to germinate, was expressed as mean germination time (MGT). Mean germination time was calculated by the following equation:

$$MGT = \frac{\sum gn}{G}$$

where g is the number of seeds newly germinated (just germinated criterion) at time n, n is the hours or days from when set to germinate and G is the total number of seeds germinating during the 7th day[13].

The control was treated as above but without sonication. All experiments were performed in triplicate.

Table II. Inner array (L9).

	Columns	
Trial number	**1**	**2**
1	1	1
2	1	2
3	1	3
4	2	1
5	2	2
6	2	3
7	3	1
8	3	2
9	3	3

RESULTS AND DISCUSSION

Effect of ultrasound on increasing the sample weight and the germination rate

The efficacy of ultrasonic waves on the increase of sample weight after 36 h and 44 h of soaking was investigated using cavitation levels between 20 and 100% power setting of the device (Table III). Maximum germination was achieved with sonication at 100% for 15 min. Increasing sonication intensity and irradiation time improved germination, showing an increase from only 34% germinated seeds without sonication to 91% for seeds treated for 5 min at 100% of the power setting. Seeds that were treated for 15 min achieved near complete imbibition (increase in seed weight 96% on a dry weight basis) after 44 h of steeping. In comparison, non-sonicated seeds increased seed weight by 34% after 36 h and approached complete imbibition only after 44 h, achieving a 52% seed weight increase. For all three treatments, near doubling of seed weight following the ultrasonic treatment indicated seeds imbibed water readily, and physical dormancy, if present, was overcome.

Moreover, as the irradiated barley absorbed water faster than the control, the steeping period was shortened to 25-30 h from the usual 46 h and during this time the barley seeds absorbed 41 to 45% of water necessary for germination (Table IV). Under the influence of ultrasound, normal steeping (or germination) occurs but several additional factors, as will be explained more fully below, contributed towards improvements in efficiency.

A number of factors have been proposed to account for more water retention capacity in dry grains. One of the possible explanations could be that the mechanical effects of ultrasonication produced numerous small holes in the coating and after steeping in the water a significant rise in seedling moisture resulted. It has been suggested that the sonication process accelerates the imbibition of water through the pericarp. Sonication may create or enlarge fissures in the protective coating surrounding the seed and pericarp. The superiority of sonication may be due to a higher holding capacity and higher porosity, which increase oxygen availability.

Table III. Percent increase of sample weight (average of 3 repetitions).

Time (min)	T = 36 hours after steeping				T = 44 hours after steeping			
	Power setting (%) of 460W							
	0	20	60	100	0	20	60	100
5	34	36	53	58	52	73	81	91
10	34	41	59	64	52	80	88	93
15	34	43	61	77	52	85	92	96

Table IV. Moisture percentage of samples (average of 3 repetitions).

Ultrasonic power Power setting(%) of 460W	Time (hours)	
	25	30
0	30.9	33.5
20	34.8	41.4
60	41.3	44.2
100	45.1	45.5

Effects of ultrasonic treatment on the final germination percentage and mean germination time

The efficacy of ultrasonic waves on the germination percentage of barley seed and mean germination time was investigated at 30°C and at cavitation levels between 20 and 100% power setting of the device. Fig. 1 and 2 illustrate the final germination and mean germination time of treated seeds by ultrasonic irradiation against time respectively. Concerning the sonication process, the following results have been obtained:

The highest seed germination (approximately 100%) was recorded at the 100% power setting. Seeds sonicated for 5, 10, and 15 min at full power of device increased in germination from ~93.3% (non-sonicated seeds) to 97.2%, 98% and 99.4%, respectively. These results may be attributed to mechanical effects due to ultrasonically induced cavitation increasing water uptake by the cell walls. The most probable mechanism for ultrasonic enhancement of germination is the intensification of mass transfer and easier access of the water to the interior of the cell wall structure. The collapse of cavitation bubbles near cell walls would be expected to produce cell disruption together with good penetration of water into the cells, through the ultrasonic jet.

The method considerably reduced the time required to initiate the germination of seeds. Hair roots appeared fast-

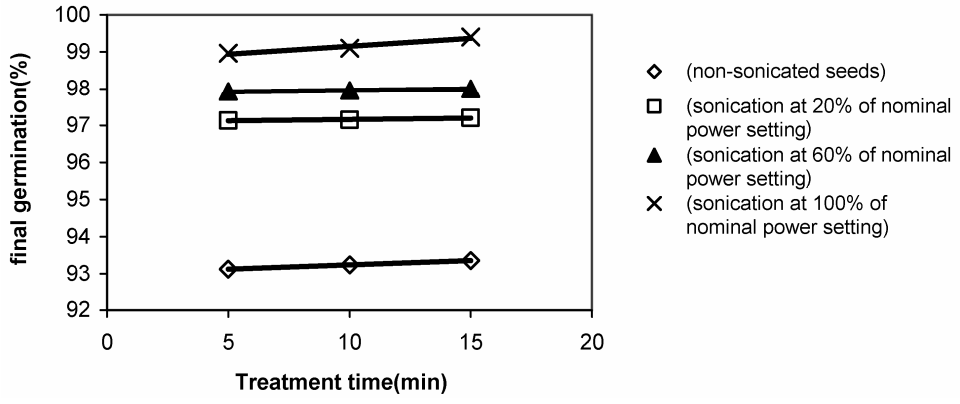

Fig 1. Effect of ultrasonic power and irradiation time on final % germination.

er in the treated samples and grew abundantly compared to the untreated seeds. When using barley treated as above, the germination period was shortened to 4 to 5 days (depending upon the ultrasonic power and exposure time to ultrasonic waves) from the usual 7 days. Moreover, the mean germination time decreased from 6.66 days for the 20% power setting to 4.04 days for the ultrasonic power setting of 100% after the 15 min processing time. The analysis of the resulting data indicates that the extent of germination and the mean germination time were significantly affected by the different ultrasonic power settings during the germination test. All resulted in increased germination of the barley seeds as compared to the control (Fig. 1). Maximum mean germination time was recorded for the 20% power setting and minimum mean germination time was recorded for the 100% power setting (Fig. 2).

Results of the germination test demonstrated that germination of barley seeds was improved by ultrasonication. The decrease in mean germination time with sonication may be due to initiating metabolic events in primed seeds. The well-known cavitation phenomenon, without doubt, contributes to this fact. Since the mixture of barley grains and water form a heterogeneous system, the most pertinent effects of ultrasound for these systems (i.e., mechanical effects) are attributed to symmetric and asymmetric cavitation. Because the barley seed surface is several orders of magnitude larger than the cavitating bubble, symmetric cavitation is hindered and the bubble collapse occurs asymmetrically near a seed-surface. Cavitation bubble collapse generates intense local heat and high pressure, with a very short lifetime and large-amplitude shock waves and microjets. When this collapse occurs close to a barley seed surface, these intense disturbances generate highly localized and transient surface stresses. This stresses and repeated bubble collapses would be expected to damage cell walls and cause shell fragmentation of barley seeds. Shell fragmentation is the major reason for a diminished germination period together with an increasing percentage of germination. Shell fragmentation dramatically increases surface areas and the mass transfer rate of water into the cell wall[31]. Therefore, as a result of increasing the mass transfer rate of the target components, the sonicated tissues absorb an extra volume of water. The extra absorbed water reacts freely and readily with the cell embryo, in a manner that releases gibberellic acid and causes a speed up of metabolic processes in aleurone cells. Since the process of converting the starch to sugar in barley seeds is dose dependent, the amount of gibberellic acid present affects the rate and yield of germination. Consequently, imbibition of water, and gibberellic acid, which is naturally synthesized as a part of plant metabolism, will significantly reduce the mean germination time and will increase the rate and yield of germination. The results clearly indicate that the mean germination time in the sonicated grains is much less, compared to nonsonicated seeds at a constant interval time. The yield of the process was increased by 6.53%. These are probably the main reasons for the germination time reduction together with the increased final germination percentage, as a result of ultrasonic treatment of seeds, but not the only ones. It is also possible that the electrical and light energy (i.e., sonoluminescence effect)[29] released by the cavitation effect produces physiological or biochemical changes in the seed which promote the germination process[26]. Moreover, the mobilization of endosperm nutrients

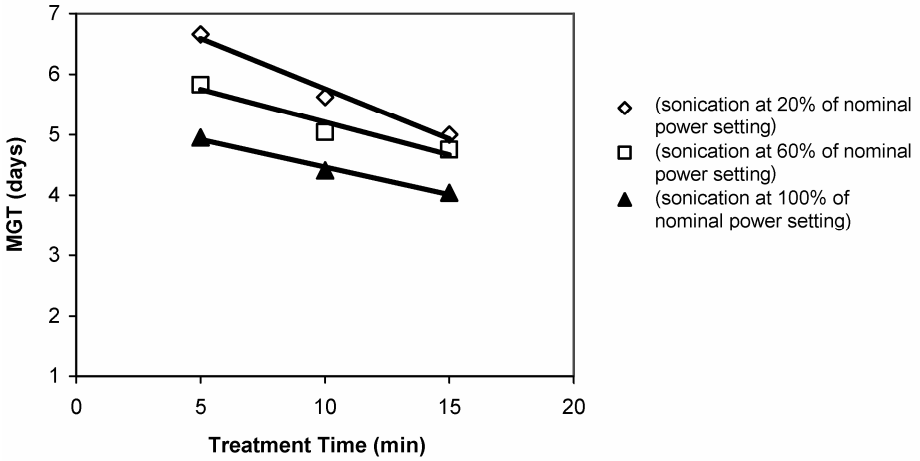

Fig 2. Effect of ultrasonic power and irradiation time on mean germination time. MGT = Mean Germination Time.

Table V. Analysis of variance (ANOVA) showing the effect of ultrasonic power (P) and the variable of time (t) as significance of the main effects.

Number	Factors	DOF	Sums of squares	Variance	F-Ratio	Pure sum	Percent
1	P	2	7.727	3.863	186.343	7.685	51.397
2	t	2	6.77	3.385	163.266	6.729	44.997
Other/error		22	0.456	0.02			3.606
Total		26	14.954				100.000%

by the action of ultrasound may underlie the enhancement of the percentage of germination in treated seeds. Obviously, at a high cavitation intensity level, there will be more damage to the barley's external surface, resulting in a higher percentage of germination, no matter what mechanisms might be involved.

The ANOVA results for the overall evaluation criteria (mean germination time) are given in Table V. According to the ANOVA results for the mean germination time, ultrasonic power is the most significant factor followed by irradiation time. The relative percentage contributions of errors are only 3.6%, which indicates that all the major contributing factors related to the objective of the study were considered.

Furthermore, Fig. 3 also indicates that the relative contributions of ultrasonic power and treatment time increased with the increasing levels. This figure depicts the main effects of ultrasonic power and treatment time. The term "main effects" is the average of obtained results (as mean germination time), in which each factor is at a given level. The average effects of interaction between time and ultrasonic power are presented in Fig. 4.

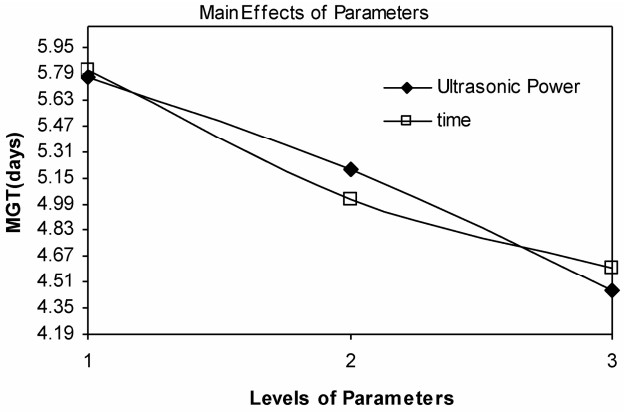

Fig. 3. Average effect of ultrasonic power and exposure time by the Taguchi method using Qualitek4 software. MGT = Mean Germination Time.

Determination of optimal condition using the Taguchi method

The Taguchi method was used to identify the optimal conditions and to select the parameters having the greatest principle influence on the mean germination time. Using the Taguchi method, optimum conditions were determined as follows: 100% power setting for ultrasonic waves and 15 min for treatment time at constant temperature and frequency (Table VI). The experiment corresponding to the optimum conditions was performed during the experimental work. The corresponding mean germination time with working conditions was 3.92 with an estimated S/N value of -14.321 using Qualitek4 software.

Conformation experiments

Once the optimal level of the process parameters was selected, the final step was to predict and verify the improvement of the performance characteristic using the optimal level of the process parameters. Since the error of variance was only 3.6% and all the major effects of the two parameters were significant, the optimum design parameter fell into the combination of t3p3 that was implemented during the experimental work. Thus there was no need to conduct additional experiments or to look for better combinations. However, confirmation experiments were performed using the optimal design parameter combination of t3p3 with the 99% confidence interval. The confirmation experiments were carried out three times at the same working conditions. The results indicated that the corresponding mean germination time was 3.92 with an estimated S/N value of -11.958. These values represent an improvement over the original results, and hence this

Table VI. Optimum condition and performance.

Number	Factors	Level description	Level	Contribution
1	P	100	3	–0.683
2	t	15	3	–0.543
Total contribution from all factors.				–1.226
Current grand average of performance.				5.146
Expected result at optimum condition.				3.92

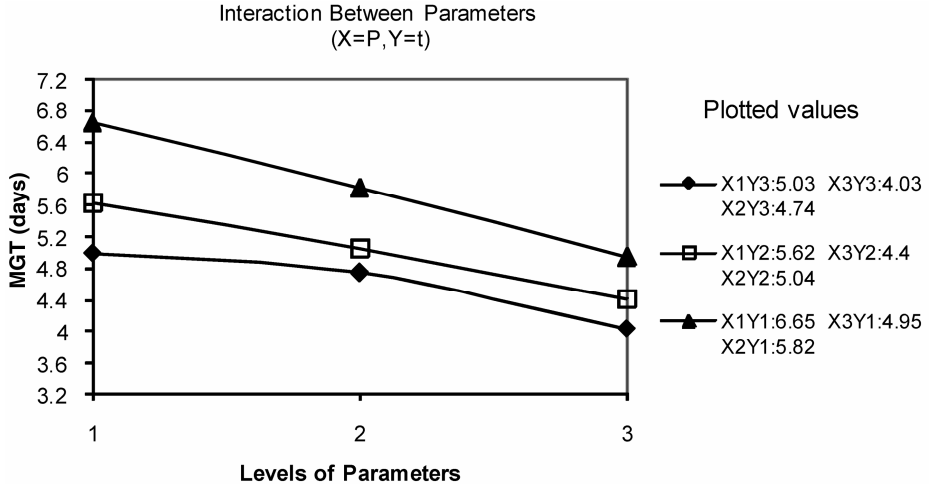

Fig. 4. Average effects of interaction between time and ultrasonic power by Taguchi method using Qualitek4 software. MGT = Mean Germination Time.

verification exercise enhanced confidence in the technique.

Commercial perspectives

Subjecting the barley seeds to direct sonication substantially reduced the time for germination and increased the percentage of final germination, but it may be questionable as to whether or not the present study provides a method of promoting the germination of seeds, when processing a large quantity of seeds at one time, thereby providing a commercially viable scale of operation. One must also consider that although the possibility of increasing the rate of barley germination by ultrasonic waves has been proven on a laboratory scale, the same may be not true for industrial applications. Moreover, the difficulty with this method is in obtaining equal ultrasonic wave exposure in all areas of treated seed lots. The reasons to date for the non-development on an industrial scale of this technique are numerous, and in part non-development is due to a lack of basic information needed for the design and scale-up procedure[17].

CONCLUSIONS

It has been shown that a promising ultrasonic priming technique improved the performance of low vigor barley seed and induced an early and synchronized crop stand. It is speculated that the ultrasonic treatment of barley seeds in aqueous media resulted in an advanced hydration process. This is a common explanation for the increase in percentage of germination together with the reduction in the time required for germination of the sonicated seeds. Ultrasound is effective for stimulating the germination of barley, suggesting that this technique has interesting possibilities in horticulture and for the brewing industry. Seeds treated by this method could be dried, stored, and germinated at a later date, while maintaining the accelerated germination characteristics.

In summary, the results obtained in this study have implications for the brewing industry. Ultrasound has the potential to be used in malting processes as a method of treating seeds to reduce the germination period and improve the percentage of total germination.

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