OPTIMIZATION OF SOAKING CONDITIONS FOR IMPROVING THE GERMINATION RATE, TOTAL POLYPHENOL CONTENT, ANTIOXIDANT ACTIVITY OF NEWLY RESTORING VD20 RICE

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ABSTRACT

This study is aimed to optimize soaking conditions, including soaking temperature, pH of soaking solution, and soaking time for maximizing the rice germination rate and biological properties [total polyphenol content (TPC) and Ferric Reducing Antioxidant Power (FRAP) as know as antioxidant activity] of VD20 germinated rice. The Box-Behnken design by using response surface methodology was used to build experimental design. The results showed that the significant impact of each treatment on the sprouting rate as well as content of TPC and value of FRAP in VD20 rice. The desirability function approach was applied to find the optimal soaking conditions leading to germination with the highest phenolic content, value of FRAP and germination rate. The optimum conditions were determined as soaking time of 6.36 hours, soaking temperature of 26°C and pH of 4.65. In these optimized conditions, the expected total phenolic content, antioxidant activity and germination rate of VD20 rice were 33.42 mgGAE/100 g, 36.9 μM TE/g and 97.55%, respectively, which was further values confirmed by experimental test. This work showed that soaking process has a significant impact on polyphenols, which could enhance the nutrition of the product. The obtained product with high antioxidant activity could be further utilized in nutraceutical foods.

Keywords: *Germination, phenolic, response surface methodology, rice, soaking.*

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1. INTRODUCTION

Rice, scientifically known as *Oryza sativa*, is a collective term for over 20 types of grass that grow annually. It serves as the primary source of sustenance for about 50% of the global population. The rice seed, also known as caryopsis, primarily comprises the seed coat, embryo and endosperm [1]. Rice bran, which refers to the seed coat of rice, is rich in protein, B complex vitamins and vitamins E and K. On the other hand, polished rice, which lacks the seed coat, primarily consists of approximately 25% carbohydrates. It also contains small amounts of iodine, iron, magnesium, and phosphorus, as well as limited quantities of protein and fat [2]. Rice bran is rich in beneficial compounds, including vitamin E (α-tocopherol and tocotrienol) and γ-oryzanol. Rice bran contains a significant amount of α-tocopherol, a form of vitamin E. This compound acts as an antioxidant and has the potential to reduce the likelihood of developing cancer and coronary heart disease [1, 2]. Research studies have indicated that eating whole grains and foods made from them can lower the chances of developing noncommunicable diseases such as type II diabetes, cardiovascular disease, cancer and obesity [3]. Brown rice is classified as whole grain rice since only the inedible husk is removed, but the exterior thin layer (bran) surrounds the endosperm and embryo. The substance includes a variety of essential nutrients and bioactive substances, including dietary fiber, unsaturated fatty acids, phenolic compounds, γ-oryzanol and phytosterol [3 - 5]. Germinated brown rice addresses the drawbacks of regular brown rice (BR) by offering improved texture, nutritional value (gamma-aminobutyric acid and ferulic acid) and nutraceutical quality, which could br decreased the fasting plasma glucose (FPG), 0.5, 1 and 2 hours postprandial plasma glucose (PPG), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c) in metabolic syndrome (MS) patients after one month consuming [6]. Water is quickly absorbed during the soaking process, leading to various biochemical reactions. These reactions cause the texture to become softer, polymers to degrade and the synthesis and accumulation of certain phytochemical compounds to increase [7 - 10]. The process of germination is a cost - effective and efficient method for enhancing the quality of cereals. Various factors, including as the specific method and circumstances used for soaking, as well as any pre - treatment applied, might have an impact on the germination process. The germination process can be either facilitated or impeded by these circumstances, which are contingent upon the nutritional composition contained inside the seed. During germination, some seed reserves undergo breakdown and are used for respiration and the synthesis of new cellular components for the developing embryo. This process results in significant changes in the biochemical, nutritional and sensory characteristics of the cereal [10]. Adequate levels of moisture and pH are crucial for the timely initiation of seed germination [11]. The germination percentage plays a crucial role in determining the amount and viability of seeds. Prolonged soaking resulted in insufficient growth and germination due to an excessive amount of water [12]. According to Chatchavanthatri *et al.* (2020) [13], the optimal temperature for boosting germination percentage through moisture soaking is 28ºC. The germination rate of seeds from numerous plant species was adversely impacted by a high pH [14]. The research conducted on red clover seeds demonstrated that the pH level of 4 yielded the greatest average seedling and hypocotyls length, whereas the lowest germination rates were seen at pH levels 6 and 7 [15]. The length of time that seeds are soaked has a significant effect on the overall amount of phenolic compounds in germinated seeds. The germination efficiency was found to be regulated by the seed moisture content, which in turn was affected by the period of soaking. Prior research has discovered a positive association between the rate at which seeds germinate and the amount of phenolic chemicals present [16, 17]. It also exhibited similarities to this study. Furthermore, extended soaking might result in the loss of phenolic compounds through the leaching process, as well as oxidation caused by changes in temperature and pH [18]. Germination rate and GABA content were increased when extended the soaking temperature and time [19]. The optimal process conditions could affect by types of grain. VD20 rice referred to one of the most popular rice varieties in Tien Giang province (Vietnam) because of its pleasant aroma and soft texture [1, 10]. It also was known as the newly restoring variety. However, it was limited about study to utilize this material. The germination process could be potential for it, can be divided into two-steps; soaking and sprouting (germinating) processes. An optimized the soaking conditions could maximize the phytochemical and antioxidant compounds in VD20 brown rice. Therefore, the objective of this study was to determine the effect and optimization of soaking process (i.e., temperature, time and pH) on the rate of germination and antioxidant properties of the VD20 rice.

2. MATERIALS AND METHODS

2.1. Materials

Paddy of VD20 variety (*Oryza sativa* L. cv. VD20) was received from Tien Giang province, Vietnam. The paddy was sun dried to get a moisture content of 12 - 14% (wet basis). The paddy was dehulled using a rice dehuller to obtain brown rice (BR). The paddy and BR were then vacuum packed and stored at 4°C for further experiment. In this study, all chemicals were of analytical grade.

2.2. Preparation of germinated rice

Germinated rice was prepared based on the results of our preliminary studies. Brown rice was soaking in soaking solution with ratio of 1: 2 for a period of time as below experimental design. Then, brown rice was germinated for 20 hours at 35°C. At the end of germination period, root hairs were removed from the germinated rice grains. Germinated rice grains were dried in an oven at 60°C to a moisture content of 10%, and separately ground into flour using hammer mill. The samples were sieved through 100 μm sieve size and packaged in PA airtight containers for analysis.

2.3. Box-Behnken design

Table 1. The experimental range of independent variables

Variables	Range of value
X_1: soaking time (hour)	5 - 7
X_2: soaking temperature (°C)	20 - 30
X_3: pH of soaking solution	4 - 6

Response surface methodology was chosen for experimental design in this study. Three independence variables, including soaking time (X_1, min), soaking temperature (X_2, °C) and pH of soaking solution (X_3), were used in this study. For controlling temperature, sample was located in the incubator with setting temperature and soaking solution was change every 2 hours at he same setting temperature and pH. The pH value was adjusted by citric acid and sodium citrate. The range of each variable was shown in table 1. The design was based on the Box-Behnken model with 6 center points and three replicates for predicting three responses as known as germination rate, total phenolic content and antioxidant activity. The total runs were 54 treatments. The analysis and design were used Statgraphics Centurion XV.I program. Multi-desirable function also was used for finding the optimal condition to maximize response.

2.4. Germination rate, total phenolic content and antioxidant activity

Germination rate. The germination rate is defined by ratio of number of germinated seeds and the total number of seeds used for the germination test [20].

Total Phenolic Compound Analysis. Total phenolic content of the germinated rice flour samples were determined by the Folin-Ciocalteu method described by Ragaee and Abdel-Aal (2006) [21] with some modifications. The sample (250 μL) was allowed to react with Folin-Ciocalteu reagent (2.5 mL) and 2 mL of sodium carbonate (7.5% w/v). The mixture was kept in darkness for 2 hours and vortexed (2 min). Optical density was recorded at 765 nm. Standard curve was constructed using gallic acid. Results were presented as gallic acid equivalent of dry weight of sample (mgGAE/g).

FRAP (Ferric Reducing Antioxidant Power) Assay. The FRAP assay was conducted as described by Wong *et al.* (2006) [22] with slight modifications. FRAP reagent consisted of acetate buffer (pH 3.6), TPTZ solution (10 mmol) in HCl (40 mmol) and iron (III) chloride solution (20 mmol) in proportions of 10: 1: 1 (v/v), respectively. The samples (100 μL) were added to FRAP solution (2.85 mL) and allowed to react for 30 min in the dark. The colored products were vortex (1 min) and the optical density was recorded at 593 nm. Standard curve was constructed using Trolox. Results were presented in μM Trolox equivalent of dry weight of sample (TE/g).

3. RESULTS AND DISCUSSION

3.1. Effect of soaking conditions on VD20 rice germination rate

Germination process could enhance the nutritional profile as well as the biological properties of various grains. The soaking process is an important step, which lead to improve the efficiency of germination process. In this study, different soaking conditions were significantly affected on the germination rate of VD20 rice. The developed model and analysis of variance in terms of linear (X_1), square (X_1^2, X_2^2, X_3^2) and interaction (X_2X_3) were significant influence on the regression of germination rate (Table 2). The lowest P-value of linear term was found on term of X_1 (soaking time), which could be considered as the most influence factor affects germination rate of VD20 germinated rice. The model also showed the p-value less than 0.05 and non-significant of lack-of-fit test, which presented that the obtained equation could fully prediction the change of germination rate at different germination conditions in this study. The predicted equation was present in Equation 1 with high value of coefficient determination (R^2 = 98.34%).

$$\text{Germination rate (\%)} = -354.64 + 61.89X_1 - 4.66X_1^2 - 0.094X_2^2 - 0.79X_2X_3 - 3.17X_3^2 \quad (1)$$

Table 2. Analysis of Variance for VD20 rice germination rate

Source	Sum of Squares	P-Value
X_1	35.69	0.0270
X_2	11.14	0.0794
X_3	9.64	0.0902
X_1^2	80.14	0.0123
X_1X_2	3.63	0.1975
X_1X_3	11.93	0.0748
X_2^2	20.27	0.0461
X_2X_3	62.97	0.0156
X_3^2	37.15	0.0260
Lack-of-fit	2.33	0.6057
Pure error	2.01	
Total (corr.)	260.77	

Note: X_1: soaking time (min), X_2: soaking temperature (^oC), X_3: pH of soaking solution.

Various methods for overcoming seed dormancy and improving germination rates have been proposed by many researchers [23, 24]. Soaking rice in water also enhanced biochemical compounds in rice [25]. Nevertheless, the process of seed dormancy breaking differs across different species. Hence, it is crucial to ascertain the appropriate method and condition for each plant species. In this study, soaking could enhance the rate of germination of VD20 rice (Figure 1). Certain seeds, such as rice, require light as it plays a crucial role in the generation of energy by plants and serves as a signal for several physiological reactions [26]. Dziki *et al.* (2015) [26] reported that germination commences when the grain is exposed to favorable conditions, including a moisture content ranging from 15% to 45%, a temperature over 4°C, the lack of germination inhibitors and the presence of atmospheric air. Consequently, the initial phase of the germination process involves immersing the grains/seeds in water, allowing water to enter the seed through the pores and micropyle and being retained within the grain mostly through interactions with proteins and fibers. The water absorption index becomes stable during the second stage, which involves the breaking of the outer layer of the seed in the area where the embryo is located, followed by the emergence of the root. During the third stage, the rate of water absorption increases once more and the cells of the radical multiply as they grow [27]. It also could be seen that the main factor affected on sprouting rate of VD20 rice was soaking time, which led to grain absorbed enough water for germinating process. Warm water soaking and low pH also promoted the sprouting of rice, which was similar trend with the study of Sagehashi and Sato [24] on seeds of Hokkaido rice cultivars. Additionally, soaking at 30°C for 80 minutes resulted in similar germination rates to seeds soaked at 12˚C for 5 days, indicating the importance of the initial soaking temperature for rice seed germination [23]. Moreover, from the contour plots and prediction of regression model, the optimal soaking conditions were a soaking time of 6.32 hour at 28ºC in soaking solution with pH of 4.75. At these conditions, the highest germination rate was observed at value of 97.97%. Most seeds that were soaked in water before planting saw a fast process of germination. Sufficient moisture and pH are essential for prompt seed germination [11]. Higher germination percentage is a significant factor for determining the quantity and viability of seeds. Excessive water produced inadequate development and germination due to prolonged soaking [12]. Chatchavanthatri *et al.* (2020) [13] also reported that moisture appropriate soaking temperature for promoting germination percentage is 28ºC. High pH was found to negatively affect the germination rate of seeds from multiple plant species [14].

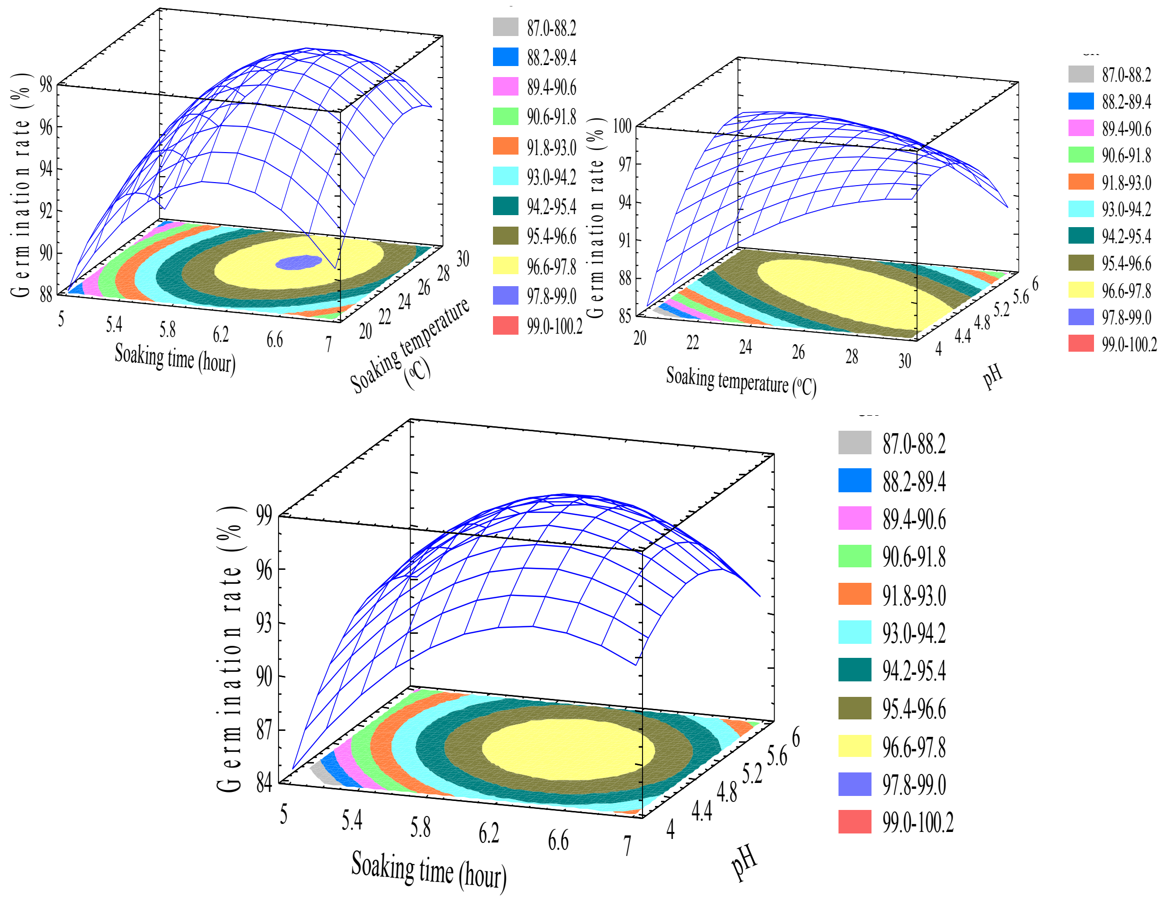


Figure 1. Contour plots the effect of soaking conditions on VD20 rice germination rate

3.2. Effect of soaking conditions on total phenolic content in VD20 germinated rice

Germination enhances the accessibility of nutrients including carbohydrates and proteins, as well as the presence of bioactive substances such as gamma-aminobutyric acid (GABA) and antioxidants. Germination also results in elevated fiber levels and promotes the synthesis of beneficial substances. Additionally, it can lead to a decrease in molecules that are unnecessary or detrimental to human nutrition, such as phytates [19]. Sprouting is a complex process during which the seed must quickly recover physically from maturation drying, resume a sustained intensity of metabolism, complete essential cellular events to allow for the embryo to emerge, and prepare for subsequent seedling growth [28]. From the other hand, sprouting may be regarded as a natural bioreactor or biotechnological module and could be used to improve the nutrition value in food products [28]. The results of total phenolics content (TPC) for sprouts obtained from VD20 grain under different soaking conditions are presented in figures 2. The obtained model also showed the best fit as shown in table 3 with high value of R^2 (92.74%). Among three factors, it could be seen that soaking time is mainly affected the content of polyphenol of VD20 germinated rice, with significant influence of linear, interaction with other factors and square term in regression model. Plant phenolics have potential health benefits mainly due to their antioxidant properties such as reactive oxygen species scavenging and inhibition, free radicals scavenging, and metal chelation [29]. Research and use of therapies for enhancing the growth of plants in seeds and seedlings are under underway. Commercial seed treatment technologies are now recognizing the potential of seed treatments to significantly enhance the value of the seed and enhance plant development and production [30]. Throughout the process of germination, there is a significant alteration in the composition and biological activity of molecules that possess nutraceutical properties. These changes can be greatly influenced by the conditions under which germination takes place. The highest values of TPC were obtained for rice grain soaking in solution pH of 4 for 6.5 hour at 27°C. Initially, TPC content of raw material was 28.39 mgGAE/100 g [18]. TPC increased in the early stage and then decreased in the late stage. Soaking duration has a substantial impact on the total phenolic content of germinated seeds. Seed moisture content, influenced by soaking duration, was found to affect germination efficiency. Positive correlation between germination rate and content of phenolic compounds in previous studies was found [16, 17]. It also presented similar with this study. Moreover, long time soaking could loss phenolic compounds due to leaching process as well as the oxidation promoted by temperature and pH [18]. The total phenolics content (TPC) in relation to soaking time soaking temperature and pH of soaking solution for all tested samples of sprouts can be described by the following Equation 2.

$$\text{TPC (mgGAE/100 g)} = -351.44 + 62.18X_1 + 41.17X_3 - 4.46X_1^2 + 0.25X_1X_2 - 2.55X_1X_3 - 0.14X_2^2 - 0.39X_2X_3 - 1.73X_3^2 \quad (2)$$

Table 3. Analysis of Variance for total phenolic content of VD20 germinated rice

Source	Sum of Squares	P-Value
X_1	35.9257	0.0078
X_2	0.878954	0.2205
X_3	13.8419	0.0199
X_1^2	73.4561	0.0038
X_1X_2	6.22746	0.0427
X_1X_3	26.1015	0.0107
X_2^2	44.9903	0.0062
X_2X_3	15.589	0.0177
X_3^2	11.029	0.0248
Lack-of-fit	16.1785	0.0504
Pure error	0.567688	
Total (corr.)	230.592	

Note: X_1: soaking time (min), X_2: soaking temperature (°C), X_3: pH of soaking solution.

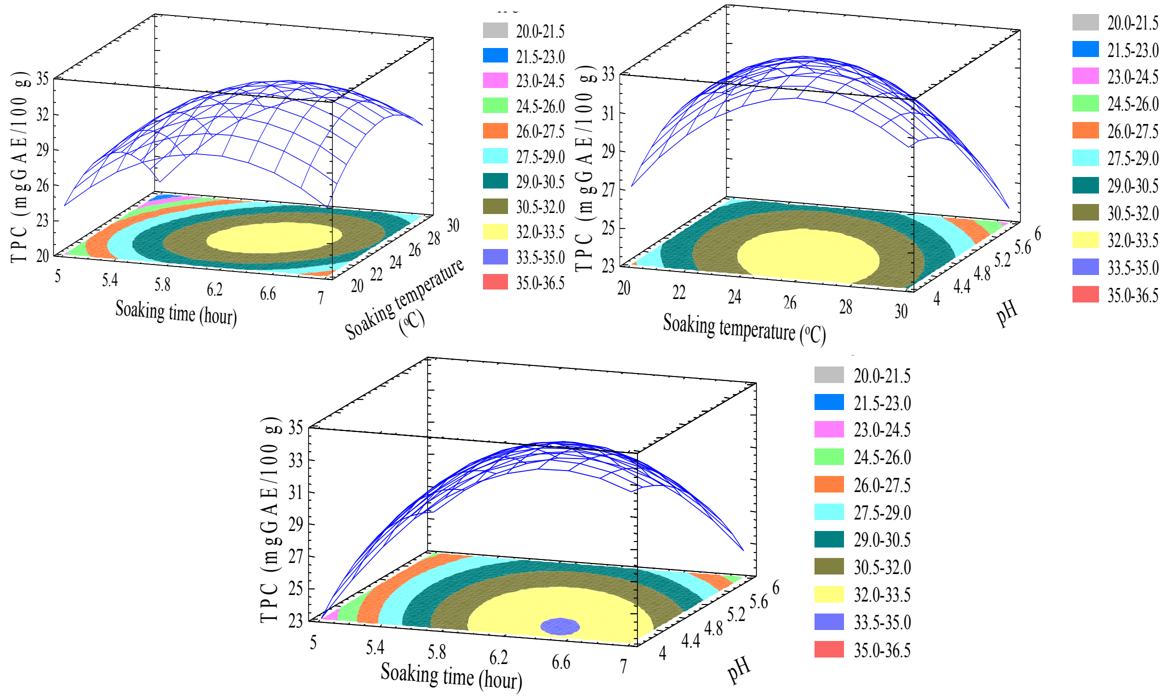
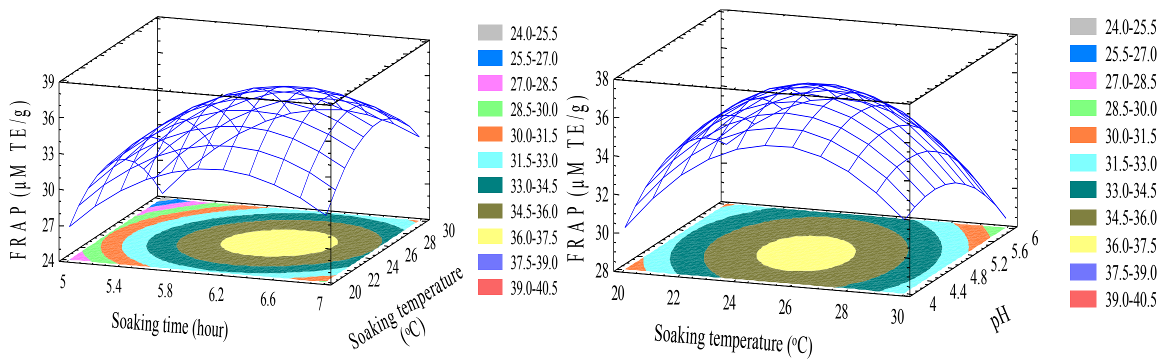


Figure 2. Contour plots the effect of soaking conditions on total phenolic content in VD20 germinated rice

3.3. Effect of soaking conditions on antioxidant activity in VD20 germinated rice

Similarly to the antioxidant content in VD20 rice, the antioxidant activity through the value of FRAP also showed the same trending. The linear (X_1), square (X_1^2, X_2^2) and interaction (X_1X_3) were mainly affect on FRAP value of germinated rice, which clearly presented in table 4 and figure 3. The lowest value of P-value was found on X_1^2, followed by X_2^2, X_1, X_1X_3, which indicated the order of effect power on the response (FRAP value). The correlation between content of antioxidant compounds and their activity was found in many research [31, 32]. It also related to the germination rate, which mainly phenomenon for enhancing the biological properties of germinated product [7, 28]. High coefficient determination (95.01%) was found in the regression model (Equation 3). The lack of fit test was not significant different which leading to the correctness of model for predicting. The model predicted that the highest value of FRAP (36.99 μM TE/g) was observed when grain was soaking in solution with pH of 4.58 at 25°C for 6.4 hours.

$$\text{FRAP } (\mu\text{M TE/g}) = -345.77 + 60.88X_1 - 4.32X_1^2 - 2.31X_1X_3 - 0.16X_2^2 - 0.21X_2X_3 \quad (3)$$



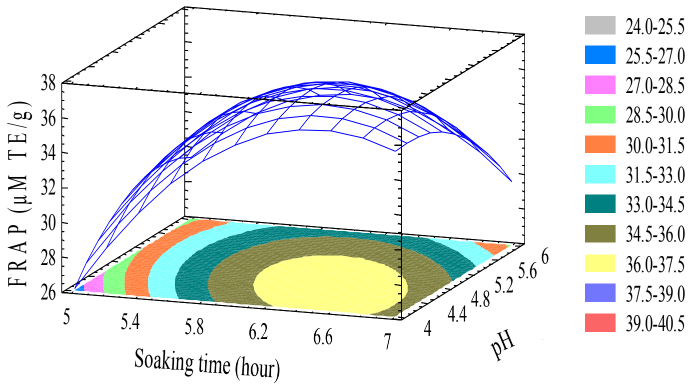


Figure 3. Contour plots the effect of soaking conditions on antioxidant activity in VD20 germinated rice rice

Table 4. Analysis of Variance for FRAP value in VD20 germinated rice

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
X_1	44.4974	1	44.4974	20.63	0.0062
X_2	0.454304	1	0.454304	0.21	0.6655
X_3	2.92613	1	2.92613	1.36	0.2967
X_1^2	68.8284	1	68.8284	31.91	0.0024
X_1X_2	3.72681	1	3.72681	1.73	0.2457
X_1X_3	21.3052	1	21.3052	9.88	0.0256
X_2^2	62.497	1	62.497	28.98	0.0030
X_2X_3	4.4247	1	4.4247	2.05	0.2115
X_3^2	13.2665	1	13.2665	6.15	0.0558
Total error	10.7843	5	2.15685		
Total (corr.)	216.32	14			

Note: X_1: soaking time (min), X_2: soaking temperature (^oC), X_3: pH of soaking solution.

3.4. Stimulated multi-objective for optimal conditions of soaking process and model validation

The correctness of the BBD model used was assessed using the model validation and desirability function technique. Upon evaluating the solutions using the desirability function, the solution with the highest desirability score of 0.96 is depicted in figure 4. Figure 4 demonstrates an exact match with the experimental condition of run 18 in the BBD matrix. Hence, further duplicate tests were conducted under the same optimal experimental settings to validate the findings. The outcomes from the BBD and desirability method are soaking time of 6.36-hours, soaking temperature of 26°C and pH of 4.65. The observed germinated rice has total phenolic content of 33.42 mgGAE/100 g, FRAP value of 36.9 µM TE/g and germination rate of 97.55%. The experimental findings closely align with the expected results of the desirability functions, providing more evidence of the BBD model's validity and accuracy.

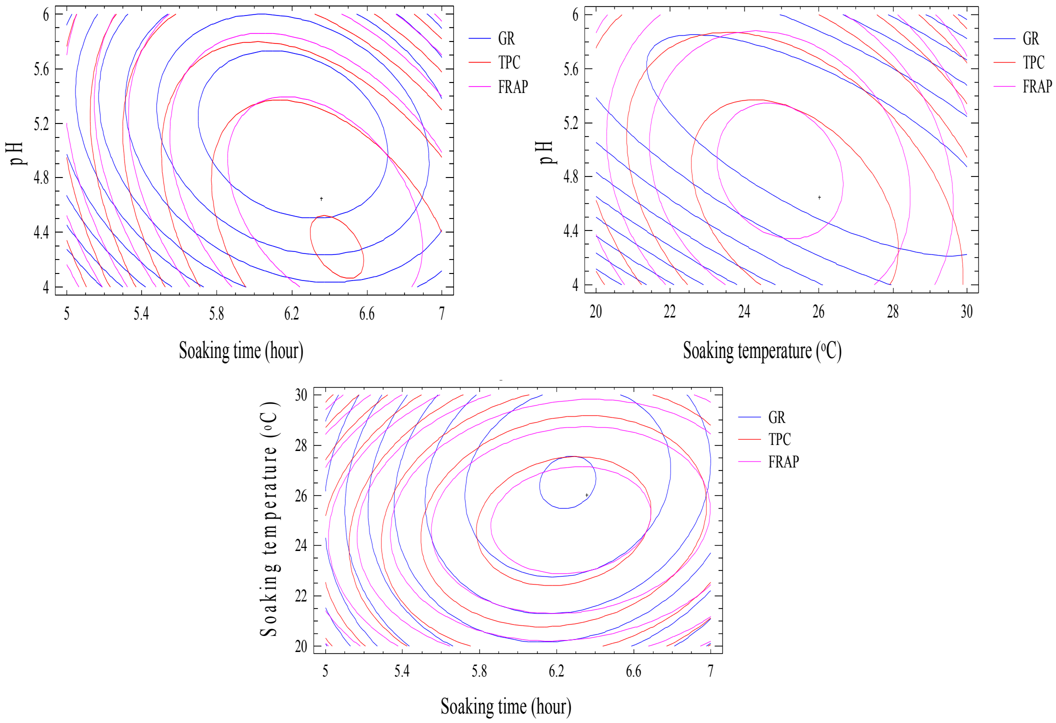


Figure 4. Contour plots of the effect of soaking conditions on rice germination rate (GR), TPC and FRAP value of VD20 germinated rice

4. CONCLUSION

This study investigated the influence of soaking process (i.e., soaking time, soaking temperature and pH of soaking) on the germination rate and antioxidants of germinated rice and then optimized soaking conditions to maximize rice germination rate and the TPC and FRAP value in germinated rice. All factors were significantly affected on the responses. A validated model was found for each response and the optimum conditions for soaking process were determined. Based on multi-response desirability, maximum polyphenolic content, FRAP value and sprouting rate can be obtained through a soaking time of 6.36 hours, using a solution with pH of 4.65 and a temperature of 26°C as know as optimal soaking conditions. The rice flour from the highly germinated kernel presented a higher concentration of phenolic compounds, showing good nutrition to be further utilized to produce a nutraceutical food.

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