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Enhancement of Nutritional Quality by Optimization of Germination Parameters in Oats (*Avena sativa* L.) using Response Surface Methodology

Anupreet Kaur Sobti^{1*}, Ritu Pradhan², Vasudha Bansal³, Shashank Garg^{4*}

^{1,2,3} Department of Foods and Nutrition, Government Home Science College, Chandigarh (Affiliated to Panjab University, Chandigarh), India. 160011

⁴School of Bioengineering and Biosciences, Lovely Professional University, Punjab, India. 144411

*Corresponding author: Anupreet Kaur Sobti, Shashank Garg
Email ID: anupreetsobti@gmail.com, shashank.21902@lpu.co.in

ABSTRACT: The germination process enhances the nutritional attributes of cereals by improving bioavailability of nutrients and reducing content of anti-nutritional factors. This study aims to optimize the germination process variables of oats (*Avena sativa* L.) using Response Surface Methodology (RSM) for enhancing its nutritional value. A Central Composite Design (CCD) was employed to optimize the effects of soaking time (6-18 hrs), germination time (24- 72 hrs) and germination temperature (15-30 °C), on two response variables i.e. total protein content and phytate content. The developed models were found to be significant with high coefficients of determination ($R^2=0.87$, protein content; 0.945, phytate content) and low p values (<0.05) in ANOVA, indicating good model adequacy. The optimized conditions obtained at 6 hr soaking, 61.25 hr germination at 15 °C temperature yielded 21.2 % (w/w) protein and 0.760 mg/g phytate, achieving an overall desirability of 0.935. The validation experiment yielded a good correspondence with the predicted result in optimized condition, and the experimental values are falling within 95% prediction interval. Optimized germination significantly improved the nutritional quality of oats, highlighting its potential in functional food development.

INTRODUCTION

1.1 Background and Significance of Oats

Oats (*Avena sativa* L.) are an important cereal grain globally recognized for their exceptional nutritional profile and diverse health benefits [1]. They rank among the top cultivated cereals worldwide, demonstrating significant economic and dietary importance, with an increasing shift from animal feed to human consumption [2]. Oats are a rich source of essential macronutrients, including high-quality protein, beneficial unsaturated fats, and soluble dietary fiber, particularly β -glucan [3-5]. Beyond these, they provide a wealth of micronutrients such as vitamins (B, C, E, K), minerals (e.g., P, K, Mg, Ca), unique galactolipids, and a variety of bioactive compounds, including polyphenols, avenanthramides, and antioxidants [4, 6]. The consumption of oats has been strongly associated with numerous positive health outcomes. The β -glucan content is particularly noteworthy for its functional properties, contributing to the reduction of blood cholesterol and glucose absorption, thereby playing a crucial role in preventing cardiovascular diseases, dyslipidemia, hypertension, and type 2 diabetes [3, 7].

Beyond their dietary fiber content, oats are distinguished among cereals for their notable protein content and superior protein quality [8]. Importantly, oat proteins possess an amino acid profile that is often considered more balanced and better suited to

human nutritional requirements compared to other cereals, being relatively rich in essential amino acids like lysine and threonine [9- 11]. This high-quality protein, which primarily consists of globulins, also contributes to the grain's digestibility and overall biological value [10]. Consequently, oats are emerging as a valuable and sustainable alternative protein source, particularly for the development of innovative plant-based foods [12].

Despite their significant nutritional advantages, like many plant-based foods, oats naturally contain certain compounds known as anti-nutritional factors [13, 14]. These ANFs, although often present in small amounts, can interfere with the digestion and absorption of essential nutrients, thereby reducing the overall bioavailability of vitamins, minerals, and macronutrients [13, 14]. Among the various anti-nutrients found in cereals and legumes, phytic acid (myo-inositol hexakisphosphate) is one of the most prominent [13, 14]. Phytate forms insoluble complexes with essential minerals such as iron, zinc, calcium, and magnesium, making them unavailable for absorption in the human digestive tract [15]. This chelation effect can pose a nutritional challenge, especially in populations where cereals form a major part of the diet. Furthermore, phytates can also interact with proteins and digestive enzymes, potentially inhibiting their activity and thus reducing protein digestibility [13]. Therefore, strategies to mitigate the effects of these anti-nutritional factors are crucial for enhancing the nutritional impact and health benefits of oat consumption.

1.2 Importance of Germination in Oat Processing and Nutritional Enhancement

Germination is a traditional and effective bioprocess that initiates profound physiological, morphological, and biochemical transformations within cereal grains [16, 17]. This metabolic activation leads to the breakdown of complex macromolecules and the *de novo* synthesis of various bioactive compounds, significantly enhancing the nutritional and functional properties of the grain [16, 18]. For oats, germination is instrumental in improving several key nutritional attributes. It leads to an increase in protein content and modification of amino acid profiles, along with an enhancement in the bioavailability of essential minerals like iron and zinc [17, 19, 20].

Crucially, germination effectively reduces the levels of anti-nutritional factors (ANF) such as phytates, which are known to chelate minerals and inhibit their absorption [13, 15, 21]. The activation of endogenous phytase enzymes during germination degrades phytic acid, thereby improving mineral bioaccessibility [13,15]. Furthermore, germination contributes to increased digestibility by breaking down starch and protein components, forms new bioactive compounds, and can improve flavor characteristics, offering a versatile approach to developing healthier and more palatable oat-based products [17, 22](Budhwar et al., 2020;; Siddiqua et al., 2019).

1.3 Need for Process Optimization

The extent of nutritional enhancement (protein) and ANF reduction during germination is highly dependent on various process parameters, including soaking time, germination time, and temperature [6, 23]. Suboptimal or uncontrolled germination conditions can lead to variable or even undesirable outcomes, such as the degradation of beneficial compounds if germination is prolonged excessively [15]. Therefore, precise control and optimization of these variables are critical to maximize the desired nutritional improvements while minimizing any potential negative effects.

Response Surface Methodology is a powerful statistical and mathematical technique widely employed in food science and engineering for modeling and optimizing complex processes [24]. RSM enables the investigation of the relationships between multiple input variables and one or more response variables, facilitating the identification of optimal processing conditions with fewer experiments compared to traditional one-factor-at-a-time approaches. Its application allows for a comprehensive understanding of variable interactions, leading to improved process efficiency, cost reduction, and superior product quality.

1.4 Research Gap and Rationale

While the benefits of oat germination are well-documented, the optimization of specific germination parameters to achieve targeted nutritional enhancements, particularly in terms of protein content and phytate reduction, remains an area requiring systematic investigation [19]. Existing studies often highlight the variability in processing conditions and their impact on oat composition, emphasizing the need for tailored optimization specific to desired outcomes and raw material characteristics [19, 23]. A comprehensive, multi-response optimization approach is crucial to identify the most suitable germination conditions that simultaneously boost desirable nutritional properties and mitigate ANFs in *Avena sativa* L. The lack of a robust, systematically optimized protocol for oat germination focusing on maximizing protein content and minimizing phytate using a Central

Composite Design within RSM presents a significant research gap. Such an investigation would provide valuable insights for the development of superior functional food ingredients from oats.

1.5 Objectives of the Study

Building upon the identified research gap, this study aims to optimize the germination process variables of oats (*Avena sativa* L.) using Response Surface Methodology. Specifically, a Central Composite Design was employed to investigate and optimize the effects of soaking time, germination time, and soaking/germination temperature on two key response variables: total protein content and phytate content. The ultimate objective is to determine the optimal conditions that significantly enhance the nutritional value of oats for potential application in functional food development.

2. MATERIALS AND METHODS

2.1. Materials and Reagents

Whole oats (*Avena sativa* L.), specifically Kent variety, were procured from National Seeds Corporation Ltd, Central State Farm, 10th km, Sirsa Road, Hisar, to ensure uniformity in quality and characteristics for all experimental phases. The Kent variety is selected for the present investigation due to its wide adaptability, consistent grain yield, and favourable nutritional composition, making it suitable for both processing and value-added product development. All chemicals and reagents used in the study, including those for biochemical analyses, were of analytical grade. Distilled and deionized water was used throughout the experiments.

2.2 Sample preparation

The oat grains were manually cleaned to remove dust, broken grains, and extraneous matter. The cleaned grains were washed thoroughly with distilled water and sterilized by soaking in 1% sodium hypochlorite solution for 5 minutes, followed by rinsing with distilled water thrice to eliminate possibility of residual chlorine. The cleaned and sterilized oat grains were soaked in distilled water according to the soaking durations specified in Table 1. The soaked grains were drained and spread evenly on sterilized germination trays lined with moist muslin cloth. Germination was carried out in an incubator (Orbitek), ensuring programmable temperature control (± 0.1 °C accuracy, ± 0.1 °C uniformity) maintained at the required temperature as in table 1. The germinating grains were rinsed with sterile distilled water every 12 hours to prevent microbial growth and maintain moisture. After germination time specified as per table 1, the sprouts were dried at room temperature until constant weight was achieved. The dried germinated oats were ground to a fine powder using Mixer Grinder (Sujata MG01, 25,000 R.P.M), sieved through a 500 μ m mesh screen, packed in airtight containers, and stored at 4°C until further analysis.

2.3. Analytical methods

Nutritional content of the oats was evaluated by quantitative estimation of proteins and phytic acid in the samples.

Determination of Protein Content

Protein content was estimated using the Kjeldahl method ($N \times 6.25$) (Kjeldahl method for crude protein determination- AOAC 981.10) [25]. The process was carried out in Kjeldron Automatic Protein Estimation System (Model: KDIGB 6M, KjeldIST EA).

Digestion: Approximately Two hundred and fifty mg of the sample was accurately weighed, wrapped in filter paper, and transferred into Kjeldahl digestion tubes. To each tube, 10mL of concentrated sulphuric acid (H_2SO_4) was added along with 3 g of catalyst mixture consisting of copper sulphate and potassium sulphate in a 1:5 ratio. The digestion unit was preheated to 250 °C before loading the samples. After placing the tubes in the digestion block, the temperature was gradually raised to 360-400°C and maintained until a clear solution was obtained, indicating complete digestion. The digested samples were then allowed to cool at room temperature for approximately 1 hour.

Distillation: Reagents including 4% boric acid, 40% NaOH and 0.1% N HCl were prepared prior to analysis. For ammonia collection, 25 mL of boric acid solution containing mixed indicator (5 drops each of methyl red and bromocresol green) was taken in a 250 mL conical flask and positioned at the receiving end of the distillation unit. The digested samples were subjected to distillation after the system reached operational readiness. Sodium hydroxide was added to liberate ammonia, which was distilled and absorbed into the boric acid solution, resulting in a colour change depending on the indicator.

Titration: Upon completion of distillation, the collected distillate was titrated against standard 0.1 N HCl. A blank determination was performed under identical conditions. The volume of acid consumed was recorded, and protein content was calculated accordingly.

Calculations:

$$\% \text{ of Nitrogen} = \frac{14 \times \text{Normality of Acid} \times \text{Actual Titrant Value} \times 100}{\text{Sample Wt} \times 1000}$$

$$\% \text{ of Protein} = \% \text{ of Nitrogen} \times \text{Power Factor (6.25 in case of solid sample)}$$

Determination of Phytic Acid

Phytic acid was determined using colorimetric technique based on the decolorization of the Wade reagent (iron-sulfosalicylic acid complex) by phytate [26]. One gram of dried and finely ground sample was weighed. 10 - 50 mL of the HCl extraction solvent (0.2 N HCl) was added and shaken for 1 hour at room temperature. The mixture was centrifuged at 1100g for 30 minutes to collect the clear supernatant. 3 mL of the sample supernatant was mixed with 1 mL of Wade reagent (solution containing 0.03% ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and 0.3% sulfosalicylic acid in distilled water) in a test tube. The solution was vortexed for approximately 5 seconds to ensure complete mixing. The optical density of the resulting solution was measured at 500 nm using a UV-Vis spectrophotometer. A standard curve using pure phytic acid concentrations was prepared using different concentrations of sodium phytate as sample. The decrease in pink colour intensity is proportional to the phytic acid concentration.

2.4. Experimental Design for optimization of process variables

Three independent variables i.e. Soaking time (6-18 hours), germination time (24-72 hours), and soaking and germination temperature (15-30 °C) were selected based on preliminary trials and a comprehensive review of existing literature on oat and cereal germination [23]. These parameters were identified as having a significant influence on the biochemical and nutritional characteristics of germinated oats.

The key response variables studied included protein content (% w/w) and phytic acid content (mg/g). These parameters were chosen to evaluate the nutritional enhancement and reduction of anti-nutritional factors following germination.

Statistical Experimental Design: Response Surface Methodology was employed to optimize the germination conditions [24]. Specifically, a Randomized Central Composite Design (CCRD) was used as the statistical experimental design. The design comprised a total of 20 experimental runs with six center points to evaluate experimental error and model adequacy using Design-Expert® software (V 13.0). The experiments were performed as per the run table generated by the software. The responses obtained from the experiments were fed again to the software for further analysis.

Analysis of variance was performed to test the significance of model terms and regression coefficients. Model was reduced and modified based on the p values of different terms appearing in the second order model maintaining the hierarchy of the system. Model adequacy was determined based on R^2 , adjusted R^2 , predicted R^2 , p-values of different coefficients, and lack-of-fit tests. Response surface plots were generated to visualize the interaction effects among variables and identify optimum germination conditions.

General form of the second-order polynomial model (Equation 1):

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{jj=i+1}^k \beta_{ijj} X_i X_{jj} + \epsilon \dots \dots (1)$$

Where:

Y = the predicted response (Total Protein Content, Phytate Content)

β_0 = the intercept constant

β_i = the linear coefficient

β_{ii} = the quadratic coefficient

β_{ijj} = the interaction coefficient

XX_i and XX_{jj} = the coded independent variables

k = the number of independent variables (in this case, k=3)

ε = the error term

2.5 Optimization and validation: The optimization tool of the Design Expert was utilized to determine the predicted values of process parameters that will give the maximum values of protein content and minimum value of phytic acid content in the given range of independent variables. 95% Confidence intervals (CI) and prediction intervals (PI) were determined with the analysis of experiment values. A set of validation experiment was run with the values of independent variables as proposed by the software to determine the experimental validity of the predicted model.

3. RESULTS AND DISCUSSION

The stage of germinating of oats are shown in the actual pictures. Figure 1(A) represents the stage of soaking of oats at controlled temperature. Seeds during the germination are shown in Figure 1(B). After drying and milling, the oats are converted into powders as shown in Figure 1(C).

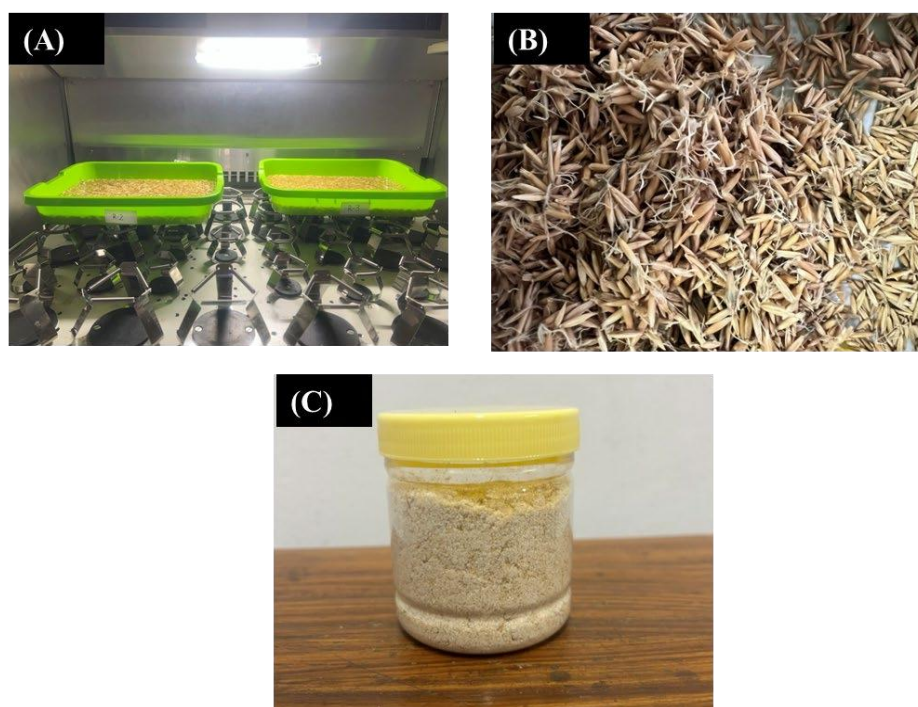


Fig. 1: Stages of processing of sample; (A) soaking of oats kernels, (B) germination of kernels, (C) milled and sieved sample for final use in experiments

The process was modelled and analysed on the basis of ANOVA table for both the response variables. Diagnosis is presented with normal plot of residuals, predicted versus actual plot, the plot of residual versus runs. Coded equation of the model is also presented to observe the relative effect of parameters of the response. Three-dimensional response surfaces and contour plots are presented for all the combinations to observe the individual and interactive effects of the process variables.

3.1 Protein Content: The ANOVA of the model for protein content indicates that the modified quadratic model was highly suitable with a very low p-value (<0.05) and a high F-value of 11.41 (Table 1). The model demonstrated strong predictive capability with an R^2 of 0.87, an Adjusted R^2 of 0.831, and a Predicted R^2 of 0.793. The low difference (<0.2) between the adjusted and predicted R^2 value is desirable for model accuracy. The adequate precision of 9.34 suggests an adequate signal-to-noise ratio, affirming the model's reliability for navigating the design space. The non-significant lack of fit ($p=0.162$) further confirmed the model's suitability to represent the experimental data. ANOVA table suggest that among the model terms,

temperature (C), interaction between soaking time and germination time (AB) and all the quadratic terms (A^2 , B^2 , C^2) are significant.

The model predicted to be most suitable is a modified form of quadratic equation that is given (coded form) by equation 1.

$$\text{Protein (g)} = 20.088 + 0.194A + 0.09B + 0.387C - 0.5 AB - 0.962A^2 - 0.935B^2 - 0.648C^2 \dots\dots\dots (1)$$

The response surface plot of the interaction (AB, BC, AC) and its corresponding contour plot is shown in Figure 2 (A-F). Both soaking time and germination time were found to have a maxima independently in the tested range for the protein content. This is in coherence with their respective p values (for A^2 and B^2 terms) from ANOVA table (<0.05). Independently, germination temperature was also found to have a maximum in the given range that is revealed from p value of the C^2 term (<0.05). The individual effects of soaking time and germination time were found to be not significant as depicted by their low p value. A-B interaction was found to be the significant ($p<0.05$) that means the change in one of these will be affected by change in another and the effect depends on combinations of variables, not just individual effects.

The normal plot of residuals was also found to follow and almost linear order that suggest that the model has been fitted properly Figure 3(A). The predicted values of the responses are presented in Table 1 as well as Figure 3(B). The scatter plot reveals an appreciable agreement between the experimental and predicted responses. The distribution of residuals with experimental runs is given in Figure 3(C). which was found to be random that further strengthens the adequate fitting of the model

Table 1: Run table obtained from CCRD design used in experiment with experimental and predicted responses

Experiment	Factors			Responses Experimental		Responses Predicted	
	A	B	C	Protein (% w/w)	Phytate (mg/g)	Protein (% w/w)	Phytate (mg/g)
1	12	48	22.5	20.44	1.33	20.09	1.41
2	12	48	22.5	19.89	1.35	20.09	1.41
3	12	48	22.5	19.34	1.46	20.09	1.41
4	12	48	35.1134	19.34	1.18	18.91	1.18
5	12	88.363	22.5	18.33	1.44	17.60	1.36
6	12	48	22.5	20.33	1.38	20.09	1.41
7	18	24	15	17.86	0.96	17.76	0.9374
8	12	48	22.5	19.86	1.51	20.09	1.41
9	18	72	30	17.25	1.32	17.71	1.40
10	6	72	30	18.56	1.26	18.33	1.28
11	6	72	15	16.36	0.98	17.55	1.02
12	18	24	30	18.43	1.19	18.53	1.19
13	12	48	9.88655	17.85	0.78	17.60	0.7468
14	12	48	22.5	20.55	1.4	20.09	1.41
15	6	24	15	16.86	0.78	16.37	0.8193
16	22.0908	48	22.5	17.86	1.35	17.69	1.32
17	6	24	30	16.36	1.15	17.15	1.08
18	1.90924	48	22.5	17.56	1.12	17.04	1.12
19	12	7.63697	22.5	17.24	0.98	17.29	1.02
20	18	72	15	16.74	1.12	16.94	1.14

Table 2: ANOVA table for Protein Content modelling

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	31.58	7	4.51	11.41	0.0002	significant
A-Soaking Time	0.5121	1	0.5121	1.3	0.2773	
B-Germination Time	0.1113	1	0.1113	0.2817	0.6053	
C-Soaking & Germination Temperature	2.05	1	2.05	5.18	0.0421	
AB	2	1	2	5.06	0.0441	
A ²	13.32	1	13.32	33.68	< 0.0001	
B ²	12.59	1	12.59	31.85	0.0001	
C ²	6.06	1	6.06	15.32	0.0021	
Residual	4.74	12	0.3953			
Lack of Fit	3.7	7	0.5285	2.53	0.162	not significant
Pure Error	1.04	5	0.2089			
Cor Total	36.32	19				

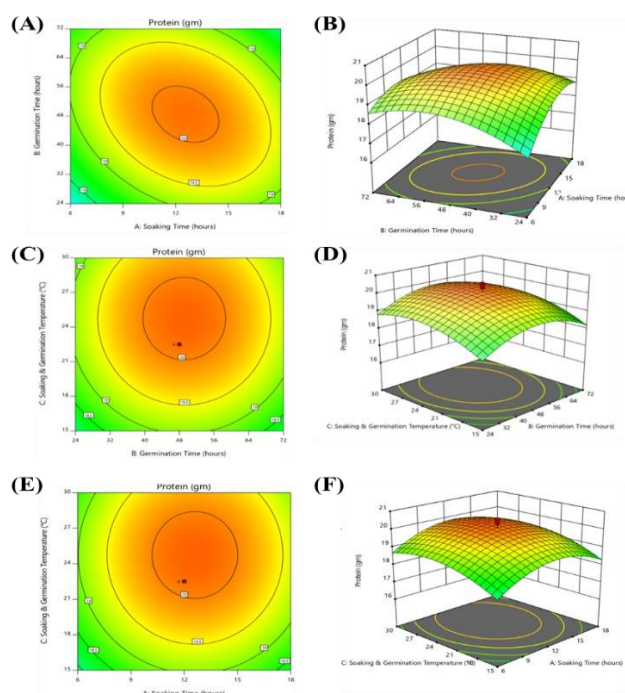


Fig. 2 Contour plots 3D RSM plots for protein content; (A) Contour plot for soaking time and germination time, (B) 3D response surface plot for soaking time and germination time, (C) contour plot for germination time and temperature, (D) 3D response surface plot for germination time and temperature, (E) contour plot for soaking time and temperature, (F) 3D response surface plot for soaking time and temperature

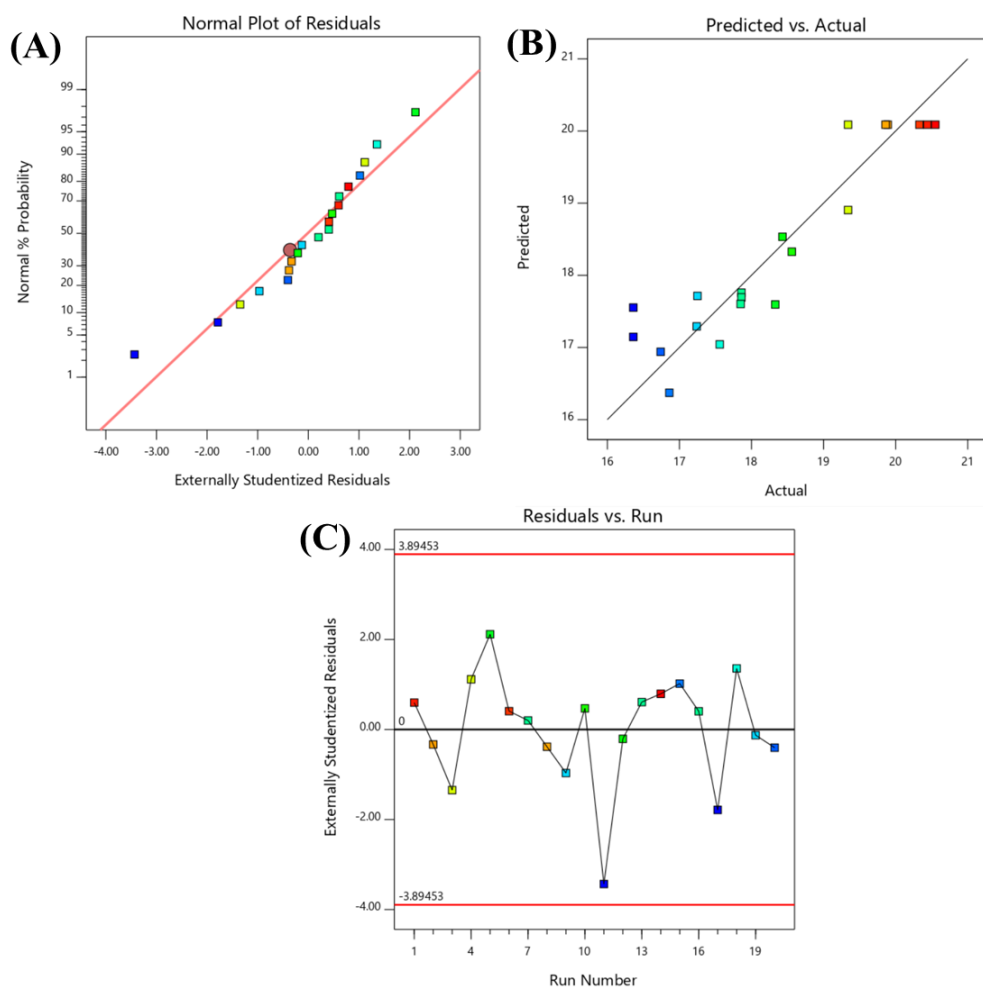


Fig. 3 Diagnostic curves for protein content model; (A) Normal plot of residuals, (B) predicted versus actual plot, (C) scatter of residual with run number

3.1.2. Phytate Content: For phytate content, the quadratic model was modified and the terms included in the model are given as in Table 3. The linear term for soaking/germination temperature (C) and the quadratic term for germination time (B^2) were identified as significant factors for phytate reduction ($p < 0.05$). However, none of the reductions (A-B, B-C, A-C) were found to be significantly affecting the phytate content as revealed from their high p values in ANOVA and hence are removed during model improvement. The lack of fit was found to be non significant that is a desirable trait as significant lack of fit points towards underfitting of the data to proposed model. The R^2 value of the model was found to be 0.945 that illustrates a good fit. The adjusted R^2 was 0.92 and predicted R^2 was 0.854. The difference of these two was less than 0.2 that suggest another proof of a good fit of the model. Also, no transformation was required to perform modelling of the data. The modelled equation is given in coded form in eq. 2.

$$\text{Phytate } \left(\frac{\text{mm}}{\text{mm}} \right) = 1.406 + 0.059A + 0.1B + 0.128C - 0.66A^2 - 0.0754B^2 - 0.1567C^2 \dots \dots \dots (2)$$

The contour plots and 3D response surface plots for all the interactions are given in Figure 4. Although the interactions were not significant, but the nature of the curve reveals that the point of maximum response is lying in the range of experimental design. Although the curvature of the contours are pointing towards interactive effects of the variables involved, but the high p values in ANOVA table is suggesting that these interactions are not significant.

Observations over the predicted versus actual curve (Figure 5A) suggest that the model is having a sufficient predictability that is supported by a good R^2 value (0.854) of predicted curve. The normal plot of residuals is given in Figure 5(B). The nearness of the points to the central line suggests a good fit and even distribution of residuals. The plot of residual (Figure 5C) versus runs

further strengthens the adequacy of the model as the residual were found to scatter randomly

Table 3: ANOVA table for Phytate Content model

Source	Sum of	df	Mean	F-value	p-value	
Model	0.84748	6	0.141247	37.443	<0.00001	significant
A-Soaking Time	0.047664	1	0.047664	12.635	0.003525	
B-Germination Time	0.138161	1	0.138161	36.62	<0.00001	
C-Soaking & Germination Temperature	0.224943	1	0.224943	59.63	<0.00001	
A ²	0.063886	1	0.063886	16.935	0.001218	
B ²	0.081974	1	0.081974	21.73	0.000445	
C ²	0.354035	1	0.354035	93.85	<0.00001	
Residual	0.04904	13	0.003772			
Lack of Fit	0.02569	8	0.003211	0.6876	0.697	not significant
Pure Error	0.02335	5	0.00467			
Cor Total	0.89652	19				

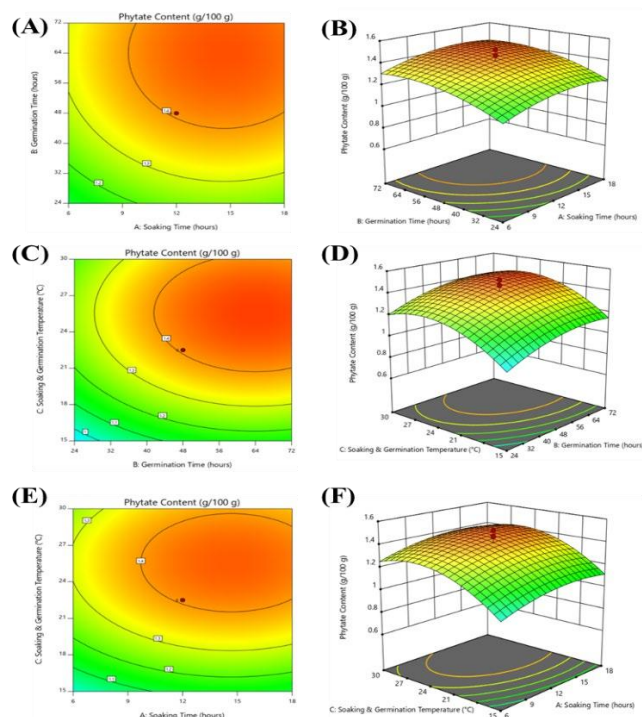


Fig. 4 Contour plots 3D RSM plots for phytate content; (A) Contour plot for soaking time and germination time, (B) 3D response surface plot for soaking time and germination time, (C) contour plot for germination time and temperature, (D) 3D response surface plot for germination time and temperature, (E) contour plot for soaking time and temperature, (F) 3D response surface plot for soaking time and temperature

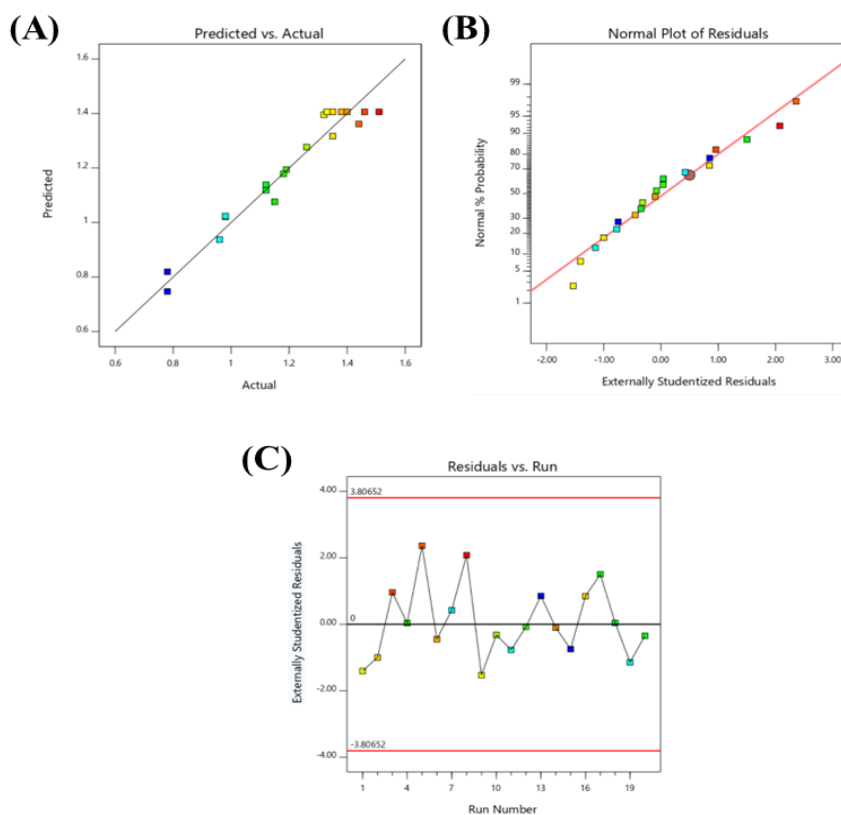


Fig. 5 Diagnostic curves for phytate content model; (A) Normal plot of residuals, (B) predicted versus actual plot, (C) scatter of residual with run number

3.2. Optimization

Multi-response optimization using the desirability function approach was performed to identify the optimal germination conditions that simultaneously maximized protein content and minimized phytate content. The criteria set for optimization aimed for the highest protein percentage and the lowest phytate concentration within the experimental design space.

The optimization revealed an overall desirability of 0.935, indicating a high degree of satisfaction for both desired responses under the identified optimal conditions. These conditions are detailed in Table 4.

Table 4: Optimized Germination Parameters and Predicted Responses

Soaking Time (hrs)	Germination Time (hrs)	Soaking and Germination Temperature (°C)	Predicted Protein (% w/w)	Predicted Phytate (mg/g)	Desirability
6.00	61.246	15.00	21.218	0.760	0.935

These optimal conditions i.e. 6 hours soaking, 61.2 hours germination, and 15 °C soaking/ germination temperature, are predicted to yield a maximum protein content of 21.2 % (w/w) and a minimum phytate content of 0.760 mg/g. These findings are consistent with the general understanding that controlled germination can significantly enhance nutritional attributes and reduce anti-nutritional factors in cereals [16, 17].

3.3 Model Validation

The optimized conditions identified by RSM were subjected to a validation experiment to confirm the predictive capability of the developed models. The validation experiment yielded a good correspondence with the predicted results (Table 5) for both protein and phytate content at the optimal parameters (6 hours soaking, 61.2 hours germination, 15 °C temperature). This alignment between predicted and experimental values validates the reliability of the RSM models in optimizing the germination process for oats. The predicted values were found to fall within 95% prediction interval (Table 5).

Table 5: Predicted and Experimental Values for the response variables at optimum conditions

	Protein (% w/w)	Phytate (mg/g)
Predicted	21.2	0.760
Experimental	21.05 ± 0.18	0.772 ± 0.015
95% Prediction interval	18.63-21.58	0.71-1.27

4 DISCUSSIONS

Soaking time has been widely reported as a critical factor influencing water uptake and metabolic activation in grains. Studies have demonstrated that soaking durations ranging from 8 to 24 h significantly enhance germination efficiency and biochemical transformations, including improved enzymatic activity and germination rates [27, 28]. Similarly, soaking for approximately 12 h has been shown to optimize bioactive compound accumulation such as γ -aminobutyric acid (GABA) in cereal grains [29, 30].

Germination duration plays a decisive role in enzymatic hydrolysis and synthesis of bioactive compounds. Previous studies on oats and other cereals have reported significant improvements in α -amylase activity, β -glucan modification, and antioxidant properties within 1–5 days of germination ([20, 31, 32]. However, with preliminary trials, it was observed that germination periods extending beyond 72 hrs was leading to undesirable changes causing quality deterioration. Therefore, a range of 24–72 h was selected to capture the optimal biochemical transformation window.

Temperature is another key determinant regulating enzymatic activity, germination kinetics, and accumulation of functional compounds. Optimal germination in oats has been reported between 20–30 °C, with higher temperatures adversely affecting germination rate and seedling growth [33, 34]. Additionally, bioactive compound synthesis such as polyphenols and avenanthramides has been shown to be highly temperature-dependent, with optimal accumulation observed around 20, 25 and 30 °C [35].

Germination is known to induce proteolytic activity, leading to improve protein digestibility and modification of amino acid profiles. Additionally, changes in measurable protein content reflect metabolic activation and synthesis of functional peptides during germination, making it a sensitive indicator of nutritional improvement. Phytic acid was selected as a key anti-nutritional factor due to its strong mineral chelating properties, which significantly reduce the bioavailability of essential micronutrients. Germination activates endogenous phytase enzymes, resulting in the hydrolyses of phytic acid and subsequent improvement in mineral bioavailability. Thus, its reduction serves as a reliable marker for evaluating the effectiveness of germination in enhancing nutritional quality.

Implications of Optimized Germination for Oat Utilization

The successful optimization of germination parameters demonstrates a practical approach to significantly enhance the nutritional quality of oats. The identified optimal conditions result in a substantial increase in protein content and a marked reduction in phytate levels, addressing two critical aspects of nutritional improvement. Higher protein content makes germinated oats a more valuable ingredient for protein-fortified foods, especially important in plant-based diets. The reduction in phytate, a known anti-nutritional factor, improves the bioavailability of essential minerals like iron and zinc, leading to a more nutritionally dense food product [13].

These findings highlight the immense potential of optimized germinated oats for various applications in functional food development. Such oats can be incorporated into breakfast cereals, baked goods, snacks, and plant-based beverages, offering consumers products with enhanced nutritional profiles and improved digestibility. This study contributes to the growing body of knowledge on cereal processing and provides a scientific basis for the industrial application of optimized oat germination to create healthier and more sustainable food options.

5.CONCLUSION

The present study optimized the germination conditions of oats (*Avena sativa* L.) using Response Surface Methodology coupled with a Central Composite Design to enhance nutritional quality. The developed quadratic models demonstrated a strong fit for protein as well as phytate content. Multi-response optimization identified 6 hours of soaking, 61.2 hours of germination, and 15 °C as optimal conditions, yielding a predicted protein content of 21.2% (w/w) and phytate content of 0.760 mg/g, with an overall desirability of 0.935. Experimental validation confirmed good agreement with predicted values.

Therefore, this study provides a scientifically optimized protocol for improving the nutritional profile of germinated oats by enhancing protein content and reducing anti-nutritional phytate levels, thereby improving mineral bioavailability. While the focus was limited to protein and phytate, the findings establish a strong foundation for broader nutritional optimization and support the application of germinated oats in functional and value-added food products.

Ethic Approval and Consent to Participate

Not Applicable. This study did not involve human participants, human data, human tissues, or animals. Therefore, ethics approval and informed consent were not required.

Consent for Publication

Not Applicable

Availability of Data and Materials

All data generated or analysed during this study are included in this article. No additional datasets were generated or analysed.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contribution

AKS: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft; RP: Conceptualization, Methodology, Supervision, Validation, Writing – review & editing; VB: Supervision, Resources, Validation, Writing – review & editing; SG: Formal analysis, Data curation, Visualization, Writing – review & editing.

All authors read and approved the final manuscript.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REFERENCES

- [1] Morcia C., Finocchiaro F., Delbono S. et al. (2023) Oats: Nutritional Uniqueness and Breeding of a Healthy Superfood. In: Kole, C. (eds) *Compendium of Crop Genome Designing for Nutraceuticals*. Springer, Singapore.153. https://doi.org/10.1007/978-981-19-4169-6_5
- [2] Vilvert E., Olson Å., Wallenhammar A.-C. et al. (2021) *Environ. Evid.* 10: <https://doi.org/10.1186/s13750-021-00239-7>
- [3] Butt M.S., Tahir-Nadeem M., Khan M.K.I., Shabir R., Butt M. (2008) Oat: unique among the cereals *Eur. J. Nutr.* 47: 68-79. <https://doi.org/10.1007/s00394-008-0698-7>
- [4] Kim I.-S., Hwang C., Yang W., Kim C. (2021) Multiple Antioxidative and Bioactive Molecules of Oats (*Avena sativa* L.) in Human Health. *Antioxidants* 10: 1454. <https://doi.org/10.3390/antiox10091454>
- [5] Sood V.K., Sanadya S.K., Kumar S., Chand S., Kapoor R. (2022) Health benefits of oat (*Avena sativa*) and nutritional improvement through plant breeding interventions. *Crop Pasture Sci.* 74: 993. <https://doi.org/10.1071/cp22268>
- [6] Aparicio-García N., Martínez-Villaluenga C., Frías J., Peñas E. (2020) Changes in protein profile, bioactive potential and enzymatic activities of gluten- free flours obtained from hulled and dehulled oat varieties as affected by germination conditions. *LWT- Food Science and Technology* 134: 109955. <https://doi.org/10.1016/j.lwt.2020.109955>
- [7] Leszczyńska D., Wirkijowska A., Gasiński A. et al. (2023) Oat and Oats processed products- Technology, Composition, Nutritional Value, and Health. *Appl. Sci.* 13: 11267. <https://doi.org/10.3390/app132011267>
- [8] Mathias-Ramwel M., Salvo-Garrido H., Reyes-Rebolledo M., Montenegro-Barriga A. (2016) Jupiter- INIA: A new oat variety with improved β - glucan and protein contents. *Chil. J. Agric. Res.* 76: 401. <https://doi.org/10.4067/s0718-58392016000400002>
- [9] Ibrahim M.N.G., Andreson H., Othman S.B., Jöudu I. (2024) Effect of neutral and acidic protease processing intervals on optimising nutritional value and enhancing physico- chemical properties of oat drink. *Foods* 13, 2285. <https://doi.org/10.3390/foods13142285>
- [10] Kumar L., Sehrawat R., Kong Y. (2021) Oat proteins: A perspective on functional properties. *LWT- Food Science and Technology* 152: 112307. <https://doi.org/10.1016/j.lwt.2021.112307>
- [11] Rafique H., Dong R., Wang X. et al. (2022) Dietary- Nutraceutical properties of Oat protein and peptides. *Front. Nutr.* 9: 950400. <https://doi.org/10.3389/fnut.2022.950400>
- [12] Holopainen-Mantila U., Pentikäinen S., Lehtinen P., Sözer N. (2024) Oats as a source of nutritious alternative protein. *J. Cereal Sci.* 116: 103862. <https://doi.org/10.1016/j.jcs.2024.103862>
- [13] Samtiya M., Aluko R.E., Dhewa T. (2020) Plant food anti- nutritional factors and their reduction strategies: an overview. *Food Prod. Process. Nutr* 2. <https://doi.org/10.1186/s43014-020-0020-5>
- [14] Tunçel N., Kaya H.P., Andaç A.E. et al. (2025) A comprehensive review of antinutrients in plant- based foods and their key ingredients. *Nutr. Bull.* 50: 171. <https://doi.org/10.1111/mbu.12732>
- [15] Hübner F., Neil T. O', Cashman K.D., Arendt E.K. (2010) The influence of germination conditions on beta- glucan, dietary fibre and phytate during the germination of oats and barley. *Eur. Food Res. Technol.* 231: 27. <https://doi.org/10.1007/s00217-010-1247-1>
- [16] Minchán-Velayarce, H.H.; Bustos, A.-S.; Paucar-Menacho, L.M.; Vidaurre-Ruiz, J.; Schmiele, M. (2025) New Frontiers in Cereal and Pseudocereal Germination: Emerging Inducers for Maximizing Bioactive Compounds. *Foods* 14: 3090, <https://doi.org/10.3390/foods14173090>
- [17] Siddiqua A., Ali M., Ahmed S. (2019) Functional properties of germinated and non- germinated cereals: A comparative study. *Bangladesh J. Sci. Ind. Res.* 54: 383. <https://doi.org/10.3329/bjsir.v54i4.44573>
- [18] Jianfei J., Ma H., Wang S. (2019) Application of ultrasound, microwaves, and magnetic fields techniques in the germination

- of cereals. *Food Sci. Technol. Res.* 25: 489. <https://doi.org/10.3136/fstr.25.489>
- [19] Aparicio-García N., Martínez-Villaluenga C., Frías J., Peñas E. (2020) Sprouted oat as a potential gluten free ingredient with enhanced nutritional and bioactive properties. *Food Chem.* 338: 127972. <https://doi.org/10.1016/j.foodchem.2020.127972>
 - [20] El-Refai A., Amal E.-B., Ashaal E.E. (2012) Effect of germination process on the chemical and biological active compounds of barley and oat grains. *J. Food Dairy Sci.* 3: 553. <https://doi.org/10.21608/jfds.2012.81667>
 - [21] Burgos V., Armada M. (2020) Implications of processing on the levels of antinutritional factors in cereal grains. *CABI Rev:* 1- 20. <https://doi.org/10.1079/pavsnr.201914054>
 - [22] Budhwar S., Sethi K., Chakraborty M. (2020) Efficacy of germination and probiotic fermentation on underutilized cereal and millet grains. *Food Prod. Process. Nutr.* 2. <https://doi.org/10.1186/s43014-020-00026-w>
 - [23] Sergiacomo A., Bresciani A., Marti A. (2025) Sprouting of oats: a review on the relationship between processing conditions and chemico- physical properties of flour. *Crit. Rev. Food Sci. Nutr.* 65: 7774. <https://doi.org/10.1080/10408398.2025.2479651>
 - [24] Palanikumar K. (2021) Response Surface Methodology in Engineering Science. IntechOpen. <https://doi.org/10.5772/intechopen.90965>
 - [25] Prakash G.G., Shinde M. (2024) Nutritional analysis and glucosidase inhibition activity of kodo millet. *Int. J. Agric. Biol. Res* 40: 942- 944. <https://doi.org/10.35248/0970-1907.24.40.942-944>
 - [26] Roust N., Taherzadeh M.J. (2026) Measuring phytic acid in complex and fermented food matrices: Modified protocol using enzymatic analysis. *JSFA Rep.* 6: 4- 13. <https://doi.org/10.1002/jsf2.70020>
 - [27] Nadeem M.K. et al. (2017) Effect of seed soaking time on germination of maize (*Zea mays*). *Biol. Res.* 2: 46. <https://psmjournals.org/index.php/biolres/article/download/56/32>
 - [28] Tanwar H. et al. (2023) Optimization of 'on farm' hydropriming conditions in wheat: Soaking time and water volume have interactive effects on seed performance. *PLoS One* 18. <https://doi.org/10.1371/journal.pone.0280962>
 - [29] Abhilasha P., Pal U., Nayak R. et al. (2022) Standardisation of soaking and germination time for preparation of germinated brown rice. *Oryza* 59: 126- 130. <https://epubs.icar.org.in/index.php/OIJR/article/view/122860>
 - [30] Chung H., Jang S., Cho H.Y., Lim S. (2009) Effects of steeping and anaerobic treatment on GABA (γ - aminobutyric acid) content in germinated waxy hull-less barley. *LWT- Food Science and Technology* 42: 1712. <https://doi.org/10.1016/j.lwt.2009.04.007>
 - [31] Tang S., Mao G., Yuan Y. et al. (2020) Optimization of oat steeping and germination temperatures to maximize nutrient content and antioxidant activity. *J. Food Process. Preserv.* 44: 14683. <https://doi.org/10.1111/jfpp.14683>
 - [32] Bazhay-Zhezherun S., Simakhina G., Bereza-Kindzerska L., Naumenko N. (2019) Qualitative indicators of grain flakes of functional purpose. *Ukr. Food J.* 8: 7- 17. <https://doi.org/10.24263/2304-974x-2019-8-1-3>
 - [33] Sulaiman S. et al. (2023) Germination process of oat (*Avena sativa* L.) to temperature and salinity using halothermal time model. *Plant Stress* 10: 100263. <https://doi.org/10.1016/j.stress.2023.100263>
 - [34] Radford B., Key A. (1993) Temperature affects germination, mesocotyl length and coleoptile length of oats genotypes. *Aust. J. Agric. Res.* 44: 677- 688. <https://doi.org/10.1071/ar9930677>
 - [35] Figueroa-Pérez M.G., Reynoso-Camacho R., Ramos-Gómez M. et al. (2023) Impact of temperature and humidity conditions as abiotic stressors on the phytochemical fingerprint of oat (*Avena sativa* L.) sprouts. *Food Chem.* 439: 138173. <https://doi.org/10.1016/j.foodchem.2023.138173>.