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# Bioactive compounds, antioxidant capacity, functional and sensory properties of optimized complementary weaning flour processed from sorghum, soybean, and karkade (*Hibiscus sabdariffa* L.) seeds

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ABSTRACT

The study aimed to formulate and optimize complementary weaning flours for better antioxidants and sensory acceptability. D-optimal constrained mixture design was used to generate Eighteen formulations with a range of malted sorghum 40–60%, blanched soybean 20–30%, boiled karkade seeds 10–20%, and 10% premix (5% figl leaves, 4.5% sugar, and 0.5% salt) added in equal proportion to all formulations were evaluated. The sensory properties of the gruel made from the selected complementary flour were evaluated using untrained panellists. The result indicated that high malted sorghum supplement levels in the formula were associated with increased bioactive compounds and antioxidant capacity. The optimum values of bioactive compounds, antioxidant capacity, and functional properties of the formulated product were 51.0%, 22.0%, and 17.0% for sorghum, soybean, and karkade seeds flour with 10.0% premix, respectively. The sensory acceptability of the gruel samples evaluated from the optimized complementary flour rich in antioxidants was significantly ($p < 0.05$) liked in terms of aroma and mouthfeel compared to the control sample. The result found that supplemented weaning flour made from malted sorghum, blanched soybean, and boiling karkade seeds flour may be processed to produce high levels of health-promoting bioactive compounds with desirable sensory qualities.



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## Introduction

In developing nations, it is a common practice to develop complementary food to overcome the undernutrition problems of children under five. However, research works to address both the malnutrition problems and other non-nutritious health-promoting compounds are limited. The World Health Organization recommends the consumption of complementary foods rich in bioactive compounds and antioxidants with desired functional properties. The organization suggested that such food be developed for community-based management of uncomplicated types of severe acute malnutrition and maintaining good health for vulnerable groups (infants, children, pregnant and lactating women, and the elderly) [23]. Among vulnerable groups, adequate nutrition, particularly during infancy and early childhood, is vital for optimal health, growth, and survival [34].

Diets with adequate bioactive compounds are essential to fight against oxidative stress caused by free radicals damaging human health [38]. Vitamin C, vitamin E, $\beta$-carotene, polyphenols, and other dietary bioactive compounds, such as omega -3 fatty acids, are well-known dietary bioactive substances that helps in decreasing oxidative damage and preventing disease vulnerability in children [17]. Several neglected plants in many developing countries have high bioactive compounds and antioxidant capacity.

There is a massive potential for growing early maturing underutilized vegetable figl (*Raphanus sativus* L.) leaves in Ethiopia's western area, particularly in the Benishangul Gumuz region. The figl leaves are sources of considerable $\beta$-carotene, L- ascorbic acid, and polyphenolic compounds of significant nutritional and antioxidant potential [28]. Karkade (*Hibiscus sabdariffa*) seeds flour is one of the underutilized plants rich in nutrients, high in proteins and fats with low antinutrient contents [26]. The karkade seeds also bear health-enhancing bioactive antioxidants with desirable functional properties for complementary foods processing [27].

Sorghum is a staple food widely grown and consumed in underdeveloped nations. Application of processing, particularly malting, can improve the nutrients and bioactive chemicals in the sorghum grain [26]. The authors above also highlighted that the inclusion of malted sorghum grains in complementary foods increases energy density and improves bioactive compounds with lower antinutrients [26], all of which benefit the gut microbiota and lower the risk of obesity, oxidative stress, inflammation, diabetes, dyslipidemia, cancer, and hypertension [13]. In addition to sorghum, soybean is one of the significant leguminous crops produced in different parts of the world, including Ethiopia. Nutrients and bioactive compounds in the soybean and their products have been strongly associated with many potential health benefits such as improved protein quality and bioactive soybean compounds that will help to reduce several chronic illnesses such as cardiovascular disease, diabetes, immune disorders, certain types of cancer, obesity and cholesterol levels [16]. With controlled processing, soybean is desirable as a component of complementary foods. It enhances protein quality and fat requirements.

Although the region is endowed with potential food crops rich in nutrients and bioactive compounds, childhood diarrheal illness was recorded as highly prevalent among children under-five years [5].

So far, supplementation of under-exploited plants such as figl leaves and karkade seeds with staple sorghum grain and soybean for bioactive compounds and antioxidants is not yet explored well for possible use to suppress malnutrition and support health. In this study the development of complementary flour from underutilized plants and staple foods rich in bioactive compounds, antioxidant capacity, and functional properties with better sensory acceptability are reported.

## Materials and methods

### *Experimental materials*

Improved Sorghum Assosa I and Soybean Clark 63k cultivars were obtained from the Assosa and Jimma Agricultural Research Centers, respectively. The brown seeds of karkade were harvested from Ethiopia's Benishangul Gumuz region's, Assosa zone. The seeds of figl were purchased from the Benishangul Gumuz region and then cultivated at the Jimma University College of Agriculture and Veterinary Medicine research farm as described in Keyata et al. [27] and the harvested leaves were used as a premix to improve $\beta$-carotene, L-ascorbic acid, polyphenol compounds, and antioxidants. Karkade local seeds were obtained from the region's local market. Premixes of iodized salts and sugar were added to all formulated items to improve the flavor.

### *Raw material preparation*

Sorghum was cleaned, washed, and steeped after then at 28 °C, the grains were allowed to germinate for 41 h as details described in Keyata et al. [28]. The soybeans were washed, blanched at 96 °C for 10 min to inactivate the lipoxygenase and lipase enzymes, and seed coats were removed. The karkade seeds were washed and cooked for 30 min at 96 °C [43]. Fresh ripe harvested figl leaves were used after rinsing and chopping [26]. Malted sorghum, blanched soybean, and karkade seeds were dried for 24 h at 50 °C, while the chopped figl leaves were dried at 45 °C. The dried samples were processed to pass through a 0.5 mm sieve size [1] and packed in airtight polyethene bags (RRH-200, Zhejiang, China).

*Premix*

The sorghum, soybean, and karkade seed flours were mixed with premix based on the mixture design developed for the proportion of mixing (figl leaves, iodized salt, and white sugar). The combined flours were homogenized in a planetary cake mixer (H.LB20/B, Hungary) for 5 min, and representative flour samples were sealed in polyethene bags and stored at 4 °C until further investigation.

*Experimental design*

The mixture design technique and food science concepts were integrated to enhance flour composition to attain the intended goal. D-optimal mixture design provided 18 runs for combining proportions of 40–60% for malted sorghum, 20–30% for blanched soybean, and 10–20% for boiling karkade seeds flour. The proportions of the primary ingredients were calculated to yield 90%, with the remaining 10% was premixed. As a control, 100 percent un-malted sorghum flour was used. The sensory evaluations were conducted using a randomized complete block design (RCBD), which considered diversity among panellists in the three districts of the region.

*Preparation of the methanolic extract*

The sample of composite complementary flour made from malted sorghum, blanched soybean, boiled karkade, and premix (figl leaves, iodized salt, and sugar) was extracted according to Handa et al. [22]. The maceration technique was used to soak ground samples (100 mg) in 100 mL of methanol (99.8%) to produce about 1 mg/mL of concentration, soaking in the solvents for 24 h and shaking in a mechanical shaker [Hy-2(C), Shanghai, China] at room temperature, and finally filtered through Whatman No. 1 filter paper. The filtered extract was used to determine the total phenolic, total flavonoids, and antioxidant capacity using two antioxidant assay systems (DPPH and FRAP).

*Bioactive compounds*

*Determination of total phenolic content*

The sample extract's total phenolic content (TPC) was determined using the procedures described by Singleton et al. [42]. A 0.4 mL (1 mg/mL) extract was combined with 1.6 mL 7.5% sodium carbonate solution and 2 mL Folin-Ciocalteu reagent that had been 10-fold diluted. A UV-VIS spectrophotometer (Model: JASCO V-630, Shimadzu Corporation, Tokyo, Japan) was used to detect absorbance at 765 nm after the reaction mixture had been incubated for an hour in the dark at room temperature. The total phenolic content was calculated using a standard curve (0.00, 6.25, 12.50, 25.00, 50.00, and 100.00 g/mL, $Y = 0.0135X + 0.1177$, $R2 = 0.9912$) and reported as mg of GAE/g.

*Determination of the total flavonoids content*

The total flavonoids content (TFC) was determined by the flavonoids-aluminum complex colorimetric assay method [11]. One mL of the extract (1 mg/mL) was combined with 0.3 mL of 5% sodium nitrite and 0.3 mL of 10% aluminum chloride. After 6 min, 2 mL of 1-M sodium hydroxide was added, followed by 2.4 mL of distilled water, resulting in a total volume of 10 mL. Using a UV-VIS spectrophotometer (Model: JASCO V-630, Shimadzu Corporation, Tokyo, Japan), the color intensity of the flavonoids-aluminum combination produced was evaluated at 510 nm. The total flavonoid content was determined as catechin equivalent (CE) from (0.00, 6.25, 12.50, 25.00, 50.00, and 100.00 g/mL, $Y = 0.01X + 0.1343$, $R2 = 0.9807$) calibration line.

*Beta (ß) carotene content determination*

With slight adjustments, the extraction of ß-carotene content was carried out as described by Sadler et al. [40]. One gram of sample flour was shake mixed with one gram of $CaCl_2.2H_2O$ and 50 mL extraction solvent (50% hexane, 25% acetone, and 25% ethanol, containing 0.1% BHT) for 30 min at room temperature. The solution was shaken repeatedly for another 15 min after adding 15 mL of distilled water. The organic phase containing the ß-carotene was separated from the aqueous phase using a separation funnel and filtered using Whatman filter paper No.1. The extraction technique was conducted in the dark to prevent carotenoids from degrading. The stock $\beta$-carotene (Sigma Aldrich, USA) standard solution was generated by precisely weighing 0.01 g $\beta$-carotene in the solvent (50% hexane, 25% acetone, and 25% ethanol) used to extract samples and to dilute to a volume of 100 mL with the same solvent. A UV-VIS spectrophotometer (Model: JASCO V-630, Shimadzu Corporation, Tokyo, Japan) was used to evaluate the absorbance of the sample extract and $\beta$-carotene standard solutions at 450 nm wavelength. A calibration line was constructed using standard solutions (0.10, 0.20, 0.40, 0.60, 0.80, and 1.00 g/mL, $Y = 0.2234X + 0.0521$, $R2 = 0.9955$) from which $\beta$-carotene was calculated and expressed in mg/g.

*Determination of L-ascorbic acid content*

The 2,6-dichloroindophenol titration method was used to determine the L-ascorbic acid concentration ([4], method 967.21). A 0.1 g sample was extracted in 500 mL of deionized $H_2O$ with 40 mL of 15 g metaphosphoric acid ($HPO_3$) and 40 mL of acetic acid (Ac). Whatman filter paper No. 1 was used to filter the extracted sample. The filtrated sample was

titrated directly with a 2,6-dichloroindophenol standard solution by dissolving 50 mg of 2,6-dichloroindophenol sodium salt and 42 mg of $NaHCO_3$ in 200 mL of deionized water to light but distinct rose-pink endpoint lasting 5 s.

The L-ascorbic acid standard solution was made by dissolving around 50 mg of L-ascorbic acid in 50 mL of $HPO_3$-acetic acid extraction solution. The content of L-ascorbic acid was estimated using the equation below.

$$L-ascorbic\ acid\ (mg/100g) = \frac{(A-B)*C*40}{10S}*100$$

where: $A$ = volume in mL of the 2,6-dichloroindophenol sodium salt solution used for the sample.

$B$ = volume in mL of the 2,6-dichloroindophenol sodium salt solution used for the blank.
C = mass in mg of L- ascorbic acid equivalent to 1.0 mL of standard indophenol solution.
$S$ = weight of sample taken (g).
40/10: 40 = volume of extract & 10 = volume of extract used for the determination.

## In-vitro *antioxidant capacity*

### *DPPH free radical scavenging activity*

The DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity of the sample's methanolic extract was calculated using Kirby and Schmidt's [29] method with slight modifications. In methanol, a 0.004% solution of the DPPH radical was produced. Then 4 mL of this solution was combined with 1 mL of sample extracts in methanol (99.8%) at varied concentration (0.20–0.56 mg/mL). The samples were kept at room temperature for 30 min in the dark. By monitoring the decrease in absorbance at 517 nm per sample, the radical scavenging ability was evaluated using a UV-VIS spectrophotometer (AS). The absorbance of freshly generated DPPH was used as a control (blank) (AC) from which the percent inhibition of free radical DPPH was determined using the equation below.

$$Inhibition(\%) = \left(\frac{AC-AS}{AC}\right)*100$$

Where: AS is the absorbance of sample extract solution and AC is the absorbance of the control (blank) solution.

From the graph of DPPH inhibition vs extract concentration, the extract concentration that offers 50% of radical scavenging activity ($IC_{50}$) was computed [9].

### *Ferric reducing antioxidant power (FRAP)*

With minor modifications, the FRAP of sample extracts was measured as described by Dudonne et al. [14]. The assay is based on the antioxidant compound's reducing capacity in the conversion of ferric ion ($Fe^{3+}$) to ferrous ion ($Fe^{2+}$), in which absorbance increase at 593 nm is measured due to the formation of the blue complex ($Fe^{2+}$/TPTZ). Acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ were mixed at a ratio of 10:1:1 (v/v/v) to make the FRAP reagent. A vortex mixer was used to mix 100 mL of sample extract (2 mg/mL) with 3 mL of produced FRAP reagent. The sample tube was held in the dark at room temperature for 30 min, and the absorbance was measured at 593 nm using a UV-VIS spectrophotometer. The $FeSO_4.7H_2O$ standard curve (0.00, 0.28, 0.56, 0.84, 1.12, and 1.40 g/mL, $Y = 1.478X + 0.0568$, $R2 = 0.9942$) was used to calculate FRAP values in terms of mM $Fe^{2+}$/g of sample.

## *Functional properties of the flours*

### *Bulk density (BD)*

The bulk density (BD) was determined according to Gupta et al. [21]. About one gram of sample flour was placed in a 10 mL test tube and compacted by tapping it several times on the laboratory bench. The final bulk volume was recorded, and bulk density was estimated using the following equation: weight of sample powder (g) divided by final volume (mL).

$$Bulk\ density(g/mL) = \frac{weight\ of\ sample}{volume\ of\ sample\ after\ tapping}$$

### *Water and oil absorption capacity (WAC and OAC)*

The water and oil absorption capabilities (WAC and OAC) were measured according to Beuchat [6]. In a pre-weighed 50 mL centrifuge tube, one gram of flour sample was combined with 10 mL of distilled water or oil. The sample was shaken for one hour on a mechanical shaker (Hy-2(C), Shanghai, China), then centrifuged for 30 min at 5000 x g (Sigma 2-16KC, UK). With a pipette, the separated water or oil was removed, and the residues with the amount of oil or water that remained were weighed again. The absorption capacity of water or oil was measured in grams of water or oil absorbed per gram of sample.

*Swelling power (SP) and solubility (SO)*

Swelling power (SP) and solubility (SO) were determined as described in Oladele and Aina [33] with slight modification. About 0.35 g of flour was mixed with 12.5 mL of distilled water in a centrifuge tube and heated at 60 °C for 30 min, with constant stirring. The tube was removed from the bath, wiped dry, cooled to room temperature, and centrifuged for 20 min at 2500 rpm to separate gel and supernatant. The swelling power (separate gel) was calculated using the following equation, and the swollen sample (paste) was obtained from decanting the supernatant.

$$Swelling\ power(g/g) = \left(\frac{weight\ of\ wet\ mass\ of\ se\dim ent}{weight\ of\ dry\ matter\ in\ gel}\right)$$

The aqueous supernatant was removed, transferred into a tarred evaporating dish, and dried in an oven at 100 °C for 4 h. The dried residue was weighed to determine the solubility using the following equations.

$$Solubility(\%) = \left(\frac{weight\ of\ dried\ sample\ in\ \ \sup erna\tan t}{weight\ of\ original\ sample}\right) * 100$$

*Numerical optimization*

Design expert software was used to determine the optimum blend of sorghum, soybean, karkade seeds flour, and premix to produce complementary flour rich in bioactive compounds and antioxidant capacity. In the optimization, high values for total phenolic content (TPC) and total flavonoid content (TFC), $\beta$-carotene, L-ascorbic acid, percentage of free radical scavenging power (% DPPH), ferric reducing antioxidant power (FRAP), and solubility while the low value for bulk density (BD), water absorption capacity (WAC), and swelling power (SP) was used.

*Gruel preparation for sensory analysis*

Based on numerical optimization results, a blend of 51% malted sorghum, 22% blanched soybean, and 17% boiled karkade seeds flour with 10% premix was chosen from 18 formulated composite complementary flours to produce as an excellent complementary flour rich in bioactive compounds and antioxidant capacity. Sensory testing was performed on the improved formula and the control (100% un-malted sorghum grain flour). Based on the preliminary test conducted in the post-harvest management laboratory and the experience of mothers in the three study districts with gruels preparation for infants, 100 g of control (ungerminated sorghum) and formulated optimized complementary flours were mixed with 950 mL and 750 mL potable boiled tap water, respectively, and cooked at 90 °C for 27 and 16 min, respectively. The water volume was determined after preliminary work before sensory analysis to attain more or less similar gruel rheology and consistency. The mixture was swirled with a wooden spoon during mixing and cooking to avoid lump formation and achieve the desired gruels consistency. The gruels were allowed to cool to 44 °C after preparation to undertake the sensory evaluation.

*Sensory evaluation*

The gruel samples were evaluated for sensory acceptability in the Homesha, Kurmuk, and Sherkole districts of Ethiopia's Assosa zone of the Benishangul Gumuz region. A total of 53 untrained panellists were chosen randomly from a list of mothers in the selected districts who has children aged 6 to 24 months and had experience preparing infant foods. Following the orientation, panellists were randomly assigned coded products to evaluate sensory aspects such as color, aroma, taste, flavor, mouthfeel, and overall acceptability. Smile emojis were used to create a five-point hedonic scale (5 = Like significantly, 4 = Like slightly, 3 = Neither like nor a dislike, 2 = Dislike slightly, and 1 = Dislike highly).

*Data analysis*

D- Optimal mixture design expert software was used to examine the data acquired from the experiment. The statistical significance of the terms in the regression equations was investigated using analysis of variance, with the significance test level set at $p < 0.05$. All of the parameters' fitted models were created. Contour plots for the specified variables were created to establish the optimal formulation for the chosen answer. Montgomery [30] described how to perform numerical optimization using response data. One-way analysis of variance was used to assess the data collected from the sensory panellists (ANOVA). All statistical analyses were conducted using JMP 13 Pro (a SAS company software), with ANOVA F test significant difference at $p < 0.05$ and findings presented as means standard deviations.

*Ethical consideration*

Jimma University's Institutional Review Board gave their approval to this study. All human volunteer operations were conducted by the Helsinki Declaration of 1964 and its subsequent revisions. All study panellists signed a written informed consent form.

## Results and discussion

### *Model selection*

Bioactive compounds, antioxidant capacity, and functional properties evaluated are presented in Table 1. The value shows linear model can refer to reasonable changes in the TPC, L-ascorbic acid, FRAP, OAC, SO, and SP of the flours as functions of mixing ratio ($p < 0.0001$). The quadratic models have strong predictive power to indicate variations in TFC, $\beta$-carotene, and BD of the flours in the blend ($p < 0.0001$). The reduced quadratic and cubic models can explain differences in%DPPH and water absorption capacity of the flours in the formulations, respectively ($p < 0.01$). The lack of fit for responses was not significantly ($p > 0.05$) different, which confirmed that the possibility of an error occurring is low. The selected models fit well with the bioactive compounds, antioxidant capacity, and functional properties of formulated complementary flour. The selected models related to blending ratios and responses determined in the formulation are presented in Table 1. The results show a significant ($p < 0.05$) association between the blending ratio of sorghum, soybean, and karkade seeds flour in the value of total flavonoids, $\beta$-carotene, and percent DPPH and water absorption capacity.

### *Bioactive compounds*

In this study, the total phenolic content (TPC) of complementary flour was raised from 2.95 to 4.81 mg/g with an increase in the malted sorghum ratios and a decrease in ratios of soybean and karkade seeds flour (Table 2) (Fig. 1a). This increase could be associated with activating an endogenous enzyme and complex biochemical metabolism during the malting of sorghum grains [15]. Adequate consumption of sorghum-rich formulation enhances the consumption of phenolic compounds for good health to prevent diseases such as inflammation and certain cancers [39,46].

The total flavonoid content (TFC) of the 18 formulated complementary flour increased from 1.21 to 2.54 mg/g with an increase in the malted sorghum flour and a decrease of blanched soybean and boiled karkade seeds in the blend (Table 2) (Fig. 2b). A fair amount of TFC in the formulated diet is vital for health to work against oxidative stress diseases such as Alzheimer's, arteriosclerosis, cancer, and aging [36]. An increase in TFC was not in agreement with an increase in sorghum flour proportion for better TPC content which needs optimization work with the intended goal of the formulation considering other nutrient components. The result shows that the TPC and TFC found in this work could benefit the consumers as the antioxidant source.

L- ascorbic acid values (L-AAC) (11.0 to 23.9 mg/100 g) were increased as the proportion of blanched soybean and boiled karkade seeds flour decreased in the blend (Table 2) (Fig. 1c). The reduction of L-AAC in the formulation could be due to the thermal degradation nature of L-AAC that could be associated with heat treatment of soybean and karkade seeds during preparation phases. However, according to these results, the complementary flour's L-AAC content was relatively higher than the control (13.1 mg/100 g). Except for formulations 3, 5, 9, 14, and 16 (Table 2), all the formulations can meet the recommended value of 15 mg/day of L-AAC for children aged from one to three years [24].

The $\beta$-carotene contents in the complementary flour increased from 16.65 to 28.25 mg/100 g with an increase in the proportion of malted sorghum flour and decreased with blanched soybean and boiled karkade seeds flour increase in the formulation (Table 2) (Fig. 1d). The $\beta$-carotene content in the formulated flour was significantly ($p < 0.05$) higher than the control sample of un-malted sorghum (1.10 mg/100 g) (Table 2). The high $\beta$-carotene contents of the formulated complementary flour are contributed mainly from the presence of 5% figl leaves added in the formulation (42 mg/100 g) [27]. A similar increase in the $\beta$-carotene content was found after supplementing moringa leaves powder [19]. The $\beta$-carotene content found in this study was more than sufficient compared to the recommended dietary allowances for children by Codex Alimentary Commission (400 µg/day) [10]. The result found in this study was significantly higher than $\beta$-carotene content (0.53 mg/100 g) in complementary foods formulated from 50% sweet potato, 15% millets, and 35% soybean. The higher $\beta$-carotene content could be due to supplementing 5% of figl leaves in the complementary flour. The findings indicated that the formulations of complementary flour could improve pro-vitamin A ($\beta$-carotene) and assist with the problem of vitamin A deficiency, which causes night blindness and sensitivity to infection.

### In-vitro *antioxidant capacity and* $IC_{50}$ *value*

With an increasing malted sorghum in the blend from 41.0% to 59.1%, the percentage of DPPH radicals scavenging activity of complementary flour increased from 62.20 (high value to achieve IC50 = 0.341 mg/mL) to 74.34% (low value to achieve IC50 = 0.192 mg/mL) at the DPPH concentration 0.43 mg/mL (Table 3). The antioxidant capacity in the formulated complementary flour is probably associated with the activation of endogenous enzymes during the malting of sorghum grains [41], which increases the concentration of antioxidant compounds. The findings highlighted that consumption and utilization of malted sorghum as one of the ingredients in the formulation of complementary flour contributes to enhancing the antioxidant capacity of the food.

The ferric reducing antioxidant power of the formulation increased from 56.3 to 78.3 mM/g with increasing in the proportion of malted sorghum (Table 3) but combined with other components. The ferric-reducing antioxidant capacity of all the formulations was better than the control sample (100% un-malted sorghum) (53.9 mM/g) (Table 3). The compounds in

**Table 1**
Analysis of variance (ANOVA) p-value for the proportion mixes of 18 runs of phytochemical contents, antioxidant capacity and functional properties.

| Source | Bioactive compounds | | | | Antioxidant capacity | | | Functional properties | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | TPC | TFC | $\beta$ Ca | L-AAC | IC50 | %DPPH | FRAP | BD | WAC | OAC | SO | SP |
| Model | Linear*** | Quadra*** | Quadra*** | Linear*** | Linear*** | RQuadra*** | Linear*** | Quadra*** | Cubic** | Linear*** | Linear*** | Linear*** |
| Linear | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| A*B | - | 0.5197 | 0.5436 | - | - | - | - | 0.0774 | 0.3447 | - | - | - |
| A*C | - | 0.0236 | < 0.0001 | - | - | 0.0053 | - | 0.6758 | 0.0001 | - | - | - |
| B*C | - | 0.9761 | 0.0031 | - | - | - | - | 0.6075 | 0.0007 | - | - | - |
| ABC | - | - | - | - | - | - | - | - | 0.0008 | - | - | - |
| $A^2BC$ | - | - | | - | - | - | - | - | - | - | - | - |
| $AB^2C$ | - | - | | - | - | - | - | - | - | - | - | - |
| $ABC^2$ | - | - | | - | - | - | - | - | - | - | - | - |
| AB (A-B) | - | - | - | - | - | - | - | - | 0.0025 | - | - | - |
| AC (A-C) | - | - | - | - | - | - | | - | 0.0001 | - | - | - |
| BC (B-C). | - | - | - | - | - | - | - | - | 0.0083 | - | - | - |
| $R^2$ (adj) | 0.9715 | 0.9813 | 0.9768 | 0.9689 | 0.9642 | 0.9528 | 0.9583 | 0.9370 | 0.9460 | 0.9199 | 0.9524 | 0.9376 |
| Lack of fit | 0.9574 | 0.5943 | 0.0014 | 0.5342 | 0.4164 | 0.6638 | 0.3546 | 0.3805 | 0.6277 | 0.2156 | 0.4774 | 0.4647 |

*** Highly significant ($P < 0.0001$), ** significant ($P = 0.0003$); $R^2$ (adj): adjusted coefficient of determination;
A: Malted sorghum flour, B: Blanched soybean flour. C: Boiled karkade seeds flour.

**Table 2**
Bioactive compounds (db) of formulated complementary flours.

| Formulation | Malted sorghum (%) | Blanched soybean (%) | Boiled karkade (%) | Premix (%) | TPC (mg/g) | TFC (mg/g) | $\beta$-carotene (mg/100 g) | L-AAC (mg/100 g) |
|---|---|---|---|---|---|---|---|---|
| 1 | 46.7 | 30.0 | 13.3 | 10 | 3.56 | 1.55 | 25.51 | 15.22 |
| 2 | 54.8 | 20.0 | 15.2 | 10 | 4.53 | 2.40 | 23.73 | 22.33 |
| 3 | 45.4 | 24.6 | 20.0 | 10 | 3.73 | 1.36 | 27.63 | 13.31 |
| 4 | 54.8 | 20.0 | 15.2 | 10 | 4.46 | 2.22 | 24.05 | 22.45 |
| 5 | 45.4 | 24.6 | 20.0 | 10 | 3.67 | 1.40 | 27.85 | 14.16 |
| 6 | 50.0 | 30.0 | 10.0 | 10 | 3.73 | 1.86 | 20.49 | 19.25 |
| 7 | 50.0 | 25.5 | 14.6 | 10 | 3.89 | 1.80 | 24.02 | 16.77 |
| 8 | 59.1 | 20.9 | 10.0 | 10 | 4.81 | 2.54 | 16.65 | 23.91 |
| 9 | 46.4 | 27.1 | 16.6 | 10 | 3.62 | 1.45 | 26.25 | 13.87 |
| 10 | 59.1 | 20.9 | 10.0 | 10 | 4.66 | 2.68 | 16.88 | 25.01 |
| 11 | 50.0 | 20.0 | 20.0 | 10 | 4.00 | 1.65 | 25.72 | 16.22 |
| 12 | 50.0 | 25.5 | 14.6 | 10 | 3.94 | 1.78 | 24.87 | 16.67 |
| 13 | 50.0 | 25.5 | 14.6 | 10 | 3.82 | 1.70 | 24.99 | 18.16 |
| 14 | 41.0 | 30.0 | 19.0 | 10 | 3.23 | 1.21 | 28.25 | 11.02 |
| 15 | 51.5 | 22.1 | 16.4 | 10 | 4.01 | 1.92 | 25.22 | 19.50 |
| 16 | 41.0 | 30.0 | 19.0 | 10 | 2,95 | 1.27 | 28.01 | 12.47 |
| 17 | 55.4 | 23.2 | 11.4 | 10 | 4,36 | 2.25 | 17.86 | 23.46 |
| 18 | 52.4 | 26.5 | 11.1 | 10 | 3,96 | 2.14 | 19.78 | 20.06 |
| Control | 90 | 0 | 0 | 10 | 6,27 | 1.79 | 23.89 | 21.07 |
| | 0 | 90 | 0 | 10 | 2,32 | 1.38 | 25.4 | 11.13 |
| | 0 | 0 | 90 | 10 | 4.31 | 2.02 | 30.87 | 8.46 |
| | Control (unmalted sorghum) | | | | 3.50 | 1.09 | 1.10 | 13.10 |
| | Minimum RDA | | | | - | - | | |
| | Adj R-Squared (%) | | | | 0.97 | 0.98 | 0.98 | 0.97 |
| | Lack of fit | | | | 0.96 | 0.59 | 0.001 | 0.53 |

Where: Premix is 5% figl leaves, 4.5% sugar and 0.5% iodized salt

**Table 3**
*In-vitro* antioxidant capacity of formulated complementary flours.

| FM | Malted sorghum (%) | Blanched soybean (%) | Boiled karkade (%) | Premix (%) | $IC_{50}$ of DPPH mg/mL | %DPPH (0.43 mg/mL) | FRAP (mM/g) |
|---|---|---|---|---|---|---|---|
| 1 | 46.7 | 30.0 | 13.3 | 10 | 0.303 | 66.3 | 61.18 |
| 2 | 54.8 | 20.0 | 15.2 | 10 | 0.248 | 70.47 | 71.53 |
| 3 | 45.4 | 24.6 | 20.0 | 10 | 0.31 | 63.41 | 59.89 |
| 4 | 54.8 | 20.0 | 15.2 | 10 | 0.241 | 71.5 | 73.49 |
| 5 | 45.4 | 24.6 | 20.0 | 10 | 0.312 | 64.92 | 61.04 |
| 6 | 50.0 | 30.0 | 10.0 | 10 | 0.275 | 67.27 | 63.88 |
| 7 | 50.0 | 25.5 | 14.6 | 10 | 0.263 | 68.12 | 64.22 |
| 8 | 59.1 | 20.9 | 10.0 | 10 | 0.192 | 74.34 | 78.3 |
| 9 | 46.4 | 27.1 | 16.6 | 10 | 0.294 | 66.55 | 63.55 |
| 10 | 59.1 | 20.9 | 10.0 | 10 | 0.206 | 72.65 | 76.54 |
| 11 | 50.0 | 20.0 | 20.0 | 10 | 0.264 | 68.54 | 67.33 |
| 12 | 50.0 | 25.5 | 14.6 | 10 | 0.262 | 69.93 | 64.9 |
| 13 | 50.0 | 25.5 | 14.6 | 10 | 0.275 | 70.23 | 65.51 |
| 14 | 41.0 | 30.0 | 19.0 | 10 | 0.335 | 62.32 | 56.31 |
| 15 | 51.5 | 22.1 | 16.4 | 10 | 0.269 | 70.35 | 68.48 |
| 16 | 41.0 | 30.0 | 19.0 | 10 | 0.341 | 62.2 | 58.27 |
| 17 | 55.4 | 23.2 | 11.4 | 10 | 0.233 | 70.89 | 72.82 |
| 18 | 52.4 | 26.5 | 11.1 | 10 | 0.273 | 69.75 | 66.79 |
| Control | 90 | 0 | 0 | 10 | 0.288 | 62.14 | 82.15 |
| | 0 | 90 | 0 | 10 | 0.39 | 55.62 | 65.1 |
| | 0 | 0 | 90 | 10 | 0.318 | 59.48 | 74.17 |
| | Control (unmalted sorghum) | | | | 0.33 | 59.7 | 53.87 |
| | Adj R-Squared | | | | 0.96 | 0.95 | 0.96 |
| | Lack of fit | | | | 0.42 | 0.66 | 0.36 |

the diet that contribute to high ferric reducing antioxidant capacity may potentially reduce oxidative damage and accompanying clinical problems such as obesity, hypertension, dyslipidemia, and type 2 diabetes [12,37].

*Functional properties*

The bulk density (BD) in this study decreased from 0.59 to 0.50 g/mL with a decrease in malted sorghum and increased with soybean and karkade seeds flour in the blend (Table 4). The lower the bulk density of food is always preferred for

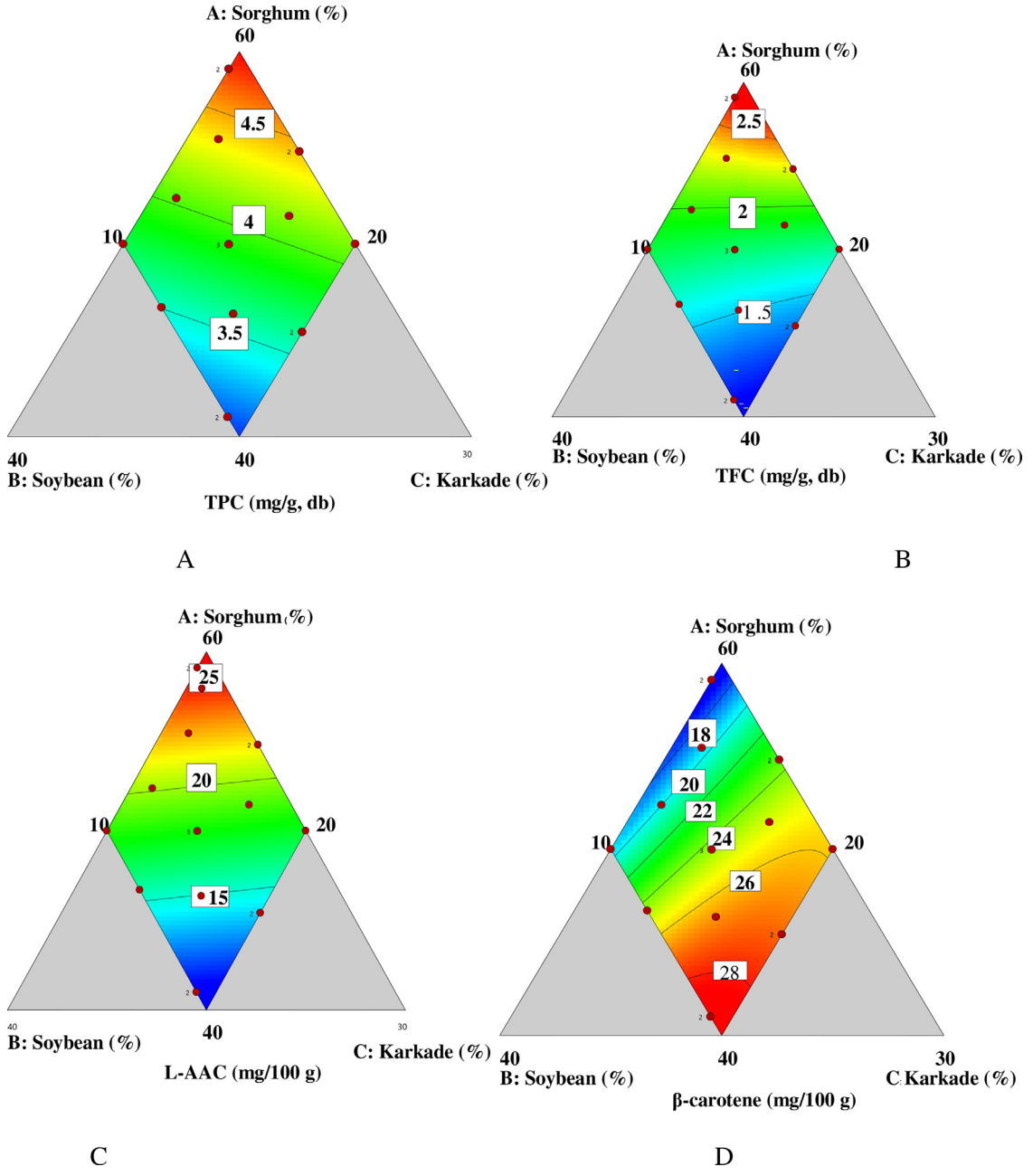


**Fig. 1.** . Contour plot of bioactive compounds of formulated complementary flour. (A) TPC (total phenolic content) (mg/g), (B) TFC (total flavonoid content) (mg/g), C) L-AAC (L-ascorbic acid) (mg/100 g), D) $\beta$-carotene (mg/100 g),.

better nutrition of children. The rise in BD on a high proportion of sorghum flour could be linked to the germination process's creation of structural carbohydrates like cellulose and hemicelluloses [35]. The decrease in BD with an increased proportion of soybean flour might be associated with removing seed coats after blanching of soybean seeds. Bolarinwa et al. [8] reported a similar decrease in the BD of the composite flour with an increase in the proportion of soybean flour. The BD density of all formulations made from the components is lower than the BD value of un-malted sorghum flour, resulting in a better-quality diet for children (Table 4).

The water absorption capacity (WAC) of complementary flours increased from 1.66 to 3.09 g/g with an increased proportion of blanched soybean and boiled karkade flours (Table 4). An increase in WAC of complementary flour blend was reported from cooked soybean and ginger-modified cocoyam starch [32]. However, with a rise in malted sorghum, the WAC of the complementary flour decreased, which could be advantageous for making thinner gruel with a high caloric density

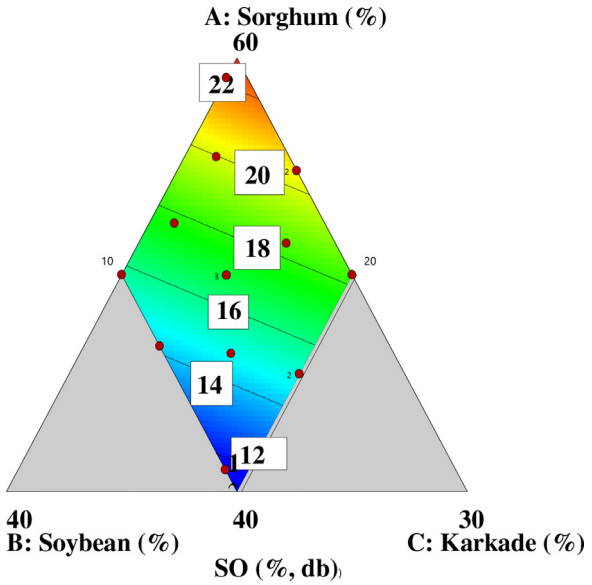


**Fig. 2.** . Contour plot of solubility (SO) of formulated complementary flour.

**Table 4**
Functional properties of formulated complementary flours.

| FM | Malted sorghum (%) | Blanched soybean (%) | Boiled karkade (%) | Premix (%) | BD (g/ml) | WAC (g/g) | OAC (g/g) | SP (g/g) | SO % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 46.7 | 30.0 | 13.3 | 10 | 0.53 | 2.19 | 1.46 | 4.64 | 13.6 |
| 2 | 54.8 | 20.0 | 15.2 | 10 | 0.57 | 1.87 | 1.03 | 4.16 | 20.4 |
| 3 | 45.4 | 24.6 | 20.0 | 10 | 0.53 | 2.14 | 1.32 | 4.74 | 14.4 |
| 4 | 54.8 | 20.0 | 15.2 | 10 | 0.58 | 1.83 | 1.09 | 4.22 | 19.7 |
| 5 | 45.4 | 24.6 | 20.0 | 10 | 0.54 | 2.22 | 1.37 | 4.82 | 14.9 |
| 6 | 50.0 | 30.0 | 10.0 | 10 | 0.55 | 2.05 | 1.27 | 4.26 | 15.5 |
| 7 | 50.0 | 25.5 | 14.6 | 10 | 0.56 | 3.09 | 1.22 | 4.62 | 17.9 |
| 8 | 59.1 | 20.9 | 10.0 | 10 | 0.59 | 1.75 | 0.99 | 3.71 | 23.3 |
| 9 | 46.4 | 27.1 | 16.6 | 10 | 0.54 | 2.16 | 1.42 | 4.69 | 14.0 |
| 10 | 59.1 | 20.9 | 10.0 | 10 | 0.59 | 1.66 | 0.96 | 3.9 | 21.6 |
| 11 | 50.0 | 20.0 | 20.0 | 10 | 0.57 | 1.94 | 1.29 | 4.70 | 18.4 |
| 12 | 50.0 | 25.5 | 14.6 | 10 | 0.56 | 1.97 | 1.25 | 4.56 | 16.3 |
| 13 | 50.0 | 25.5 | 14.6 | 10 | 0.55 | 2.01 | 1.16 | 4.62 | 16.9 |
| 14 | 41.0 | 30.0 | 19.0 | 10 | 0.51 | 2.47 | 1.62 | 4.93 | 12.7 |
| 15 | 51.5 | 22.1 | 16.4 | 10 | 0.56 | 2.06 | 1.17 | 4.38 | 18.9 |
| 16 | 41.0 | 30.0 | 19.0 | 10 | 0.5 | 2.28 | 1.68 | 5.14 | 11.8 |
| 17 | 55.4 | 23.2 | 11.4 | 10 | 0.59 | 1.84 | 1.13 | 4.02 | 19.3 |
| 18 | 52.4 | 26.5 | 11.1 | 10 | 0.58 | 1.85 | 1.19 | 4.06 | 18.4 |
| Control | 90 | 0 | 0 | 10 | 0.59 | 1.47 | 1.12 | 3.6 | 26.9 |
| | 0 | 90 | 0 | 10 | 0.5 | 2.59 | 1.81 | 5.42 | 17.3 |
| | 0 | 0 | 90 | 10 | 0.55 | 2.13 | 1.51 | 7.06 | 18.6 |
| | Control (unmalted sorghum) | | | | 0.69 | 1.30 | 1.10 | 5.30 | 24.6 |
| | Adj R-Squared | | | | 0.94 | 0.95 | 0.92 | 0.95 | 0.94 |
| | Lack of fit | | | | 0.38 | 0.63 | 0.22 | 0.48 | 0.47 |

Where: Premix is 5% Figl leaves, 4.5% sugar and 0.5% iodized salt

per unit volume [25]. Besides this, the low WAC could allow the addition of more flour to help process nutrient-dense products.

The oil absorption capacity (OAC) in the formulated complementary flours ranged from 0.96 to 1.68 g/g (Table 4). The OAC was increased with an increase in the proportion of blanched soybean and boiled karkade seeds flours and decreased with an increase in malted sorghum flours in the blend. The result showed that the flour blend, which has high OAC, could probably be due to the hydrophobic character of protein in the flour [31]. The presence of proteins exposes more non-polar amino acids to the fat and enhances hydrophobicity that absorption of more oil which is desired characteristic to prepare energy and nutrient-dense food, especially for infants and young children [45].

The swelling power (SP) of the complementary flours decreased from 5.14 to 3.71 g/g with increased supplementation of malted sorghum in the formulation (Table 4). The low swelling capacity observed in the blend of high malted sorghum

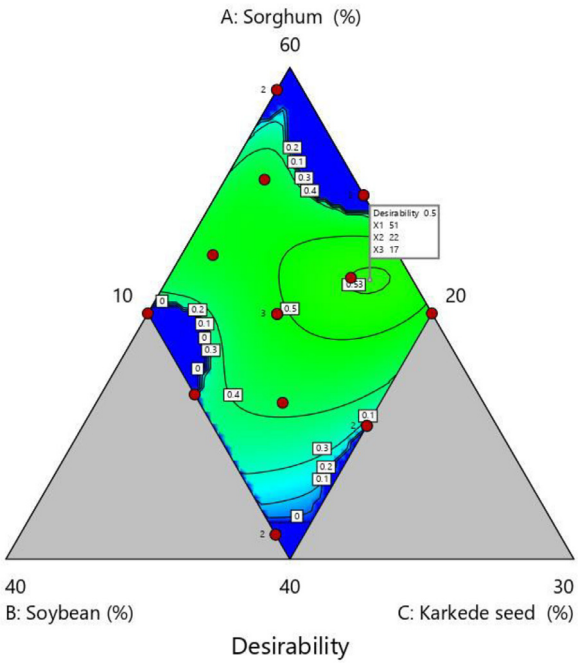


**Fig. 3.** . Numerical optimization of bioactive compounds, antioxidant capacity and functional properties of formulated complementary flour.

could be possible because of starches breakdown by amylase enzymes on the malting to sugars known to swell less than the intact starch granules [3]. For supplemental food gruel, prepared complementary flour with a low swelling capacity is chosen because it increases the nutrient density of the food [25].

Water solubility (SO) of the complementary flours increased from 11.81 to 23.32% with an increase in the malted sorghum ratio and decreased with the increase of blanched soybean and boiled karkade seeds flours (Fig. 2). A similar result was also observed in the complementary meal supplemented with malted sorghum flour [2]. The high solubility of malted sorghum-based complementary flour can be attributed to the high sugar content generated from enzymatic hydrolysis starches on sorghum malt production [44]. Malting cereal grains reduce viscosity and boost solubility in gruel made from complementary food formulations [7]. The findings imply that developing supplementary flour with a high solubility power is essential for newborns and young children because it provides more digestible components and produces a smooth and consistent gruel ideal for infant feeding.

*Optimization of complementary flour rich in bioactive compounds, antioxidant capacity, and desirable functional properties*

The best formulation with a desirability value of 0.50 was a blend of 51% malted sorghum, 22% blanched soybean, and 17% boiled karkade seeds flour with 10% premix (Fig. 3). This mix proportion could provide complementary flour rich in health-enhancing bioactive compounds, antioxidant capacity, and good functional properties among evaluated formulations and the control.

*Sensory evaluation of optimized complementary gruel*

A sensory evaluation was made between the optimized complementary formulation and the control or traditionally used gruel made from un-malted sorghum flour.

The panellists evaluation results showed that there was no significant ($p > 0.05$) difference in the taste, flavor, and overall acceptability between the control and optimized gruel from the formulated complementary flour rich in bioactive compounds and antioxidant capacity (Table 5). However, there were significant ($p < 0.05$) differences in the color, aroma, and texture. The control was more preferred in color (4.19) than the optimized formulation (3.34) (Fig. 4), which could be associated with the addition of 5% green figl leaves powder in the pre-mix to make the gruel color greener. Gebretsadikan *et al.* [20] also indicated the lesser acceptance of gruel color due to the addition of green moringa leaf powder.

However, the aroma of gruel from the optimized formulation got better (3.66) as compared with the control (2.57) (Table 5). An improved aroma of the optimized formulation could be associated with germination of sorghum flour and heat treatment of soybean and karkade seeds. Bolarinwa et al. [8] also reported that sorghum malting plays a vital role in enhancing aroma. In addition, reducing sugars from germinated sorghum and amino acids from soybean and karkade could favor Maillard reactions to develop a better aroma [18].

**Table 5**
Sensory scores of complementary gruels along with control sample ($N = 53$).

| FM | Color | Aroma | Taste | Flavor | Mouthfeel | Overall acceptability |
|---|---|---|---|---|---|---|
| Control | 4.19 ± 1.00 | 2.57±1.00 | 3.47 ± 0.99 | 3.38 ± 1.12 | 2.49 ± 1.12 | 3.32 ± 1.08 |
| FM1 | 3.34 ± 0.89 * | 3.66±0.89 ** | 3.40 ± 1.03 ns | 3.34 ± 0.89 ns | 3.25±0.79 ** | 2.85 ± 0.81 ns |

Where: ns: non-significant ($p > 0.05$), * significant ($p < 0.05$), and ** significant ($p < 0.0001$).
Control sample: 100% unmalted sorghum.
FM1 (formulation 1): 51% malted sorghum, 22% blanched soybean, and 17% boiled karkade seeds flour with 10% of premix (5% of figl leaves, 0.5% salt, 4.5% sugar).


**Fig. 4.** . Gruel developed for sensory evaluation from optimized complementary flour along with a control sample.
Where control sample: 100% unmalted sorghum and FM1 (formulation one): 51% malted sorghum, 22% blanched soybean, and 17% boiled karkade seeds flour with 10% of premix (5% of figl leaves, 0.5% salt, 4.5% sugar).

In terms of mouthfeel, gruel made from optimized flour showed better acceptance (3.25) than the gruel made from the control sample (2.49) (Table 5). The pre-milling processes of the ingredients could contribute to modifying the macromolecules structures for better mouthfeel, which could be associated with improvement in specific functional properties are indicated above.

## Conclusion

In this study, bioactive compounds, antioxidant capacity, functional and sensory properties of optimized complementary weaning flour processed from malted sorghum (40–60%), blanched soybean (20–30%), and blanched karkade seeds (10–20%) with 10% premix (5.0% figl leaf powder, 4.5% sugar and 0.5% iodized table salt) flours along with control sample (un-malted sorghum) are reported. The findings showed that increasing the proportion of malted sorghum levels in the formula increased bioactive compounds (total phenolic, flavonoid, L-ascorbic acid, and beta carotene contents) and antioxidant capacity (percent of free radical scavenging activities and ferric reducing power). The study showed formulation from 51.0% malted sorghum, 22.0% blanched soybean, and 17.0% boiled karkade seeds flour plus 10.0% premix was found as the optimum values of bioactive compounds, antioxidant capacity, and functional properties. The sensory acceptability of the gruel samples evaluated from the optimized complementary flour rich in antioxidants was significantly liked in terms of aroma and mouthfeel compared to the control sample. In general, the result found that supplemented weaning flour made from malted sorghum, blanched soybean, and boiling karkade seeds may be processed to produce high levels of health-promoting bioactive compounds with desirable sensory qualities.

## Declaration of Competing Interest

There are no conflicts of interest declared by the authors.

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