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Process Standardization of Functionally Enriched Millet-Based Nutri-Cereal Mix Using D-Optimal Design Approach for Enhancing Food and Nutritional Security

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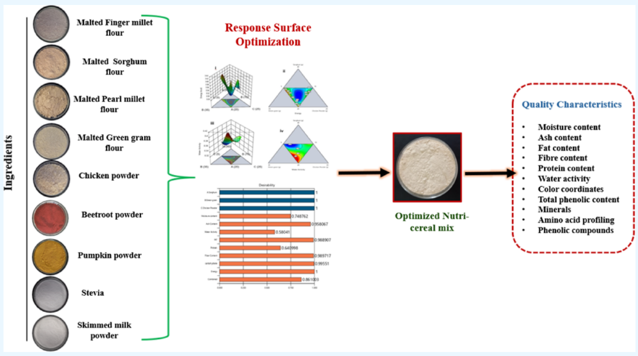
ABSTRACT: Millets are currently employed in a variety of ways, including direct consumption and usage in the manufacture of certain cuisines or snacks. The present investigation was aimed at optimizing functionally enriched millet-based nutri-cereal mix comprising chicken and vegetable for a nutrition-deficient population. A total of 16 experiments were carried out by using optimal (custom) design model of mixture design with 60% major ingredients, including malted sorghum flour (20−30%), malted green gram flour (15−25%), and boiled chicken powder (5−15%). To make 100% of the total nutri-cereal mixture, other ingredients such as malted pearl millet (10%), finger millet flour (10%), beetroot powder (2.5%), pumpkin powder (7.5%), skimmed milk powder (9.5%), and stevia powder (0.5%) were added. Numerical optimization was done using Design Expert software, version 13. The optimized ratio was 30% malted sorghum flour, 15% malted green gram flour, and 15% chicken powder. The predicted values of responses 5.101%, 3.616%, 1.963%, 11.165%, 28.005%, 50.149%, 330.282 kcal, and 0.373 were in accordance with experimental values 6.426%, 3.455%, 1.714%, 11.432%, 29.12%, 47.853%, 323.318 kcal, and 0.385 for moisture, ash, fat, fiber, protein, carbohydrates, energy, and water activity, respectively, with a small error percentage. The results of mineral content, phenolic content, and amino acid profiling revealed that the optimized Nutri-cereal mix have higher amounts of these components. The results also suggested that the optimized Nutri-cereal mix of these malted millet flours can potentially enhance the nutritional deficiency as well as improve food and nutritional security.



1. INTRODUCTION

India is stated as the largest producer of millets, an underutilized crop that has occupied an important place in Indian culture as well as in various regions of the world. Due to the lack of awareness of its nutritional benefits, its consumption has been dropping for many years, earning the name "orphan crops." As global agrifood confronts issues in feeding an ever-rising global population, millets are known to be recognized as "nutri-cereals," providing an affordable and nutritious powerhouse with minimal fertilization requirements. Following persistent research and various studies, this unpopular cereal crop came into existence in the year 2023, which has been declared by the United Nations as the International Year of Millets 2023 (IYOM23) to strengthen small-holder farmers and promote their cultivation, accomplish sustainable growth, completely eradicate hunger, cope with climate change, promote biodiversity, and transform agrifood supply systems, which are the biggest threats to global food security.[1]

Millets are currently employed in a variety of ways, including direct consumption and usage in the manufacture of certain cuisines or snacks. Millets are rich in nutritional, functional, antioxidant, and phytochemical properties that play an important role in human health, such as low digestibility, which aids in providing a feeling of fullness, blood pressure control, diabetes, obesity, malnutrition, menstrual cramps, and antimicrobial, anticancerous, and antiallergic properties.[2] Millets are rich in protein content, including essential amino acids like methionine, but lack other amino acids, while polyphenolic substances include gallic acid, hydrobenzoic acid, hydroxycinnamic acid, vanillic acid, ferulic acid, syringic acid,

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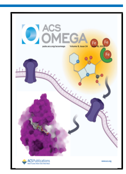

Table 1. Experimental Design per 100 g

run	malted sorghum flour (g)	malted green gram flour (g)	chicken powder (g)	malted pearl millet flour (g)	malted finger millet flour (g)	beetroot powder (g)	pumpkin powder (g)	skimmed milk powder (g)	stevia (g)
1	30	15	15	10	10	2.5	7.5	9.5	0.5
2	28.3333	18.3333	13.3333	10	10	2.5	7.5	9.5	0.5
3	30	25	5	10	10	2.5	7.5	9.5	0.5
4	30	25	5	10	10	2.5	7.5	9.5	0.5
5	25.8333	23.3333	10.8333	10	10	2.5	7.5	9.5	0.5
6	25	25	10	10	10	2.5	7.5	9.5	0.5
7	28.3333	20.8333	10.8333	10	10	2.5	7.5	9.5	0.5
8	20	25	15	10	10	2.5	7.5	9.5	0.5
9	25	20	15	10	10	2.5	7.5	9.5	0.5
10	30	20	10	10	10	2.5	7.5	9.5	0.5
11	20	25	15	10	10	2.5	7.5	9.5	0.5
12	30	20	10	10	10	2.5	7.5	9.5	0.5
13	25	20	15	10	10	2.5	7.5	9.5	0.5
14	30	15	15	10	10	2.5	7.5	9.5	0.5
15	28.3333	23.3333	8.33333	10	10	2.5	7.5	9.5	0.5
16	23.3333	23.3333	13.3333	10	10	2.5	7.5	9.5	0.5

flavonoids, etc.[3] Millets, because of their health-promoting properties, help in reducing the risk of a variety of major and minor health disorders.[4] Studies have revealed that owing to its low cost, it offers several advantages that might easily replace rice, wheat, or refined wheat-based food products. Millets might be a boon to humanity in the future, helping to tackle hunger and other health issues.

Major millets, including finger millet (ragi), pearl millet (bajra), and sorghum (jowar), are used to manufacture a variety of snacks, bakery goods, flour-based beverages, animal fodders, pet treats, etc.[5] They contain all the necessary components for the body's acquisition of certain minerals such as Ca, P, Fe, Mn, Mg, Na, K, Zn, Cu, and Al. In addition, millets have β-carotene and vitamin C, which are required for child and infant weaning foods.[6−9]

Keyata et al.[10] have revealed that sorghum has high levels of antinutritional factors like tannin, phytate, and oxalate. To improve protein digestibility, macro- and micronutrient profiles, and enhance nutritional and functional properties, millet grains were subjected to germination. With all these considerations in mind, the present study aimed to prepare a nutri-cereal mix for a nutrition-deficient population using major ingredients, including malted sorghum flour, malted green gram flour, and boiled chicken powder. Furthermore, such a combination of millets and chicken powder for a nutri-cereal mix for enhancing the nutrient quality developed in the present investigation reveals the potential for a sustainable food system, which will lead to a holistic solution to meet nutritional requirements effectively.

2. MATERIALS AND METHODS

Finger millet, pearl millet, sorghum, chicken, beetroot, and pumpkin were purchased from a local market in Dehradun. The ingredients for the nutri-cereal mix were selected on the basis of their nutritional quality, as reviewed from the existing literature. All of the chemicals used in this study were of analytical grade and purchased from Hi-Media, India.

2.1. Preparation of Raw Materials. *2.1.1. Preparation of Flour-Malted Finger Millet Flour.* The method of Kumar et al.[11] was adopted, with some modifications. In brief, cleaned and washed finger millets were soaked in drinking water in a ratio of 1:7 (w:v) for 36 h at room temperature. After soaking, the whole water was drained out, and the grains were left for 24 h at room temperature for germination. Germinated grains were dried in a tray dryer (Thermolife Sciences) at 55 °C for 10 h and milled into flour. Malted finger millet flour was stored in an airtight container for further analysis.

2.1.1.1. Malted Pearl Millet Flour. The pearl millet flour was prepared using the method of Adebiyi et al.[12] Thoroughly cleaned and washed pearl millet grains were soaked in drinking water at a ratio of 1:5 for 8 h at room temperature. After soaking, the whole water was drained out, and the grains underwent germination process at room temperature for 24 h. Germinated pearl millet grains were dried in a tray dryer (Thermolife Sciences) at 50 °C for 8 h and milled into flour. Malted pearl millet flour was stored in an airtight container for further analysis.

2.1.1.2. Malted Sorghum Flour. The method of Keyata et al.[10] was used to prepare sorghum flour. Sorted, cleaned, and washed sorghum grains were soaked in drinking water at a ratio of 1:5 for 12 h at room temperature. After soaking, the whole water was drained out, and sorghum grains were left for 36 h for germination at room temperature. Germinated pearl millet grains were dried in a tray dryer (Thermolife Sciences) at 50 °C for 6 h and milled into flour. Malted pearl millet flour was stored in an airtight container for further analysis.

2.1.1.3. Malted Green Gram Flour. Malted green gram flour was developed by following the procedure of Shingote et al.[13] Cleaned and washed green gram grains were selected and soaked in drinking water at a ratio of 1:4 for a period of 12 h at room temperature. After soaking, the whole water was drained out, and the grains were left for 24 h at room temperature to undergo the germination process. Furthermore, the germinated green gram grains were subjected to drying in a tray dryer (Thermolife Sciences) at 65 °C for 8 h and milled into flour. The malted green gram flour was then stored in an airtight container for further analysis.

2.1.2. Preparation of Chicken Powder. Chicken powder was produced using the method described by Saini et al.[14] with some modifications. Boneless chickens were cleaned four to five times with potable water to remove the blood and feathers. After washing, the meat was chopped into small pieces and boiled in a pressure cooker for 20 to 25 min. The boiled meat was dried in a tray dryer (Thermolife Sciences) at 55 ± 5 °C

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for 8–10 h and then ground into a fine powder. The chicken powder was stored in an airtight container at 4 °C for further analysis.

2.1.3. Preparation of Beetroot Powder and Pumpkin Powder. The methods of Bunkar et al.[15] and Nanthachai et al.[16] were adopted to prepare the beetroot powder and pumpkin powder, respectively. Beetroot was washed thoroughly with potable water and then peeled. After peeling, it was washed again with potable water, cut into small pieces, and then dried in a tray dryer (Thermolife Sciences) at 50 °C for 8 h. The dried beetroot was crushed in a grinder to make a fine powder and stored in an airtight container at 4 °C for further analysis. Peeled pumpkin was cut into small pieces (1–2 cm) and dried in a tray dryer (Thermolife Sciences) at 60 °C for 9–10 h. Dried pumpkin was ground to a fine powder and stored in an airtight container at 4 °C for further analysis.

2.1.4. Experimental Design and Methodology. The experiments were carried out using the optimal (custom) design model of mixture design with 60% major ingredients, including malted sorghum flour (20–30%), malted green gram flour (15–25%), and boiled chicken powder (5–15%). To make 100% of the total nutri-cereal mixture, other ingredients such as malted pearl millet (10%), finger millet flour (10%), beetroot powder (2.5%), pumpkin powder (7.5%), skimmed milk powder (9.5%), and stevia powder (0.5%) were added (Table 1). A total of 16 randomized runs along with experimental conditions were designed using Design Expert software, version 13, and the quality characteristics of the nutri-cereal mix in terms of moisture content (% wb), ash content (%), fat content (%), fiber content (%), protein content (%), carbohydrate content (%), energy (kcal), and water activity were analyzed. Furthermore, to compare the quality parameters of the nutri-cereal mixture, a control mixture was prepared with the similar set of ingredients including wheat flour in place of millets.

2.2. Proximate Analysis of the Nutri-Cereal Mix. For the quantitative assessment of proximate analysis of the nutri-cereal mix and control sample, the moisture content was analyzed as per the standard procedure of AOAC[17] using hot air oven (Thermo LifeScience, India). Two grams of the sample was placed at 105 ± 5 °C and dried until a constant weight was achieved. The ash content was evaluated using muffle furnace (Ambassador, India) at 550 °C until reaching a persistent weight (AOAC).[17] The fat content was determined using automatic fat analyzer (SoxTRON-SOX 4, Tulin, India) at 70 °C. The fiber content of the sample was assessed by automatic fiber analyzer (FibroTRON-FRB 4, Tulin, India). The protein content of the sample was analyzed by automatic nitrogen analyzer (KjelTRON, Tulin, India, equipped with KDIGB 8M, KjelSCRB, KjelDIST SA components). For calculating the protein content, the nitrogen content was multiplied by a protein factor of 6.25. Carbohydrate content and energy were calculated as per eqs 1 and 2 used by Kumar et al.[18]

$$\text{Carbohydrates (\%)} = [100 - (\text{Moisture content} + \text{Fat content} + \text{Ash content} + \text{Protein content} + \text{Fiber content})] \quad (1)$$

$$\text{Energy (kcal)} = [(4 \times \text{Carbohydrates}) + (9 \times \text{Fat}) + (4 \times \text{Protein})] \quad (2)$$

2.3. Water Activity. Water activity of the nutri-cereal mix was determined at room temperature using a water activity meter (Rotronic-HC2- Aw, Switzerland).

2.4. Total Phenolic Content (TPC). The TPC was measured as per the method described by Kumar et al.[19] For the preparation of ethanolic extract, the sample and 80% ethanol solvent were mixed in a ratio of 1:10 and kept in a shaker overnight at 120 rpm. Then, the solution was centrifuged at 8000 rpm for 10 min, and the supernatant was collected and concentrated using a rotary vacuum evaporator (DLAB, RE-100) at 50 °C. The dry extract was reconstituted in 80% ethanol with a concentration of 1 mg/mL. For the determination of TPC, 1 mL of ethanolic extract was added to 0.5 mL of Folin Ciocalteu (10%) and 2.5 mL of Na_2CO_3 (20%) and mixed for 1 min using a vortex mixture. Absorbance was noted at 725 nm after 45 min of incubation in the dark using a UV spectrophotometer (Shimadzu 1900). The standard curve of gallic acid was used to calculate the TPC (mg of GAE/100 mg).

2.5. Total Flavonoid Content (TFC). The TFC was measured as per the method previously given by Chang et al.[20] and adopted by Kumar et al.[19] In brief, 1 mL of ethanolic extract of nutri-cereal mix was added to 0.3 mL of $NaNO_2$ (5%), 0.3 mL of $AlCl_3$ (5%), 2 mL of NaOH (1M), and 2.4 mL of distilled water and mixed using a vortex mixture. The absorbance was noted at 510 nm immediately using a UV spectrophotometer (Shimadzu 1900). Standard curve of Quercetin was used to calculate TFC (mg QE/100 mg).

2.6. Color Analysis. The color index, lightness (L*), redness to greenness (a*), and yellowness to blueness (b*) of the nutri-cereal mix were analyzed using a colorimeter (Hunter's color lab, Virginia, USA). The White Index (WI), Hue Angle (h), Yellow Index (YI), Browning Index (BI), and Chroma (C) were calculated using eqs 3–8, as reported by Kumar et al.[18]

$$\text{WI} = \sqrt{(100 - L^{*2}) + a^{*2} + b^{*2}} \quad (3)$$

$$h = \tan^{-1}\left[\frac{b^*}{a^*}\right] \quad (4)$$

$$\text{YI} = \frac{142.86b^*}{L^*} \quad (5)$$

$$\text{BI} = \frac{100(X - 0.31)}{0.17} \quad (6)$$

where

$$X = [a^* + 1.75L^*]/[5.645L^* + a^* - 3.012b^*] \quad (7)$$

$$C = \sqrt{a^{*2} + b^{*2}} \quad (8)$$

2.7. Mineral Analysis. The mineral composition analysis of the nutri-cereal mix utilized the iCE 3400 Atomic Absorption Spectrometer from Agilent Technologies (USA), following the procedure outlined by Ghumman et al.[21] Initially, 1 g of the nutri-cereal mix was subjected to a crucible for charring and ashing at 600 °C in a muffle furnace. Furthermore, the resultant ash was dissolved in a solution of 1N nitric acid (2.5 mL), filtered, and subsequently diluted with Milli-Q water. The diluted filtrate was then analyzed for Zn, Ca, Fe, Mg, and Mn content by employing Atomic Absorption

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Table 2. Proximate Analysis of the Nutri-Cereal Mix[a]

run	moisture content (%)	ash content (%)	fat content (%)	protein (%)	fiber (%)	carbohydrate (%)	energy (kcal)	water activity (%)
1	5.1	3.6	1.98**	27.98	11.2**	50.14	330.3	0.3733
2	5.3	2.8	0.618	27.16	9.61	54.512	332.25	0.3645
3	5.9	2.1	0.424*	21.8*	7.88	61.896	338.6	0.3534
4	5.8	2.1	0.424*	21.8*	7.88	61.996**	339	0.3534
5	6**	2.7	0.836	26.68	9.35	54.434	331.98	0.3894
6	5.9	2.6	0.666	27.81	7.83*	55.194	338.01	0.3899
7	5.6	2.2	0.837	25.75	11.03	54.583	328.865	0.3453
8	4.9	1.7*	1.475	28.87	9.55	53.505	342.775**	0.374
9	4.8*	3.7	1.542	26.05	9.25	54.658	336.71	0.367
10	5.6	2.1	0.922	25.75	11.17	54.458	329.13	0.3473
11	4.9	2.3	1.674	31.48**	9.55	50.096*	341.37	0.3941**
12	5.4	2.1	0.43	28.32	9.2	54.55	335.35	0.3596
13	5.1	3.7**	1.542	26.05	9.25	54.358	335.51	0.367
14	5.1	3.6	1.98**	27.98	11.2**	50.14	330.3	0.3733
15	5.7	3.1	0.534	25.63	8.6	56.436	333.07	0.3446*
16	5.8	2.9	0.662	27.6	9.82	53.218	329.23*	0.3836
control	4.81	2.165	0.645	18.16	5.139	69.708	357.304	0.456

[a]The labels * and ** represent the minimum and maximum values.

Spectrometer. The instrument was calibrated by using mineral standard stock solutions.

2.8. Polyphenolic Profile. The phenolic compound in the nutri-cereal mix was assessed using Shimadzu LC-30 HPLC system well equipped with a DAD-PDA detector, a C18 column, a rapid separator autosampler, and a binary pump. The mobile phase was used to separate the sample, which consisted of two components, A and B. The mobile phase A contained 1% acetic acid, while B comprised acetic acid, acetonitrile, and distilled water mixture in a 1:32:67 ratio with a defined gradient program. The phenolic compounds were observed at 275 nm, and their profiles were determined by assessing their retention time to standard compounds.[22]

2.9. Amino Acid (AA) Profile. The amino acid (AA) profile of the nutri-cereal mix was estimated by using Shimadzu LC-30 HPLC system well equipped with a fluorescence detector and a C18 column, as described by Kaur et al.[23] Initially, 100 mg of the nutri-cereal mix was placed for digestion until 24 h at 110 °C with 6 N HCl, anaerobically. Furthermore, to enhance the detectability of amino acids, derivatization was carried out using o-phthaladehyde, mercaptopropionic acid, and 9-fluorenylmethyl chloroformate. Subsequently, derivatized amino acid was extracted using 0.1 N HCl (1 mL) and placed in HPLC system. Additionally, a standard amino acid mixture was run to calibrate and validate the analysis. The amino acid content is expressed in mg/100 g.

2.10. Optimization Methodology. Optimal (custom) design model of mixture design was used to determine the optimized value of the major ingredients, including malted sorghum flour (20−30%), malted green gram flour (15−25%), and boiled chicken powder (5−15%), and eight responses including moisture content (%), ash content (%), fat content (%), protein content (%), fiber content (%), carbohydrate (%), energy (kcal), and water activity were taken into consideration. The adequacy of the designed models was determined using the statistical parameters, which are regression coefficient (p and Fisher's F-value), lack of fit test, R^2 (coefficient of determination), and coefficient of variation (C.V. %). For better illustration, response surfaces and contour plots were generated by using Design Expert software, version 13.

3. RESULTS AND DISCUSSION

3.1. Model Fitting and Adequacy of Experimental Data. Table 2 represents the experimental data of quality characteristics, which are moisture content (%), ash content (%), fat content (%), fiber content (%), protein content (%), carbohydrate content (%), energy (kcal), and water activity of the nutri-cereal mix obtained from the 16 experiments, which were analyzed by calculating analysis of variance (ANOVA), coefficient of determination (R^2), standard deviation, mean, and C.V. (%) using Design Expert software, version 13. Special quartic model equations were used to check the potency of the experimental data. Eqs 9−16 show the model equations for moisture content (% wb), ash content (%), fat content (%), fiber content (%), protein content (%), carbohydrate content (%), energy (kcal), and water activity of the nutri-cereal mix.

$$\text{Moisture content} = +1.21A - 1.08B - 9.48C + 23.14AB + 36.94AC + 40.71BC - 138.52A^2BC - 40.52AB^2C - 6.22ABC^2 \quad (9)$$

$$\text{Ash content} = -1.49 - 0.5535B + 20.56C + 12.56AB - 23.68AC - 31.96BC + 46.72A^2BC + 154.82AB^2C - 91.77ABC^2 \quad (10)$$

$$\text{Fat content} = +4.51A + 2.65B + 16.81C - 12.70AB - 34.78AC - 32.68BC + 70.97A^2BC + 110.42AB^2C - 56.95ABC^2 \quad (11)$$

$$\text{Fiber content} = -4.60A - 40.87B - 27.49C + 122.33AB + 108.85AC + 174.87BC - 445.46A^2BC - 144.64AB^2C - 269.20ABC^2 \quad (12)$$

$$\text{Protein content} = +53.90A + 63.07B + 35.18C - 146.65AB - 66.14AC - 75.76BC + 263.48A^2BC + 165.63AB^2C + 180.47ABC^2 \quad (13)$$

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Table 3. ANOVA and Regression Analysis of Quality Characteristics of the Nutri-Cereal Mix[i]

	special quartic model															
	moisture content (%)		ash content (%)		fat content (%)		protein content (%)		fiber content (%)		carbohydrate (%)		energy (kcal)		water activity (%)	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
model	27.02	0.00013[a]	14.254	0.001[a]	9.283	0.0040[a]	9.721	0.0035[a]	5.011	0.0235[b]	24.870	0.00017[a]	9.7620	0.0035[a]	4.3757	0.0335[b]
linear mixture	69.16	0.00002[a]	26.677	0.0005[a]	27.413	0.0004[a]	26.644	0.0005[a]	13.835	0.003[a]	80.341	0.00001[a]	19.102	0.0014[a]	9.9250	0.0090[a]
AB	3.45	0.10550[c]	0.203	0.665	0.194	0.672	1.489	0.261	2.505	0.157	0.0001	0.9906	9.5315	0.0176[b]	1.1987	0.3098
AC	9.61	0.01732[b]	0.792	0.403	1.597	0.246	0.330	0.583	2.166	0.184	0.0412	0.8448	9.4422	0.0179[b]	0.0215	0.8875
BC	10.48	0.01431[b]	1.29	0.292	1.265	0.297	0.389	0.552	5.019	0.060[c]	0.4662	0.5166	15.572	0.0055[a]	0.2236	0.6506
A^2BC	17.65	0.00403[a]	0.402	0.545	0.868	0.382	0.685	0.434	4.739	0.065[c]	0.4938	0.5049	17.621	0.0040[a]	0.1341	0.7250
AB^2C	1.69	0.23520	4.938	0.061	2.347	0.169	0.302	0.599	0.558	0.479	0.8095	0.3981	1.4237	0.2716	0.0368	0.8531
ABC^2	0.039	0.84912	1.701	0.233	0.612	0.459	0.352	0.5714	1.895	0.211	0.7805	0.4062	4.3140	0.0764[c]	2.4363	0.1625
lack of fit	0.271	0.77264	2.87	0.147	4.8490	0.0674[c]	0.192	0.830	1.350	0.339	0.0343	0.9664	0.5049	0.6313	3.4306	0.1153
lack of fit	NS		NS		NS		NS		NS		NS		NS		NS	
R^2	0.9686		0.9422		0.9139		0.9174		0.8513		0.9660		0.9177		0.8334	
std. dev.	0.105		0.235		0.2431		1.0160		0.653		0.921		1.90		0.0097	
mean	5.431		2.70		1.0341		26.669		9.523		54.63		334.53		0.3675	
C. V. (%)	1.94		8.69		23.50		3.81		6.86		1.69		0.5694		2.64	
F	27.02		14.25		9.28		9.72		5.01		24.87		9.76		4.38	

[i] a, b, and c represent 1, 5, and 10% level of significance, respectively.

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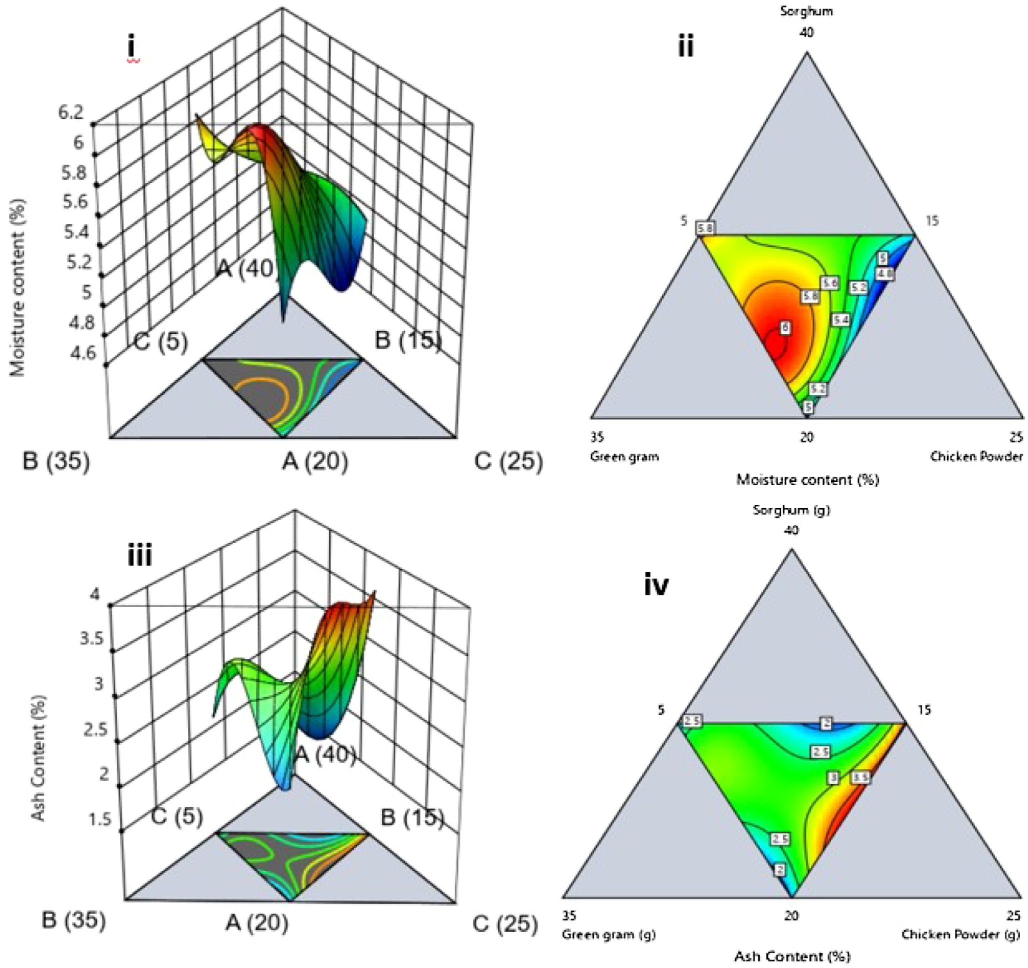

Figure 1. 3D response surface and 2D contour graph showing the effect of sorghum, green gram, and chicken powder on moisture content (i-3D and ii-2D) and ash content (iii-3D and iv-2D).

$$\text{Carbohydrate content} = +46.47A + 76.78B + 64.42C + 1.32AB - 21.18AC - 75.17BC + 202.80A^2BC - 245.71AB^2C + 243.68ABC^2 \quad (14)$$

$$\text{Energy} = +442.05A + 583.25B + 549.69C - 695.60AB - 662.35AC - 897.84BC + 2503.86A^2BC + 673.49AB^2C + 1184.03ABC^2 \quad (15)$$

$$\text{Water activity} = +0.5602A + 0.7741B + 0.2670C - 1.26AB - 0.1610AC - 0.5479BC - 1.11A^2BC + 0.5519AB^2C + 4.53ABC^2 \quad (16)$$

Very promising coefficients of determination (R^2) of 0.9686, 0.9422, 0.9139, 0.8513, 0.9174, 0.9660, 0.9177, and 0.8334 were obtained for moisture content (% wb), ash content (%), fat content (%), fiber content (%), protein content (%), carbohydrate content (%), energy (kcal), and water activity of the nutri-cereal mix, respectively (Table 3). The R^2 of more than 80% explained a good relationship between experimental as well as predicted values.[24] The C.V. (%) for moisture content (% wb), ash content (%), fat content (%), fiber content (%), protein content (%), carbohydrate content (%), energy (kcal), and water activity of the nutri-cereal mix was 1.94%, 8.69%, 23.52%, 6.86%, 3.81%, 1.69%, and 0.5694%, respectively, which shows minimum variation in data suited to the model due to higher mean values of 5.431, 2.70, 1.0341, 9.523, 26.669, 54.63, and 334.53, respectively. The reliability of experimental data relies on C.V. values; lower the C.V, higher the reliability and vice versa.[25,26] The C.V. (%) values in this study were observed to be 1.94%, 8.69%, 23.52%, 3.81%, 6.86%, 1.69%, 0.5694%, and 2.64% for moisture, ash, fat, fiber, protein, carbohydrate, energy, and water activity, respectively. The p values for all the quality characteristic parameters of the special quartic model were lower than 0.05, and higher p value of lack of fit shows the nonsignificance of lack of fit.[27]

3.2. Effect of Mixture Ingredients on Responses.

3.2.1. Moisture Content. The moisture content of any food material plays a vital role in determining its shelf life, water activity, and textural properties, which can increase the microbial growth as higher moisture food materials can easily deteriorate due to microbial propagation and thus become less acceptable for consumption.[28,29] Moisture content of the nutri-cereal mix ranged between 4.8% and 6.0%, which was within the acceptable limit (3.25% to 4.95%) (Table 2). Moisture content of the control was found to be 4.81%. From eq 9, it was observed that the positive coefficient of sorghum (A) and the quadratic terms AC and BC, whereas the negative coefficients of green gram (B), chicken powder (C) and special quartic terms A^2BC, AB^2C, and ABC^2 have positive and negative contributions on the moisture content of the nutri-

Table 4. Constraints for Numerical Optimization, Predicted and Experimental Values at Optimized Value

name	goal	lower limit	upper limit	predicted	experimental	error (%)
sorghum	maximize	20	30	30	30	
green gram	is in range	15	25	15	15	
chicken	maximize	5	15	15	15	
moisture content (%)	minimize	4.8	6	5.10	6.42 ± 0.18	5.89
ash content (%)	maximize	1.7	3.7	3.61	3.45 ± 0.17	4.45
fat (%)	maximize	0.424	1.98	1.96	1.71 ± 0.32	12.68
fiber content (%)	maximize	7.83	11.2	11.16	11.43 ± 0.21	2.33
protein (%)	maximize	21.8	31.48	28.00	29.12 ± 0.10	3.82
carbohydrate (%)	minimize	50.096	61.996	50.14	47.85 ± 0.08	4.57
energy (kcal)	is in range	328.865	342.775	330.28	323.31	2.10
water activity (%)	minimize	0.3446	0.3941	0.37	0.38	3.11

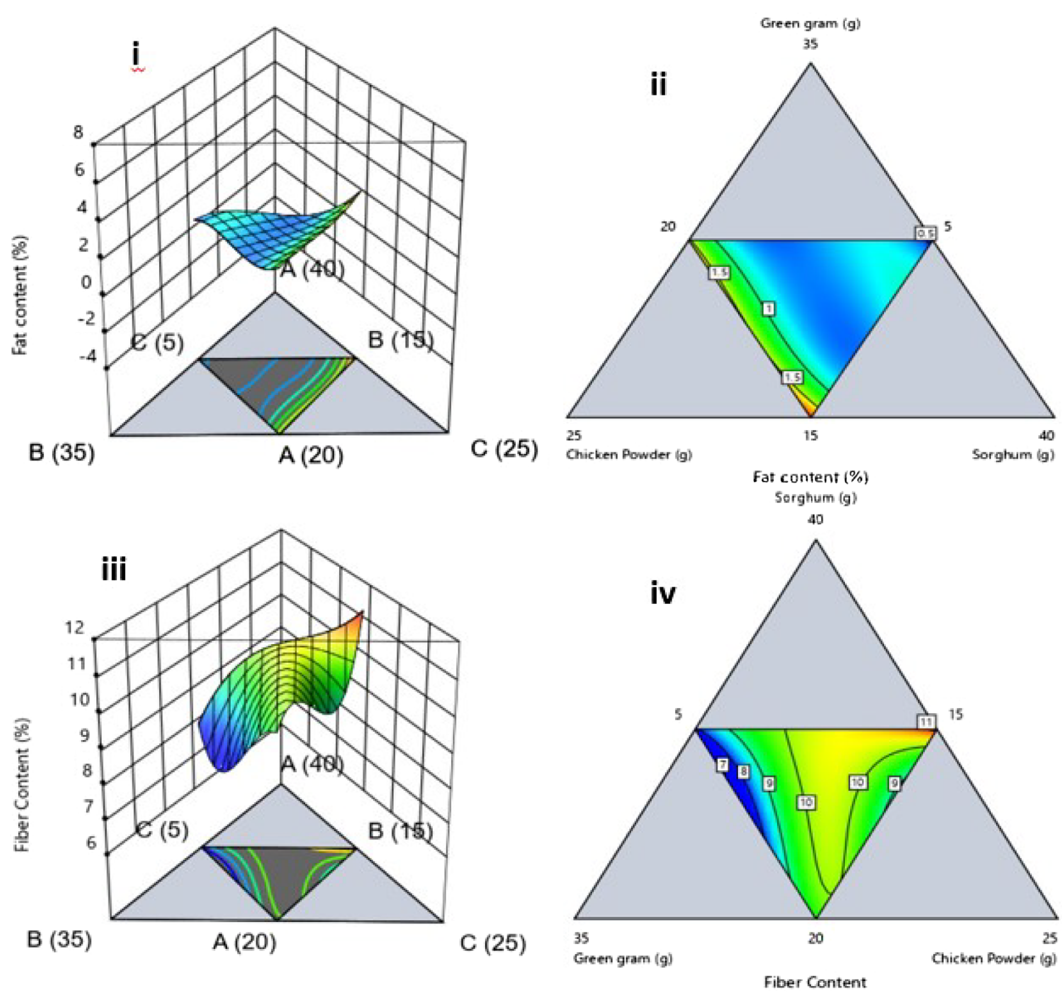

Figure 2. 3D response surface and 2D contour graph showing the effect of sorghum, green gram, and chicken powder on fat content (i-3D and ii-2D) and fiber content (iii-3D and iv-2D).

cereal mix. The moisture content of the nutri-cereal mix in the present study increased from 4.8% to 6.0%, with an increase in the portion of green gram and sorghum and with a reduction in the portion of chicken powder (Figure 1i,ii. The moisture content of the optimized nutri-cereal mix (6.426%, Table 4) of this study was higher than the complementary foods made from sorghum and premix reported by Keyata et al.[10]

3.2.2. Ash Content. From Table 2, it was analyzed that the ash content of the nutri-cereal mix ranged between 1.70% and 3.70%, and the ash content of the control was 2.165%, which was within the acceptable limit (2.22%−3.30%).[30] From eq 10, it was observed that the positive coefficient of chicken powder (C), quadratic term AB, and special quartic terms A^2BC and AB^2C have a positive contribution on the ash content of the nutri-cereal mix, whereas the negative coefficients of sorghum (A), green gram (B), and quadratic terms AC and BC and special quartic terms ABC^2 have a negative contribution on the ash content of the nutri-cereal mix. The ash content of the nutri-cereal mix in this study increased from 2.0% to 2.5%, with an increase in portions of chicken powder and sorghum and with a reduction in the portion of green gram flour (Figure 1iii,iv. The ash content of the optimized nutri-cereal mix

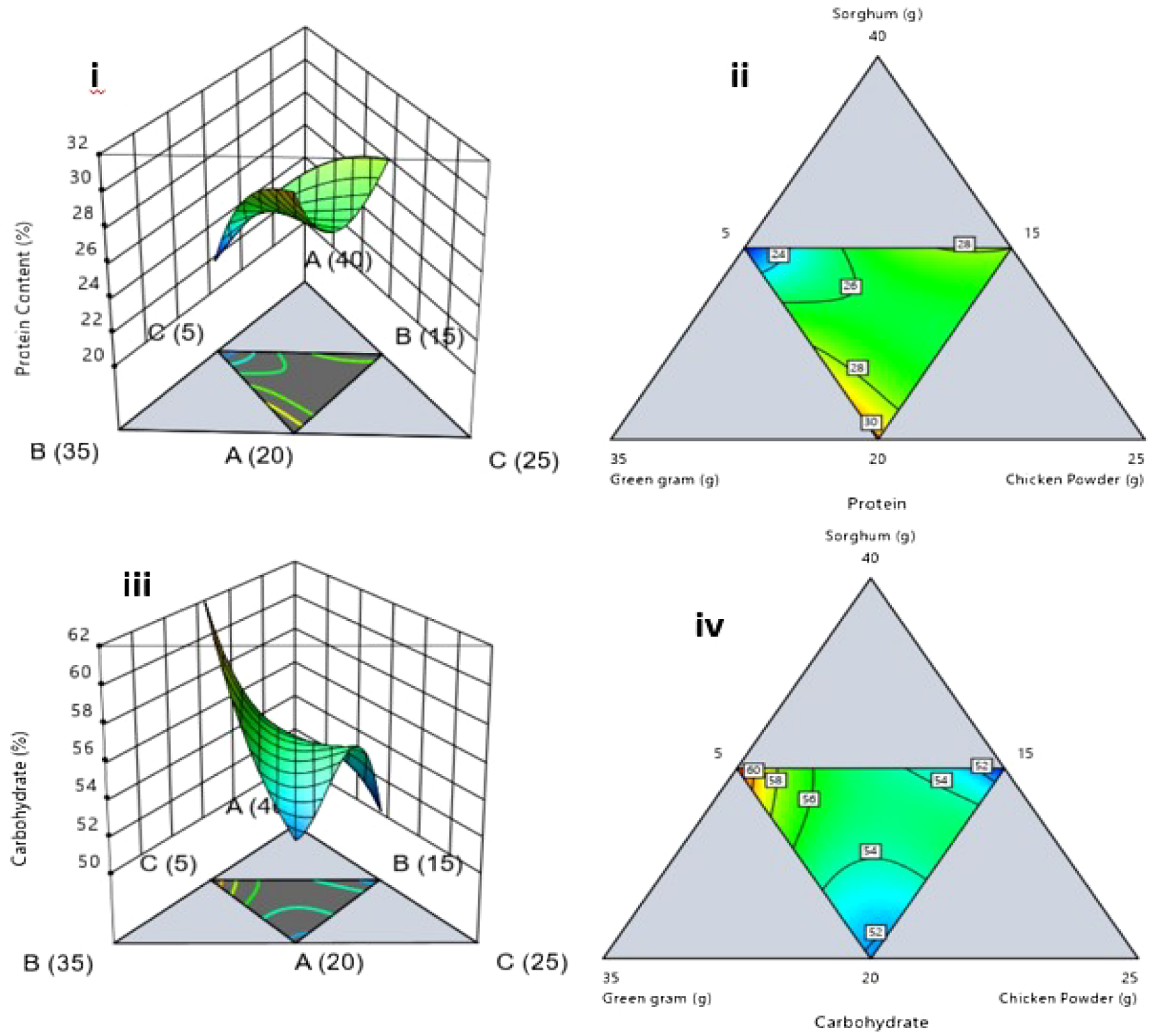

Figure 3. 3D response surface and 2D contour graph showing the effect of sorghum, green gram and chicken powder on protein content (i-3D and ii-2D) and carbohydrate content (iii-3D and iv-2D).

(3.455%, Table 4) of this study was higher than the sorghum chakki prepared with green gram, bengal gram, urad dal, and rice flour reported by Chavan et al.[30] The high ash content of the nutri-cereal mix was due to the sorghum, finger millet, green gram, and pearl millet in the nutri-cereal mix.

3.2.3. Fat Content. The fat content of the nutri-cereal mix varied from 0.424% to 1.98%, while the fat content of the control was 0.645% (Table 2). From eq 11, it can be observed that the positive coefficients of sorghum (A), green gram (B), and chicken powder (C) and special quartic terms A^2BC, AB^2C have positive input on the fat content of the nutri-cereal mix, whereas the negative coefficients of quadratic terms AB, BC, and AC and special quartic term ABC^2 have a negative input on the fat content of the nutri-cereal mix. From Figure 2i,ii, it was observed that the fat content of the nutri-cereal mix increased from 0.5% to 1.5%, with an increase in the portion of chicken powder, and decreased with an increase in the portion of green gram flour and sorghum flour. The fat content of the optimized nutri-cereal mix (1.714%, Table 4) of this study was lower than that of the chicken meat powder−incorporated patties, biscuits, and cookies reported by Naveena et al.;[31] Berwal,[32] respectively.

3.2.4. Fiber Content. The experimental results of fiber content (Table 2) showed that the fiber content of the nutri-cereal mix ranged from 7.83 to 11.2% and was found to be 5.139% for the control. From eq 12, it was observed that only the positive coefficient of quadratic terms AB, BC, and AC have a positive impact on the fiber content of the nutri-cereal mix. From Figure 2iii,iv, it was observed that the fiber content of the nutri-cereal mix increased from 7% to 10% with an increased level of sorghum and a decreased portion of chicken and green gram flour. The fiber content of the optimized nutri-cereal mix (11.432%, Table 4) of this study was found to be higher than the complementary foods made from sorghum in the premix reported by Keyata et al.[10]

3.2.5. Protein Content. As per the guidelines of WHO/FAO,[33] a protein content higher than 15% is recommended for infants and young children. From Table 2, it was observed that the protein content of the nutri-cereal mix ranged from 21.80% to 31.48%, and the protein content of the control was 18.16%. From eq 13, it was observed that except for the quadratic terms AB, BC, and AC, all the other terms, i.e., the linear term of sorghum (A), green gram (B), and chicken powder (C), special quartic terms A^2BC, AB^2C and ABC^2 have a positive contribution on the protein content of the nutri-cereal mix.

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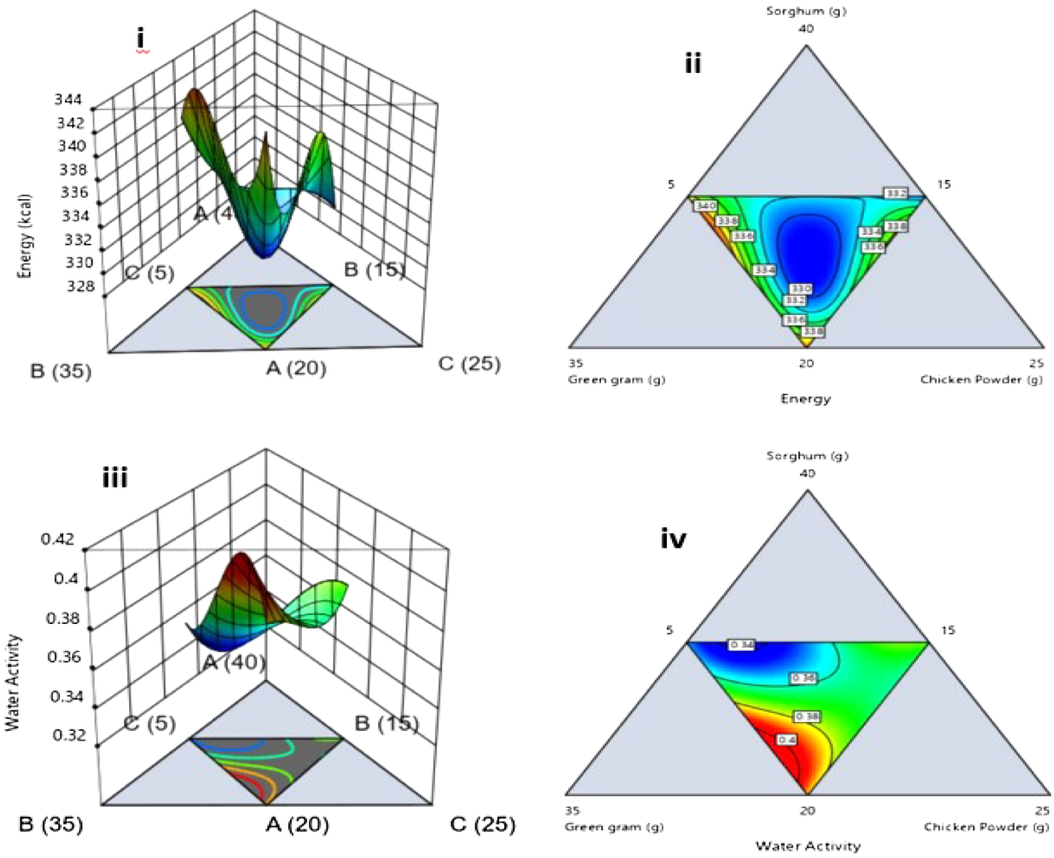

Figure 4. 3D response surface and 2D contour graph showing the effect of sorghum, green gram, and chicken powder on energy (i-3D and ii-2D) and water activity (iii-3D and iv-2D).

The protein content of the nutri-cereal mix in this study increased from 24% to 30%, with an increase in portions of chicken powder and green gram flour and a reduction in the portion of sorghum flour (Figure 3i,ii). Similar findings were reported by Keyata et al.[10] for their soybean, karkade, sorghum, and premix-based food. The protein content of the optimized nutri-cereal mix (29.12%, Table 4) of this study was found to be higher than the complementary flour formulated with maize, roasted pea flour, and barley reported by Fikiru et al.[34] High protein content of the nutri-cereal mix could be due to the high protein content and high proportion of green gram and chicken powder.

3.2.6. Carbohydrate Content. From Table 2, it was observed that the carbohydrate content of the nutri-cereal mix ranged from 50.096% to 61.996%, and the carbohydrate content of the control was 69.708%. From eq 14, it was observed that the positive coefficient of sorghum (A), green gram (B), and chicken powder (C), quadratic term AB and special quartic terms A^2BC and ABC^2 have a positive contribution to the carbohydrate content of the nutri-cereal mix, whereas the negative coefficient of quadratic terms AC, BC, and the special quartic term AB^2C have a negative contribution to the carbohydrate content of the nutri-cereal mix. From Figure 3iii,iv, it was concluded that the carbohydrate content of the nutri-cereal mix in this study increased from 52% to 60%, with an increase in the portion of sorghum and a reduction in green gram flour. Similar findings were reported by Keyata et al.[10] and Souci et al.[35] The carbohydrate content of the optimized nutri-cereal mix (47.853%, Table 4) of this study was lower than that of the millet-based composite flour developed.[9]

3.2.7. Energy. From eq 15, it can be observed that except for the quadratic terms AB, BC, and AC, all of the other terms, i.e., the linear term of sorghum (A), green gram (B), and chicken powder (C), and the special quartic terms A^2BC, AB^2C, and ABC^2 have a positive contribution to the energy of the nutri-cereal mix, whereas the negative coefficient of quadratic terms AC, BC, and AC have a negative contribution to the energy of the nutri-cereal mix. From Table 2, it was observed that the energy of the nutri-cereal mix ranged between 328.865 and 342.775 kcal and 357.304 kcal for control. The energy of the nutri-cereal mix in this study increased from 330 kcal to 340 kcal, with an increase in the portion of chicken powder and reduction in green gram and sorghum flour (Figure 3i,ii). The energy of the optimized nutri-cereal mix (323.318 kcal, Table 4) of this study was found to be quite lower than that of the millet-based weaning food reported by Thathola and Srivastava,[36] and a ragi, rice and green gram−based infant food reported by Jadhavar et al.[37] (Figure 4)

3.2.8. Water Activity. The minimum amount of water needed for microbial invasion is known as the water activity of a food. The shelf life and textural properties of food depend on its water activity. The water activity of powdered food products is an essential parameter for determining their shelf life and quality characteristics. Low water activity can prevent microbial spoilage, textural change, and rancidity.

From eq 16, it can be observed that the negative coefficient of quadratic terms AB, BC, and AC and the special quartic term A^2BC have a negative contribution to the water activity of the

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nutri-cereal mix, whereas the positive coefficient of sorghum (A), green gram (B), and chicken powder (C), and the special quartic terms AB^2C and ABC^2 have a positive contribution to the water activity of the nutri-cereal mix. From Table 2, it was observed that the water activity of the nutri-cereal mix ranged from 0.3446 to 0.3941, with 0.456 for the control and 0.385 for the optimized nutri-cereal mix. The water activity of the nutri-cereal mix was found to be in the acceptable range for enhancing the shelf life of any powdered food product. The value of water activity of the nutri-cereal mix in this study increased from 0.34 to 0.40, with a decrease in the portions of chicken powder, green gram, and sorghum (Figure 3iii,iv).

3.2.9. Optimization of Nutri-Cereal Mix Formulation and Validation of Model. The numerical optimization was done using Design-Expert software, version 13. The individual goals for major ingredients and quality characteristic parameters (responses) were fixed to get the optimized values of the ingredients and the responses (Table 4). Based on specified criteria, optimization was performed for the individual responses. Out of 3 possible solutions given by the software, only one possible solution that most suited the criteria was selected that had a desirability of 0.861 (Figure 5). The

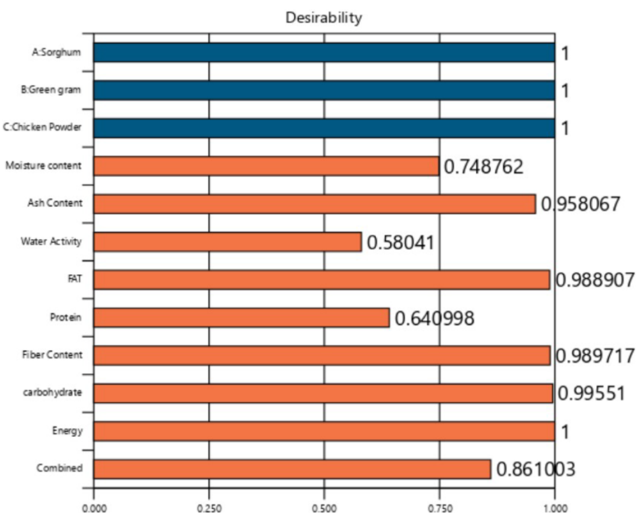

Figure 5. Desirability graph.

optimized values of the ingredients and their corresponding responses are listed in Table 4. The predicted values of responses 5.101%, 3.616%, 1.963%, 11.165%, 28.005%, 50.149%, 330.282 kcal, and 0.373 (Figure 5) were in accordance with the experimental values 6.426%, 3.455%, 1.714%, 11.432%, 29.12%, 47.853%, 323.318 kcal, and 0.385 for moisture, ash, fat, fiber, protein, carbohydrates, energy, and water activity, respectively, with a small error percentage (Table 4).

3.2.10. Antioxidant Study of Control and Optimized Nutri-Cereal Mix. The phenolic compounds as well as flavonoids play a vital role in the antioxidant activity due to the availability of redox potential and their tendency to donate hydrogen atom in free radical reaction.[38,39] From Table 5, it was observed that the TPC and TFC of the control and optimized nutri-cereal mix were 60.3 mg GAE/100g, 25.53 mg QE/100g and 56.00 mg GAE/100g, 32.54 mg QE/100g, respectively. The optimized mix had a higher TFC and lower TPC as compared to the control. The variation in TPC and TFC of the control and optimized nutri-cereal mix could be due to the ingredients used. Our findings are in line with those observed by Keyata et al.[40] who reported that the TPC of control formulation is higher than formulated flours with different ingredients. The fair amount of TPC and TFC in optimized nutri-cereal mix could play a protective role against diseases such as inflammatory diseases, aging, and Alzheimer's.[41−43] Overall, the amount of TPC and TFC in the nutri-cereal mix could provide a healthy source of antioxidant-rich food product.

Table 5. Antioxidants, Color Index, Minerals, and Phenol Profiling of Control and Optimized Nutri-Cereal Mix

parameters	control	optimized nutri-cereal mix
TPC (mg GAE/100 g)	60.3 ± 0.86	56.00 ± 0.49
TFC (mg QE/100 g)	25.53 ± 0.16	32.54 ± 0.13
L^*	56.5 ± 0.45	76.99 ± 0.22
a^*	1.4 ± 0.04	3.57 ± 0.03
b^*	30.0 ± 0.63	18.42 ± 0.08
total color difference (ΔE)	0	23.63 ± 0.09
hue index (h)	−89.96 ± 0.38	53.75 ± 0.43
saturation index (SI)	20.65 ± 0.23	18.77 ± 0.08
white index (WI)	51.84 ± 0.06	70.30 ± 0.12
yellow index (YI)	52.08 ± 0.13	34.19 ± 0.06
brown index (BI)	41.92 ± 0.04	30.24 ± 0.05
Ca (mg/L)	3739.09 ± 0.06	10994.09 ± 0.09
Fe (mg/L)	217.32 ± 0.11	375.40 ± 0.08
Mg (mg/L)	1509.22 ± 0.10	3568.65 ± 0.09
Zn (mg/L)	29.87 ± 0.17	39.65 ± 0.13
trans-ferulic acid (ppm)	220.26 ± 0.12	92.27 ± 0.16
sinapic acid (ppm)	13.99 ± 0.15	6.12 ± 0.12
kaempferol (ppm)	44.56 ± 0.11	25.46 ± 0.13

3.2.11. Color Analysis of Control and Optimized Nutri-Cereal Mix. The consumer satisfaction and demand totally depend on the food product's color characteristics. Table 5 represents the various parameters of color like the total color difference (ΔE), hue angle (h), saturation index (SI), white index (WI), yellow index (YI), and brown index (BI) of the control and the optimized nutri-cereal mix. TCD of the optimized nutri-cereal mix increased significantly.

From Table 5, it was observed that the optimized nutri-cereal mix had high values of a* and a lower value of b* compared to the control. High values of a* and lower values of b* above zero signified that the red and yellow tones are predominating over the green and blue tones, respectively.[44] The lower L* value of the control as compared to the optimized nutri-cereal mix indicated that the optimized nutri-cereal mix is darker in color than the control.

The (h) value of the optimized nutri-cereal mix was found to be more than that of the control, indicating that the millet flour−based optimized nutri-cereal mix had more yellow character. The (h) positive value also stipulated that the optimized nutri-cereal mix did not deviate from the color.[45] The saturation index is a measure of the difference between hue and gray color. The value of the saturation index of the optimized nutri-cereal mix was found to be lower than that of the control. The high SI value of the control suggested that the control had more color intensity than the optimized nutri-cereal mix. The white index of any food product indicates the whiteness of that food product due to discoloration during processing. The highest YI and BI were observed for the control, possibly due to the heat-induced browning of wheat

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flour in the control.[46] The color change in the optimized nutri-cereal mix may be due to the composition of millet flour.

3.2.12. Mineral Analysis of Control and Optimized Nutri-Cereal Mix. Mineral plays a crucial role in the proper operation of various organs in the body. The mineral composition of control and optimized nutri-cereal mix is summarized in Table 5. Nearly all the calcium in the body is located in the bones and teeth, where the skeletal system is a reservoir for calcium needed within the bloodstream and other areas. The adequate amount of calcium intake is must during childhood and adolescence stage for proper growth and calcification of bones.[47] In this study, the optimized nutri-cereal mix exhibited a significantly higher calcium content, measuring 10 994.09 mg/L. The higher calcium content may probably be due to the presence of a good proportion of finger millet, sorghum, and green gram in the optimized nutri-cereal mix.[48,49] However, similar results were reported for pearl millet−based ready-to-eat product and sorghum-based karkade and premix by Aande et al.[50] and Keyata et al., respectively.[10]

Iron (Fe) is a crucial element for the human body, ensuring homeostasis. It is essential for the various cellular processes like oxygen transport, immune response, and protein metabolism and serves as an important component in various metabolic enzymes.[51] The control showed 217.32 mg/L concentration of Fe, which is lower than the optimized nutri-cereal mix measuring 375.40 mg/L. The Fe content in the optimized nutri-cereal mix was approximately 72.74% higher than that in the control. The increment in Fe content was seen due to the ingredients of the optimized nutri-cereal mix, which consists of a higher percentage of iron within. Similar results were observed by Tadesse et al.[52] and Yadav et al.[53] where pumpkin and germinated amaranth flour−based complementary food and gluten-free flour mix was developed.

Magnesium (Mg) is an essential mineral for living beings as it plays a crucial role in various physiological functions.[54] The control exhibited a lower level of Mg (1509.22 mg/L), while the optimized nutri-cereal mix consisted of a higher (3568.65 mg/L) concentration. A substantial increase in Mg content carries key implications for combating Mg deficiency, particularly in susceptible populations. Comparable findings were noted in multigrain bhakari premix by Rekha et al.[55]

Zinc (Zn) is an indispensable mineral for playing critical roles in cellular functioning, division, growth, and other metabolic processes.[56] There was a notable increase in the zinc content when compared to that in the control. The control exhibited 29.87 mg/L concentration, while the optimized nutri-cereal mix consisted of 39.65 mg/L. An increase of 32.74% of zinc content is noted compared to that of the control. Such a considerable increment was due to the presence of millets, green gram, and chicken in the right proportion in the optimized nutri-cereal mix. Additionally, it underscores the efficacy of the optimized nutri-cereal mix in upgrading the zinc content, which is crucial for nutritional enhancement. A similar result was observed by Ashwath et al.[57] where multigrain premix-biscuit was prepared.

3.2.13. Phenol Analysis of Control and Optimized Nutri-Cereal Mix. Phenolic compounds possess potential health-benefiting properties such as antioxidant activity, antiproliferative effect, anti-inflammatory effect, antidiabetic effect, and antiatherogenic effects.[58] In this study, quantification of a few phenolic compounds such as *trans*-ferulic acid, sinapic acid, and kaempferol were focused, where the optimized nutri-cereal mix had higher concentrations measuring 220.26, 13.99, and 44.56 ppm than the control mix, as summarized in Table 5. This substantial enhancement indicates that the ingredients present in the optimized nutri-cereal mix were effective in upgrading the concentrations of *trans*-ferulic acid, sinapic acid, and kaempferol.

3.2.14. Amino Acid Analysis of Control and Optimized Nutri-Cereal Mix. Amino acid compositions of control and optimized nutri-cereal mix are summarized in Table 6. In this study, optimized nutri-cereal mix showed a higher proportion of aspartic acid, glutamic acid, asparagine, glycine, argainine, alanine, DOPA, tyrosine, cysteine, leuocine, and lysine, whereas a lower proportion of theonine and ornithine was observed as compared to control. Glycine is crucial for the synthesis of key biomolecules like collagen, while arginine is important for immune functioning and in healing of wounds.[59] Glutamic acid, DOPA, and cysteine were substantial in larger amounts in optimized nutri-cereal mix when compared to the other amino acid compounds, which provides the potential health benefits correlated with neurotransmitter regulation and antioxidant defense.[60] Additionally, this rise in amino acid concentration suggests an improvement in the control and optimized nutri-cereal mix protein quality. Similarly, least changes were observed in glycine, alanine, tyrosine, and leucine in the optimized nutri-cereal mix than in the control. Similarly, results of lentils and horse gram flour showed enhancement of amino acid concentration, as reported by Ghumman et al.[21]

Table 6. Amino Acid Profiling of Control and Optimized Nutri-Cereal Mix

	content (mg/g)	
amino acid	control	optimized nutri-cereal mix
aspartic acid	0.727	0.983
glutamic acid	3.221	3.985
asparagine	0.132	0.165
glycine	0.122	0.133
theonine	0.415	0.377
arginine	0.031	0.059
alanine	0.151	0.164
DOPA	0.511	0.693
tyrosine	0.052	0.059
cysteine	0.4	0.891
leucine	0.497	0.533
ornithine	1.053	0.974
lysine	0.075	0.091

4. CONCLUSION

The optimized nutri-cereal mix developed in the current study from a combination of some major ingredients, i.e., 30% malted sorghum flour, 15% malted green gram flour, and 15% chicken powder, and other ingredients such as malted pearl millet flour (10%), finger millet flour (10%), beetroot powder (2.5%), pumpkin powder (7.5%), skimmed milk powder (9.5%), and stevia powder (0.5%), can potentially enhance the nutrient deficiency in the population, as suggested by the study results. The developed mix had a higher nutritional profile as compared to the control and may be used as a substitute over the other health mix available in the market for a growing proportion of the population, who require a cereal mix that is wholesome in terms of nutritive, functional, and sensory attributes.

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S.S. contributed to the methodology and writing original draft. P.G. and S.K. contributed to the conceptualization and supervision. A.P.K. contributed to the supervision, review, and editing. V.K., W.A., and A.D. contributed to the formal analysis, review, and editing.

Notes

The authors declare no competing financial interest.

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