



Optimization and Development of *Bacopa monnieri* Infused Nutri-cereals Pizza Base

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ABSTRACT

Background: Pizza, an Italian culinary delight, is relished by diverse groups of individuals. In this study, pizza base was innovatively crafted by substituting refined wheat flour (RWF) by combining with little millet flour (LMF) and proso millet flour (PMF) along with *Bacopa monnieri*.

Methods: A meticulous investigation was conducted to assess the impact of different quantities of RWF (20-40 g), LMF (20-40 g) and PMF (20-40 g) on the quality attributes of the pizza base, using a D-optimal design for optimization.

Result: The findings revealed that an increased proportion of refined wheat flour (RWF) enhanced the overall acceptability of the pizzabase. Further more, the hardness of the pizzabase was influenced by higher RWF quantities and reduced levels of proso millet flour (PMF) and little millet flour (LMF). Similarly, an increase in the amount of PMF led to a significant increase in both protein and antioxidant content. The optimized proportions for RWF, LMF and PMF were determined to be 40 g, 33.3334 g and 26.6666 g, respectively. This optimization yielded the following outcomes: An overall acceptability score of 8.33, an antioxidant content of 92.2727%, a pizza base texture (hardness) of 2388.88 g and a protein content of 10.3131%. These results are in concurrence with the predictions made by the significantly ($p < 0.05$) fitted quadratic models.

Key words: *Bacopa*, D-optimal design, Little millet, Pizza base, Proso millet, Refined wheat flour.

Abbreviation: ADHD: Attention-deficit/hyperactivity disorder, DPPH: 2,2-diphenylpicrylhydrazyl, LMF: Little millet flour, PMF: Proso millet flour, RSM: Response surface methodology, RWF: Refined wheat flour.

INTRODUCTION

Recent research shows that people of all ages are increasingly relying on indulgent foods like pizza and burgers, despite knowing the health risks involved. These choices are often made during special occasions, leading to a regular consumption of items like soft drinks, chips and burgers. People tend to prioritize the sensory appeal of these foods, such as taste and appearance, over considering the potential health dangers of fast food. Consuming excessive pizza causes various health risks, including excess calories, insulin resistance, high blood pressure, bloating, depression, dental issues, weight problems and high cholesterol. It can also negatively impact learning abilities, brainpower and increase the risk of non-treatable conditions like digestive problems, fatigue, weakness, cancer and kidney disease (Yadav and Kaur, 2019). The distinction among fast food, a regular meal and a homemade dish lies in the substantial amount of calories and fat they provide to the body. According to the United States Department of Agriculture's 2009 guidelines, a typical adolescent should consume around a calories of 2100 and a maximum of 93 g of fat daily. A single meal from a fast food restaurant, including a pizza, fries, a drink, burger and dessert, can nearly meet these daily limits (Bayol *et al.*, 2009).

What if processed foods are tailored to people's preferences while also enhancing their nutritional value?

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Milletts are currently undergoing a significant transformation as they are incorporated into products like biscuits, cookies and extruded items. Minor millets, such as kodo millet, foxtail millet, barnyard millet, little millet and proso millet, are recognized as "nutri-cereals" due to their richness in dietary fiber, calcium, polyphenol and protein content. These millets also possess nutraceutical properties, including antioxidants that can reduce the risk of health issues like cancer, heart disease, diabetes, high blood pressure and tumors (Bhat *et al.*, 2018). Further more, emerging patterns of micro-nutrient deficiency suggest an impending public health crisis in India, despite the implementation of various policies aimed at addressing

these issues (Goel *et al.*, 2021). Hence, an attempt has been made to develop pizzabase by partially replacing refined wheat flour using millet and incorporating water hyssop as functional ingredient.

Proso millet (*Panicum miliaceum*) is a warm-season grass with a cultivation period ranging from 60 to 100 days. This millet variety is prized for its high nutritional value, serving both as a grain for human consumption and as feed for birds (Sandhvi and Singh, 2022). It has been used to make drinks and pastries in the past. This grain is rich in nutrients and doesn't have gluten. It's different because foods made from proso millet don't raise blood sugar as much as other foods. This makes it a good choice for making foods that are healthy for blood sugar and don't contain gluten. People are interested in dietary fiber because it helps control things like blood sugar and fats, being a healthier choice (Xiao *et al.*, 2023). Little millet (*Panicum sumatrense*) serves as a valuable addition to the ever-growing array of healthful foods, attributed to its nutritional excellence. Consistent inclusion of little millet in the diet holds considerable advantages for postmenopausal women grappling with symptoms of cardiovascular ailments such as elevated blood pressure and cholesterol levels. Moreover, it harmoniously complements both traditional and innovative culinary creations, without imposing any distinct flavors of its own (Biradar *et al.*, 2021). Bacopa monnieri, an ancient traditional ayurvedic medicine in India and the key compounds in Bacopa monnieri are saponins, which play a crucial role in enhancing nerve impulse transmission. (Shankar *et al.*, 2018). To harness the benefits of Bacopa monnieri, value-

added products, including nutritional balls, puttu mix, cookies, soup mix and health drink mix have been developed using bacopa monnieri. These value-added products have the potential to enhance the cognitive abilities of children dealing with ADHD (Amaravathi *et al.*, 2020).

This study seeks to create a pizza crust by partly replacing refined wheat flour with little and proso millet. The research utilizes RSM software to find the suitable pizza base composition. The resulting pizza base was then assessed for its physical, chemical and nutritional attributes.

MATERIALS AND METHODS

The research took place in September 2023 at the Department of Food Technology, Kongu Engineering College in Erode.

Raw materials

Refined wheat flour (RWF), Little millet flour (LMF), Proso millet flour (PMF), Bacopa powder, yeast, sugar, salt, oil was procured from local stores.

Preparation of pizza base

The millet-based pizza dough preparation process involves several distinct steps. Initially, the acquired millets were roasted at a 160°C for 15 minutes. After roasting, the millets were milled to achieve a fine consistency. To activate the yeast, 3 g of sugar are dissolved in lukewarm water at a temperature of 43°C, followed by the addition of 3 g of yeast. This yeast-sugar mixture is left to incubate for 15 minutes at room temperature. Subsequently, ingredients like refined wheat flour (RWF), little millet flour (LMF), proso

Table 1: Experimental data.

Trial	Independent variables			Dependent variables			
	Refined Wheat flour	Little Millet flour	Proso Millet flour	Overall acceptability	Antioxidant	Texture	Protein
	g	g	g	Hedonic Scale	%	g	%
1	40	40	20	8.2	91.96	2645.87	13.27
2	26	37	37	6.9	94.63	4368.59	13.5
3	34	33	33	7.8	93.85	3127.25	12.56
4	40	33	27	8.4	92.66	2458.69	12.98
5	40	40	20	8.3	91.48	2358.47	13.95
6	40	27	33	8.3	93.91	2344.27	9.98
7	20	40	40	6.2	94.36	4987.21	13.57
8	37	26	37	7.2	94.86	2963.84	10.57
9	30	30	40	6.8	95.67	4158.69	11.967
10	30	30	40	7.3	95.23	3985.69	11.689
11	40	20	40	8.6	95.88	2356.78	9.667
12	40	30	30	8.7	92.15	2351.89	11.36
13	40	20	40	8.7	95.78	2345.26	9.56
14	20	40	40	6.3	95.11	4758.32	13.91
15	30	40	30	7.9	93.23	4154.21	14.15
16	30	40	30	7.7	93.47	3365.4	14.35

Note: Dependent variable values represent the mean of three repeated measurements.

millet flour (PMF) were blended as given in Table 1 along with 0.3 mg of bacopa powder. Additionally, salt is added at a ratio of 2% per 100 g of flour. The activated yeast is then introduced into this mixture, along with 10 ml of oil for every 100g of flour, ensuring thorough mixing for uniform distribution. The resulting dough is kneaded for 10 minutes to evenly distribute all components. The prepared dough undergoes a proofing process at room temperature for 2 hours, with a knocking-back step after 1.5 hours. Finally, the pizza dough is spread to the required size, docked to release any trapped gas and baked at 160°C for 15 minutes (Fig 1).

Experimental design

This study utilizes the statistical software Design-Expert (Stat-Ease, Version 13) to employ RSM for optimizing the formulation. The selection of D-optimal design for experimental trials was made due to its effectiveness in foreseeing interactions among various factors affecting dependent responses and preventing extreme conditions. The independent variables like refined wheat flour (20-40 g), little millet flour (20-40 g) and proso millet flour (20-40 g), were denoted as RWF, LMF and PMF, respectively. The dependent variables encompassed overall acceptability (rated on a Hedonic Scale), antioxidant content (%), texture (measured in hardness, g) and protein content (%).

Proximate analysis

The proximate composition was determined in accordance with standard AOAC (2005).

Antioxidant property

The DPPH scavenging activity of the pizza base were evaluated by same method as in previous study (Xiao *et al.*, 2023).

$$\text{DPPH scavenging activity (\%)} = 1 - \frac{A1-A2}{A0} \times 100\%$$

Here,

A1= Absorbance of the sample (0.5 mL sample + 2.5 mL DPPH solution),

A2= Absorbance of the control group (0.5 mL sample + 2.5 mL ethanol).

A0= Absorbance of the blank group (0.5 mL ethanol + 2.5 mL DPPH solution).

Texture

The textural attributes of the pizza base were assessed using a Brookfield CT3 texture analyser in compression mode. In this mode, 5 cm × 5 cm sections of the base were sliced and positioned on a sample holder. A double compression test was conducted at 40% of the sample's height, employing a 21 mm diameter aluminium cylindrical probe. The testing speed was configured at 1.7 mm/s. The software provided data on hardness, cohesiveness, chewiness and resilience.

Sensory analysis

A sensory analysis was carried out with a group of 30 semi-trained volunteer assessors, whose ages ranged from 20 to 50 years. These assessors judged the color, taste, aroma, texture, flavor and overall acceptability of samples that were presented to them in a random order. The samples were served at room temperature (24±1°C) and the evaluations were conducted under standard lighting conditions. To ensure accurate assessments, the panelists cleansed their palates with water between each sample. Their judgments were recorded on 9-point hedonic scale, with a rating of 1 representing the lowest score (Dislike Extremely) and a rating of 9 signifying the highest score (Like Extremely).

RESULTS AND DISCUSSION

Effect of independent variables on overall acceptability

The pizza base's overall acceptability was evaluated by considering sensory attributes such as color, taste, aroma,

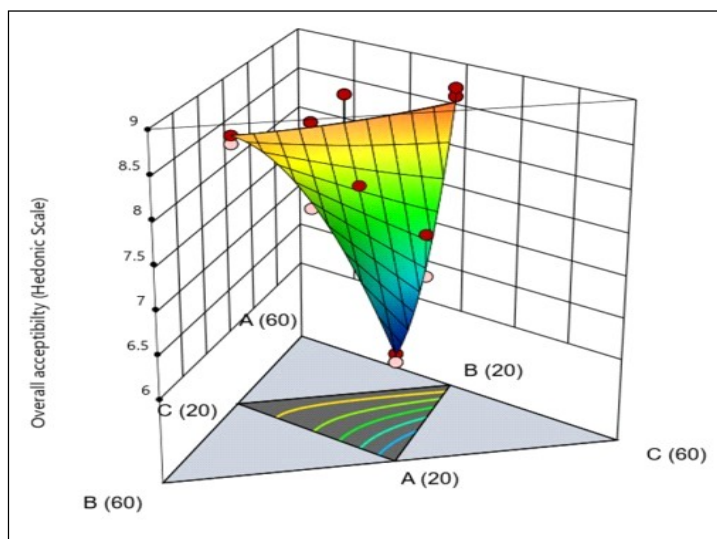


Fig 1: Effect of different composition of flour on overall acceptability.

flavor and texture. Ratings on a 9-point hedonic scale ranged from 6.2 to 8.7 as depicted in Fig 1 and these variations were influenced by changes in the independent variables (Table 1). The quadratic model was found to be highly significant, as evidenced by an F-value of 19.57 ($p < .0001$) as shown in Table 2 and there was no significant lack of fit. The model is deemed appropriate due to its high coefficient of determination (0.9073). The quadratic model that describes the relationship between the significant variables and overall acceptability in terms of coded values is expressed in the following equation:

Overall acceptability =

$$10.4515 * A + 10.2033 * B + 2.73613 * C - 8.11191 * AB + 7.73057 * AC - 0.736872 * BC$$

In the depicted figure, it's evident that RWF is the primary influencing factor on overall acceptability, with LMF

and PMF flours following in importance. The data illustrates that as the quantity of refined wheat flour increases and millet flours decrease, the overall acceptability of the pizza base increases, reaching a maximum limit. This observation is consistent with findings from prior research (Biradar *et al.*, 2021). Conversely, the decline in overall acceptability with a higher proportion of millets can be attributed to an increase in the hardness of the base and the emergence of a bitter sensation associated with millets.

Effect of independent variables on antioxidant

The capacity to scavenge DPPH in the pizza base ranged from 91.48% to 95.88% and these variances were impacted by alterations in the independent variables, as detailed in Table 1. The quadratic model was determined to be remarkably significant, possessing an F-value of 44.42 ($p < .0001$), with a lack of fit that was not considered significant as shown in Table 2. The model is deemed

Table 2: Analysis of variance for second-order polynomial model of dependent variables.

Source	F value and fit statistics			
	Overall acceptability	Antioxidant (%)	Texture (Hardness,g)	Protein (%)
Model	19.57*	44.42*	60.17*	49.41*
Linear mixture	44.79*	104.62*	147.07*	120.10*
AB	4.02	0.5156	6.11	0.8393
AC	3.59	0.2956	0.0260	0.2218
BC	0.0366	10.99	0.2691	5.76
Lack of fit	5.02	1.46	0.1306	2.11
Coefficient of determination (R^2)	0.9073	0.9569	0.9678	0.9611
Adjusted R^2	0.8609	0.9354	0.9517	0.9416
Predicted R^2	0.8214	0.8829	0.9010	0.9021
Standard deviation	0.3103	0.3603	210.96	0.3383
Coefficient of variance (%)	4.03	0.3833	6.40	2.82

Notes: *Significant with 5% level of significance; A- Refined Wheat flour; B- Little millet flour; C- Proso millet flour; AB- interaction between RWF and LMF; AC- Interaction of RWF and PMF; BC- Interaction between LMF and PMF.

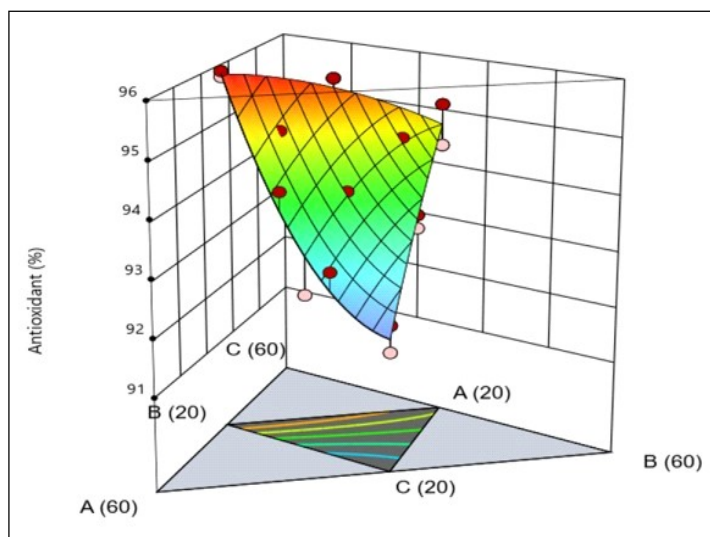


Fig 2: Effect of different composition of flour on antioxidant.

appropriate due to its elevated coefficient of determination (0.9073). The quadratic model, delineating the relationship between the pivotal variables and antioxidants in terms of coded values, is articulated in the subsequent equation:

Antioxidant =

$$87.6341 * A + 94.1213 * B + 102.776 * C + 3.37244 * AB + 2.57498 * AC - 14.8314 * BC$$

The F-value analysis suggests that PMF is the predominant factor influencing the antioxidant property, while RWF shows no significant impact as shown in the Fig 2. Previous research has consistently demonstrated that polyphenols exhibit remarkable biological activity and antioxidant capabilities. Nevertheless, the typical decline in bioactivity observed in the baking of fortified foods primarily results from the thermal degradation of these functional components (Li *et al.*, 2022). Antioxidant reduces

with decrease in quantity of PMF and a increase in the ratio of LMF. This suggests that augmenting PMF can enhance the pizza's antioxidant capacity. Similar results was reported by (Xiao *et al.*, 2023) in which cake prepared using proso millet flour fermented with dietary fiber had increased antioxidant activity followed by cake made using proso millet flour. These results showed PF-based cakes had improved the antioxidant activity of the cakes. These findings imply that including PMF in pizza may contribute to the protection against oxidative stress and may play a preventive role in chronic diseases.

Effect of independent variables on hardness

The pizzabase hardness exhibited a range of fluctuations, ranging from 2344.27 g to 4987.21 g and these fluctuations were influenced by variations in the independent variables as presented in Table 1. The quadratic model was

Table 3: Physio-chemical analysis of optimized composition.

Parameters	Control	Optimized sample
Moisture (%)	10.3	10.56
Ash (%)	1.23	1.96
Carbohydrate (%)	74.45	71.23
Protein (%)	7.3	10.3131
Crude fiber (%)	0.3	1.26
Fat (%)	5.70	4.56
Energy value	378.3 kcal	367.2124 kcal
Texture	Hardness (g)	2109
	Cohesiveness (%)	0.75
	Chewiness (g)	1685
	Resilience (%)	0.32
Colour	L*=85.23	L*=56.32
	a*=-1.23	a*=7.23
	b*=16.36	b*=9.78
Antioxidant (%)	23.56	92.2727
Sensory analysis	8.7	8.3

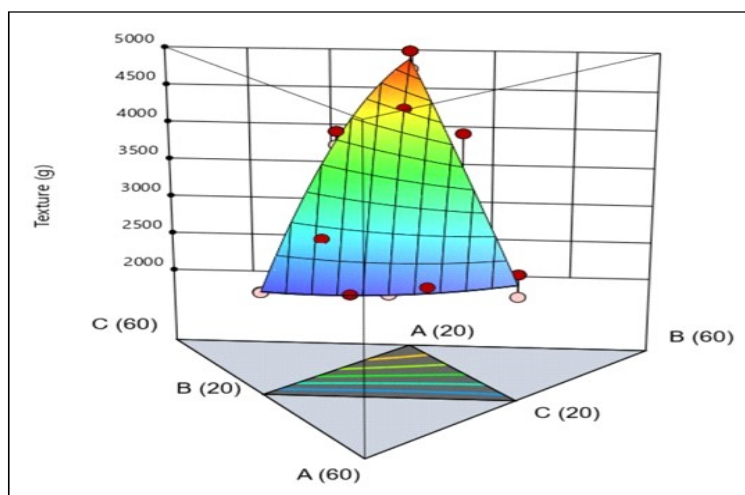


Fig 3: Effect of different composition of flour on texture (Hardness).

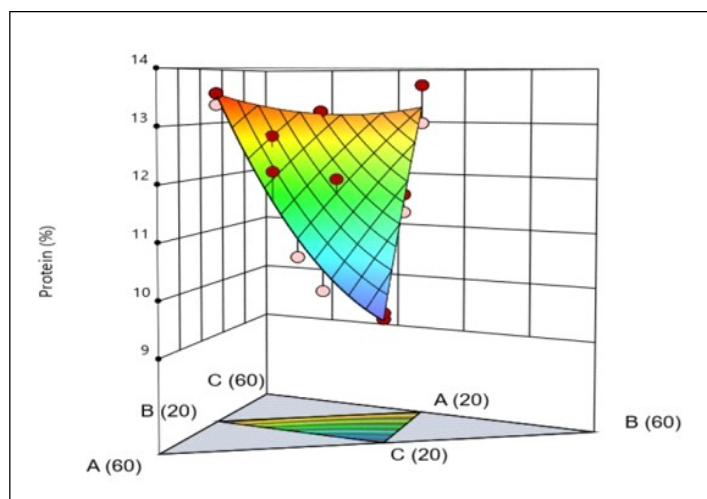


Fig 4: Effect of different composition of flour on protein.

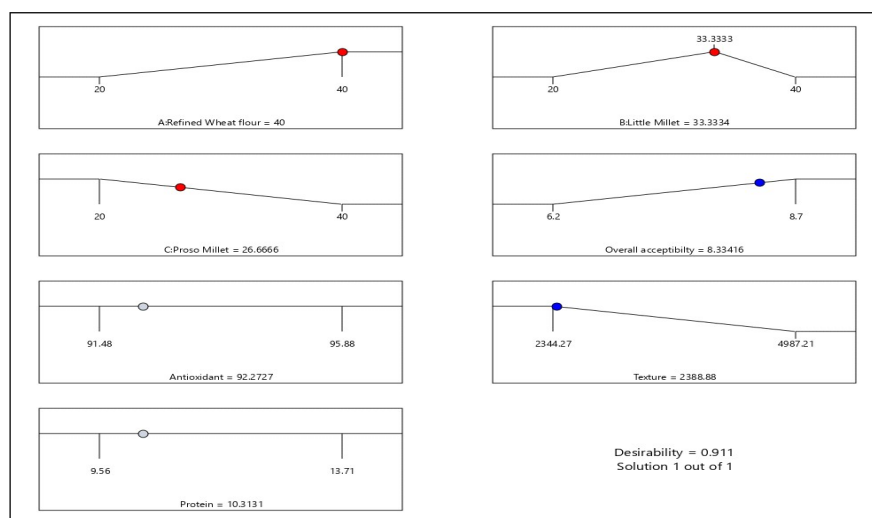


Fig 5: Optimized composition.

determined to be highly significant, with an F-value of 60.17 ($p < .0001$) and the lack of fit was deemed not significant. The model is considered appropriate due to its substantial coefficient of determination (0.9678) as shown in Table 2. The quadratic model, which describes the connection between the significant variables and antioxidants in coded values, is expressed by the following equation:

Texture =

$$-2158.51 * A + 3789.78 * B + 6662.27 * C + 6796.42 * AB + 446.896 * AC - 1358.66 * BC$$

RMF emerges as the primary factor influencing the firmness of the pizza base, with LMF and PMF following in significance as shown in Fig 3. Firmness is chiefly associated with density and chewiness reflects the internal resistance of the food structure, both of which are notably essential indicators for assessing the quality of flour-based products, as highlighted in the study by (Li *et al.*, 2020) As the proportion of RMF increases, there is a reduction in the

pizza base's firmness. Conversely, an increase in the presence of millet results in heightened firmness of the pizza base. The correlation between increased firmness and elevated chewiness in the product is primarily due to the positive relationship between firmness and chewiness, as noted by (Xiao *et al.*, 2023). The increase in firmness can be attributed to millet's lack of gluten, which leads to reduced elasticity and structural formation, in accordance with the findings of (Upadhyaya *et al.*, 2016). When mixing dough, gluten plays a crucial role in creating a structure that traps carbon dioxide produced during fermentation. When gluten-free flours replace wheat flour, the reduced gluten content results in a weaker dough network, leading to denser and harder pizzabase compared to pizzabase made with gluten-containing flour, as shown in a study by (Mannuramath *et al.*, 2015). Further more, the fiber content in LMF and PMF can interact with starch through its water-binding and embedding capacity. This interaction may delay the aging process of starch and assist in preserving the

textural characteristics of flour-based products. The composition involving a 40% replacement exhibited favorable texture, aligning with the conclusions of (Mastrascusa *et al.*, 2021).

Effect of independent variables on protein

The protein content in the pizza base exhibited a range of fluctuations, ranging from 9.56% to 13.71% and these changes were impacted by alterations in the independent variables (as outlined in Table 1). The quadratic model was determined to be remarkably significant, boasting an F-value of 39.87 ($p < .0001$) and there was no notable lack of fit as shown in Table 2. This model is deemed appropriate due to its elevated coefficient of determination (0.9522). The quadratic model that delineates the association between the significant variables and antioxidant content in terms of coded values is expressed in the following equation:

Protein=

$$7.76469 * A + 13.3239 * B + 18.2907 * C + -4.04048 * AB + 2.09445 * AC + -10.0821 * BC$$

The analysis of the F value indicates that protein content is predominantly influenced by PMF, followed by LMF and RWF as shown in Fig 4. A reduction in the millet content of PMF corresponds to a noticeable decrease in protein levels. Notably, LMF exhibited significantly lower protein content when compared to PMF. (Mannuramath, Yenagi *et al.*, 2015). The increased contribution of PMF can be attributed to variations in nitrogen content and Proso millet proteins are characterized by their high amino acid content, which makes them valuable resources for the food industry in the development of emerging gluten-free protein sources (Kumar *et al.*, 2020; Wang *et al.*, 2021).

Optimized composition

Using the design expert software, the desirability of 0.911 was obtained. The preferred ration were the desirability was maximum was RWF: 40 g, LMF: 33.3334 g, PMF: 26.6666 g which is being depicted in Fig 5. The optimized composition was subjected to further physio-chemical analysis whose results were shown in Table 3.

CONCLUSION

Utilizing nutriceals and incorporating bacopa into pizza base preparation can transform it into a functional food, offering a plethora of health benefits. It was observed that increasing the quantity of refined wheat flour (RWF) improved overall acceptability. The hardness of the pizza base was influenced by higher RWF amounts and reduced levels of proso millet flour (PMF) and little millet flour (LMF). Similarly, an raise in PMF led to a rise in protein and antioxidant content. The optimized proportions of RWF, LMF and PMF were determined to be 40 g, 33.3334 g and 26.6666 g, respectively. This optimization resulted in the following responses: overall acceptability (8.33), antioxidant

content (92.2727%), texture (hardness) (2388.88 g) and protein content (10.3131%). Consequently, incorporating underutilized minor millets like little millet and proso millet, along with functional ingredients like bacopa powder, not only enhances the functional properties of the pizza base but also augments its nutritional qualities. Consequently, these underutilized millets can serve as valuable ingredients in the food industry for creating innovative products, such as pizza bases.

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Conflict of interest

All authors declared that there is no conflict of interest.

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