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Development and Optimization of Black Rice-based Instant Idli Mix Powder

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Abstract

The present study aimed to develop a convenient instant mix for a traditional Indian breakfast dish, idli, by incorporating black rice—a potential "superfood"—as a substitute for conventionally used polished rice. The optimization of black rice and black gram content in the Black Rice-Based Instant Idli Mix Powder (BRIIP) was done using a two-factor Central Composite Rotatable Design. The formulation comprising 20 g (34%) of black rice and 38.24 g (66%) of black gram was found to be optimum. Nutritional and physico-chemical assessments of the optimized BRIIP were performed. The BRIIP showed DPPH inhibition activity, total phenolic content, and total flavonoid content of 58.24%, 402.33±7.85 mg GAE/100g, and 50.75±5.11 mg QE/100g, respectively. The idli prepared using reconstitution of BRIIP showed satisfactory sensory properties. The shelf-life analysis of the BRIIP stored in LDPE pouches at room temperature (25 °C) revealed a 207.14% increase in free fatty acid and 574.41% increase in thiobarbituric acid reactive substances (TBARS) content from day 0 to day 28. Microbiological quality assessment revealed a 67.52% increase in the total plate count, while yeast, mold, and coliform counts remained nil throughout the storage. All the parameters of shelf-life evaluation remained within the acceptable range throughout the storage period.

Article History

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Keywords

Black Rice;
Instant Food Mixes;
Food Fermentation;
Idli;
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Abbreviations

DPPH:	2, 2-diphenyl-1-picrylhydrazyl
TPC:	Total phenolic content
TFC:	Total flavonoid content
GAE:	Gallic acid equivalent

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QE: Quercetin equivalent
BRIIP: Black rice-based instant idli mix powder
LDPE: Low-density polyethylene
FFA: Free fatty acid
TBARS: Thiobarbituric acid reactive substances
MDA: Malondialdehyde
MMT: Million metric tons
USD: United States Dollar
CCRD: Central composite rotatable design
RSM: Response surface methodology
AOAC: Association of Official Analytical Chemists
CC: Coliform count
YMC: Yeast and mold count
ANOVA: Analysis of variance
N: Newton
N. s: Newton second
RTE: Ready-to-eat
CFU: Colony-forming unit

Introduction

Rice (*Oryza sativa*) is indisputably a staple food for the majority of the world's population and serves as a primary source of energy in their diets. The global milled rice production was estimated to be approximately 522.62 MMT in 2023-24, comprising 34.07% of the total grain production. China, with a production of 144.62 MMT, and India, with 137.83 MMT, stand as the leading rice-producing countries, contributing an estimated 28.43% and 26.46%, respectively, to the total global rice production.[1] The International Rice Research Institute, Philippines, holds more than 132,000 different accessions of rice, including cultivated, wild and related varieties. Black rice, also commonly known as "forbidden", "heavenly", and "prized" rice, etc. has been recognized as a 'super food'. The total market revenue generated by black rice exceeded 181 million USD in 2022; with the market trend projecting a Compound Annual Growth Rate (CAGR) of 5.13% by 2030.[2] China is the largest producer of black rice, comprising 62% of the total production and is followed by Sri Lanka, Indonesia and India.[3] Among the 200 different varieties of black rice available, Jasmine black rice constitutes around 25% of the total share.[2,4]

The black/purple color of the black rice is because of the presence of an important class of polyphenols i.e., the anthocyanin. Anthocyanins are found in abundance in many fruits and vegetables especially, blueberries, blackberries, purple cabbage, etc. and are well-known for their anti-oxidant, neuro-protective, anti-cancerous, anti-cardiovascular disease, and anti-hypertensive properties.[5] Black rice contains approximately 0.65 times the anthocyanin content compared to blueberries whereas, white rice is completely devoid of it.[6,7] Additionally, black rice contains higher amounts of polyunsaturated fatty acids, total polyphenols, total antioxidants, vitamin E, carotenoids, folic acid, oryzanol than white and red rice.[8] Previous studies have shown that black rice possesses various health benefits, including the regulation of glucose and lipid metabolism,[9] neuroprotective effects,[10] and hepatoprotective effects,[11] among others. The ever-growing popularity of black rice can be attributed to its nutritional superiority compared to white rice and the increasing health-consciousness of consumers. This trend has led to the expansion of the novel functional food market, resulting in the introduction of various black rice products, such as cookies fortified with black rice,[12] pasta made with partially substituted wheat flour using black rice,[13] and black rice cake,[14] to name a few.

Idli, a beloved cereal-based fermented food in South India, has gained popularity beyond its region of origin. It serves as a good source of energy, protein, vitamins and minerals.[15] The traditional method of making idli involves natural fermentation of soaked and ground white, parboiled rice and decorticated black gram, resulting in a white color, soft, spongy texture, and mild, appealing flavor. While traditional methods of preparing Indian cuisines, including idli, can be time-consuming, instant mixes offer a convenient alternative for those with busy lifestyles. These mixes cater to the needs of modern individuals, saving both time and effort.

While instant mixes address the need for convenience, their potential to incorporate functional ingredients, such as black rice, remains largely untapped. Despite its well-documented nutritional and functional benefits, black rice is underutilized in instant food products, particularly in traditional dishes like idli. Research on optimizing black rice formulations to achieve desirable sensory and textural qualities is limited. Furthermore, data on the storage stability of black rice-based instant mixes, including lipid oxidation, microbial safety, and sensory quality, is insufficient. Consumer acceptance of these products, particularly regarding taste, texture, and color, also requires further study. Addressing these gaps can lead to the development of innovative, nutritionally superior, and convenient food products that align with modern consumer preferences. This study aims to bridge these gaps by optimizing and developing a black rice-based instant idli mix powder (BRIIP). The study evaluates its physico-chemical properties, sensory acceptability, and storage stability.

Material and Methods

Materials

Organic, unpolished black rice (Manipuri black rice, also known as “Chakhao”) and dehulled, split black gram (Pant Urd-31) were procured from a local market in Varanasi, India. TATA®Salt, Weikfield® baking powder and Amul® dahi (Composition: Carbohydrates- 4.4%; protein- 4%; fat- 3.1%), food grade low-density polyethylene (LDPE) zip pouches (thickness: 250 gauge/ 63.50 microns and size 10×14 inches) were purchased from a local convenience store, Varanasi, India. All the chemicals (unless stated otherwise) were of analytical grade from HiMedia, Pvt. Ltd., Mumbai, India and the glassware was from Borosil®, Pvt. Ltd., Varanasi, India.

Preparation of Black Rice-based Instant Idli Mix Powder (BRIIP)

Figure 1. presents the flowchart for the preparation of black rice-based instant idli mix powder (BRIIP). Cleaned and destoned black rice and black gram were washed, and soaked in approximately 5 times their quantity of water (30 ± 2 °C) for 2 and 4 hours, respectively. After draining the water, black rice was ground into coarse particles or grits (approximately 500 microns) and black gram was ground into a fine paste in a mixer grinder (Make: Philips, Model HR 7629). The paste and grits were blended together along with 3% salt to form batter. The prepared batter was fermented in a closed stainless steel container approximately 15 times larger than the batter volume to allow for proper fermentation and expansion over 18 hours at 30°C. The fermented batter was spread in plastic sheets and dried in a stainless steel tray drier (Khera Instrument Pvt. Ltd.) at 60°C for 24 hours (until 5% moisture content). Finally, the dried product was ground into powder and blended with 1% baking powder. The prepared mix was packaged in food-grade LDPE zip pouches and kept for storage.

Reconstitution of the Prepared Black Rice-based Instant Idli Mix Powder (BRIIP)

Equal amounts of BRIIP and curd were mixed evenly. The mixture was left undisturbed for 15 minutes. One and a half volumes of water were added until the desired consistency was reached to form the batter.

Preparation of Idli from the Batter

The prepared batter was poured in a stainless steel idli maker/pan (Make Hawkins) and steamed for 15 min.

Optimization of Process Variables for the Development of BRIIP

Central Composite Rotatable Design (CCRD) of the Response Surface Methodology (RSM) was employed for the optimization of the product using the Design Expert software version 8.0. The independent variables taken into consideration for optimization were black rice and black gram. The lower and upper limits for both variables were set as 20g and 60g respectively, based on preliminary study conducted using sensory evaluation. Sensory characteristics (Color, flavor, texture, taste and overall acceptability) were taken as the responses. The effect of independent variables on these responses were evaluated. A combination of 13 trials were generated as shown in Table 1. Salt (3%) and baking powder (1%) were kept constant for all the formulations. The primary objective of the optimization was to maximize the incorporation of black rice in the formulation while simultaneously enhancing the desirable sensory properties, aiming to develop a functional food product with appealing sensory characteristics. The experiments were performed and responses were fitted in the design framework and analyzed to assess the effect of independent variables on them.

Proximate Analysis

The proximate analysis (moisture, total carbohydrates, fats, protein, dietary fiber and ash) of BRIIP was conducted according to the methods outlined by AOAC.[16]

pH and Titratable Acidity

The pH of BRIIP batter was determined using an electronic pH meter (Hitech lab, India), calibrated using a pH 7 and pH 4 standard buffer. The Titratable acidity of BRIIP batter was determined by AOAC method.[17] 1g batter was mixed thoroughly with 10ml deionized water. The mixture was filtered using a Whatman no. 42 filter paper and the filtrate was titrated against N/100 NaOH using phenolphthalein indicator. The % Titratable acidity was calculate using the following formula:

% titratable acidity (as lactic acidity)=(Vol.of titrant (NaOH)×Normality (NaOH)×0.09×100)/(Weight of batter taken)

Determination of Antioxidant Potential

The antioxidant potential of BRIIP was evaluated through its % DPPH inhibition activity and the quantification of polyphenols and flavonoids present in the sample.[18]

Preparation of Extract

2 g BRIIP was added to 25 ml of 80% ethanol and thoroughly mixed in an incubator shaker (Infors HT Ecotron, UK) at 150 ×g for 12 h. The mixture was then centrifuged (Thermo Scientific ST16R Refrigerated Centrifuge) at 10,000 ×g and 4 °C for 15 min. The supernatant (extract) was separated and stored at -20 °C for further analysis.

%2, 2-diphenyl-1-picrylhydrazyl (DPPH) Inhibition Activity

2 ml of freshly prepared DPPH solution (0.1 mM DPPH in 80 % ethanol) was mixed with 300 µL of sample extract. Simultaneously, a control sample was prepared (2 ml DPPH with 300 µL of 80 % ethanol). The solution mixtures were kept in dark for 30 min, following which absorbance was taken using UV-visible spectrophotometer (Shimadzu Corporation, Japan) at 517nm. The % DPPH inhibition activity was calculated using the following formula:

% DPPH inhibition=(Absorbance of control-Absorbance of sample)/(Absorbance of control)×100

Total Phenolic Content (TPC)

Folin–Ciocalteau (FC) method was employed to determine the TPC. A 300 µL sample extract was combined with 2 ml of 0.02 N FC reagent, thoroughly mixed, and kept undisturbed for 6 min at room temperature. Subsequently, 2 ml of 7.5% sodium carbonate was added to this mixture, followed by incubation at room temperature for an hour. Following this, the absorbance was measured at 750 nm. A gallic acid (0 -700 µg/ml) calibration curve was constructed using the same procedure as the sample. The TPC was calculated using the following equation as obtained from the curve.

Absorbance=0.002201 gallic acid (µg)+0.6365 ($R2$=0.9914)

Total Flavonoid Content (TFC)

To determine theTFC, 300µL sample extract was combined with 0.4 ml of 5% sodium nitrite, thoroughly mixed, and kept undisturbed at room temperature for 6 min. Subsequently, 0.4 ml of 10% Aluminium chloride was added to the mixture and again incubated at room temperature for 6 min. Then, 3 ml of 1M NaOH was added, mixed and incubated at room temperature for an hour. A quercetin (0 -50 µg/ml) calibration curve was constructed using the same procedure as the sample. The TFC was calculated using the following equation as obtained from the curve.

Absorbance=0.008 quercetin (µg)+0.042 (R2=0.990)

Viscosity

Viscosity of the batter was measured using a Viscometer (DVPlus, AMETEK Brookfield). 400-500 ml batter (25 °C temperature) was taken in a beaker and placed under the viscometer. The spindle, LV-03 (63) was lowered to dip into the sample up to its immersion mark on the spindle shaft. The viscosity was measured as the torque required to rotate the spindle immersed in the sample.

Texture Profile Analysis

The textural properties of the idli prepared from the BRIIP, i.e., hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience, were determined with the TA.TX plus Stable Micro Systems, Surrey, UK Texture Analyzer using TA- 42 probe. Freshly prepared idli (5cm diameter; 0.6 cm thickness) was cooled to room temperature and placed on the heavy-duty platform of the texture analyzer. Test mode was compression type. The Pre-test speed, Test speed and Post-test speed were 1.00 mm/sec, 5.00 mm/sec and 5.00 mm/sec respectively. Target distance and trigger force were set at 10.000 mm and 5.0 g respectively. The textural properties were evaluated using Texture Exponent Lite Software.

Sensory Evaluation

Sensory evaluation of idli prepared from BRIIP was conducted by 30 semi-trained panelists, including food/dairy technology professors, research scholars, and postgraduates from the Dairy Science and Food Technology Department, BHU, India. The idli was evaluated for various sensory parameters (color and appearance, taste, texture, flavor, and overall acceptability), and the responses of the panelists were recorded as scores on a 9-point hedonic scale, with 9 indicating extremely liked and 1 indicating extremely disliked.[19]

Shelf-Life Evaluation

The BRIIP was packaged in LDPE zip pouches and stored at 25°C. Shelf life was assessed by monitoring changes in sensory parameters, free fatty acid content, thiobarbituric acid reactive substances (TBARS) content, and microbiological stability. These analyses were conducted every 7 days over a period of 28 days.

Free Fatty Acid (FFA) Analysis

FFA analysis of BRIIP was performed according to AOAC method No. 940.28.[16]

Thiobarbituric acid Reactive Substances (TBARS) Analysis

TBARS analysis was conducted according to previous method with some modifications.[20] 2 g BRIIP was mixed with 25 ml of 50 % glacial acetic acid and left to stand at room temperature for 10 minutes, followed by centrifugation (10,000 × g; 15 min; 10 °C) and filtration using Whatman 42 filter paper. 2 ml of the filtrate obtained was mixed with 3 ml of 0.02 M Thiobarbituric acid (TBA) reagent (Sigma-Aldrich, Darmstadt, Germany) prepared in glacial acetic acid. The mixture was heated in a water bath at 95 °C for 1 hour, cooled to room temperature and the absorbance was measured at 532 nm. A malondialdehyde (MDA) (0 -1 µM) calibration curve was constructed using the same procedure as the sample. The TBARS was calculated using the following equation as obtained from the curve.

$$\text{Absorbance} = 1.2447\ \text{MDA}\,(\mu M) + 0.0965\ (R2=0.995)$$

Microbiological Analysis

The microbial quality of the BRIIP was assessed by determining the total plate count (TPC), yeast and mold count (YMC), and coliform counts (CC). This was done by plating various dilutions of the sample on nutrient agar, potato dextrose agar, and Violet Red Bile Agar, respectively. The TPC and CC plates were then incubated at 37°C for 24 hours, while the YMC plates were incubated at 25°C for 48 hours. The resulting colonies were counted, and the results were recorded as log Colony Forming Units (CFU) per gram of the BRIIP.

Statistical Analysis

The experimental data obtained from the study were analyzed using Design Expert® version 8.0 (Stat Ease, Inc., Minneapolis, USA). Analysis of variance (ANOVA) was performed to evaluate the impact of independent variables on the responses at linear, quadratic, and interaction levels. A significance level of $p \leq 0.05$ was considered for all analyses. A second-order polynomial equation was generated through ANOVA, and the optimization toolbox was employed to determine the optimal levels of the independent variables. The objective of the optimization process was to develop the BRIIP with the best possible sensory properties. Additionally, the accuracy of the design was assessed by evaluating the standard deviation and R-squared values.

All assays were conducted in triplicate, and the results were reported as mean values with standard errors. Tukey's test was utilized to identify significant differences among the means of different groups, with a confidence level of 95%. The statistical analyses were performed using Microsoft Excel and GraphPad Prism version 6.

Results and Discussion

Optimization of Black Rice and Black Gram Levels in the Formulation

Figure 1. presents the general flowchart for the preparation of BRIIP. Table 1 presents the data regarding the responses observed in the idli made from BRIIP using different levels (20-40 g) of independent variables (black rice and black gram). To investigate the influence of black rice and black gram levels on the sensory profile of the BRIIP idli, the collected data was subjected to analysis utilizing different models (linear, quadratic, and two-factor interaction) as shown in Table 2. The quadratic response surface models were chosen for all responses based on their suitability. The positive or negative sign of the coefficients, along with their magnitude, indicates the direction and intensity of the

variable's effect on the responses. Various statistical measures such as F-ratio, mean, standard deviation, coefficient correlation, and lack of fit were computed to assess the adequacy of the model.

Fig. 1: Flowchart for the preparation of Black rice based instant idli mix powder

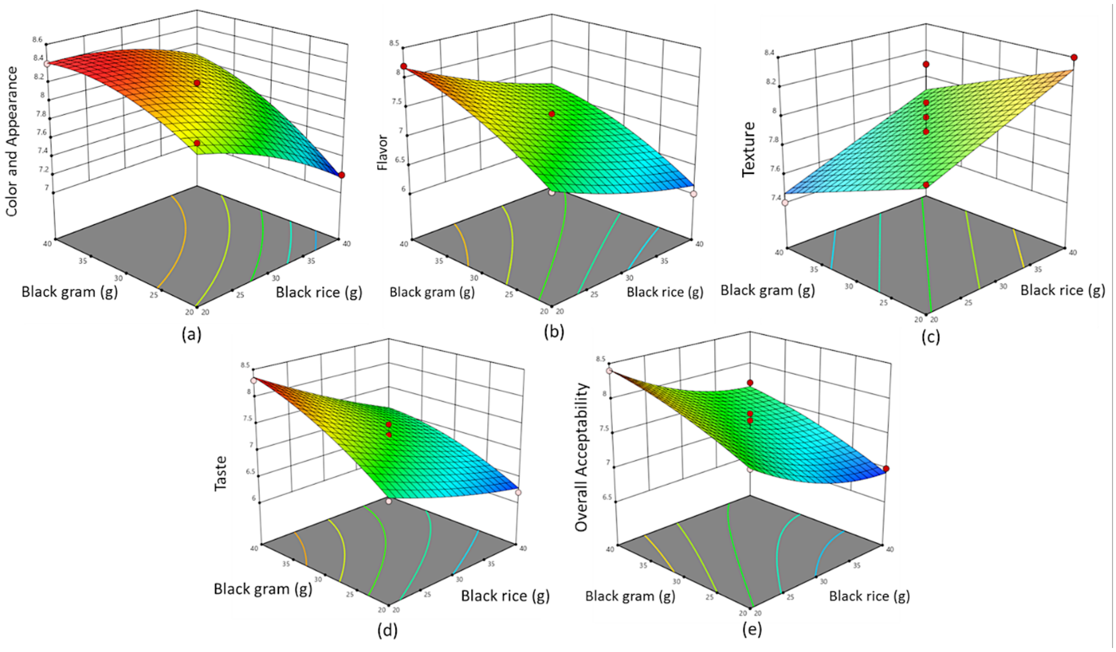

Fig. 2: Response plots showing effect of black rice and black gram on various sensory parameters

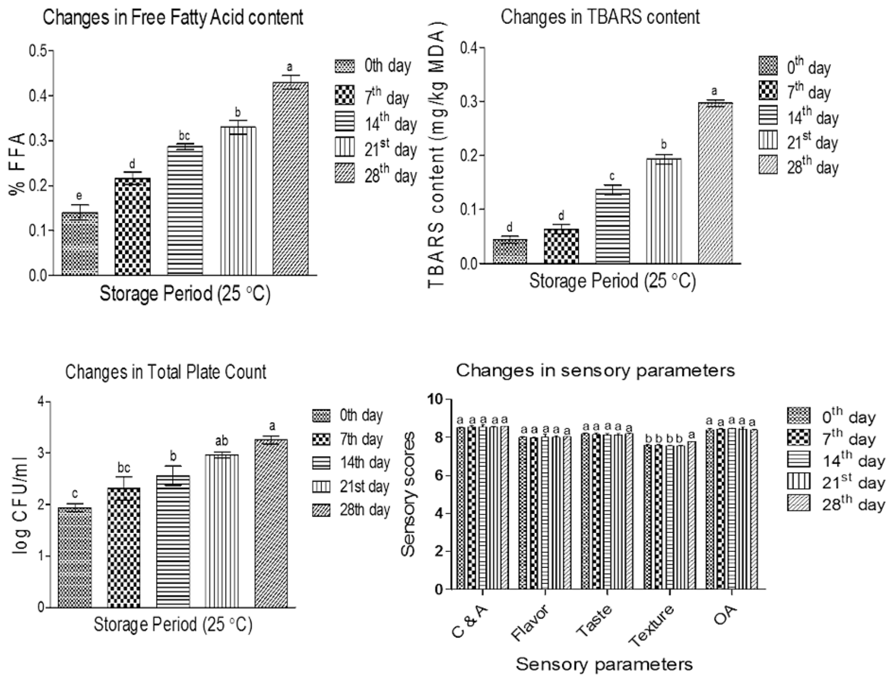

Fig. 3: Shelf-life analysis of Black rice based instant idli mix powder stored in LDPE pouches at ambient temperature (25 °C)

Table 1: Experimental design and responses obtained for BRIIP

Process variables			Responses				
Trial no.	Black rice (g)	Black gram (g)	Color and Appearance	Flavor Acceptability	Texture	Taste	Overall
1	40	40	8	7.1	8.1	7	7.7
2	30	30	8.2	7.2	8	7.3	7.5
3	20	40	8.4	8.2	7.4	8.3	8.4
4	30	30	8.2	7.4	8.1	7.5	7.5
5	30	15.8579	7.3	6.2	8.1	6.3	6.9
6	30	30	8	7.2	7.8	7.3	7.8
7	30	30	8.2	7.1	7.8	7.1	7.4
8	30	44.1421	8.4	7.7	7.5	7.8	7.9
9	40	20	7.2	6	8.4	6.2	7
10	44.1421	30	7.6	6.9	8	6.8	7.3
11	15.8579	30	8.3	8	7.6	8.2	8.5
12	30	30	8.2	7.2	7.9	7.3	7.7
13	20	20	8.1	7	7.9	7	7.7
Predicted[a]	20	38.24	8.41	8.12	7.5	8.3	8.37
Actual[b]	20	38.24	8.51	8	7.58	8.2	8.41
Difference[c]	-	-	0.1	-0.12	0.08	-0.1	0.04

Note: Response values are expressed as the mean of scores obtained from the panelists; a: values obtained using the optimization software; b: values obtained after the preparing and evaluating the BRIIP idli using the optimized conditions in laboratory; c: these values indicate the accuracy of experiment

Table 2: ANOVA and regression coefficients for the responses

	Intercept	A	B	AB	A^2	B^2
Color and Appearance	8.16	-0.286244*	0.331954*	0.125*	-0.0987*	-0.14875*
Flavor	7.22	-0.456954*	0.552665*	-0.025	0.08375	-0.16625*
Texture	7.89231	0.220711*	-0.206066*			
Taste	7.3	-0.509987*	0.527665*	-0.125	0.0625	-0.1625*
Overall Acceptability	7.58	-0.387132*	0.351777*	3.21352E-15	0.1725*	-0.0775

Note:*-Significant at 5% level; A- black rice at linear level; B- black gram at linear level (where variable is effecting the response independently); A2–black rice at quadratic level; B2–black gram at quadratic level (where a variable interacts with itself); AB- interaction between black rice and black gram

Effect of variables on the Color and Appearance of BRIIP Idli

Color and appearance (C & A) of a food are basic sensory aspect which affects the product's acceptability. A typical idli is white or off-white in color. However, the prepared BRIIP idli had a purplish-black color due to the presence of anthocyanin pigment in the black rice.[6] Figure 2(a) represents the effect of varying black rice and black gram amounts on the sensory scores for C & A. The value of responses for C & A ranged from 7.3 to 8.4 (Table 1), with a mean value of 8.01. The coefficient of estimate (Table 2) shows that both black rice (A) and black gram (B) significantly ($p< 0.05$) affected C & A scores for the idli at all levels (linear, quadratic and interaction). Black rice had a negative effect at both linear and quadratic levels, whereas black gram showed a positive effect at linear and a negative effect on quadratic level. The negative impact of black rice on C & A may be due to consumer expectations, as idli is traditionally white, and the purple-black color may not be as visually appealing. Processing conditions, including heating and interactions with other components of the food matrix, may result in the fading of bright color of anthocyanins.[21] A similar decrease in color sensory scores was observed in pasta with the addition of black rice bran.[22] However, the trend is shifting toward health foods with unique appearances, driven by the incorporation of healthier, unconventional ingredients like black rice. A consumer perception study demonstrated that consumer acceptance of color in food products (bread, yogurt, and pâté) improved significantly when participants were informed that the color changes were due to the addition of antioxidant-rich plant-based extracts, compared to when no such information was provided.[23] Notably, the interaction (AB) between black rice and black gram resulted in significant positive effect on the C & A scores of the idli. The equation for predicting C & A scores at different variable levels is as follows:

$$C \& A = 8.16 – 0.2862A + 0.3320B + 0.1250AB – 0.0987A2 – 0.1487B2$$

Effect of Variables on Flavor of BRIIP Idli

The flavor of food is the result of the intricate interaction of various volatile and high molecular weight components, stimulating both the odor and taste senses of an individual.[24] A typical idli has a pleasant, mildly acidic to somewhat bland flavor. Black rice has a distinct flavor compared to white rice, which might be due to the higher aromatic and nitrogen-containing compounds in black rice.[25] Figure 2(b) shows the effect of varying black rice and black gram amounts on the sensory scores for flavor. The value of responses for flavor ranged from 6 to 8.2 (Table 1) with a mean value of 8.01. The coefficient of estimate (Table 2) shows that black rice and black gram had a significant ($p < 0.05$) negative effect on flavor at the linear and quadratic levels, respectively, whereas black gram showed a significant positive effect on the flavor of the idli at the linear level. The interaction between the two variables did not significantly affect the flavor. The nutty, fragrant flavor of black rice, compared to the bland flavor of conventional idli, was not favored by the panelists. In a study, sweet bread made by substituting black rice for wheat flour showed decreasing flavor scores with increasing black rice concentration.[26] In contrast, a study found that black rice cookies scored better in flavor than wheat flour control cookies.[27] This indicates that the sensory acceptability of black rice varies depending on the product and formulation. These variations highlight the need for product-specific optimization to improve flavor profiles and overall consumer acceptance. In this study, adding flavor additives, such as spices, to the mix could help mask the flavor of black rice and improve the flavor profile of the idli. The equation for predicting flavor scores at different variable levels is as follows:

$$Flavor=7.22-0.4570A+0.5527B-0.0250AB+0.0837A^2 – 0.1662B^2$$

Effect of Variables on Texture of BRIIP Idli

The texture of idli is a critical factor in determining its consumer acceptability. Typically, idli has a soft, spongy, and fluffy texture that is highly desirable among consumers.[28] Figure 2(c) shows the effect of varying black rice and black gram amounts on the sensory scores for texture. The value of responses for texture ranged from 7.4 to 8.4 (Table 1) with a mean value of 7.89. The linear model was best suited for defining the effects of the variables on the texture of the idli. The coefficient of estimate (Table 2) shows that black rice had a significant positive effect, while black gram had a significant negative effect on the texture of the idli. The texture of an idli is dependent on the amylose, amylopectin, starch, and protein content, as well as the soaking and fermentation time.[28]Achieving an optimum ratio of the variables, along with carefully controlling other factors, may help improve the texture profile of BRIIP idli. The equation for predicting texture scores at different variable levels is as follows:

$$Texture = 7.89 + 0.2207A – 0.2061B$$

Effect of Variables on Taste of BRIIP Idli

Typically, idli has a somewhat bland or mildly acidic taste, which results from the fermentation process.[28] Figure 2(d) shows the effect of varying black rice and black gram amounts on the sensory scores for taste. The value of responses for taste ranged from 6.2 to 8.3 (Table 1) with a mean value of 7.24. The coefficient of estimate (Table 2) shows that black rice and black gram had a significant ($p < 0.05$) negative effect on taste at the linear and quadratic levels, respectively, whereas black gram showed a significant positive effect on the taste of the idli at the linear level. The interaction between the two variables did not significantly affect the taste scores. Similarly, another study noted that the distinct taste of black rice posed challenges for the acceptability of gluten-free bread made from black glutinous rice.[29] The equation for predicting taste scores at different variable levels is as follows:

$$Taste = 7.30 - 0.5100A + 0.5277B - 0.1250AB + 0.0625A^2 - 0.1625B^2$$

Effect of Variables on Overall Acceptability of BRIIP Idli

Overall acceptability of a product refers to the degree to which a product is found satisfactory or pleasing by consumers, considering various attributes such as taste, texture, appearance, aroma, and overall quality. This measure is often used in sensory evaluation to determine the success and marketability of food products.[30] Figure 2(e) shows the effect of varying black rice and black gram amounts on the sensory scores for overall acceptability. The value of responses for overall acceptability ranged from 6.9 to 8.5 (Table 1) with a mean value of 7.64. The coefficient of estimate (Table 2) shows that black rice had significant negative effect on overall acceptability whereas black gram had significant positive effect on the overall acceptability of the BRIIP idli. These findings suggest that while black rice may detract from overall acceptability due to its distinct sensory properties, black gram can enhance the appeal of the product by contributing positively to key sensory attributes. Balancing the proportions of these ingredients is crucial to optimize the overall acceptability and ensure consumer satisfaction with the BRIIP idli.

The equation for predicting OA scores at different variable levels is as follows:

$$OA = 7.58 - 0.3871A + 0.3518B + 0.0000AB + 0.1725A^2 - 0.0775B^2$$

Numerical Optimization

Following the specified objectives, each variable and response was carefully investigated to optimize the product parameters and identifying the best possible formulation. Utilizing the software, optimal formulation was generated, comprising 20 g (34%) black rice and 38.24 g (66%) black gram having a desirability of 0.873. A comparison among the predicted and actual values was conducted to assess the effectiveness of the optimized conditions, as outlined in Table 1.

Physico-Chemical Properties and Proximate Composition

Table 3 presents the physico-chemical properties and proximate composition of the BRIIP.

Table 3: Physico-chemical attributes of BRIIP

Parameters	Amount
Moisture content (%)	4.42±0.6
Carbohydrates (%)	60.60±2.8
Protein (%)	22.76±0.8
Fat (%)	2.04±0.1
Fiber (%)	6.22±0.3
Ash (%)	3.96±0.3
Viscosity[a] (cP)	2279±47
pH[a]	4.34±0.5
Titratable acidity[a] (as lactic acid)	1.01±0.1
DPPH inhibition activity (%)	58.24±5.6
Total Phenolic Content (mgGAE/100g)	402.33±7.85
Total Flavonoid Content (mgQE/100g)	50.75±5.11

Data is presented as Mean±SD (n=3); a: physico-chemical properties relating to the fermented batter

Antioxidant Potential

The antioxidant potential of BRIIP was evaluated based on its total polyphenol and flavonoid content, as well as its capacity to scavenge DPPH free radicals. BRIIP demonstrated a significant DPPH inhibition rate of 58.24±5.6%. Previous research also indicates strong antioxidant activity in black rice products.[31-33] For instance, incorporating 8.75% black rice into cookies enhanced their antioxidant potential, achieving a 79.8% higher DPPH inhibition compared to a control sample without black rice.[31] Similarly, in another study an increasing trend was observed in antioxidant activity with higher concentrations of black rice in rice noodles.[34] Polyphenols, known for their health benefits including antioxidant properties, are bioactive components prevalent in plant products.[35] The total polyphenol and flavonoid content of BRIIP was

402.33±7.85 mg GAE/ 100 g and 50.75±5.11 mg QE/ 100 g, respectively. Pigmented rice varieties, such as black rice, are rich in polyphenols and flavonoids, particularly anthocyanins.[6] Therefore, substituting white rice with black rice in food products introduces these beneficial bioactives. Incorporating black rice into biscuits increased the total polyphenol and flavonoid content by approximately 5.5 times compared to biscuits made with white rice.[31] Another study also reported significant increase in total phenolic content with increasing addition of black rice flour in black rice fortified flaky rolls.[36] These polyphenols present in black rice are associated with numerous health benefits anti-inflammatory, anti-diabetic and cardiovascular disease risk reduction.[37]

Texture Profile Analysis

The texture profile analysis of the optimized BRIIP idli was conducted to obtain objective and quantifiable measurements of its textural properties. Hardness, defined as the minimum force required to cause deformation,[28] was measured at 34±3.605 N. Adhesiveness, indicated by the negative peak on a texture profile curve and corresponding to the stickiness of the food, was recorded at -1.33 N·s. Springiness, the ability of the food to return to its original state after the deformation force is removed, was 0.997±0.345. Cohesiveness, which describes how well the food maintains its shape from the first chew to the second, was 0.520±0.082. Chewiness, calculated as hardness × cohesiveness × springiness, was 3.128±0.628 N. Resilience, the ratio of the area under the curve before and after the peak force, was 0.891±0.315. All values obtained were comparable to a previous study on idli made from various pigmented rice varieties.

Sensory Analysis

Sensory analysis of the optimized BRIIP idli was conducted, and the results are presented in Table 1. The sensory scores for color and appearance, flavor, and taste were all above 8 on the hedonic scale, while the texture received a score of 7.58± 0.15. The overall acceptability was also rated above 8. These results indicate that the product was well-received by the panelists and deemed fairly acceptable.

Shelf-Life Analysis

The shelf-life analysis of BRIIP, packaged in LDPE pouches and stored at 25°C, was conducted by evaluating changes in sensory parameters, free fatty acid content, thiobarbituric acid reactive substances (TBARS) content, and microbiological stability over a 28-day storage period.

Changes in Free Fatty Acid (FFA) Content

The changes in free fatty acid (FFA) with storage is presented in figure 3 (a). A significant ($p< 0.05$) increase in FFA content was observed with the storage time. The FFA content of the freshly prepared mix initially measured 0.14 ± 0.017 %, increased to 0.43 ± 0.015% after 28 days of storage. Similar results were observed in another study on popped pearl millet ready-to-eat (RTE) snacks packaged in metallized pouches, where the FFA content rose from 0.25% to 0.61% over a 90-day storage period at 25 °C.[38] Another study also reported an increase in FFA content in their study on the quality and sensory evaluation of gatta instant mix, with values rising from 0.3% to 0.9% over a 30-day storage period at room temperature.[39] FFA formation occurs due to lipolysis, which is triggered by factors such as photooxidation, enzymatic activity, and other processes. This can result in the production of hydroperoxides and volatile flavor compounds, negatively affecting the flavor and overall quality of the product.[40] Such degradation impacts sensory attributes and significantly reduces shelf life. To minimize these effects, it is crucial to ensure proper packaging and optimal storage conditions.

Changes in Thiobarbituric Acid Reactive Substances (TBARS) Content

The TBARS (Thiobarbituric Acid Reactive Substances) content was assessed to monitor the extent of secondary oxidation products formed due to lipid degradation during the storage period. The changes in TBARS content over time are presented in Figure 3 (b). Initially, the TBARS content was 0.043 ± 0.007 mg/kg MDA, which increased to 0.29 ± 0.006 mg/kg MDA after 28 days of storage. Despite this increase, the value remained below the threshold of 1 mg/kg MDA.[41] The increase in TBARS content during storage may be attributed to the inability of LDPE packaging to fully prevent exposure to light and oxygen. Similar significant increases in TBARS content were reported in the shelf-life analysis of an instant soup mix stored at room temperature for 90 days,[42] and in the shelf-life analysis of a pearl millet-based instant kheer mix.[43] In the latter study, the increase in TBARS content was correlated with the rise in storage temperature along with the storage period.[43]

Changes in Microbiological Quality

Microbiological quality analysis during storage was performed by assessing changes in the total plate count (TPC), yeast and mold count (YMC), and coliform count (CC) in the BRIIP. The changes in TPC are presented in Figure 3 (c). The TPC increased from an initial 1.94 log CFU/g to 3.25 log CFU/g on the 28th day, which remained within the limits prescribed by the Food Safety and Standards Authority of India.[44] Yeast, mold, and coliform were undetected in the samples throughout the storage period. The microbiological stability of BRIIP is likely due to the low moisture content (<5%) of the instant mix, as well as the antimicrobial effects of black rice constituents.[45] Other research on instant mixes, has also demonstrated good microbiological stability.

Changes in Sensory Parameters

The changes in the various sensory parameters are presented in Figure 3(d). Sensory scores for all parameters, except texture, remained unaffected throughout the storage period. However, texture scores significantly improved on the 28th day. This enhancement in texture may be attributed to the decrease in rice stickiness during storage, likely due to the increase in amylose content.[47] Overall, the sensory scores for all parameters remained within the acceptable range (>7) throughout the storage period.

Conclusion

This study focused on optimizing the content of black rice and black gram in black rice based instant idli mix powder (BRIIP) to create a functional and convenient food product. The optimized BRIIP idli demonstrated satisfactory sensory quality and notable antioxidant potential. Additionally, BRIIP stored in LDPE pouches at ambient temperature remained shelf-stable over a 28-day period. The higher dietary fiber and bioactive components of the developed black rice-based idli offer significant benefits compared to conventional white or milled rice idli. Moreover, the development of an instant mix for the idli enhances its convenience, catering to fast-paced lifestyles and providing improved shelf stability. Future research could explore the use of other pigmented rice varieties and their combinations to develop even more functionally superior products. Investigating the impact of different packaging materials on shelf life could further extend the product's longevity. Moreover, studies focusing on the nutritional profile, glycemic index, and bioavailability of the bioactive compounds in BRIIP will provide a deeper understanding of its health benefits.

In conclusion, the optimized BRIIP idli represents a promising addition to the functional food market, offering health benefits, convenience, and shelf stability. Continued research and development will enhance its nutritional profile and consumer appeal, making it a valuable option for health-conscious consumers seeking convenient and nutritious food choices.

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to Reproduce Material from other Sources

Not Applicable.

Author Contributions

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- **Zakarya Al-Zamani:** Data Collection, Analysis, Writing – Review & Editing.
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References

1. USDA. Production- Rice. https://www.fas.usda.gov/data/production/commodity/0422110 Accessed 26 Aug 2024.
2. Black rice market. Credence Research. https://www.credenceresearch.com/report/black-rice-market. Published 1 Nov 2023. Accessed 26 Aug 2024.
3. Kushwaha U.K.S. Black rice. 1st edition. 2016.
4. Goswami K., Thapa D. B., Sandilya J., Deka N. An assessment of economic profitability of black rice (*Oryza sativa* L. indica) production in Assam, India. *J Appl Res Med Aroma.* 2023; 34: 100488. DOI: https://doi.org/10.1016/j.jarmap.2023.100488.
5. Li D., Wang P., Luo Y., Zhao M., Chen F. Health benefits of anthocyanins and molecular mechanisms: Update from recent decade. *Crit Rev Food Sci Nutr.* 2017; 57(8): 1729-1741. DOI: https://doi.org/10.1080/10408398.2015.1030064.
6. Jeong I. H., Oh M. S., Jeon J. S., Kim H. T., Hong S. R., Park K. H., Yoon M. H. A comparative study on anthocyanin and polyphenol contents in colored agricultural products. *J Food Hyg Saf.* 2017; 32(5): 371-380. DOI: https://doi.org/10.13103/JFHS.2017.32.5.371.
7. Limtrakul P., Semmarath W., Mapoung S. Anthocyanins and proanthocyanidins in natural pigmented rice and their bioactivities. In: Rao V, Mans D, Rao L, eds. Phytochemicals in Human Health. London, UK: BoD – Books on Demand; 2019: 1-24. Accessed August 26, 2024.
8. Biswas S. K., Kim D. E., Keum Y. S., Saini R. K. Metabolite profiling and antioxidant activities of white, red, and black rice (*Oryza sativa* L.) grains. *J Food Meas Charact.* 2018; 12: 2484-2492. DOI: https://doi.org/10.1007/s11694-018-9865-6
9. Zhao F., Ren C., Li L., Huang C., Zhao Q., Zhong Y., Hu Q., Liao W., Xia H., Yang L., Wang S. Effects of germinated brown rice and germinated black rice on people with type 2 diabetes mellitus combined with dyslipidaemia. *Food Funct.* 2024. DOI: https://doi.org/10.1039/D3FO05070J.
10. Bayazid A. B., Jeong S. A., Azam S., Oh S. H., Lim B. O. Neuroprotective effects of fermented blueberry and black rice against particulate matter 2.5 μm-induced inflammation i*n vitro* and *in vivo*. *Drug Chem Toxicol.* 2024: 1-11. DOI: https://doi.org/10.1080/01480545.2024.2367559.
11. Laorodphun P., Panya A., Swe M. T., Arjinajarn P. Black rice bran extract exerts hepatoprotection via attenuating inflammation and apoptosis in obese-insulin-resistant rats. *Heliyon.* 2024; 10: 19. DOI: https://doi.org/10.1016/j.heliyon.2024.e38538.
12. Bagchi Banerjee B., Janghu S. Effect on rheological behavior and composition of wheat cookies fortified with black rice (Chakhao) flour. J Culinary Sci Technol. 2024: 1-26. DOI: https://doi.org/10.1080/15428052.2024.2416436.
13. Subanmanee N., Sarasuk C., Wongtom R., Khampaen W. Changes in the physical, chemical, and sensory properties of pasta made by partially substituting wheat flour with black glutinous rice flour. Afr J Food Agric Nutr Dev. 2024; 24(8): 24138-24160. DOI: https://doi.org/10.22004/ag.econ.348048.
14. Cheirsilp B., Mekpan W., Sae-Ear N., Billateh A., Boukaew S. Enhancing functional properties of fermented rice cake by using germinated black glutinous rice, probiotic yeast, and enzyme technology. Food Bioprocess Technol. 2023; 16(5): 1116-1127. DOI: https://doi.org/10.1007/s11947-022-02985-z.

15. Roopashri A. N., Savitha J., Divyashree M. S., Mamatha B. S., Rani K. U., Kumar A. Indian traditional fermented foods: The role of lactic acid bacteria. In: Lactobacillus - A Multifunctional Genus. IntechOpen; 2023. DOI: https://doi.org/10.5772/intechopen.110741. Accessed August 29, 2024.
16. AOAC. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists; 2000.
17. Finley J., Barrett W., Betz J. AOAC SMPR 2015.009. AOAC Int. Published 2015.
18. Jakha K. H., Goyal C., Dhyani P., Rai D. C., Saharan B. S., Dhull S. B., Duhan J. S. Characterization of Langra mango peel powder and assessment of its prebiotic and antioxidant potential. *J Food Biochem.* 2024; 1: 9026617. DOI: https://doi.org/10.1155/2024/9026617.
19. Amerine M. A., Pangborn R. M., Roessler E. B. Principles of Sensory Evaluation of Food. ; 2013.
20. Zeb A., Ullah F. A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances in fried fast foods. *J Anal Methods Chem*. 2016; 2016(1): 9412767. DOI: https://doi.org/10.1155/2016/9412767.
21. Oancea S. A review of the current knowledge of thermal stability of anthocyanins and approaches to their stabilization to heat. *Antioxidants*. 2021; 10(9): 1337. DOI: https://doi.org/10.3390/antiox10091337.
22. Sethi S, Nanda S. K., Bala M. Quality assessment of pasta enriched with anthocyanin-rich black rice bran. *J Food Process Preserv*. 2020; 44(12): e14952. DOI: https://doi.org/10.1111/jfpp.14952.
23. Bordim J., Lise C. C., Marques C., Oldoni T. C., Varela P., Mitterer-Daltoé M. L. Potential use of naturally colored antioxidants in the food industry—a study of consumers' perception and acceptance. *J Sensory Stud.* 2021; 36(4): e12657. DOI: https://doi.org/10.1111/joss.12657.
24. Ohloff G., Flament I., Pickenhagen W. Flavor chemistry. *Food Rev Int.* 1985; 1(1): 99-148. DOI: https://doi.org/10.1080/87559128509540767.
25. Yang D. S., Lee K. S., Jeong O. Y., Kim K. J., Kays S. J. Characterization of volatile aroma compounds in cooked black rice. *J Agric Food Chem.* 2008; 56(1): 235-240. DOI: https://doi.org/10.1021/jf072360c.
26. Widodo S., Hudiah A., Qur'ani B., Nurramadhan T. Acceptance of sweet bread with black rice flour as a substitute ingredient. *BIO Web Conf.* 2024; 98:04002. DOI: https://doi.org/10.1051/bioconf/20249804002.
27. Bolea C. A., Turturică M., Enachi E., Vizireanu C., Stănciuc N. Development and characterization of added value appetizer biscuits based on black rice flour. Ann Univ Dunarea de Jos Galati Fascicle VI *Food Technol.* 2021; 45(2): 48-61. DOI: https://doi.org/https://doi.org/10.35219/foodtechnology.2021.2.04.
28. Durgadevi M., Shetty PH. Effect of ingredients on sensory profile of idli. *J Food Sci Technol.* 2014; 51: 1773-1783. DOI: https://doi.org/10.1007/s13197-012-0686-z.
29. Burešová I., Červenka L., Šebestíková R., Augustová M., Jarošová A. Applicability of flours from pigmented and glutinous rice in gluten-free bread baking. *Foods*. 2023; 12(6): 1324. DOI: https://doi.org/10.3390/foods12061324.
30. Love J. Product acceptability evaluation. In: Quality Attributes and Their Measurement in Meat, Poultry, and Fish Products. *Boston,* MA: Springer US; 1994:337-358. DOI: https://doi.org/10.1007/978-1-4615-2167-9_13.
31. Hwang E. S., Tai N. D. Quality characteristics and antioxidant activities of black rice cookies with added dried laver (Porphyra tenera). *Korean J Food Cookery Sci.* 2014; 30(4): 472-479. DOI: https://doi.org/10.9724/kfcs.2014.30.4.472.
32. Rocchetti G., Chiodelli G., Giuberti G., Masoero F., Trevisan M., Lucini L. Evaluation of phenolic profile and antioxidant capacity in gluten-free flours. *Food Chem.* 2017; 228:367-373. DOI: https://doi.org/10.1016/j.foodchem.2017.01.142.
33. Bedier D. F., Salem R. H., Almashad A. A., Barakat E. H. Quality characteristics of noodles containing various levels of black rice flour. *J Food Dairy Sci.* 2020; 11(5):141-146. DOI: https://doi.org/10.21608/jfds.2020.106997.
34. Park J., Park H. Y., Kim H. S., Choi H. S., Song H, Chung H. J., Oh S. K. .Effects of

using black rice on quality and functional properties of "Dodamssal" rice noodles containing resistant starch. *J Korean Soc Food Sci Nutr*. 2021;50 (10):1040-1048. DOI: https://doi.org/10.3746/jkfn.2021.50.10.1040.

35. Abbas M., Saeed F., Anjum F. M., Afzaal M., Tufail T., Bashir M. S., Suleria H. A. R. Natural polyphenols: An overview. *Int J Food Prop.* 2017; 20 (8):1689-1699. DOI: https://doi.org/10.1080/10942912.2016.1220393.
36. Lu W. C., Chiu C. S., Chan Y. J., Li P. H. Risk assessment and characterizations of flaky rolls fortified with black rice (*Oryza sativa* L.). *Food Chem*. DOI: https://doi.org/10.21203/rs.3.rs-1637528/v2.
37. Wongsa P. Phenolic compounds and potential health benefits of pigmented rice. In: *Recent Advances in Rice Research.* Vol 4. 2020:19-21.
38. Singh R., Singh K., Nain M. S., Singh R. Storage stability of popped pearl millet-based ready-to-eat breakfast cereal. *Indian J Agric Sci.* 2020; 90 (10):1915-1920. DOI: https://doi.org/10.56093/ijas.v90i10.107902.
39. Saha R., Dunkwal V., Goyal M. Quality and sensory evaluation of value-added gatta instant mix. Food Sci Res J. 2011; 5(3):50-52.
40. Shahidi F., Hossain A. Role of lipids in food flavor generation. *Molecules.* 2022; 27(15):5014. DOI: https://doi.org/10.3390/molecules27155014.
41. Ockerman, H. W. (1985). Quality control of post-mortem muscle tissue (Vol. 3, pp. 65-70). Department of Animal Science, Ohio State University.
42. Sarkar B., Upadhyay S., Gogoi P., Das A., Hazarika M., Rahman Z., Datta A. Development and quality evaluation of instant soup mix incorporated with spent hen meat shred. *Int J Livest Res.* 2019; 7:24-30.
43. Bunkar D. S., Jha A., Mahajan A., Unnikrishnan V. S. Kinetics of changes in shelf life parameters during storage of pearl millet-based kheer mix and development of a shelf life prediction model. *J Food Sci Technol.* 2014; 51:3740-3748. DOI: https://doi.org/10.1007/s13197-012-0892-8.
44. Food Safety and Standards Authority of India. FSSAI Manual on Methods of Analysis: Microbiological Examination of Food and Water (File No. 11014/07/2021-QA). Food Safety and Standards Authority of India; May 16, 2024.
45. Chandramouli B., Latha M. M., Narendra K., Mallikarjuna K. Phytochemical and antimicrobial investigations of methanolic seed extract of black rice (*Oryza sativa* L.) mentioned in an ancient palm leaf manuscript (Talapatra). *Food Sci Res J.* 2017. DOI: https://doi.org/10.20959/wjpr20183-10797.
46. Kour K., Sood M., Bandral J. D., Gupta N., Kumari A. Physical, functional and microbiological parameters of tomato-broccoli blended instant vegetable soup mix. *Eur J Nutr Food Saf.* 2024; 16(4):102-109. DOI: https://doi.org/10.9734/ejnfs/2024/v16i41412.
47. Tulyathan V., Leeharatanaluk B. Changes in quality of rice (*Oryza sativa* L.) cv. Khao Dawk Mali 105 during storage. *J Food Biochem.* 2007; 31(3):415-425. DOI: https://doi.org/10.1111/j.1745-4514.2007.00125.x.

Highlights

1. Optimized BRIIP had 34% black rice and 66% black gram, having 0.87 desirability and 8.41 acceptability.
2. Nutritional, physicochemical and textural analysis of BRIIP/ BRIIP idli was performed.
3. DPPH inhibition TPC and TFC were 58.24%, 402.33 mg GAE/100g and 50.75 mg QE/100g, respectively.
4. Shelf-life parameters (FFA, TBARS, Sensory and Microbial load) remained within acceptable range.