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Multi-Response Optimization of Soaking, Germination and Drying for Malting Barley (RD-2715) using Central Composite Design

Shweta Wattamwar
Maharashtra Institute of Technology - Art, Design and Technology University
Amit Kulthe
amitkulthe.ft@gmail.com

Maharashtra Institute of Technology - Art, Design and Technology University
Anjali Bhoite
Maharashtra Institute of Technology - Art, Design and Technology University
Bhagwan Sakhale
Dr. Babasaheb Ambedkar Marathwada University
Gauri Athawale
Maharashtra Institute of Technology - Art, Design and Technology University
Rajkumar Dagadkhair
Food Safety and Standards Authority of India

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Abstract

The investigation explores the RSM-driven optimization of barley (RD-2715) malting process with major emphasis on the influence of the malting processes on the physico-chemical and bio-functional quality characteristics of barley malt. Barley malting, comprising of soaking, germination and drying steps, changes the biochemical composition of the barley grains. The study undertaken aimed to predict the best malting conditions for the production of high-quality barley malt by virtue of Response Surface Methodology-Central Composite Design (RSM-CCD). The optimization measured the impact of three key parameters of malting, *viz*. soaking time (24 to 72 h), germination time (24 to 72 h), and drying temperature (50 to 70°C), on protein content, β-glucan content, and antioxidant activity in malted barley. The results with 1.00 desirability coefficient for the barley variety under consideration depicted high accuracy across all responses. On the basis of results obtained, the predicted ideal conditions for the barley (RD-2715) malting were a soaking time of 24.01 h, a germination time of 46.89 h, and a drying temperature of 62.65°C. The resultant barley malt produced under such ideal malting conditions of soaking (24 h), germination (48 h) and drying (60°C) exhibited a protein content of 9.40%, a β-glucan level of 6.12%, and an antioxidant activity of 64.81% as DPPH assay.

1. Introduction

Barley (*Hordeum vulgare* L.) finds itself fairly rated at being the fourth most cultivated cereal crop around this world, yet food industrial applications of barley have little significance with the greatest amount being served as either animal feed or as malt for brewing purposes; thus, these restrictions have arisen mainly due to marketing problems related to taste or appearance. Malting is complicated and involves different types of enzymes: it starts with cleaning and sorting raw barley, followed by steeping, germination, and kilning. Noteworthy, barley lacks some important enzymes such as α-amylase but this incrementally produced in malting process. Barley is particularly recognized for its high β-glucan content, a form of soluble fiber that provides numerous health benefits. The average concentration of β-glucan in barley can reach as high as 6%, categorizing it as a soluble dietary fiber.[1] The β-glucan content in malt and barley cell walls typically varies from 2 to 8 grams per 100 grams, positioning barley as the second richest cereal in β-glucan, surpassed only by oats.[2] Although the β-glucanase enzymes generated during germination may contribute to the breakdown of β-glucan, careful management of malting conditions can help maintain or even improve its availability.[3]

Due to the absence of requisite enzymatic action for β-glucan hydrolysis within the human gastrointestinal tract, this polysaccharide traverses to the colon unchanged. Within the colonic environment, saccharolytic bacteria, notably those belonging to the *Bifidobacterium* genus (renowned for their probiotic effects), as documented[4], can metabolize β-glucan. Research indicates a positive correlation between β-glucan consumption and improved intestinal health, modulation of the gut microbiome composition, and augmented immune system functionality. Its prebiotic action stems from its role as a fermentable substrate, nourishing advantageous gut bacteria and consequently stimulating

the production of short-chain fatty acids (SCFAs), which play a critical role in supporting digestive homeostasis.[5]

The malting procedure, which includes steeping, germination, and kilning, finds widespread application across the brewing and food industries. Both effective and inexpensive methods of improving the nutritional qualities of seed grains for further digestibility and physiological advantages are the familiar approach in seed malting.[6][7] Not only does malting improve digestibility, but it also enhances bioavailability of important nutrients, and decreases anti-nutritional factors. Some beneficial nutritional improvements may result from the malting process as it can be seen from the degradation of complex molecules leading to less complex forms and later converted into crucial components.[8] From germination onward, the total protein concentration in cereal grains increases marginally, usually concomitant with an ever-increasing utilization of other kernel components in respiration processes.

Response Surface Methodology (RSM) describes a number of mathematical and statistical techniques applied in approximating and optimizing stochastic models.[9] Response Surface Experiments are experiments centring around generating a predictive model for multiple continuous variables contextually within RSM design.[10] The experimental designs, majorly comprising of screening and optimization processes, restrict the number of trials necessary to evaluate a very large combination of independent variables.[11] Modern applications of RSM cut across a variety of contemporary industries such as food, materials, and chemical engineering, especially in process optimization. In food processing, RSM enhances techniques such as blanching, dehydration, extraction, enzymatic hydrolysis, clarification, fermentation, synthesis of microbial metabolite and food formulation.[12] Other applications have been documented in mining studies using RSM.[13][14] Therefore, the current research was undertaken to study the impact of malting conditions on the physico-chemical and bio-functional quality characteristics of barley malt using RSM to predict the ideal conditions for barley malting.

2. Materials and Methods

Barley

The barley (RD-2715 variety) was purchased from the local market in Pune, Maharashtra, India. The grains were cleaned, sorted and packed in LDPE pouches for further in-depth research.

Malting Procedure

The barley grains were subjected to a systematic process given in literature with slight modification of soaking, germination, and drying, with controlled variations in time and temperature to determine their effects on malt characteristics. The soaking and germination conditions were aligned with the specifications provided in the CCD, with adjustments indicated in Table 2. Post-germination, the samples were dried at different temperatures according to the RSM until the moisture content was reduced to between 4% and 5%. The detail procedure of malting is represented in Fig. 1.

Optimisation through RSM

The research study was carried out to examine the relative impacts of three predictor variables *viz*. soaking time, germination time, and drying temperature, on the quality of barley malt, utilizing Response Surface Methodology (RSM). The software Design Expert 13 (Stat-Ease Corporation, Minneapolis, USA) facilitated the development of a Central Composite Design (CCD). A total of twenty (20) malting runs were performed according to the specifications of the CCD for the selected variables. Table 1 presents the independent variables along with their corresponding actual values. The range for these independent variables was established based on the operational parameters employed in the commercial production of barley malt. The response variables assessed during the malting process included protein content, β-glucan content, and antioxidant activity.[10]

Table 1
Independent variables (factors) and their actual values design

Factor	Name	Units	Type	Coded Low	Coded High	Mean	Std. Dev.
A	Soaking Time	h	Numeric	-1 ↔ 24.00	+1 ↔ 72.00	48.00	17.41
B	Germination Time	h	Numeric	-1 ↔ 24.00	+1 ↔ 72.00	48.00	17.41
C	Drying Temperature	℃	Numeric	-1 ↔ 50.00	+1 ↔ 70.00	60.00	7.25

Protein content

A quantity of 0.3 grams of flour was accurately weighed into Kjeldahl digestion flasks, and to each flask, 1 gram of a catalyst mixture (K_2SO_4 and $CuSO_4 \cdot 5H_2O$ in a 10:1 ratio) was added. Following this, 5 millilitres of concentrated H_2SO_4 was added to each flask, and the flasks were placed in a digester, where they were heated to 350°C for approximately three h until a clear white solution was achieved. Once the digestion was complete, the flasks were removed from the digester and allowed to cool. After cooling, 50 millilitres of distilled water was added to each flask and mixed thoroughly, followed by the addition of 50 millilitres of 40% NaOH. The distillation process involved positioning the digestion tube with a receiver flask that held 25 ml of a 4% boric acid solution. The distillate produced was titrated with a standardized 0.1N hydrochloric acid until the endpoint was achieved. The amount of hydrochloric acid used to reach this endpoint was recorded from the burette, allowing for the nitrogen content to be calculated using a specific formula. The calculation of the protein amount was, in turn, achieved by multiplying the percentage of nitrogen with 6.25 as per the standard method.[15]

β-glucan Content

Barley grains were finely crushed in a laboratory mill to obtain a particle size of ≤ 500 µm. 1 g of ground barley flour was taken into a clean beaker for further evaluation. 50 ml of hot distilled water at 80°C was

added to the flour, and the mixture was stirred continuously for 30 to 60 minutes to solubilize the β-glucan. After stirring, the extract was cooled to room temperature. To precipitate the β-glucan, three volumes of 95% ethanol were added with vigorous shaking into the cooled extract. The solution then been placed in refrigerator at 4°C for a period up to 12 to 24 h to initiate the precipitation. The precipitated β-glucan was isolated by filtration through Whatman No. 1 filter paper. The remaining residue was then washed in a 70% ethanolic solution for the elimination of impurities. The isolated β-glucan was then further dried in an oven temperature range of 50–60°C until it achieved stable weight. The dried precipitate was weighed, while the content of β-glucan was estimated gravimetrically determining the result as the percentage of β-glucan based on the initial weight of the sample.[16]

$$\beta - \text{glucan}\,(\%) = \frac{Weightofdried\beta - \text{glucan}}{Sampleweight} X100$$

Antioxidant Activity

The procedure is a modified methodology and adopted with slight modification.[17] Under this methodology, a methanol solution containing DPPH free radicals was utilized. Each extract sample (0.2 ml) was added to 1 ml of the DPPH solution and 2 ml of methanol. The resulting mixture was then shaken and kept in the dark for 30 minutes. A blank of methanol was used as a reference to measure absorbance (A) of the solution at 517 nm. The antioxidant activity was expressed as percentage inhibition percentage of DPPH free radical according to the mentioned equation below:

Antioxidant activity (%) = [1 – (A of sample $_{t=30}$ /A of control $_{t=0}$)] × 100

4. Results and Discussion

The study assessed the effects of three distinct soaking durations (24, 48, and 72 h), three germination periods (24, 48, and 72 h), and three drying temperatures (50, 60, and 70°C) on malt quality. The response variables analysed included protein levels, β-glucan content, and antioxidant activity. The response surface and central composite design for the malting parameters and malt quality characteristics of each sample are presented in Table 2. The primary results from the statistical analysis regarding the impact of the varying malting conditions, along with the corresponding model fits, are illustrated in Table 2. A ρ-value < 0.05 indicated that the soaking, germination, and drying temperature significantly influenced the response variables. The model demonstrated high R^2 values and $p < 0.05$, confirming its significance due to the majority of values being statistically significant. All measured attributes conformed to a quadratic model. Run 2 (S 72h, G 24h and D 70°C) recorded highest protein content (9.40%) whereas run 20 (S 24h, G 24h and D 50°C) recorded the least protein content (7.04%) The maximum β-glucan (6.12%) content was recorded for run 10 (S 24h, G 48h and D 60°C) whereas the lowest values for β-glucan and protein and were noted for run 4 (S 72h, G 72h and D 70°C) and antioxidant activity for run 11 (S 24h, G 24h and D 70°C) with 4.67%, 6.58% and 60.79% respectively.

Table 2
Response surface-based central composite design (CCD) for barley malting.

Run	A: Soaking Time (h)	B: Germination Time (h)	C: Drying Temperature (ºC)	Protein Content (%)	β-glucan (%)	Antioxidant Activity (DPPH) (%)
1	24	72	50	8.27	5.34	65.73
2	72	24	70	9.40	5.85	63.11
3	72	48	60	9.04	5.75	64.89
4	72	72	70	6.58	4.69	66.40
5	24	72	70	8.15	5.65	65.65
6	48	48	60	9.18	5.67	64.25
7	48	72	60	7.67	5.01	66.01
8	48	48	60	9.01	5.59	64.30
9	48	48	60	9.20	5.70	64.45
10	24	48	60	9.37	6.12	63.69
11	24	24	70	9.14	5.95	60.79
12	48	24	60	8.65	5.73	61.79
13	72	24	50	8.30	5.75	62.30
14	48	48	50	8.98	5.54	63.98
15	48	48	60	9.15	5.60	64.47
16	48	48	70	9.20	5.69	64.08
17	48	48	60	9.25	5.72	64.47
18	48	48	60	9.16	5.64	64.36
19	72	72	50	7.85	4.76	66.30
20	24	24	50	7.04	5.51	60.87

Effect of malting on protein content of barley malt

The interactive 3D plot illustrated in Fig. 2a reveals the correlation between soaking time (from 24 to 72 h) and germination time (from 24 to 72 h) with respect to protein content (%) in barley malt. As expected, protein levels rose with increased durations of both soaking and germination, reaching their highest values at the maximum time combinations for each parameter. This suggests that prolonged soaking

followed by germination stimulated metabolic activities such as proteolysis and amino acid mobilization, resulting in a more effective breakdown of native proteins and the production of new enzymatic proteins essential for seed development.

In the early stages, the protein content showed a minor reduction as germination time increased under limited soaking conditions, probably due to the use of nitrogenous compounds in the initial metabolic processes. But with the continued soaking of barley, the action of enzymes accelerated proteolysis, liberating more soluble protein and in turn increasing the protein content of malted barley. These observations support the earlier work which noted similar increase in soluble nitrogen and free amino nitrogen (FAN) during prolonged germination of barley.[24] In the same way, some researchers emphasized that malting conditions, especially the extent of germination, are essential for changes in protein during the malting of barley and for its accumulation.[20] The protein content of the unmalted grains was stated to approximate 9.80% to 13.17%, which, in malting, significantly increased ($p < 0.05$) to 10.90-13.33% in different cultivars.[26] In the present study, it can be suggested that the protein content may be triggered due to the biosynthesis of various amylolytic enzymes during germination.

Figure 2 Effect of Malting (a) soaking & germination (b) soaking & drying (c) germination & drying on protein content of barley malt

The 3D surface plot in Fig. 2b describes the interactions of soaking time (from 24 to 72 h) and drying temperature (50 to 70°C) on the percentage of protein content in barley malt. The plot shows positive relationships between the two independent variables and protein content. It was observed that the protein content increased with increase in both soaking time as well as drying temperature. The protein content varied from about 8.0% to 10.0%, with maximum levels recorded at the 72 h of soaking and 70°C of drying temperature.

The soaking of seeds not only induced the seeds rehydration, but extensive soaking also increased the enzymatic (proteolytic) activity resulting in degradation of native insoluble proteins into soluble forms. On the other hand, higher drying temperatures caused further concentration of the material with rapid moisture loss arresting the enzyme activity, thus enabling preservation of products from proteolysis. High-temperature drying may also pose the chances of enzyme denaturation or protein damage. Moderate to high drying temperatures, 50–70°C as observed in this work, seem to hold the best combination for ease of protein retention and quality enhancement.

Hydration starts with soaking, where the activation of endogenous enzymes gets underway, including proteolytic ones from the seeds, which begin degrading the complex native proteins into simpler peptides and amino acids. Crude protein content more or less remains unchanged, while enzymatic hydrolysis commonly increases protein quality and solubility, eventually increasing the protein digestibility by leaching of some anti-nutritional factors, such as phytic acid and tannins.[27][28]

In addition, literature evidences regarding parallel trends in malt quality at different drying temperatures confirm the significance of proper thermal treatment in preserving protein integrity of malt.[25] The protein content of the sprouts dried at 60°C was higher than that dried at either 50°C or 70°C, although statistically, the differences when comparing 60 with 50 degrees Celsius were not significant. Thus, a drying temperature range of 50–70°C may be optimal for reducing the unfolding of polypeptide chains through denaturation, which enhances their vulnerability to digestive enzymes.[29]

Figure 2c illustrates that both the germination time and the temperature during drying have a substantial effect on the final protein content of barley malt. The optimal protein levels were observed at moderate drying temperatures, specifically between 60–65°C, and with germination times of approximately 48 to 60 h. The depicted curved surface indicates a non-linear relationship. For instance, at shorter germination periods, rise in drying temperature resulted in increase in protein content, likely due to the concentration effects from moisture loss. Conversely, extended germination times combined with elevated drying temperatures may result in protein degradation or denaturation, ultimately decreasing the total protein content that can be measured.

During the process of germination, there is a substantial increase in the enzyme activity such as proteases, which enable the breakdown of native proteins through hydrolysis. This enhancement is crucial for the solubility and digestibility of proteins, particularly in brewing applications. On the other hand, if germination is extended beyond the optimal period, it can result in excessive protein degradation, negatively impacting their nutritional and functional attributes.[30]

Certain literature also reported a comparable increase in soluble protein during the sprouting of finger millets.[28] Similarly rise in protein content in cereals was identified, with red sorghum showing a 12% increase and millets a 9% increase.[27] Moreover, 5% rise in protein content was observed after 48 h of sprouting at 30°C in two varieties of millets from Tanzania.[31]

Once germination occurs, the subsequent drying phase, referred to as drying, stops enzyme activity and maintains the integrity of the malt. Elevated drying temperatures induce flavor enhancement during the malting process and increase malt shelf life; however, they can also partially denature proteins and initiate Maillard browning type reactions with amino acids, leading to lower values of measurable protein content.[32] This supports the findings of declining protein concentration with increasing drying temperature as shown in the Fig. 2c.

It further mentions that quick and/or uneven drying can interfere with the protein structure as well as that of other nutrients in the barley malt. Thus, researchers have also emphasized on proper optimization of kiln parameters to ensure the solubility of proteins that is extremely important for wort and yeast functioning in brewing.[33]

It is also reported that the germination phase influences the final protein concentration, as well as the subsequent drying conditions, which in turn may affect enzymatic stability and other nutrient retentions.

[23]

Table 3
ANOVA for quadratic model of protein content during malting of barley

Source	Sum of Squares	df	Mean Square	F-value	*p*-value	Remark
Model	12.36	9	1.37	143.10	< 0.0001	Significant
A-Soaking Time	0.0640	1	0.0640	6.67	0.0273	
B-Germination Time	1.61	1	1.61	167.59	< 0.0001	
C-Drying Temperature	0.4121	1	0.4121	42.95	< 0.0001	
AB	1.54	1	1.54	160.50	< 0.0001	
AC	0.5778	1	0.5778	60.22	< 0.0001	
BC	2.63	1	2.63	274.47	< 0.0001	
A^2	0.0024	1	0.0024	0.2502	0.6278	
B^2	2.84	1	2.84	295.53	< 0.0001	
C^2	0.0201	1	0.0201	2.09	0.1786	
Residual	0.0960	10	0.0096			
Lack of Fit	0.0633	5	0.0127	1.94	0.2430	Not Significant
Pure Error	0.0327	5	0.0065			
Cor Total	12.45	19				

The quadratic model utilized for the evaluation of protein content in barley malting displayed a very high F value, being 143.10 and an extremely low *p* value ($p < 0.005$), as summarized in Table 3, which defines the considerable importance to this model. The significant terms within the model include A, B, C, AB, AC, BC, and B^2. The close alignment of the experimental and predicted values is supported by the determination (R^2) and adjusted determination (R^2adj) values of 0.9923 and 0.9854, respectively. A value of R^2 approaching 1, along with an R^2adj that is close to R^2, indicates a good fit of the model to the actual data.[34] Additionally, an adequate precision ratio of 40.81 reflects a strong signal. Thus, the model is considered satisfactory, highlighting the potential of RSM in the selection of malting parameters.

Effect of Malting on β-glucan content of barley malt

The primary component of the cell walls in barley endosperm is β-glucan, or mixed linkage (1–3,1–4)-β-D-glucan, which constitutes roughly 75% of its structure, while arabinoxylan represents a smaller segment at around 20%. The total β-glucan content in barley grains varies from 2% to 10% of their overall mass.[18] In its raw state, barley has a β-glucan content of 8.8%. After malting, all samples demonstrated a significant reduction ($p < 0.05$) in β-glucan levels, with the degree of decrease differing according to the malting conditions applied. The malt produced showed β-glucan concentrations to be highest (6.12%) at 24 h soaking time while it decreased to 4.69% at 72 h soaking time (Table 2). This reduction may be primarily attributed to the enhanced activity of the enzyme β-(1–3), (1–4)-glucan-4-glucanhydrolase (EC 3.2.1.73), known as β-glucanase, which is synthesized during germination and serves to hydrolyze β-glucan.[19]

The effect of soaking and germination on malt β-glucan content is illustrated in Fig. 3a. At lower soaking times, higher β-glucan levels were associated with longer germination times. Conversely, at higher soaking times, β-glucan was reduced, while very long soaking times were associated with an increase in β-glucan content. This indicated that, short soaking time might be too short to fully hydrolyze β-glucan, while prolonged soaking could lead to structural damage of β-glucan, affecting its bioavailability. The duration of germination also plays important role because increased germination time promotes the enzymatic activity that hydrolyzes β-glucan. Moreover, prolonged germination could increase adverse metabolic changes, reducing the β-glucan content under optimal conditions.[20]

Model fit (Fig. 3a) shows significant interactions between soaking and germination durations and shows the need for the process to be optimized to achieve the desired levels of β-glucan. The contour variations again at the bottom of the plot indicate a likely optimal range for these variables. In conclusion, results present confirmatory evidence that both soaking and germination affect β-glucans contents in the malt, whose final concentration in the malt post-processing is ultimately contingent to the interaction of these two variables. Further, these results corroborate previously carried out statistical analyses indicating that respective durations of germination and soaking largely affect β-glucan levels, and the purpose in consideration can be optimized on their combination.

The surface plot indicates the soaking duration and drying temperature effect on barley malt β-glucan content and presents quite a complex relationship between these two processing factors (Fig. 3b). It is evident from the response surface analysis that soaking period and drying temperature exert a non-linear effect on β-glucan concentrations. The response surface analysis indicates a non-linear correlation between soaking time and drying temperature in influencing β-glucan concentrations. Typically, longer soaking times result in decreased β-glucan levels, especially at elevated drying temperatures. Nevertheless, excessive soaking beyond the optimal threshold can cause over hydration, which may adversely affect enzymatic activity.

β-glucan degradation is highly dependent on drying temperature. This indicates that at lower drying temperatures (50–55°C), the content of β-glucan remains relatively high, indicating lower activity of the enzyme. An increasing drying temperature to 60–65°C leads to significantly increased degradation of the β-glucan, presumably resulting from increased enzymatic degradation. However, under very hot (over 65°C) conditions, enzymes could deactivate, leading to a slower reduction in β-glucan storage. In double-floor kilns, β-glucans degradation is also present and continues during withering (the first period of low-temperature drying) but is most pronounced in the top layer of the kiln where lower temperature and high moisture level promotes extended β-glucan degradation. [21]

These findings are further corroborated by the interplay between soaking and drying. The plot at the base of the 3D surface highlights that optimal β-glucan reduction occurs at moderate soaking durations (approximately 48–56 h) in combination with drying temperatures between 60–65°C. This interaction suggests that controlled soaking, followed by carefully regulated drying, enhances enzymatic hydrolysis, leading to lower β-glucanlevels in malt.

This 3D response surface plot (Fig. 3c) visualizes the interactive effects of germination time (B) and drying temperature (C) on β-glucan content (%) of malt. The graph shows a clear interactive influence of these two variables on β-glucan degradation. β-glucan needs to be solubilized (released from cell walls) before it can be effectively degraded by β-glucanases. During the initial stages of germination, both solubilization and degradation occur. However, if germination proceeds for too long, and especially if the enzymes are later inactivated by heat during drying, the initially solubilized β-glucan might not be fully broken down, potentially leading to higher levels in the final malt. As germination time increases from 24 to 72 h, there is a noticeable decrease in β-glucan content, particularly when combined with lower drying temperatures (50–60°C). This trend can be attributed to the activation of endogenous β-glucanase enzymes during germination, which facilitates the hydrolysis of β-glucans into simpler sugars.[22]

For β-glucan to be effectively degraded by β-glucanases, it must first be solubilized, which involves its release from the cell walls. This process of solubilization, along with degradation, occurs during the early stages of germination. Typically, longer periods of germination promote increased enzymatic activity, resulting in a more extensive breakdown of β-glucans. Nonetheless, it is essential to be aware that prolonged germination can lead to excessive modification of the malt, which may adversely affect other quality factors and could even result in a reduction of total fiber content.[23] Germination contributes to a decrease in the overall β-glucan content. Subsequently, drying functions as a regulatory point for the enzymes involved in this reduction. It was observed that at higher temperature conditions, i.e. above 65°C, the content of β-glucan seemed to be constant or decreasing very slowly and, therefore, it indicated that hydrolysis remained incomplete due to inhibition of enzymatic activity before completion of hydrolysis.

β-glucan exhibits a surface gradient pattern following a high decrease with long germination and then moderate drying temperatures. Thus, malt should be conditioned for effective degradation of the grain under kiln time-temperature controlled condition for enzyme work to take place.[21] According to

literature, β-glucan content of malted barley decreased from 8.4 to 5.5% on account of β-glucan degradation during the whole process of malting.[24] Furthermore, the amount of β-glucan for dried malted barley following varied drying temperatures amounts to between 10.32 and 11.32%.[25] Thus, the complexity in such interrelations indicates that the longest germination times and moderate drying temperatures (55–60°C) maximized the reductions in β-glucan. This is further amplified and stated that ideal germination conditions reduced the β-glucan content of both oats and barley substantially and enhanced the nutrition and processing quality of malt.[23] Thus, it brings in the need to have the appropriate duration of germination compared with drying temperature so that easy processing of malts promotes maximum breakdown of β-glucan improving the malting quality.

Table 4
ANOVA for quadratic model of β-glucan during malting of barley

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remark
Model	2.46	9	0.2736	90.34	< 0.0001	Significant
A-Soaking Time	0.3133	1	0.3133	103.45	< 0.0001	
B-Germination Time	1.12	1	1.12	368.36	< 0.0001	
C-Drying Temperature	0.0865	1	0.0865	28.56	0.0003	
AB	0.3528	1	0.3528	116.50	< 0.0001	
AC	0.0648	1	0.0648	21.40	0.0009	
BC	0.0112	1	0.0112	3.71	0.0828	
A^2	0.1304	1	0.1304	43.05	< 0.0001	
B^2	0.3316	1	0.3316	109.51	< 0.0001	
C^2	0.0288	1	0.0288	9.50	0.0116	
Residual	0.0303	10	0.0030			
Lack of Fit	0.0164	5	0.0033	1.17	0.4324	Not Significant
Pure Error	0.0139	5	0.0028			
Cor Total	2.49	19				

The results obtained from the analysis of variance (ANOVA) based on experimental data for β-glucan are presented in Table 4. The model *F* value of 90.34 implies the model is significant. There is only a 0.01% chance that an *F* value this large could occur due to noise. Statistical noise is unexplained variability within a data sample that is rendered meaningless by the existence of too many variables. *P* values less than 0.05 indicate model terms are significant. In this case, A, B, C, AB, AC, A^2, B^2, C^2 are significant model terms. Adequate precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. A ratio of 35.51obtained in this analysis indicates an adequate signal. The model can be used to navigate the design space.

Effect of Malting on Antioxidant activity (DPPH) of barley malt

The 3D response surface plot (Fig. 4a) depicts the interactive influences of soaking and germination duration on the antioxidant activity of barley malt, as quantified by DPPH scavenging activity (%). The graph shows a pronounced upward trajectory, indicating that longer soaking and germination periods significantly boost antioxidant activity. At shorter durations (e.g. 24 h), the antioxidant activity was approximately 60%, while extending the treatment to 72 h resulted in levels exceeding 66%. This increase may be attributed to the biochemical changes that occur during the malting process, which involve the activation of hydrolytic enzymes with the biosynthesis of various compounds, including flavonoids, phenolic acids and melanoidins.

Soaking of barley grains in water promote rehydration triggering the metabolic processes that actually soften the seed matrix itself and cause mobilization of nutrients. Once this is done, germination enhances enzymatic activities, resulting in the breakdown of complex molecules and formation of bioactive compounds. Studies have shown that during germination, there is a great increase of phenolic content, which is of prime importance in antioxidant activity, due to both the enzymatic breakdown of bound phenolics along with synthesis of new phenolic compounds.[35] Studies showed that extended germination improve antioxidant capacity by virtue of both phenolic extraction as well as increased activity of antioxidant enzymes superoxide dismutase and catalase.[36] This is particularly relevant for functional food development, as in, such malt ingredients with high antioxidant content would be favorable for health improvements. The trend observed would imply optimizing the parameters of soaking and germination in barley malting to increase greatly the antioxidant potential. Such findings are consistent with earlier research pointing out the advantageous effects of malting on the nutraceutical attributes of cereals thus emphasizing the need for specialized malting processes in functional foods.[37] [38]

The 3D surface chart shown in Fig. 4b shows the interactive effect of soaking time and drying temperature on the antioxidant activity as DPPH % of barley malt. It can be noticed that a slight improvement of the antioxidant activity was observed with increased soaking durations and drying temperatures; however, this increase was more gradual and subtle when compared to earlier germination responses seen on the surfaces. The antioxidant activity was observed to range from

roughly 60% to 66%, with the highest activity noted at extended soaking times (approximately 64–72 h) and moderately high drying temperatures (> 60°C).

The soaking process is pivotal in commencing the metabolic activities of barley grains, leading to the activation of hydrolytic enzymes such as amylases and proteases. These enzymatic activations play a significant role in breakdown of cellular matrices, releasing bound antioxidant compounds, particularly phenolic acids and flavonoids. [35] Moreover, prolonged soaking can enhance the hydration of the grains, a necessary condition for effective germination and the following biochemical transformations.

The temperature at which drying occurs significantly affects the final concentration and stability of bioactive substances. Previous research indicated that moderate heat treatments could enhance the concentration of antioxidant compounds or leading to the development of Maillard reaction products that possess antioxidant properties. [39] Alternatively, under high heat, the thermolabile compounds degrade, necessitating meticulous optimization. Herein, the increasing antioxidant activity in response to higher drying temperatures in the present study suggested that the drying temperature range between 50 and 70°C was appropriate to maintain or improve the concentration of antioxidant compounds. This response surface, therefore, underscores the synergistic yet subtle influence of pre- and post-germination treatments on the malt antioxidant potential of barley critical in the context of functional food ingredients or nutritionally enriched malt-based product development.

Figure 4c represents a three-dimensional plot showing the interaction of germination time and drying temperature in regard to antioxidant activity (DPPH %) of barley malt. From the plot, it is clear that both longer germination time and higher drying temperature enhanced antioxidant activity, with DPPH percentages increasing from just above 60% to nearly 67% with the respective levels considered in the study. This indicates that both long germination and high drying temperatures trigger either the biosynthesis or liberation of antioxidant compounds in barley grains.

These finding found to be consistent with earlier researches that indicated germination driven triggering of essential enzymatic pathways, including phenylpropanoid metabolism, aiding the production of phenolic compounds recognized for their antioxidant capabilities.[38] Furthermore, the drying process, especially at moderate to elevated temperatures, could lead to Maillard reactions synthesizing melanoidins large molecular compounds known for their notable antioxidant effects.[40] Nevertheless, extremely high temperatures may compromise heat-sensitive antioxidants; however, for instance, the temperature range seemed to stay within a favorable limit.

The enhancement of DPPH activity with respect to germination time displayed more effective enzymatic breakdown of cellular walls, releasing bound phenolics and flavonoids, rendering them more bioavailable. Moreover, drying post-germination aided in concentrating these compounds, especially when carried out under optimal temperature conditions.[36]

From a research perspective and in the realm of functional food development, these findings are highly significant. They offer empirical support for the optimization of processing parameters related to barley malt, aimed at maximizing its antioxidant activity, which has the latent to enhance the nutritional and health benefits of barley-derived products.

Table 5
ANOVA for Quadratic model of Antioxidant Activity (DPPH) for malting of barley

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remark
Model	50.92	9	5.66	286.18	< 0.0001	significant
A-Soaking Time	3.93	1	3.93	198.86	< 0.0001	
B-Germination Time	45.07	1	45.07	2279.86	< 0.0001	
C-Drying Temperature	0.0723	1	0.0723	3.65	0.0850	
AB	0.7381	1	0.7381	37.34	0.0001	
AC	0.1431	1	0.1431	7.24	0.0227	
BC	0.0630	1	0.0630	3.19	0.1045	
A^2	0.0123	1	0.0123	0.6211	0.4489	
B^2	0.2872	1	0.2872	14.53	0.0034	
C^2	0.1026	1	0.1026	5.19	0.0459	
Residual	0.1977	10	0.0198			
Lack of Fit	0.1530	5	0.0306	3.42	0.1017	not significant
Pure Error	0.0447	5	0.0089			
Cor Total	51.12	19				

Table 5 shows the outcomes of the analysis of variance for quadratic model of antioxidant activity (DPPH) for malting of barley. F-value for overall model is 286.18, with a $p < 0.0001$, indicated that the model was highly significant. A (Soaking Time) had the significant effect, B (Germination Time) had strongest effect whereas, C (Drying Temperature) had non-significant effect on antioxidant activity of malt. The interaction effects revealed that the combination of Soaking Time and Germination Time (AB) was highly significant, as well as the interaction between Soaking Time and Drying Temperature (AC), also showed significant effect. However, the interaction between Germination Time and Drying Temperature (BC) found to be not significant. Additionally, the quadratic terms indicate that Germination

Time squared (B^2) and Drying Temperature squared (C^2) had significant effects, while Soaking Time squared (A^2) had not significant effect. Regarding model fit, the residual sum of squares was 0.1977 with 10 degrees of freedom. The lack of fit test was not significant (F = 3.42, p = 0.1017), indicating that the model adequately explained the variation in the data.

Table 6
Summary Statistics for all models

Model	**Protein Content**	**β-glucan**	**Antioxidant Activity**
	Quadratic	**Quadratic**	**Quadratic**
F-value	143.10	90.14	286.18
p-value	< 0.0001	< 0.0001	< 0.0001
R^2	0.9923	0.9879	0.9961
Adjusted R^2	0.9854	0.9769	0.9927
Lack of Fit (p-value)	0.2430	0.4324	0.1017

In developing models within the adopted methodology, attention must be paid to the lack of fit test, a statistical indicator used to check the model's adequacy in predicting response surfaces.[41] Replicated runs at the centre of the experimental region enabled to check model adequacy by means of the lack-of-fit test. A significant outcome ($p < 0.05$) indicates an unreliable model in adequately representing a response of interest.[42] Fitted models presented a non-significant lack of fit, so they were considered suitable for making predictions and drawing conclusions from the response surfaces (Table 6). The analysis of variance (ANOVA) results indicated that the developed quadratic models were highly significant for predicting β-glucan, Protein Content, and Antioxidant Activity, as evidenced by their respective F-values of 90.14, 143.10, and 286.18, with p-values all below 0.0001. These results confirm that the independent variables contribute significantly to the response variables. The coefficient of determination (R^2) values for protein content (0.9923), β-glucan (0.9879), and antioxidant activity (0.9961) suggested a strong correlation between the model predictions and the observed data. Additionally, the adjusted R^2 values, which account for the number of predictors, remained high for protein content, β-glucan and antioxidant activity (0.9769, 0.9854, and 0.9927, respectively), further supporting the robustness of the models. Most importantly, the tests for lack of fit for all models returned non-significant *p*-values (0.4324, 0.2430, and 0.1017, respectively), meaning that the models could describe the intrinsic variability of the data without systematic prejudices. Therefore, these results indicated that the models were reliable for prediction of target responses and could therefore be effectively used for optimization of the process.

Optimization for Multiple responses

The method of ‘desirability functions’ propounded by researchers has confirmed identification of the optimum combination malting parameters which can improve the concurrent responses.[43] This can transform each estimated response into a separate desirability unit, denoted as di, where $0 \leq di \leq 1$. A 'larger is better / maximum' function was used for maximizing β-glucan content, whereas an 'in range' function made the conditions that protein content and antioxidant activity should stay within those limits defined. The optimization criteria and acceptance thresholds for each response are shown in Table 7.

Table 7
Criteria for optimizing multiple responses

Responses	Desirability function	Optimization limits
B-glucan Content	Maximum	Min = 4.69, Max = 6.12
Protein Content	In range	Min = 6.58, Max = 9.40
Antioxidant activity (DPPH)	In range	Min = 60.79, Max = 66.40

Optimization of malting process for barley

A numerical optimization technique was employed to identify the ideal processing parameters, including soaking time, germination duration, and drying temperature, utilizing Design Expert software. The focus of the optimization process was on the specific quality of barley malt β-glucan, in that the malt has been produced for functional foods. To optimize such situations until the defined goals were achieved, the particular objectives were tied to a general composite function called desirability function (Myers and Montgomery, 2002). Optimal processing conditions thus identified to be ideal for producing preferred malt were soaking time of 24.01 h, germination time of 46.89 h, and final drying temperature of 62.65°C. The predicted outcomes for β-glucan content, protein content, and antioxidant activity are 6.12%, 9.40%, and 64.81%, respectively, at a maximum desirability score of 1.

Conclusion

The model required for Response Surface Methodology (RSM) was developed by statistical and graphical techniques and built on two-level-three-factor designs for the experimental results. The method has provided a very useful insight into the selected malting parameters affecting the modification of the grains studied as evidenced by response surface analysis. Key factors such as soaking time, germination duration, and drying temperature significantly influenced all measured responses. The RSM effectively identified the optimal malting conditions specifically, soaking for 24.01 h, germination for 46.89 h, and drying at 62.65°C-for producing barley malt with a β-glucan content of 6.12%, protein level of 9.40%, and antioxidant activity of 64.81%. The predicted values closely matched the experimental results, demonstrating the model's validity. However, this preliminary investigation is confined to the specific parameters of this experimental design, and varying levels and combinations of process parameters could yield different results. Nonetheless, the study sheds light on how malting parameters influence the quality of malt derived from barley.

Declarations

Conflict of Interest:

The authors of this document both assert that no financial or personal relationships exist that may cause the competing interests in the work being reported.

Consent for Publication:

All the authors have agreed with the content and gave consent for the publication.

Dual Publication

The authors affirm that the current study has not been previously published in any other journal and is the result of genuine efforts from all authors.

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Author Contribution

All the authors (Shweta Wattamwar, Amit Kulthe, Anjali Bhoite, Bhagwan Sakhale, Gauri Athawale, Rajkumar Dagadkhair) have made significant contributions to the conception of the research work, data analysis and interpretation and drafting of the manuscript.• Conception and design of research work, data collection, analysis and interpretation of data, drafting of the manuscript: Shweta Wattamwar, Amit Kulthe, Gauri Athawale, Rajkumar Dagadkhair• Critical revision of the intellectual content and approval to the manuscript for the publication: Amit Kulthe, Anjali Bhoite, and Bhagwan Sakale• Agree to be accountable for all the aspects of the research work ensuring the accuracy or integrity of the work: Shweta Wattamwar and Amit Kulthe

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Data Availability

The data generated during the current investigation is available with the corresponding author to be shared on request.

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Figures

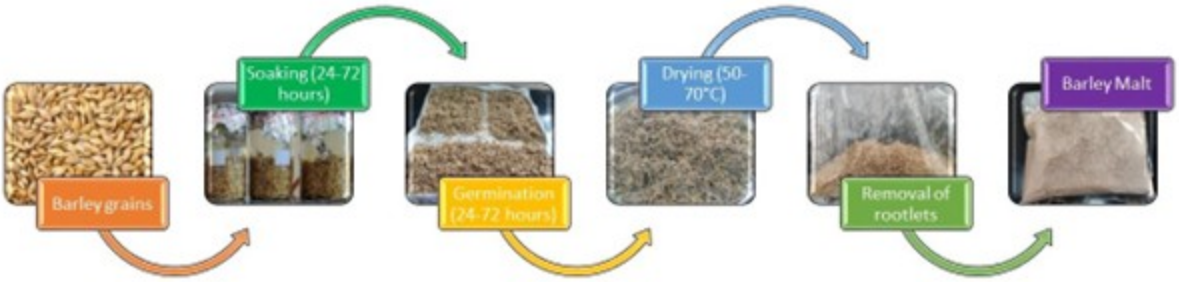


Figure 1

Preparation of barley malt

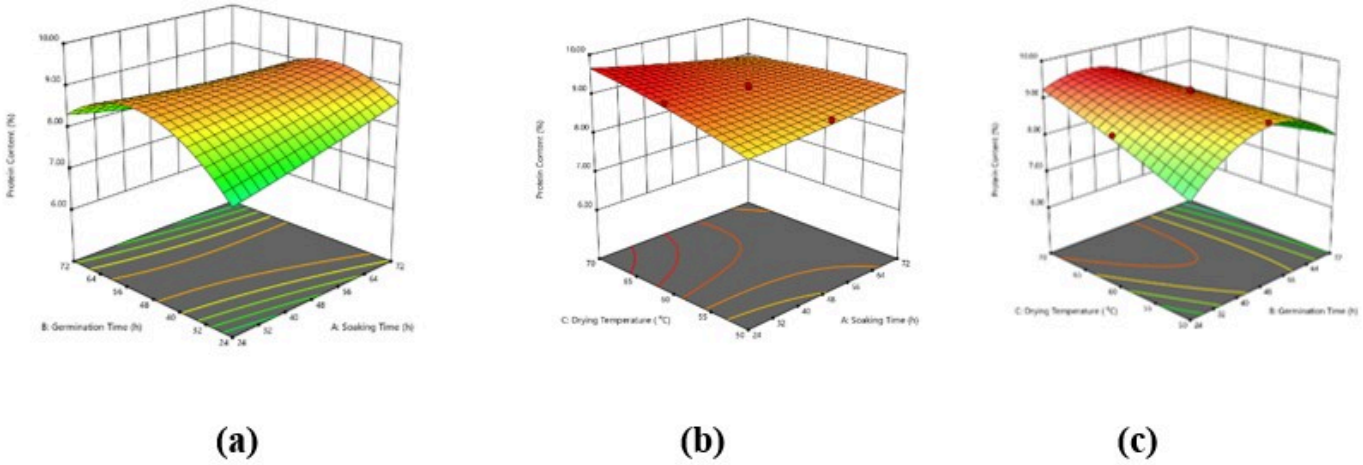


Figure 2

Effect of Malting (a) soaking & germination (b) soaking & drying (c) germination & drying on protein content of barley malt

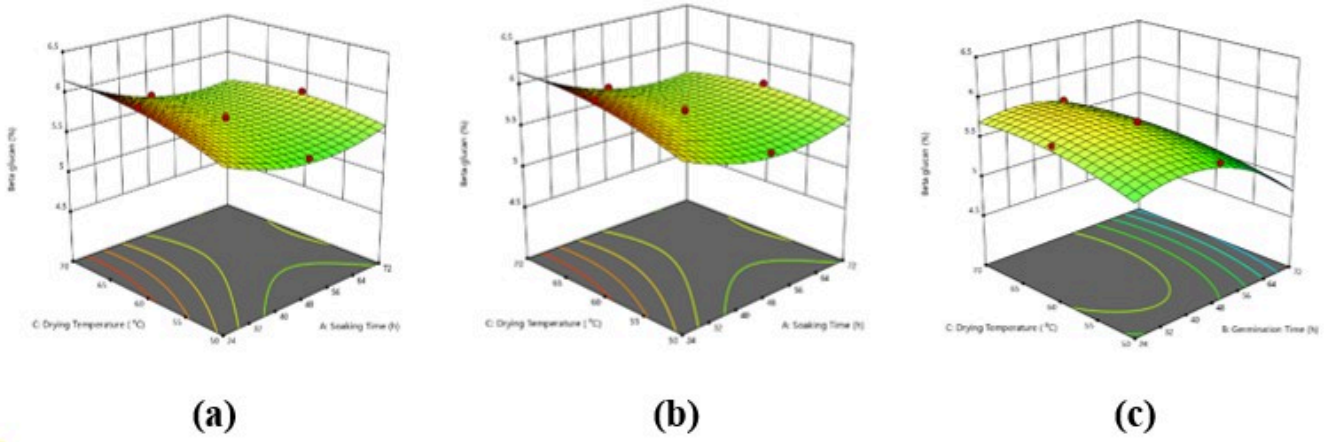


Figure 3

Effect of Malting (a) soaking & germination (b) soaking & drying (c) germination & drying on β-glucan content of barley malt

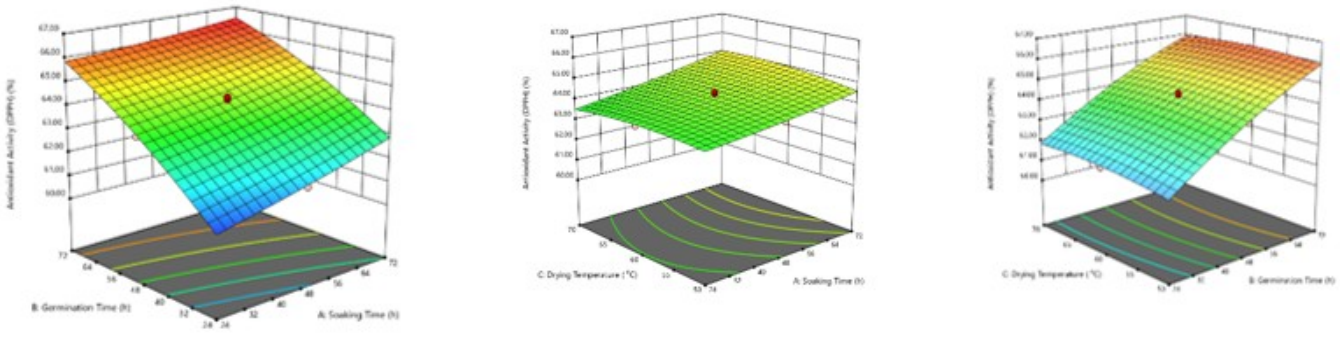


Figure 4

Effect of Malting (a) soaking & germination (b) soaking & drying (c) germination & drying on antioxidant activity of barley malt