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Optimization of functional wheat-based bread formulation using mixture response surface methodology

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Abstract

Bread is a commonly consumed staple and its formulation with functional ingredients such as dietary fiber and phenolic antioxidants could enhance its antioxidant potentials. Functional bread was formulated from composite flours of wheat (WHF), malted millet (MMF) and okara (SYF). The study was carried out using mixture design approach as an optimization technique. A simplex-centroid mixture design was used to generate fourteen (14) experimental runs based on different combinations of wheat (70-90%), malted millet (5-45%) and okara (5-45%) flours. The composite flours were processed into bread and analyzed for sensory qualities and antioxidant properties (radical scavenging activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH), total flavonoid (TFC) and total phenolic (TPC) contents. Contour plots were used to relate responses, i.e. appearance, crust color, taste, texture, and overall acceptability of bread, as well as antioxidant properties of the developed bread from different proportions of the flours. Results showed that the optimum flour combination that produced higher antioxidant properties and acceptability was 65.18% WHF, 19.43% MMF, and 15.39% SYF with a desirability function of 0.61. Wheat flour significantly ($p \leq 0.05$) influenced the sensory characteristics while the increased proportion of malted millet and okara flour significantly ($p \leq 0.05$) increased the total antioxidant properties of the bread. This study showed the potential of wheat, malted millet, and okara flour blends in the development of functional bread with acceptable sensorial qualities, and enhanced antioxidant properties.

1. Introduction

Bread is one of the baking yeast-leavened products that is accepted as a very convenient form of food greatly desirable to all population groups. It is one of the ancient and widely acceptable staples in all parts of the world (Valavanidis, 2018). From time immemorial, wheat flour is reported as the principal flour in bread making due to its utmost baking quality compared to other cereals because of the quality and quantity of its characteristic wheat protein called gluten (Goel *et al*., 2021). However, bread can be made from either refined wheat flour which is known as white flour, or wholemeal wheat flour but the most commonly consumed bread in Nigeria is white bread and it is characterized by low nutritional value and antioxidant potential. Consequently, the use of composite flour, in which a portion of wheat flour is substituted by locally grown crops such as cereals, legumes, and root and tubers for improving the nutritional quality of bread and making functional bread has been explicitly elucidated by several researchers (Oluwole *et al.,* 2017; Bolarinwa *et al.,* 2019; Ivanišová *et al.,* 2023).

In recent times, the growing consumers' demand for functional food products has been linked with the increased incidence of non-communicable diseases and diet-related health problems, and functional food could serve as a dietary intervention approach to prevent/ reduce the risk of these diseases. In view of this, the development of functional bread has attracted research interests using different biological components that provide additional health benefits beyond basic nutritional functions. Studies have shown that natural plant foods and food processing wastes are being incorporated into bread owing to their inherent bioactive components such as dietary fiber and phenolic antioxidants (Ibidapo *et al.*, 2015; Odunlade *et al.,* 2017; Wang *et al.*, 2022). Epidemiology studies on human health have underlined the beneficial roles of phenolic

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compounds in the prevention of chronic diseases as well as contributing to antioxidant activities (Taylor and Duodu, 2015; Cao *et al.*, 2019). Antioxidant activity is one of the key indicators of the health beneficial effects of food materials in the prevention and reduction of risk of diseases. Moreover, increased consumption of dietary fiber have been implicated with reduced risk of incidence of certain forms of cancer and the development of diabetes mellitus (Zhang *et al.,* 2018). Similarly, dietary fiber increases fecal bulk, improves the bowel transit of feces and the feeling of satiety, reduces blood cholesterol levels, and prevents colon cancer, obesity (He *et al.,* 2022). Consequently, the development of wheat-based functional bread supplemented with fruits and vegetables flour/ powder to improve the phenolic content and antioxidant property without having adverse effect on its sensory acceptability has been exploited (Msaddak Lotf *et al.,* 2017; Xu *et al.*, 2019; Bolarinwa *et al.,* 2019). The study conducted by Tolve *et al.* (2021) also confirmed that the development of wheat bread in achieving functional food properties does not only involve delivering the active components at an appropriate level for physiological benefits but also a product satisfying consumers' requirement in terms of overall acceptability.

Millet (*Pennisetum glaucum*) is one of the most drought-tolerant grains with a short-growing period compared to other major cereals such as rice and maize. It is a small-seeded crop with several varieties including pearl millet, foxtail millet, and finger millet. It is mainly cultivated as a traditional staple food grain and animal feed, biomass in over 40 countries in Africa and Asia (Singh and Sarita, 2016). Currently, the global millet production statistics hits about 30.73 million tons, with India contributing about 11.42 million tons (37%), securing its first position as the world leading producer (Kaur *et al.,* 2024). Scientific reports confirmed that millet contains remarkable amounts of bioactive components such as dietary fiber, minerals, vitamins, phytochemicals, polyphenols, and flavonoids, which help to promote human health and wellness, making it useful in functional foods formulation (Chandra *et al.,* 2016; Kaur *et al.,* 2024). However, some of the minerals are not readily bioavailable due to inherent antinutritional factors. Appropriate food processing techniques such as malting, germination, fermentation and soaking have been reported to improve the bioavailability of these nutrients. Malting is one of the processing techniques used to reduce antinutritional factors and improve the nutritional and organoleptic quality of foods. It is defined as limited controlled germination of grains in moist conditions. Najdi Hejazi *et al,* (2016) reported that malting significantly reduces the anti-nutrient contents of cereal grains and improve their nutrients' bio-availabilities. There have been numerous studies by previous researchers on the use of millet/malted millet in bread, cookies, complementary foods, fermented products to mention a few (Awolu *et al.,* 2017; Das *et al.,* 2019).

Okara or soy residue is a by-product obtained during the processing of soymilk. This insoluble wet soymilk by -product is regarded as waste used for landfills or as feed ingredients. However, it is an excellent source of high-quality protein, dietary fiber, and bioactive compounds such as soy saponin and isoflavones, a class of phytoestrogen phytochemical, with high antioxidant activity (Davy *et al.,* 2022). Previous studies had shown that substitution of both wet and dry okara in wheat flour for biscuit, cookies and bread production could serve as a means of increasing the dietary fiber content (Fei *et al*., 2013; Davy *et al.,* 2022). Therefore, it is expected that the combination of these flours with significant health-promoting benefits, would result in the production of functional bread with acceptable sensory quality.

Sensory evaluation is an important criterion for the quality assessment of food products using human sensory organs and acceptance of food products. Mixture experiment design is a statistical technique that can be used for evaluating the preference and acceptance of final product based on the relative proportions of the components in the mixture and consequently selecting the best components proportion through predictive equations that allow the application of mathematical algorithms, thereby establishing the optimal conditions for food formulation (Myers *et al.,* 1995; Ogundele *et al.,* 2016). Simplex-centroid design can be applied as a standard tool to find the optimal response for any mixture of the ingredients, and to obtain the influence on the response of each ingredient singly and in combination with other ingredients.

Therefore, this study aimed at formulating a functional bread with an optimized mixture ratio of wheat, malted millet, and soy residue okara flours having appreciable antioxidant properties and acceptable sensorial qualities.

2. Material and methods

2.1 Materials

The refined wheat flour (*Triticum aestivum*) milled by Golden Penny Flour Mills Nigeria, soybean (*Glycine max*), and millet (*Pennisetum glaucum*) were purchased from a local market in Lagos. All the chemicals used in this study were of analytical grade.

2.1.1 Malted millet preparation

Cleaned and sorted millet grains (*Pennisetum glaucum*) were malted for 48 h following the processing steps described by Adebiyi *et al.* (2018). The malted grains were washed and dried in a 10-

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tray electrically heated drying cabinet (Mitchell Dryers Ltd. Denton Holmes Carlisle, CA2 SDU, England) at 60°C for 5-6 h. The dried malts were devegetated and milled to obtain fine malted flour and packaged in a low-density polyethylene bag until further use.

2.1.2 Okara flour preparation

Okara is the pulpy insoluble residue obtained during soymilk processing. This wet soymilk residue is allowed to dry in a tray electrically heated drying cabinet at a temperature of 60°C for 12 h. The dried okara was thereafter milled into fine powder. Okara flour was then packaged hermetically in polyethylene film for subsequent use.

2.2 Experimental design

The Design-Expert 6.0.8 version was used to determine the optimum proportion of functional bread formulation. A simplex-centroid mixture design was used to obtain a different combination of the main flour components (wheat flour (X_1), malted millet flour (X_2), and okara flour (X_3) with the sum of the component proportion of 100%. The flour mixture components proportions were subject to constraints as follows; 50 < wheat flour < 90, 5 < malted millet < 45, and 5 < okara < 45. A total of 14 experimental runs with four replicates were generated as shown in Table 1. The flours were thoroughly mixed to obtain a homogenous blend which was consequently used to produce fourteen (14) bread samples. The studied responses were sensory attributes such as appearance, crust color, taste, texture, and overall acceptability as well as the antioxidant properties: dietary fiber, radical scavenging activity by 1,1-diphenyl -2-picrylhydrazyl (DPPH), total flavonoid, and total phenolic contents.

Mixture experimental design assumes Equation 1,

$$Yi = \beta_1X_1+ \beta_2X_2+ \beta_3X_3+ \beta_{12}X_1X_2+ \beta_{13}X_1X_3+ \beta_{23}X_2X_3 + \beta ijXij^2 \text{ ---------- (1)}$$

Where Yi is the response, the β symbols represent coefficient to be fitted via regression analysis, x_1, x_1x_2, xij^2 are the linear, interaction and quadratic effect of the components, respectively. A simplex-centroid design consists of all points that are equally weighted mixtures of 1 to q components.

For the formulation optimization, the optimal bread analysis was done using numerical and graphical optimization techniques. This technique searches the design space, using the models created during analysis to find factor settings that meet the defined goals. To find out an effective solution, a multiple response method called desirability was applied Myers *et al.* (1995). The numerical criteria were set to maximize the values for all the sensory and antioxidant properties except IC_{50} DPPH which was set at a minimum. The graphical optimization was done by generating the contour diagrams for each of the sensory and antioxidant parameters studied to determine the optimum formulation points of wheat, malted millet, and okara flour. The fitted models for all parameters were generated to develop ternary contour plots to display the effects of the ingredients on the studied properties.

2.3 Production of bread

The bread was made using the straight dough method as described by FIIRO (2008). The recipe used for bread production includes composite flour (100%), water (57-65%), fat (3%), sugar (12%), salt (1.8%) and yeast (0.5%). All the ingredients were added to the composite flours and mixed in a mixer (VM1 Multipurpose Mixer Z.1.B.P.9- 85601 Montaigu Cedex (France) Type BM 20) for 5 min then water was added and mixed for another 10 min to form smooth, non-stick, and elastic dough. The dough obtained was then kneaded and scaled into uniform sizes (300 g), placed in a lightly greased pan, and proofed in the proving chamber at 30°C and 80% RH after transferred into a heated oven (Deck oven) and baked at a temperature of 220°C for 30 min.

Table 1. Mean response values of the sensory and antioxidant properties of functional bread.

Run	Wheat flour (X1)	Millet flour (X2)	Okara (X_3)	APP	CRC	TAT	TEX	OAP	DF (%)	IC_{50} DPPH (µg/mL)	TFC (mg/g)	TPC (mg/g)
1	56.67	11.67	31.67	6.17	5.92	5.83	6.42	6.33	13.66	59.12	46.64	32.02
2	50.00	25.00	25.00	5.33	4.73	5.67	5.93	5.67	9.95	54.04	42.01	39.82
3	63.33	18.33	18.33	6.40	6.67	5.98	5.20	6.73	9.22	59.03	46.01	33.75
4	50.00	5.00	45.00	4.92	4.83	4.33	4.50	4.92	12.34	65.62	46.51	24.36
5	70.00	25.00	5.00	5.93	6.00	6.40	5.87	7.24	8.89	57.43	13.49	34.59
6	50.00	25.00	25.00	5.47	4.87	5.60	5.40	6.27	10.75	55.93	39.42	35.49
7	90.00	5.00	5.00	7.67	7.58	7.17	7.25	7.50	6.14	65.96	13.72	20.61
8	50.00	45.00	5.00	5.67	5.40	6.27	6.07	6.40	8.24	53.19	14.58	41.14
9	90.00	5.00	5.00	7.54	7.41	7.20	7.13	7.33	6.84	73.64	13.88	19.82
10	50.00	5.00	45.00	4.07	4.00	4.07	4.53	5.40	11.64	73.31	50.37	24.87
11	70.00	5.00	25.00	6.87	6.60	5.07	6.33	5.47	11.56	74.52	28.22	24.21
12	56.67	31.67	11.67	4.81	4.94	5.27	6.20	5.60	12.85	52.63	31.63	42.74
13	50.00	45.00	5.00	5.82	5.20	5.60	5.40	6.33	10.16	57.08	26.21	37.56
14	76.67	11.67	11.67	6.4	5.90	6.20	6.27	6.07	9.62	62.73	27.67	28.41

Appearance, CRC-Crust colour, TAT-Taste, TEX-Texture, OAP-Overall acceptability, DF-Dietary fiber, DPPH- Radical scavenging activity, TFC-Total flavonoid content, TPC-Total phenolic content

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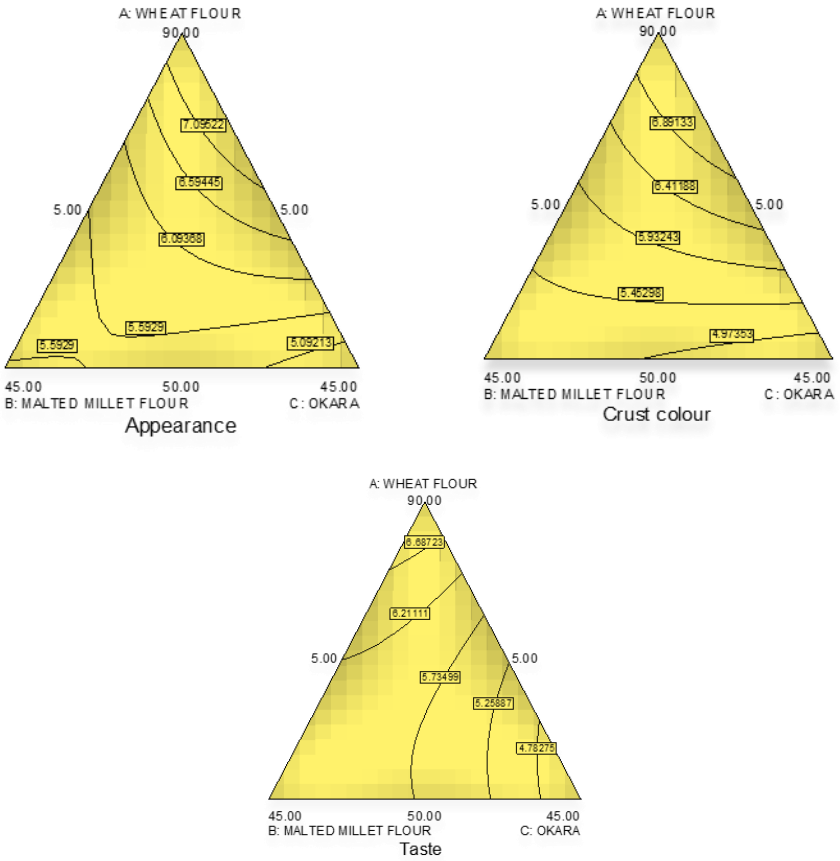

Figure 1. Contour plots showing the effect of mixture components of wheat flour, malted millet flour, and okara on the appearance, crust colour and taste of bread sample.

The bread loaves are left to cool to room temperature (32 ± 2°C) before packaging for further analysis.

2.4 Sensory evaluation of bread

Sensory evaluation was conducted to assess the consumer acceptability of the sample. Fifty (50) untrained panelists with ages between 20 and 45 years were recruited from staff and students of the Federal Institute of Industrial Research Oshodi, Lagos. Panelists were instructed to evaluate the appearance, color, taste, texture, flavor, and overall acceptability of the bread using a 9-point hedonic scale of 9 (Extremely acceptable) to 1 (extremely unacceptable) (Ihekoronye and Ngoddy, 1985).

2.5 Antioxidant properties

2.5.1 Dietary fiber (DF) determination

The enzymatic-gravimetric procedure of AOAC 999.43 described by Ibidapo *et al.* (2023) was adopted to determine the total dietary fiber fraction of the bread sample. The total dietary fiber was calculated as the weight of the non-digestible residue minus the weight of the protein and ash (protein + ash), and the result was reported as % of the original sample weight.

2.5.2 Radical scavenging activity assay using 1, 1-diphenyl-2-picrylhydrazyl (DPPH)

The DPPH radical scavenging activity of the bread samples was assessed according to a slightly modified method described by Ojewunmi *et al.* (2013). Different concentrations (20, 40, 60, 80 and 100 µg/mL) of the aqueous extract of the sample were mixed with a methanolic solution of DPPH and made up to 1 ml with distilled water. The mixture was vortex and kept at 25°C for 30 mins after which the absorbance was read against the blank sample at 517 nm on a UV- visible spectrophotometer. The result was expressed as a percentage inhibition of the DPPH radical. However, the antioxidant activity of the test sample was expressed as $IC_{50,}$ which is defined as an effective concentration of the sample required to inhibit DPPH radical formation by 50%. This implies that the higher the antioxidant activity, the lower the IC_{50}. It was calculated using linear regression analysis using the concentration-absorbance curve. Ascorbic acid was used as standard.

$$\% \text{ Inhibition of DPPH} = \text{Abs}_{\text{blank}} - \text{Abs}_{\text{extract}} \div \text{Abs}_{\text{blank}} \times 100$$

2.5.3 Total flavonoid content (TFC)

This was determined by the aluminum chloride method described by Chang *et al.* (2002). The absorbance of the reaction mixture was read at 415 nm on a UV- visible spectrophotometer and the total flavonoid content was calculated as ascorbic acid

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Table 2. Regression coefficients and analysis of variance (ANOVA) of models for sensory characteristics.

Coefficients	Sensory characteristics				
	Appearance	Crust colour	Taste	Texture	Overall acceptability
A-Wheat flour	7.56*	7.36*	7.16*	7.15*	7.33*
B-Malted millet flour	5.63*	5.23*	5.82*	5.79*	6.24*
C-Okara flour	4.59*	4.49*	4.31*	4.63*	5.28*
AB	-3.83	-1.80	-0.95	-2.94	0.67
AC	3.69	3.29	-1.45	1.75	-2.52
BC	1.13	0.30	2.51	2.19	1.09
Significance of the model (p)	0.002	0.004	0.002	0.014	0.039
Lack of fit of the model	0.145^{ns}	0.08^{ns}	0.09^{ns}	0.10^{ns}	0.05^{ns}
R^2	0.88	0.85	0.87	0.79	0.72

A, B, C correspond to the effects of wheat flour, malted millet flour, and okara flour respectively, in the model; ns significant effect at level p≤0.05; *Significant at the level of p≤0.05

equivalent (mg/g) using the equation based on the calibration curve.

2.5.4 Total phenolic content (TPC)

This was determined using the Folin-Ciocalteu reagent as described by Rani *et al.* (2019). The absorbance of the reaction mixture was read using a UV-visible spectrophotometer at 760 nm. Total phenolic content was standardized against gallic acid solution and expressed as gallic acid equivalent (GAE/100 g).

2.6 Statistical analysis

Data obtained from each run were statistically regressed and analyzed for variance using IBM SPSS Statistics 20 to generate estimated regression coefficients and coefficient of determination (R^2). Statistical significance was accepted at 5% probability levels.

3. Results and discussion

3.1 Response surface regression of effects of composite flours on the sensory properties

The appearance scores for varying formulations of the bread samples ranged from 4.07 to 7.67 as shown in Table 1. The bread sample from the blend of WHF 90.00: MMF 5.00: SYF 5.00 had the highest score while the blend with WHF 50.00: MMF 5.00: SYF 45.00 had the least sensory score. The least score obtained is probably attributed to the dilution of the gluten network of the resultant bread that consequently affected the choice of the appearance score. The regression coefficient and analysis of variance (ANOVA) of the sensory characteristics was depicted in Table 2. The ANOVA showed that the model (quadratic) and the model terms significantly ($p \leq 0.05$) influence the appearance. The contour plot revealed that appearance score increased with increasing levels of wheat flour as displayed in Figure 1. The proportion of wheat flour (Table 2) significantly influenced the appearance score. The regression coefficient (R^2) of 0.8783 obtained buttressed the assertion.

$$Appearance = 7.56A + 5.63B + 4.59C - 3.83AB + 3.69AC + 1.13BC$$

…….. *Eq 2*

The crust color scores as presented in Table 1 ranged from 4.00-7.58 following a similar trend with the appearance score having the highest score in the composite flour of WHF 90.00: MMF 5.00: SYF 5.00 and least score in the blend containing WHF 50.00: MMF 5.00: SYF 45.00. An increase in wheat flour proportion influenced the crust color score whereas an increase in okara flour proportion resulted in a decreased score (Figure 1). This result was in agreement with the findings of Akinjayeju and Adekoya (2018) who reported the least color score in composite bread with wheat (75%), soy meal (20%) and finger millet flour (5%). The ANOVA showed that the model (quadratic) and the linear mixture components are significant model terms that exhibited significant ($p \leq 0.05$) influence on the crust colour of the bread. The coefficient of determination (R^2) was 0.85. There was an interaction effect between malted millet flour and wheat flour.

$$Crust\ colour = 7.36A + 5.23B + 4.49C - 1.80AB + 3.29AC + 0.30BC$$

…….. *Eq 3*

The mean hedonic score for taste perception of the developed bread ranged from 4.07 to 7.17 with the highest score in the composite flour of WHF 90.00: MMF 5.00: SYF 5.00 and least score in the blend containing WHF 50.00: MMF 5.00: SYF 45.00. The contour presented in Figure 1 implied that the taste perception score increased with the increasing level of wheat and malted flour. The ANOVA for the taste perception score revealed that the model (quadratic) is significant ($p<0.05$) and the linear mixture components are significant ($p<0.05$) model terms. The coefficient of determination (R^2) obtained was 0.87 showing that the model was good.

$$Taste = 7.16A + 5.82B + 4.31C - 0.95AB - 1.45AC + 2.51BC$$

…… *Eq 4*

The mean hedonic score for texture exhibited by the bread samples ranged from 4.50 to 7.25. The highest scores were obtained from the composite flour of WHF 90.00: MMF 5.00: SYF 5.00 and the least score in the blend containing WHF 50.00: MMF 5.00: SYF 45.00. The contour plot (Figure 1) showed that increased wheat

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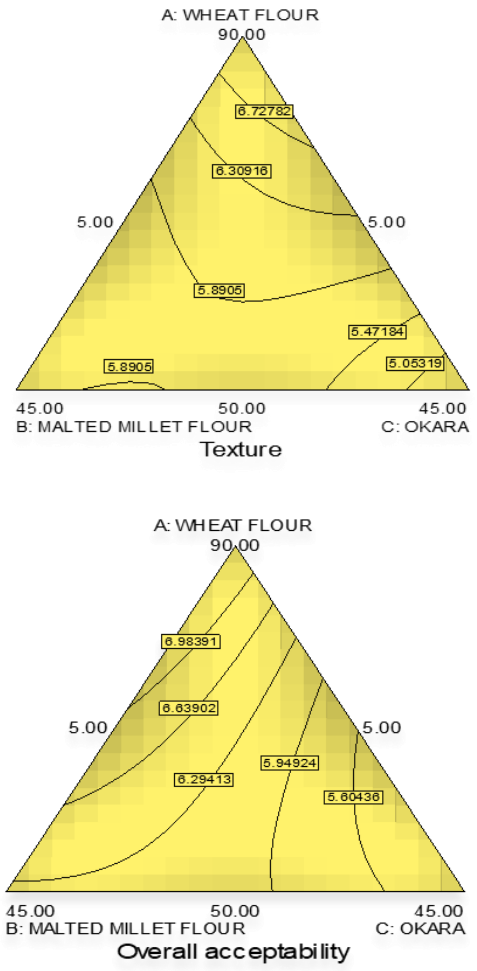

Figure 2. Contour plots showing the effect of mixture components of wheat flour, malted millet flour and okara on the texture and overall acceptability of bread sample .

flour proportion resulted in better texture composite bread. The low texture score obtained might be due to the high supplementation of *okara* flour, which contributed to a reduction in the elastic property of wheat gluten and consequently less carbon dioxide gas retention in the baked dough (Oluwole *et al.,* 2017). This result agrees with the observations of Akinjayeju and Adekoya (2018) that increased fiber content of soy meal flour and whole millet resulted in the hard crumb texture of the bread. Davy *et al.* (2022) and Porcel *et al.* (2017) also reported similar dilution effects of non-wheat flour. The ANOVA for the texture score of the bread (Table 2) revealed that the model (quadratic) is significant ($p \leq 0.05$) and the linear mixture components are significant model terms with a coefficient of determination (R^2) of 0.79, indicating that the model is fit.

$$Texture = 7.15A + 5.79B + 4.63C - 2.94AB + 1.75AC + 2.19BC$$

…… *Eq 5*

The mean hedonic score for overall acceptability ranged from 4.92 to 7.33. The highest score was obtained from the composite flour of WHF 90.00: MMF 5.00: SYF 5.00 and the least score in the blend containing WHF 50.00: MMF 5.00: SYF 45.00. The trend of overall acceptability scores of the developed bread as a function of the mixture proportion is presented in the ternary mixture plot (Figure 2). The acceptability score increased with an increased proportion of wheat flour whereas the increasing proportion of okara flour resulted in a decreased acceptability score.

$$Overall\ acceptability = 7.33A + 6.24B + 5.28C + 0.71AB - 2.67AC + 1.12BC$$

…… *Eq 6*

Previous studies involving okara in bread and noodle -making have also reported low ratings for sensory attributes as evaluated by Porcel *et al.* (2017).

ANOVA for the overall acceptability score of the bread revealed that the model (quadratic) was significant ($p \leq 0.05$) and the linear mixture components are significant ($p \leq 0.05$) model terms (Table 2). The coefficient of determination (R2) of the model predicting the overall acceptability of the bread was 0.72, indicating 72% predictive accuracy.

Table 3. Regression coefficients and analysis of variance (ANOVA) of models for antioxidant properties.

Coefficients	Antioxidant properties			
	Dietary fibre	IC_{50} DPPH radical scavenging activity	Total flavonoid content	Total phenolic content
A- wheat flour	6.37*	69.90*	13.94*	20.02*
B-malted millet flour	9.49*	55.37*	20.17*	39.69*
C- *okara* flour	12.14*	69.13*	48.33*	24.61*
AB	5.74	-27.23	-14.93	23.58*
AC	9.97	9.06	-11.45	9.52
BC	0.17	-34.02*	24.52	25.12*
ABC	-	-	474.00*	-
Significance of the model (p)	0.03	0.003	0.0001	0.0001
Lack of fit of the model	0.07^{ns}	0.73^{ns}	0.95^{ns}	0.53^{ns}
R^2	0.74	0.87	0.97	0.96

A, B, C correspond to the effects of wheat flour, malted millet flour and *okara* flour respectively, in the model; ^{ns}no significant effect at level $p \leq 0.05$; *Significant at the level of $p \leq 0.05$

3.2 Response surface regression of effects of composite flours on the antioxidant properties

3.2.1 Dietary fiber

Table 1 shows that dietary fiber content for the bread samples ranged from 6.14 to 13.66% with a blend of WHF 56.67: MMF 11.67: SYF 31.67 having the highest value and the lowest value obtained from the blend of WHF 90.00: MMF 5.00: SYF 5.00. The regression parameters for predicting the antioxidant properties of composite bread are shown in Table 3. The result showed the effects of wheat flour, malted millet, and okara flour mixture

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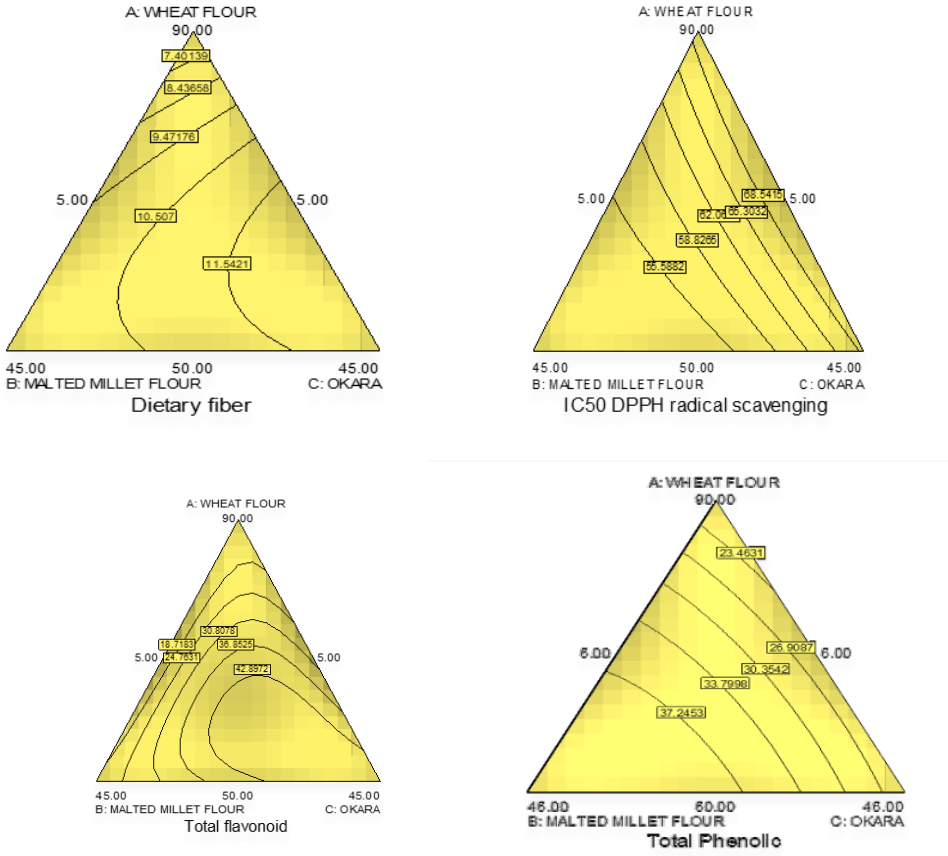

Figure 3. Contour plots showing the effect of mixture components of wheat flour, malted millet flour, and okara on dietary fiber, IC_{50} DPPH radical scavenging activity, total flavonoid and phenolic content of bread sample.

on the antioxidant properties of the bread samples. There was a significant ($p \leq 0.05$) interaction effect between malted millet flour and okara flour. The ternary mixture plot (Figure 3) which revealed increased dietary fiber content with an increasing proportion of okara flour presents the trends in dietary fiber with the blend proportions of component flours. This is in agreement with the findings of Davy *et al.* (2022). The ANOVA representation of dietary fiber content showed that the model (quadratic) and the linear mixture component are significant ($p \leq 0.05$) model terms. The significance of the coefficient of the fitted quadratic model was evaluated by using F-test and P -value. The coefficient of determination (R^2) of the model predicting the dietary fiber of the bread was 0.7413.

$$Dietary\ fiber = 6.37A + 9.49B + 12.14C + 5.74AB + 9.97AC + 0.17BC$$

…… *Eq 7*

3.2.2 IC_{50} DPPH radical scavenging activity

The IC_{50} DPPH radical scavenging activity values ranged from 52.63 to 74.52 µg/ml with the highest value in the composite flour of WHF 70.00: MMF 5.00: SYF 25.00 and lowest found in WHF 56.67: MMF 31.67: SYF 11.67 (Table 1). Table 3 presented the ANOVA which showed that the model (quadratic) and the linear mixture components are significant model terms and that interaction between malted millet and okara flour exhibited a significant ($p \leq 0.05$) effect on the IC_{50} radical scavenging activity of DPPH on the bread. Significant interaction ($p \leq 0.05$) effect between malted millet and *okara* flour on the IC_{50} radical scavenging activity of DPPH on the bread. The ability of the bread formulations to scavenge the DPPH radical increased with an increasing proportion of okara and wheat flour. This might be due to the presence of high flavonoid content. The coefficient of determination (R^2) was 0.8659, implying that the models explained 86.6% variability in the IC_{50} DPPH radical scavenging activity of the bread.

$$IC50\ DPPH = 69.90A + 55.37B + 69.13C - 27.23AB + 9.06AC - 34.02BC$$

…… *Eq 8*

3.2.3 Total flavonoid content

Results showed that the highest total flavonoid content was obtained from the composite flour of WHF 50.00: MMF 5.00: SYF 45.00 and least score in the blend containing WHF 70.00: MMF 25.00: SYF 5.00 (Table 1). This implies that increased levels of okara flour had a positive effect on the total flavonoid content of the developed bread. The study conducted by Wang *et al.* (2022) confirmed this

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Table 4. The desirable ranges for each response for optimization of wheat-based functional bread formulation.

Responses /factors	Goals	Lower limit	Upper limit	Importance	Optimum
Wheat flour	In range	50	90	3	65.18
Malted millet flour	In range	5	45	3	19.43
Okara flour	In range	5	45	3	15.39
Appearance	Maximum	4.07	7.67	3	6.04
Crust colour	Maximum	4.00	7.58	3	5.95
Taste	Maximum	4.07	7.20	3	5.90
Texture	Maximum	4.50	7.25	3	5.98
Overall acceptability	Maximum	4.92	7.50	3	6.35
Dietary fibre	Maximum	6.14	13.66	3	10.78
IC_{50} DPPH	Minimum	52.63	74.52	3	58.43
Total flavonoids content	Maximum	13.49	50.37	3	41.10
Total phenolic content	Maximum	19.82	42.74	3	34.83

result. There was a significant ($p<0.05$) interaction effect between wheat flour, malted millet, and okara flour on the total flavonoid content of the bread (Table 3). The contour plot (Figure 3) showed increased total flavonoid content with an increasing proportion of okara flour. The ANOVA for the total flavonoid content of the bread revealed that the model (special cubic) is significant ($p\leq0.05$) and the linear mixture components are significant ($p\leq0.05$) model terms. R^2 was 0.9660 and this indicated that the model is highly fit (96.60 %).

$$TFC = 13.94A + 20.17B + 48.33C - 14.93AB - 11.45AC + 24.52BC + 474.00ABC$$

…… *Eq 9*

3.2.4 Total phenolic content

The total phenolic content exhibited by the bread samples ranged from 19.82 to 42.74 mg/g (Table 1). The highest value was obtained from the composite flour of WHF 56.67: MMF 31.67: SYF 11.67 and the least score in the blend containing WHF 90.00: MMF 5.00: SYF 5.00, indicating that increased levels of malted millet and okara flour had a positive effect on total phenolic content of the developed bread. Regression analysis (Table 3) displayed significant interaction effects between wheat flour and malted millet flour as well as malted millet and okara flour respectively on the total phenolic contents. This result was in alignment with the findings of Msaddak *et al.* (2017) who reported that cladodes powder supplementation enhanced the total phenolic content and antioxidant activity of bread. The ANOVA result of the total phenolic content of the bread revealed that the linear mixture component, AB and BC are significant model terms. The coefficient of determination (R^2) was 0.9592, indicating that the model is highly fit (95.92%).

$$TPC = 20.02A + 39.69B + 24.61C + 23.58AB + 9.52AC + 25.12BC$$

…… *Eq 10*

The contour plot presented in Figure 3 describes increasing total phenolic content with an increase in the proportion of malted millet flour. This might be due to the malting of millet flour that probably enhanced the release of bound phenolic from the cell walls of millet grains (Adebiyi *et al.,* 2017). Similarly, Taylor and Duodu (2015) reported that an increase in total phenolic content might also probably be a result of the formation of Maillard reaction products and heat-induced antioxidant compounds during the baking process.

3.2.5 Optimization

Optimization of ingredients in the food's formulation is necessary for the development of a product. Table 4 showed the desirable ranges for the sensory and antioxidant properties for optimization of the developed bread formulation. Result showed that the optimum mixture ratio were 65.18% wheat flour, 19.43% malted millet and 15.39% okara flour with the desirability function of 0.61. This optimum formulation produced a functional bread containing 11.25% levels of total dietary fiber, 104.86 mg/100 g flavonoid content, and 54.69 mg/100 g phenol content with sensorial acceptability score of 6.85

Conclusion

The outcome of this research work showed that acceptable functional bread could be produced from blends of wheat, malted millet and *okara* flour. Optimum mixture ratios of malted millet (19.43%) and okara flour (15.39%) composited with wheat flour (65.18%) have been established to be the best optimal composition for developed functional bread in terms of sensory and antioxidant properties. Similarly, the findings showed that varying proportions of the three flours had different effects on the sensorial and antioxidant properties of developed bread. However, wheat flour significantly influenced the sensory acceptability of the bread while increased levels of malted millet and *okara* flours in bread formulation enhanced the total antioxidant properties. Therefore, the use of malted millet and okara flour could be explored to produce healthy or functional bread to suffice the emerging healthy food choices. Furthermore, the finding established that the optimum formulation established was able to reduce the

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inclusion of wheat flour utilization by 35% using functional ingredients.

Conflicts of interest

The authors declare no known conflicts of interest.

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