OPTIMIZATION OF PROCESS CONDITIONS FOR GERMINATING PIGMENTED RED KAVUNI RICE USING RESPONSE SURFACE METHODOLOGY (RSM)

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ABSTRACT

Recent years, people have been seeking natural and wholesome food, predominantly functional food, which plays a vital role in disease prevention. Germinated brown rice is one of the feasible techniques to improve the nutritional quality to promote brown rice consumption. To optimize the process conditions of germination, Response Surface Methodology (RSM), a statistical computation technique based on two-factor and three-level Central Composite Design (CCD), was employed to optimize the conditions of germinated brown rice. The optimized state was 16 hours of soaking and 72 hours of germination had the highest overall acceptability, shorter cooking span, and potent antioxidant activity. The results showed a good fit with the proposed quadratic model for overall acceptability (R2=0.99), cooking time (R2=0.97) and anti-oxidant activity (R2=0.98). Germinated brown rice could be added and used as a nutritional ingredient shortly. It will be in high demand because of nutritional and nutraceutical properties for the forthcoming generations.

Keywords: Red Kavuni, germinated brown rice, Response surface methodology

Introduction

Rice is the staple food for most South Asians, with 50% of consumed calories from cereals such as rice, wheat and maize. India ranks second in rice production and accounts for 22.3% of the global output (Khatkar et al., 2016). India produced a record 117.94 million tonnes of rice during 2019-2020. Rice is preferred for its easily digestible starch and high lysine content, which aptly complements the prominent rice-pulse blended consumption pattern. Traditional rice varieties like red kavuni rice are gaining popularity for their nutritional value and other health-promoting bioactive components. The bran of the red kavuni rice is rich in polyphenolic compounds, which possess potent antioxidant and anti-inflammatory properties. Dietary diversification in terms of the introduction of pigmented traditional rice varieties will pave the way for the valorization of rice in the diet owing to health-promoting compounds, moreso when consumed in the form of germinated brown rice with better cooking quality and antioxidant properties.

Brown rice

Brown rice is the unprocessed grain that holds the embryo and is developed by removing hulls from the grains. Generally, people prefer polished white rice to brown rice, but rice milling removes the fiber-rich nutritious bran. Brown rice is rich in nutrients like B vitamins, minerals, dietary fiber, and other bioactive components (Hudson et al.,2000; Kim et al., 2011). Milling is the process where the process of dehusking or dehulling removes the husk and bran. Because of the bran and germ layers, brown rice is nutritionally superior to polished white rice(Saleh et al., 2019). Regular consumption of brown rice has been reported to reduce the risk of metabolic disorders such as obesity (Ravichanthiran et al., 2018), type 2 diabetes (Hu et al., 2020), cardiovascular disease (Truswell., 2002 and Hollaender et al., 2015)and also some types of cancer (Aune et al., 2016).

Germinated Brown Rice (GBR)

Germinated brown rice is obtained by soaking the paddy in water to induce germination. Generally, it improves the nutritional compounds as well as the cooking characteristics of the grains. The germination process activated the enzymes present in the grain and germinated rice has enhanced levels of vitamins, minerals, dietary fiber and phytochemicals such as ferulic acid, gamma-aminobutyric acid (GABA), acylated steryl glycoside (ASG), γ-oryzanol, phenolic compounds and antioxidant activity. The breakdown of high molecular weight polymers causes the generation of bio-functional substances and enhancement of sensory characteristics, mainly contributing to the softening of rice texture and improvement in flavor of rice grains (Beal and Mottram, 1993).

Response Surface Methodology (RSM)

Response Surface Methodology (RSM) is a collection of mathematical and statistical data representing the behavior of data set to make statistical predictions. It is an appropriate method widely used in the food industry to optimize, develop, improve and design the food process (Ghorbannezhad et al., 2016; Kaushik et al., 2006). For the optimization process, quadratic response surfaces such as three-level factorial, central composite design and Box-Behnken are generally applied for the optimization process (Bruns et al., 2006). In this study, the central composite design of the Experiment was used to optimize the germination conditions of brown rice.

Materials and Methods

Traditional rice variety Red kavuni was procured from the central farm of Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, Tamil Nadu. The seeds were thoroughly cleaned to remove the dirt and other extraneous material. The paddy was soaked in water for 12-16 hours at room temperature. During the soaking period, the water was frequently changed every 3-4 hours to avert the undesirable aroma due to fermentation. The dripping water was drained, and the paddy was tied in a muslin cloth and incubated at room temperature for different intervals (12, 14, 16) to initiate the process of germination. The germinated paddy was dried in the cabinet drier at 50˚C for 4 hours to a final moisture content of 12 - 14%, packed in air tight container and stored at ambient condition.

Experimental design

A traditional variety of Red kavuni rice was selected for this study. Face-centered Central Composite Design (CCD) was used for the design of experiment with two independent variables, including soaking time (X1- 12-16 hours), germination time (X2 -48-72 hours); and three dependent variables, including organoleptic evaluation (9-point hedonic scale), cooking time and anti-oxidant activity. The face-centered CCD with 13 experiments is presented in Table 2. The combined effect of response variables obtained was evaluated by the quadratic model using polynomial Equation (1). ANOVA was used to calculate the linear, interaction, and quadratic coefficients at the significance level ($p < .05$) of the process parameters are shown in Table 5.

$$\mathbf{Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2} \text{ .... } \mathbf{Eqn\ (1)}$$

Table 1. Experimental design for optimization of process parameters

Parameters codes	Codes		
	Lower limit	**Upper limit**	**Units**
Soaking time	12	16	hours
Germination time	48	72	hours

Sensory evaluation of germinated brown rice

Sensory analysis was conducted to evaluate the overall acceptability of the germinated brown rice. The GBR samples conforming to the 13 experimental trial runs were cooked with the required distilled water for an optimal time (Juliano, 1982). The cooked red kavuni rice was subjected to sensory evaluation by 25 semi-trained panel members (both males and females). The quality attributes considered for evaluation were colour, flavor, texture, taste and overall acceptability. To assess the product, the most common measure of 9 point hedonic scale was employed (9 = like extremely, 8 = like very much, 7 = like, 6 = like slightly, 5 = neutral, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike and 1 = dislike extremely).

Cooking time

The cooking time was determined by Juliano (1982). The GBR samples of the red kavuni rice variety conforming to the 13 experimental trial runs were washed, soaked in distilled water for 3 – 4 hours, and further cooked. The end point of cooking was analyzed by pressing the cooked rice between the two glass slides up to the point of translucency of grain and disappearance of the white core, indicating the sample's cooking time.

Anti-oxidant activity

Extraction of sample : The germinated samples were extracted by using 70% ethanol (50 mg/ml, w/v) and kept under room temperature for 24 hours. The samples were filtered and collected for the assay of antioxidant activity by DPPH Radical Scavenging Activity (Lin et al., 2015). Extracts were mixed with 1 ml of 0.2µM DPPH methanol solution and then incubated at room temperature for 30 min. The absorbance was detected using ELICO double beam UV VIS spectrophotometer (SL 210)at 517nm wavelength. BHA and α – tocophenol were used as a standard anti-oxidants to compare the data against the sample.

Scavenging effect % = [1- (sample absorbance value) . (absorbance value of a control group without sample)$^{-1}$] X 100%

Table 2. Face centered central composite design with uncoded valueof factors and observed quality responses of Germinated Brown Rice (GBR)

Experimental Run	Independent variables		Dependent variables		
	Soaking time (hrs)	Germination time (hrs)	Overall acceptability	Cooking time (mins)	Anti-oxidant activity (%)
1.	14	60	8.25	47.28	82.17
2.	14	60	8.25	47.28	82.17
3.	12	72	8.72	45.00	81.03
4.	14	60	8.25	47.28	82.17
5.	16	60	8.34	48.25	82.83
6.	16	72	8.98	44.68	83.24
7.	12	60	8.29	48.56	80.96
8.	16	48	8.10	49.62	81.93
9.	14	60	8.25	47.28	82.17
10.	14	48	8.02	49.25	81.45
11.	12	48	8.09	50.02	80.29
12.	14	60	8.25	47.28	82.17
13.	14	72	8.85	45.02	82.98

Three-dimensional surface plots represented the interaction between the dependent and independent variables. The associated model adequacies were obtained by R2, adjusted R2, predicted R2, p-values and F-values in this experiment. Co-efficient of determination (R2) explained the variation of one factor concerning another factor. The coefficient estimate represented the expected change in a given response due to the change of variable in one unit.

Result and Discussion

Effect of variables on responses

The data generated from the face-centered CCD applied to explore the impact of process variables on overall acceptability, cooking time and anti-oxidant activity of germinated brown rice are summarized in Table 1 and 2.

Effect of soaking and germination time on organoleptic characteristics

The soaking of germinated rice positively affected organoleptic attributes, as can be inferred from Table 2 and 4. Organoleptic evaluation of the GBR samples was done by 25 semi-trained panel members, which generated a mean sensory score for overall acceptability ranging from 8.02 to 8.98. The highest sensory score was obtained for an experimental trial run no 6, which included 16 hours of soaking and 72 hours of germination. The Model F-value of 312.08 implies the model is significant. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case, A, B, A2, B2, and AB are significant model terms. The coefficient of determination (R2) 0.99 indicates that the quadratic model experiment was well fitted with the range and was highly acceptable. The "Pred R-Squared" of 0.9562 was in reasonable agreement with the "Adj R-Squared" of 0.9923." Adeq Precision" measures the signal-to-noise ratio. The ratio of 53.236 indicates an adequate signal.

Since Red kavuni is a pigmented rice, the rice color is generally appealing to the consumer. Further, the germination of the red kavuni rice revamps the rice texture and eating quality rendering pleasant flavor and mild chewy texture. The germination process can improve the taste of the GBR with a soft mouthfeel than white rice. The mean values of the organoleptic score were 8.02,8.09, 8.10, 8.25,8.34, 8.72,8.85 and 8.98. From this, it could be inferred that rice's soaking and germination time influences rice's texture and the sensory attributes of the cooked germinated rice. The comparison value between the residuals and predicted values was shown in figure 1a, and the 3D graphical representation of the effect between the independent and dependent variables was shown in Figure 1b.

Effect of soaking time and germination on cooking time of GBR

The uncoded value of factors and their observed responses with their interaction effect are shown in Tables 2 and 4. Soaking rice accelerates the hydration capacity during the cooking process, further softening the grain. The experimental runs from RSM gave 13numbers of trials; the lower and upper levels of soaking time ranged from 12-16 hours. Among the treatments, the recorded cooking time was 45.02 min for 14:48(ST: GT),47.28 for 14:60 and 49.25 mins for 14:72 hrs. Similarly, for 12 hours (3 treatments) of soaking, the observed cooking time was 45.00 mins for 12:48 hrs, 48.56 for 12:60, 50.02for 12:72 and 44.68 for 16:48, 48.25 for 16:60 and 49.62 for 16:72 hrs respectively. The Model F-value of 63.09 implies the model is significant. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case, A2, and B2 are significant model terms. Values greater than 0.5000 indicate the model terms are not significant. The R2(0.97) shows that it was fitted with the range. The "Pred R Squared" of 0.8345 is in reasonable agreement with the "Adj R-Squared" of 0.9628. "Adeq Precision" measures the signal-to-noise ratio. A ratio greater than 4 is desirable. The ratio of 24.608 indicates an adequate signal. The study revealed significant differences among the samples subjected to different soaking and cooking time. Examining the interactions between the independent and dependent variables, cooking time was positively affected by the soaking time, and no further significant interactions were found. On increasing ST to 16 hours, cooking time was significantly reduced to 45 mins. Indicating that ST is inversely proportional to the cooking time. Usually, the cooking of brown rice takes a long time to cook due to the presence of bran in the outer layer, which gives an adversely hard texture to the cooked brown rice. Soaking rice in water results in cracking of the grain structure, thereby shortening the cooking time. The germination process in brown rice softens the husk's outer layer and upgrades the rice's quality by improving water absorption, which makes the rice cook fast (Gujral et al., 2012). Similarly, the duration of germination also has a significant impact on cooking time. With the decrease in germination time, the time required for cooking is prolonged. Figure 2a. Depicts the comparative effect between the plotted residuals and predicted values. The 3-D graph represents the soaking and germination time interactions on cooking characteristics (Figure 2b). Jiamyangyuen and Ooraikul (2008) reported the effect of germination on cooking time. An increase in germination time decreases the cooking time by increasing rice kernel hydration and subsequent grain size expansion during germination. Kayahara and Tsukahara (2000) stated that the reaction between phytic acid and minerals during sprout initiation specified that germinated brown rice could be quickly cooked and digested.

Effect of soaking and germinationon anti-oxidant activity

The interaction effect between germination and anti-oxidant activity is shown in Tables 2 and 4. DPPH method was done to estimate the amount of antioxidant activity of germinated rice. The highest anti-oxidant was observed for 72 hours of germination, and the value is 83.24%. The result shows that increasing the germination time increases the antioxidant activity of germinated brown rice. The Model F-value of 75.74 implies the model is significant. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case, A, B, and A2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The coefficient of determination (R2)implies that the model was well fitted. The "Pred R-Squared" of 0.8425 is in reasonable agreement with the "Adj R-Squared" of 0.9689i.e. the difference is less than 0.2. The ratio of 30.612 indicates an adequate signal. The interactive effect between the residuals and predicted is manifested in figure 3a. The 3D graphical representation between soaking and germination time was expressed in figure 3b.Gujral et al. (2012) reported that the germination of three paddy cultivars (IR-8, PR-106 and sharbati) for up to 48 hours led to an increase in anti-oxidant activityfrom 18.2 to 37.9%. Chung et al. (2016) stated that the germination process could increase the antioxidant capacity in both pigmented and non-pigmented varieties. In this, the rice extracts from the Superjami variety showed the lowest EC50 values and the highest amount of antioxidant compounds, followed by Superhongmi and brown rice.

Optimization by Response Surface Methodology (RSM)

Fitting the model

In this study, Face-centered Central Composite Design (CCD) was employed to optimize the germination conditions of red kavuni. The experimental runs from thirteen trails were shown in table (2), and the experimental and predicted values were shown in table (3). The similarity between the experimental and predicted values reveals the level of accuracy in the fitted model. The optimized condition was obtained during run no 6 at 16 hours of soaking and 72 hours of germination in the values of overall acceptability (8.98), antioxidant activity (83.24)and shortening the span of cooking (44.68), respectively.

Table 3.Experimental and Predicted values for different levels of experiment design

Run	Overall acceptability		Cooking time (mins)		Anti-oxidant activity (%)	
	Exp	**Pre**	**Exp**	**Pre**	**Exp**	**Pre**
1.	8.25	8.25	47.28	47.41	82.17	82.21
2.	8.25	8.25	47.28	47.41	82.17	82.21
3.	8.72	8.75	45.00	45.28	81.03	81.18
4.	8.25	8.25	47.28	47.41	82.17	82.21
5.	8.34	8.36	48.25	47.91	82.83	82.74
6.	8.98	8.98	44.68	44.97	83.24	83.37
7.	8.29	8.25	48.56	48.26	80.96	80.83
8.	8.10	8.08	49.62	49.66	81.93	81.89
9.	8.25	8.25	47.28	47.41	82.17	82.21
10.	8.02	8.04	49.25	49.18	81.45	81.51
11.	8.09	8.10	50.02	50.05	80.29	80.27
12.	8.25	8.25	47.28	47.41	82.17	82.21
13.	8.85	8.82	45.02	44.45	82.98	82.70

Exp- Experimental value Pre- Predicted value

To the experimental data, multiple regression analysis was applied to express the empirical relationship between the coded and uncoded variables were shown in table (4). In this way, Y is the response value of overall acceptability, cooking time, and anti-oxidant activity:X_1 and X_2 where X_1= soaking time X_2 = germination time.

Table 4. Polynomial mathematical models fitted with interaction effect obtained in terms of coded factors for different responses

	Response	Equation
Y_1	Overall acceptability	$Y_1= +8.25*+0.05*A+0.39*B+0.06*AB+0.05*A^2+0.17*B^2$
Y_2	Cooking time (mins)	$Y_2= +47.41*-0.17*A-2.37*B+0.02*AB+0.67 *A^2-0.59 *B^2$
Y_3	Anti-oxidant activity (%)	$Y_3= +82.21*+0.95*A+0.59*B+0.14*AB-0.42*A^2-0.10*B^2$

Note: A= Soaking time B= Germination time

The ANOVA results, probability value, degrees of freedom,goodness of fit were summarized in the table (5).

	Overall acceptability					Cooking property					Anti-oxidant activity				
Factor	df	Coefficient estimate	Sum of square	F-value	p-value	df	Coefficient estimate	Sum of square	F-value	p-value	df	Coefficient estimate	Sum of square	F-value	p-value
Model	5		1.07	312.08	<0.0001	5		35.37	63.09	<0.0001	5		8.41	75.74	<0.0001
Linear															
A	1	0.053	0.017	24.86	0.0016	1	-0.17	0.18	1.58	0.2495	1	0.95	5.45	245.4	<0.0001
B	1	0.39	0.91	1329.52	<0.0001	1	-2.37	33.56	299.33	<0.0001	1	0.60	2.14	96.13	<0.0001
Interaction															
A^2	1	0.052	0.0003	10.76	0.0135	1	0.68	1.26	11.26	0.0122	1	-0.43	0.51	22.80	0.0020
B^2	1	0.17	0.081	118.66	<0.0001	1	-0.59	0.97	8.69	0.0215	1	-0.11	0.032	1.46	0.2666
Quadratic															
AB	1	0.063	0.016	22.76	0.0020	1	0.020	0.0003	0.014	0.9083	1	0.14	0.081	3.66	0.0975
Mean±SD		8.36±0.026					47.45±0.33					81.97±0.15			
R^2		0.99					0.9783					0.9819			
Adjusted R^2		0.99					0.9628					0.9689			
PredictedR^2		0.95					0.8345					0.8425			
CV		0.31					0.71					0.18			

Table 5. Regression co-efficient and ANOVA estimation for process parameters of Red kavuni Germinated Brown Rice (GBR)

Validation of the model

The optimized conditions were soaking time of (X_1) 16 hours and germination time (X_2) of 72 hours. Under the optimized condition, the observed effect of response variable was overall acceptability (8.98), cooking time (44.68 mins) and anti-oxidant activity (83.24 %). The results showed that experimental values were in good agreement with the predicted values, so the RSM model is satisfactory and accurate.

Conclusion

The worth of picking the right option for the optimization process in the food industry is a back-breaking process. Therefore, Response Surface Methodology (RSM) is a specialized statistical tool to distinguish the relationship between the independent and dependent variables. Red kavuni rice is one of the famous traditional rice varieties which requires consideration to promote utilization as a functional food. The optimized results show that the germination of paddy at 16 hours of soaking and 72 hours of germination has higher acceptability with a hedonic score of 8.98, shortens the cooking span (44.68 mins) and exhibits the higher anti-oxidant activity (83.24). From this, rice as a staple food , the germination of traditional paddy variety will be groundbreaking for the forthcoming community.

Author contribution

Kanmani. K – optimized the process conditions, cooking time and milling quality for further analysis.

Hemalatha.G – guided for selection of response variables of response surface methodology, process optimization and edited the article.

Kanchana. S – carried out the sensory analysis and assess the cooking quality of rice.

Vanniarajan.C – provided suggestion for optimization of germination process in paddy.

Mini.M.L – assist to analyze the anti-oxidant activity of germinated brow rice.

Gurusamy.A – provied the quality raw material (traditional red kavuni) based on germination percentage of paddy.

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