Food Science and Engineering
http://ojs.wiserpub.com/index.php/FSE/

Research Article

Effect of Fermentation and/or Cooking on Some Nutritional Quality of Sorghum Meals Formulated with Different Ratios of Wheat and Pumpkin Flour

Mayada A. Mustafa, Almujtaba H. M. Abdallh, Elfadil E. Babiker*

Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Khartoum North 13314, Shambat, Sudan
Email: ebabiker.c@ksu.edu.sa

Received: 24 July 2024; **Revised:** 14 October 2024; **Accepted:** 17 October 2024

Abstract: Sorghum cultivar (Tabat), defatted pumpkin seed pulp (DPSP), and wheat flour were used to prepare four meals to study the effects of component ratio and fermentation and/or cooking on various nutritional qualities. Before and after processing, the meals' chemical compositions, total calories, minerals, antinutrients, and *in vitro* protein digestibility (IVPD) were assessed. The addition of wheat and pumpkin flour resulted in a decrease in carbohydrates and antinutrients and an increase in ash, protein, fiber, fat, total and extractable minerals, and IVPD. After fermentation, the antinutrients were significantly ($P \leq 0.05$) reduced, resulting in meals of excellent quality. Cooking the fermented dough afterward, nevertheless, revealed much greater improvement. The proportion of ingredients decreased the effect of cooking on chemical composition and led to a substantial ($P \leq 0.05$) reduction in tannin and phytate, which was accompanied by a significant ($P \leq 0.05$) increase in IVPD and mineral extractability. Meals were accepted more often than the control group ($P \leq 0.05$). Out of all the formulations, the one with 10% wheat and 20% DPSP flours (M3) was found to be the most nutrient-dense and bioavailable.

Keywords**:** sorghum, wheat, pumpkin, supplementation, nutritive value

1. Introduction

Malnutrition especially micronutrient deficiencies is the main contributor of preventable is a widespread problem in underdeveloped countries that disproportionately affects the elderly, young people, and pregnant mothers, who are viewed as the weaker members of society [1]. Sorghum (*Sorghum bicolor* L.) Moench is a significant drought-resistant cereal crop in Africa that is widely turned into flour and used to feed the poorest sectors of society. However, sorghum, like other grains, has low nutritional value due to a lack of necessary amino acids and the presence of antinutrients such as phytate and tannins [2]. These antinutritional substances interact with minerals, vitamins, and proteins to generate insoluble compounds that prevent critical nutrients from being absorbed and utilized by the human body [3]. As the most important and widely produced crop in the world, wheat (*Triticum aestivum* L.) provides 20% of all calories consumed with minimal amounts of antinutrients, it is also a substantial source of minerals and protein [4]. On the other hand, pumpkin seed pulp has a high mineral content that is beneficial to both humans and animals, and it is particularly rich in protein, carbohydrates, and fats with low antinutrients [5]. According to El Salhy et al. [6], pumpkin

DOI: https://doi.org/10.37256/fse.5220245361

flour is a rich source of beta-carotene, iron, ascorbic acid, and protein. The process of fermentation has been found as a simple food processing method with the ability to increase nutritional accessibility. Microorganisms created during fermentation destroy antinutritional components, decreasing antinutrients and raising levels of iron, calcium, and zinc [7]. Fermentation can also lower pH and create soluble ligands that improve iron and zinc absorption, hence boosting the activity of phytase, a naturally occurring enzyme found in grain and legume flours [8]. Fermentation improves protein digestibility by lowering anti-nutrient levels and increasing the activity of microbial proteases generated during the breakdown of the food matrix [9]. Aromatic molecules are created during fermentation, which improves the sensory characteristics of foods [10]. The fermentation of sorghum flour extends its shelf life by producing lactic acid, which lowers the pH and inhibits microbial development [11]. Fermentation improves food safety and shelf life, which aids in food preservation. In addition, to increase the nutritional value of grains, home processing procedures such as cooking, roasting, sprouting, fermenting, and malting are excellent alternatives [12]. Several conventional food processing methods, such as cooking and malting, enhance the nutritional value of cereal grains by reducing antinutrients such as tannin, phytic acid, and oxalic acid in societies where cereals and legumes are an important part of the diet [13]. Food supplementation by the addition of micronutrient-rich foods to staple foods is a low-cost option for addressing nutrient deficiencies that has the potential for widespread use and adaptation [14]. Improving the nutritional content of sorghum by integrating underutilized nutrient-rich crops like pumpkin is a cost-effective and efficient way to alleviate micronutrient deficits while also improving the quality of staple foods. Unlike wheat, pumpkin seed flour is high in fiber, which improves intestinal function and provides a sense of satiety, which is crucial for body weight control [15]. Pumpkin seed flour has the potential to serve as a beneficial ingredient in a variety of designed foods [16]. Therefore, the use of pumpkin seed flour and wheat flour as additives in sorghum meal manufacturing has a high potential to fill the nutritional gap that may exist in sorghum. This could assist in minimizing malnutrition caused by protein-energy deficiencies in schoolchildren and adults, as the primary causes of malnutrition in Africa are a lack of nutritious foods and insufficient consumption of protein-rich diets [17]. This study aims to examine the effects of fermentation, cooking, and component ratios on the nutritional value of ready-to-eat meals made with sorghum, wheat, and DPSP flour.

2. Materials and methods

2.1 *Materials*

The sorghum cultivar (Tabat) was obtained from the Agricultural Research Corporation in Wad-Madani, Sudan. Wheat (Debaria) flour was obtained from the Seen Flour Mills in Khartoum, Sudan. Pumpkin seeds were purchased in the Omdurman local market in Sudan. Sorghum, wheat, and pumpkin seeds were cleaned from foreign matter. The pumpkin seed pulps were defatted and powdered in an electric miller until they passed through a sieve (0.4 mm), then oven-dried at 60 °C for 14 hours. All chemicals used in this study were reagent grade.

2.2 *Preparation and processing of meals*

Sorghum, wheat, and DPSP flours were utilized to make four different meals with varying ratios (M0, 100:0:0; M1, 70:20:10; M2, 70:15:15, and M3, 70:10:20). Each meal's ingredients were mixed with distilled water (1:2 W/V) and left to ferment spontaneously for 24 hours at room temperature. After the incubation period, the fermented flour was placed in aluminium plates and dried in a hot air oven for a further 24 hours at 70 °C. The dried samples were then powdered to pass a 0.4 mm screen and stored at 4 °C. Next, a part of the fermented dough was cooked on a heated steel plate. The cooked meals were stored at 4 °C for further examination after being dried at 70 °C in a hot oven and pulverized to pass a 0.4 mm screen.

2.3 *Proximate composition*

The AOAC [18] standard procedures were used to assess the crude protein, fat, and ash content. Using the difference, the carbohydrate (nitrogen-free extract) was calculated. According to Osborne and Voogt's [19] description, the energy was computed using the Atwater factors, which state that there are four calories in one gram of carbohydrates, four calories in one gram of protein, and nine calories in one gram of fat.

2.4 *Determination of mineral contents*

The mineral contents of the meal samples (2 g) were determined using Pearson's [20] method. After being heated to 550 °C for three hours, the material was cooled. About 10 ml of 50% HCl and 5 ml of 33% HNO_3 were used to treat the ash, and it was then immersed in a water bath at 100 °C for an hour. After adding 10 ml of HCl, the sample was left in the water bath for an additional fifteen minutes. Following that, the mixture was thoroughly mixed before being added to a 100 ml volumetric flask that also held 100 ml of distilled water. Using Perkin-Elmer 2380 atomic absorption spectroscopy, the Fe content was ascertained. P content was determined using the ammonium molybdate technique [21]. To determine the Ca content, the Chapman and Pratt's [22] method was applied.

2.5 *Minerals HCI-extractability*

The ingredients and meal minerals' extractability were determined by the method proposed by Kumar and Chauhan [23]. Ten milliliters of 0.03 N HCl were used to extract one gram of the material, which was left at 37 °C for three hours. After passing the clear extract through Whatman filter paper, it was oven-dried at 100 °C and heated for three hours at 550 °C in a muffle furnace. After the heated samples had cooled, 3 ml of 3 N HCI was added, and the volume was diluted with distilled water to form 50 ml. The concentrations of Fe, P, and Ca in the extract were ascertained before. The extractability of the minerals was computed as a percentage of the total mineral content of the HCl extract.

2.6 *Determination of tannin*

The method of Price et al. was performed for the quantitative estimate of tannin [24]. Ten milliliters of 1% HCl in methanol (V/V) were poured into a 100-milliliter conical flask containing 0.2 grams of each sample, thoroughly mixed, and centrifuged for five minutes at 2,500 rpm. Next, 1.0 ml of supernatant and around 5 ml of vanillin-HCl reagent were combined. Using a spectrophotometer (PD-UV, Apel, Saitama, Japan), the absorbance was measured at 500 nm following 20 minutes of incubation at 300 °C. Using a standard curve, the concentration of tannin was determined, and the results were represented as catechins equivalent (CE mg/100 g).

2.7 *Determination of phytate*

A spectrophotometer (PD-UV, Apel, Saitama, Japan) was used to measure the phytate content following Wheeler and Ferrel's [25] method. A standard curve with different $Fe(NO_3)_2$ concentrations was plotted to calculate ferric ion concentration and a 4:6 iron and phosphorus molar ratio was used to calculate the phytate phosphorus content.

2.8 *In vitro protein digestibility determination*

Monjula and John's [26] method was used to calculate the IVPD. Meals both before and after processing, weighing about 5 g of each item, were digested for three hours at 37 °C using 1.5 mg of pepsin (25,000 u/ml) in 15 ml of 0.1 M HCl. Four milligrams of pancreatin (800 u/ml) in 7.5 milliliters of 0.2 M phosphate buffer, pH 8.0, with 0.05% sodium azide, was added to the mixture after it had been neutralized with NaOH (0.5 M). The mixture was then gently agitated and incubated at 37 °C for a full day. After being incubated, the material was centrifuged at 5000xg for 20 minutes at room temperature, and then treated with 10% trichloroacetic acid (10 ml) to inactivate the enzymes. Kjeldahl's method [18] was used to measure the amount of nitrogen in the supernatant. The formula below was used to estimate the IVPD:

$$\text{Protein digestibility (\%)} = \frac{\text{N in supernatant} - \text{N in the blank}}{\text{N in sample}}$$

2.9 *Sensory evaluation*

The sensory evaluation was carried out by a panel of thirty semi-trained individuals who were chosen from the faculty staff and students. A wide range of characteristics were evaluated, such as appearance, flavor, texture, aroma, and overall acceptance. A 9-point hedonic scale was used in the study; 1 indicates strong dislike, 5 indicates neither like nor dislike, and 9 indicates high like. The panelists were given a briefing before the procedure and instructed to rinse their mouths with water before evaluating the following set of samples. Each panelist was a regular consumer of wheat and sorghum-based products. The scores for each character in each meal were averaged and the data was presented as mean ± SD.

2.10 *Statistical analysis*

Using IBM SPSS Statistics 23.0 software, an analysis of variance was conducted on three data replicates (SPSS Inc., USA). The data's means and standard deviations are displayed. To determine the significance between means, Duncan's multiple range tests were used. Statistical significance was considered acceptable at $P \leq 0.05$.

3. Results and discussion

3.1 *Nutritional quality of ingredients and meals*

Table 1 indicates that sorghum flour had a significantly ($P \leq 0.05$) higher content of carbohydrates (70.83%), phosphorus (297.85 mg/100 g), and total energy (369.87 Kcal) than other ingredients. It also had a higher content of tannin (369.87 mg/100 g) and phytate (340.02 mg/100 g), which coincided with a lower protein digestibility (45.73%). As reported by Tasie and Gebreyes [27] and Abah et al. [28], sorghum is a rich source of minerals and carbohydrates, but it also contains a high concentration of antinutrients, which reduces the bioavailability of minerals and proteins. Wheat flour, on the other hand, had better protein digestibility (49.88%) and considerably ($P \leq 0.05$) higher carbohydrate content (71.71%) than sorghum. According to Javid et al. [29], wheat is a valuable source of carbohydrates in most industrialized countries, as well as a protein and dietary fiber for human foods. It also contains minerals, lipids, and vitamins, all of which are sources of micronutrients. Furthermore, wheat showed minimal amounts of antinutrients, indicating a high nutritional value. Moreover, DPSP flour had considerably ($P \leq 0.05$) higher levels of ash, protein, fiber, calcium, and iron. It also had lower levels of phytate (184.96 mg/100 g) and tannin (22.13 mg/100 g), which coincided with comparable protein digestibility. Pumpkin seed pulp has been reported to have a high mineral content that is beneficial to both humans and animals, and it is particularly rich in protein, carbohydrates, and fats with low antinutrients [5]. In addition, El Salhy et al. [6] reported that pumpkin flour is a rich source of beta-carotene, iron, ascorbic acid, and protein.

Table 1. Chemical composition (%), minerals contents (mg/100 g), total energy (Kcal), ant-nutrients (mg/100 g) and *in vitro* protein digestibility (IVPD) of sorghum cultivar, wheat and pumpkin seeds flour samples

Flour type	Dry matter	Ash	Crude protein	Crude fiber	Fat	CHO	Ca	Fe	P	Total energy	Tannin	Phytate	IVPD (%)
Sorghum (Tabat)	$91.65^{b} \pm 0.13$	$1.99^{b} \pm 0.01$	$11.02^{b} \pm 0.11$	$2.23^{b} \pm 0.08$	$3.49^{b} \pm 0.08$	$70.83^{b} \pm 0.17$	$22.34^{b} \pm 0.15$	$4.62^{c} \pm 0.89$	$297.85^{a} \pm 1.25$	$369.87^{a} \pm 3.56$	$340.02^{a} \pm 0.98$	$229.33^{a} \pm 0.61$	$45.73^{c} \pm 1.66$
Wheat flour	$87.67^{c} \pm 0.03$	$0.89^{c} \pm 0.02$	$12.63^{b} \pm 0.14$	$0.44^{c} \pm 0.01$	$2.01^{c} \pm 0.21$	$71.71^{a} \pm 0.26$	$8.54^{c} \pm 0.69$	$10.86^{b} \pm 0.67$	$75.76^{c} \pm 0.55$	$361.12^{b} \pm 4.34$	$40.67^{b} \pm 0.64$	$43.42^{c} \pm 1.99$	$49.88^{a} \pm 0.82$
Pumpkin	$94.65^{a} \pm 0.09$	$8.67^{a} \pm 0.01$	$63.15^{a} \pm 0.13$	$7.09^{a} \pm 0.11$	$3.81^{a} \pm 0.12$	$6.92^{c} \pm 0.17$	$48.08^{a} \pm 0.55$	$26.37^{a} \pm 1.92$	$224.1^{b} \pm 0.51$	$346.59^{c} \pm 2.28$	$22.13^{c} \pm 0.86$	$184.96^{b} \pm 0.15$	$47.9^{b} \pm 0.81$

Values are mean ± SD. Values with the same superscript letter in column are not significantly different ($P \leq 0.05$). CHO = Carbohydrates.

Nutrient-dense ready-to-eat meals were produced by fermenting and cooking sorghum in different ratios with wheat and DPSP flour to compensate for nutritional deficiencies and to reduce the factors that lead to poor nutrient bioavailability. Four meals were made using various proportions of wheat, sorghum, and DPSP flour. The proximate composition and energy changes of sorghum flour combined with wheat and pumpkin are shown in Table 2. Except for the raw meal with high pumpkin flour (M3), which had considerably ($P \leq 0.05$) high dry matter, the dry matter percent of the meals did not alter significantly after adding different ratios of wheat and DPSP flour or even when they were processed. Fermentation reduced the dry matter percentage slightly for all meals. Cooking of fermented meals, however, somewhat raised the dry matter percentage. According to El Khatib and Muhieddine [30], the increased dry matter content could be attributed to the high dry matter concentration of pumpkin pulp seed flour. The ash content of the meals increased significantly ($P \leq 0.05$) with the addition of wheat and DPSP flours, with a high value noted for meals prepared with 20% DPSP flour (M3). Meals that were fermented both before and after cooking had more ash content than sorghum meal, and M3 had a significantly ($P \leq 0.05$) high value. According to Hamed et al. [31], the high ash content in DPSP flour may have contributed to the rise in ash concentration following the addition of wheat and DPSP flours. Moreover, the present study aligns with the findings of Ike et al. [32], who examined the nutritional and microbiological characteristics of pumpkin seed composite flours and deduced that the elevated ash content in the blends might be ascribed to the superior nutritional value of pumpkin seed flour in comparison to wheat flour.

Table 2. Chemical composition (%) and total energy (Kcal) of ready-to-use fermented and cooked meal

Chemical composition (%)	Composite flour			
	S:W:P	S:W:P	S:W:P	S:W:P
	100:0:0	70:20:10	70:15:15	70:10:20
Dry matter				
Raw	$91.65^{bB} \pm 0.27$	$90.82^{cA} \pm 0.31$	$91.16^{bA} \pm 0.24$	$93.86^{aA} \pm 0.26$
Fermented	$91.16^{aB} \pm 0.04$	$90.08^{bB} \pm 0.67$	$90.97^{bB} \pm 0.76$	$91.37^{aB} \pm 0.56$
Cooked	$92.45^{aA} \pm 0.17$	$91.07^{bA} \pm 0.38$	$91.56^{bA} \pm 0.31$	$91.61^{bB} \pm 0.33$
Ash				
Raw	$1.99^{cB} \pm 0.08$	$2.44^{bA} \pm 0.06$	$2.66^{bB} \pm 0.24$	$3.24^{aA} \pm 0.12$
Fermented	$2.29^{bA} \pm 0.03$	$2.65^{bA} \pm 0.18$	$3.04^{aA} \pm 0.19$	$3.45^{aA} \pm 0.29$
Cooked	$2.61^{bA} \pm 0.08$	$2.84^{bA} \pm 0.35$	$3.24^{aA} \pm 0.29$	$3.62^{aA} \pm 0.12$
Crude protein				
Raw	$11.02^{dC} \pm 0.11$	$17.67^{cC} \pm 0.94$	$20.19^{bB} \pm 0.92$	$22.86^{aC} \pm 0.87$
Fermented	$15.03^{dA} \pm 0.53$	$19.37^{cA} \pm 0.15$	$21.89^{bA} \pm 0.12$	$24.44^{aA} \pm 0.46$
Cooked	$13.31^{dB} \pm 0.59$	$18.56^{cB} \pm 0.17$	$20.49^{bB} \pm 0.12$	$24.39^{aA} \pm 0.15$
Crude fiber				
Raw	$2.23^{aA} \pm 0.75$	$2.42^{aA} \pm 0.19$	$2.78^{aA} \pm 0.21$	$2.88^{aB} \pm 0.62$
Fermented	$2.03^{aA} \pm 0.04$	$2. 22^{aA} \pm 0.63$	$2.55^{aA} \pm 0.63$	$2.64^{aB} \pm 0.14$
Cooked	$2.39^{bA} \pm 0.89$	$2.46^{bA} \pm 0.06$	$2.79^{bA} \pm 0.16$	$3.19^{aA} \pm 0.19$

Table 2. (cont.)

Chemical composition (%)	Composite flour			
	S:W:P	S:W:P	S:W:P	S:W:P
	100:0:0	70:20:10	70:15:15	70:10:20
Fat				
Raw	$3.49^{bA} \pm 0.08$	$3.73^{bA} \pm 0.07$	$4.05^{aA} \pm 0.15$	$4.41^{aA} \pm 0.17$
Fermented	$3.79^{bA} \pm 0.37$	$3.88^{bA} \pm 0.06$	$4.21^{aA} \pm 0.15$	$4.54^{aA} \pm 0.05$
Cooked	$3.78^{bA} \pm 0.27$	$3.81^{bA} \pm 0.72$	$4.18^{aA} \pm 0.12$	$4.51^{aA} \pm 0.14$
Carbohydrates				
Raw	$70.83^{aA} \pm 0.72$	$64.55^{bA} \pm 0.61$	$61.61^{cA} \pm 0.91$	$58.12^{dA} \pm 0.33$
Fermented	$68.51^{aB} \pm 0.38$	$61.97^{bC} \pm 0.33$	$59.29^{cC} \pm 0.69$	$56.32^{dB} \pm 0.04$
Cooked	$70.37^{aA} \pm 0.66$	$63.39^{bB} \pm 0.59$	$60.81^{cB} \pm 0.16$	$55.89^{dC} \pm 0.75$
Total energy (Kcal/mole)				
Raw	$358.81^{cB} \pm 1.22$	$362.45^{bA} \pm 1.72$	$363.65^{aA} \pm 1.24$	$363.61^{aA} \pm 1.55$
Fermented	$368.27^{aA} \pm 1.34$	$360.28^{cB} \pm 2.12$	$362.61^{bB} \pm 2.74$	$363.91^{bA} \pm 2.14$
Cooked	$368.74^{aA} \pm 2.02$	$362.09^{bA} \pm 2.32$	$362.82^{bB} \pm 1.52$	$361.71^{cB} \pm 1.81$

Values are mean ± SD. Values with the same superscript letter in column are not significantly different ($P \leq 0.05$). S:W:P = percent of sorghum (Tabat), wheat and pumpkin flour in the composite flour.

All ratios of ingredients showed a substantial increase in protein and fiber contents ($P \leq 0.05$) especially when DPSP flour was added. Compared to other meals, M3 had higher protein and fiber content (22.86% and 2.88%, respectively). According to Hamed et al. [31], this is largely because DPSP flour has a high protein and fiber content. Ike et al. [32] reported that as more pumpkin seed flour is substituted, the mixes' protein and fiber content rises. This projected increase served as the foundation for creating the blends, resulting in a completed product that had not only more protein but also higher protein quality. Meal fermentation increased protein content significantly ($P \leq 0.05$), with M3 having the highest protein content (24.44%) compared to other meals. Cooking the fermented meals, however, reduced the protein content of all meals, including those that had not been formed. Crude fiber decreased significantly ($P < 0.05$) during fermentation, but slightly rose after heating all meals. Mbijiwe et al. [33] blended fermented sorghum flour with pumpkin pulp and seed to produce vitamin A and iron, and the results show that pumpkin pulp and pumpkin seed can be utilized to improve the nutritional value of sorghum with high protein and fiber content.

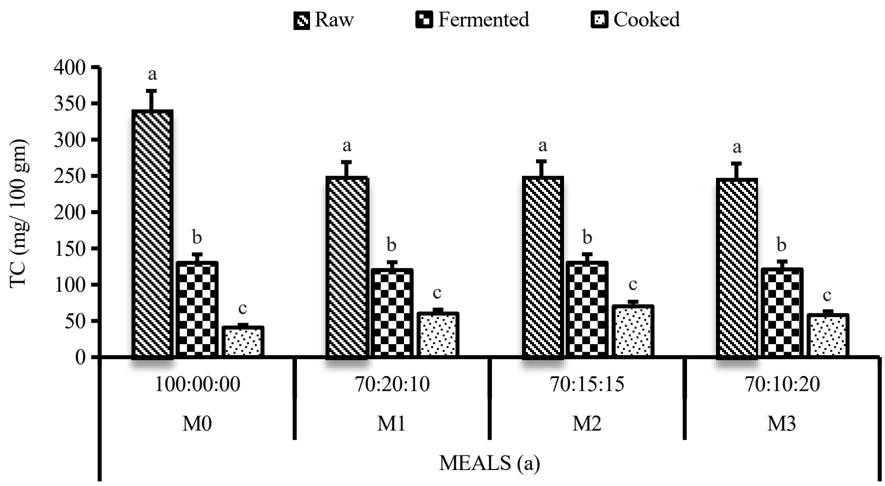
The result indicates that ingredients with high nutrient values may be the cause of the increase in protein content before processing. Furthermore, as observed by Kårlund et al. [34] and Adebo et al. [35], fermentation considerably ($P \leq 0.05$) increased protein content through the production and solubilization of metabolic intermediates by microbes. After fermentation, the amount of protein increases and the amount of fiber decreases, which is consistent with the results of Makkawi et al. [36] who examined the quality of cooked and fermented sorghum flour using baobab fruit pulp flour as a starter. Consistent with the current findings, a study by Babalola and Giwa [37] demonstrated that a considerable ($P \leq 0.05$) reduction in fiber content during fermentation may be attributable to breakdown by fermenting bacteria.

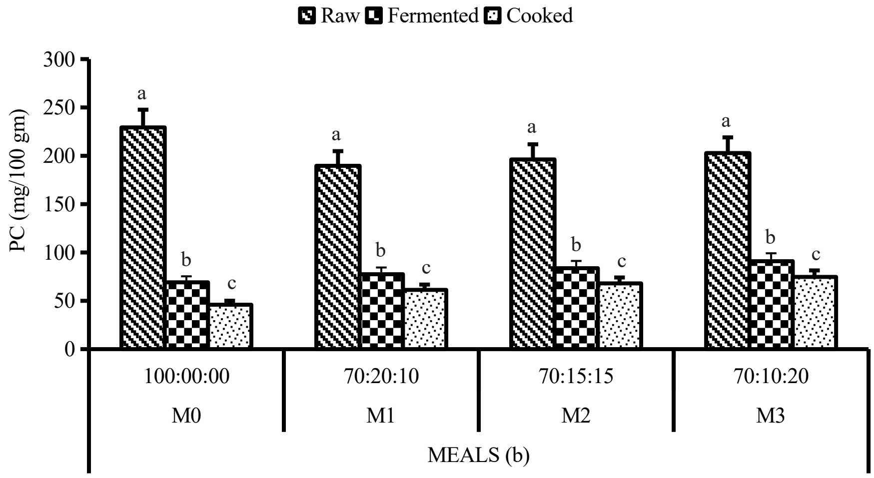
The fat content of the raw meals was significantly ($P \leq 0.05$) enhanced by the addition of DPSP flour and wheat, while it decreased slightly after processing. The high remaining fat in DPSPF may be the cause of the rise in fat content in raw meals, whereas a high rate of lipolysis to fatty acids and glycerol during fermentation may be the cause of the decrease in fat content after processing. Adebo et al. [35] report that although nutrient changes during fermentation depend on the inherent and readily available nutrients in the starting raw material, fermentation generally increases these nutrient qualities, such as fats and fatty acids in cereals and legumes, while in certain cases, a decrease in these constituents was noted. Due to the low levels of carbohydrates in both DPSP flour and wheat flour, it has been found that there was a decrease in carbohydrates after adding such ingredients. This reduction may have occurred during processing because some of these nutrients were being used by fermented microflora for energy production [38]. The overall energy of the raw meals varied both before and after the addition of DPSP flour and wheat flour, which may have been caused by variations in various energy sources.

3.2 *Changes in tannin, phytate, and in vitro protein digestibility (IVPD) of meals*

The effects of adding wheat flour and DPSP flour on IVPD and anti-nutritional elements in raw and processed meals are depicted in Figure 1. Raw sorghum flour had the greatest concentrations of phytate (229.33 mg/100 g) and tannin (340.02 mg/100 g) when compared to other ingredients. In addition, sorghum flour's IVPD (45.73%) was lower than that of the other ingredients. Tannin (Figure 1a) and phytate (Figure 1b) levels were significantly ($P \leq 0.05$) reduced with the addition of DPSP flour and wheat flour. Furthermore, Figure 1c shows a rapid increase in IVPD, which coincided with a significant ($P \leq 0.05$) decrease in antinutrients (Figures 1a & b). Moreover, when the meals were fermented, there was a substantial ($P \leq 0.05$) decrease in antinutrients and an increase in IVPD. According to Olagunju et al. [38], fermentation dramatically lowered the levels of trypsin inhibitor activity, tannin content, and phytic acid. The action of phytase, which is released during fermentation, may be responsible for this reduction. Makawi et al. [36] have reported that the enzyme tannase, which is generated by microorganisms during fermentation, may be responsible for the decrease in tannin levels in prepared meals. Annor et al. [39] state that processing increases protein digestibility by decreasing anti-nutrient levels and breaking down of proteins.

Additionally, it has been reported that the enzymatic breakdown of dietary fibers releases protein from plant tissues, and the action of microbe-produced enzymes reduces or degrades tannins, polyphenols, and phytic acid during fermentation [39]. According to Suarti and Budijanto [40], reduced pH levels during fermentation may enhance peptidase enzyme activity and activate endogenous proteases, leading to higher amounts of peptides and free amino acids as well as improved protein solubility. Furthermore, some seeds with low protein digestibility because of phytic acid, tannins, and trypsin inhibitors can be boiled or fermented to increase their digestibility; fermentation is the most effective processing method for reducing the activity of these substances [41]. Ogodo et al. [42] reported a similar effect on the protein's digestion in fermented sorghum flour. The researchers hypothesized that hydrolytic enzymes had converted the extremely insoluble storage proteins into simpler, more soluble molecules; and concluded that the pH drop that takes place during fermentation may increase the peptidase enzyme activity and protein solubility.





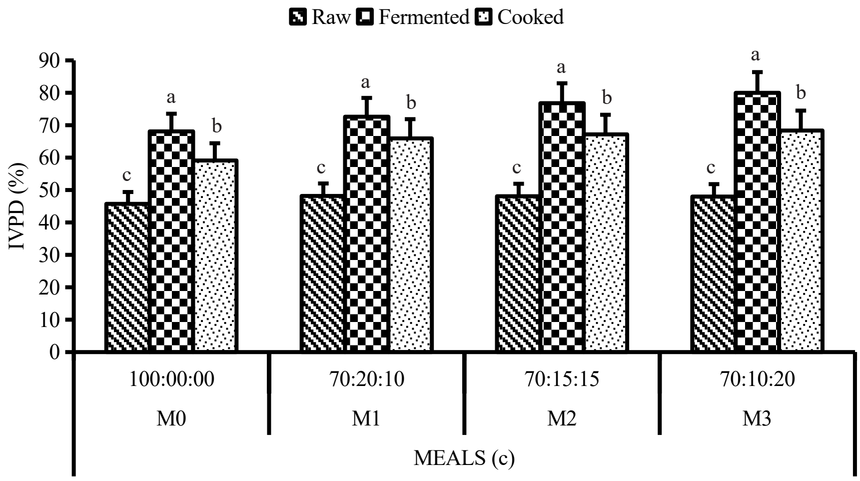


Figure 1. Tannin (TC, a), phytate (PC, b) contents and protein digestibility (IVPD, c) of ready-to-use fermented and/or cooked meal (M, Sorghum: Wheat: Pumpkin). Superscript a, b or c in a column indicate significant difference at $P \leq 0.05$ (Duncan's Multiple Range Test)

3.3 *Changes in mineral contents and extractability of meals*

After adding DPSP flour and wheat flour, Figure 2 demonstrates a significant ($P \leq 0.05$) increase in Ca content, with M3 obtaining the largest value of 27.92 mg/100 g. More increment was seen following heating, with a maximum value obtained for M3 (50.66 mg/100 g), and fermentation of the meals produced an additional and considerable increase in Ca content, with the highest value obtained for M3 (39.84 mg/100 g). With the inclusion of DPSP and wheat flour, it was discovered that the extractability of Ca in raw meals increased, with M3 obtaining the highest percentage (50.52%). Meal fermentation greatly improved the Ca extractability, with M3 obtaining the highest percentage (83.95%). Additionally, all meals' Ca extractability was greatly improved by cooking the fermented meals, with M3 obtaining the highest percentage of 78.17%. Cooking has been shown to reduce fermented foods' ability to extract calcium. The trends for Fe (Figure 3) and P (Figure 4) were comparable to those for Ca. The increase in mineral content is consistent with Liomba et al.'s study [43], which showed that pumpkin flour is an excellent source of minerals that may be used to fortify cereal crops of low nutritive value, serving as a solution to address micronutrient shortages.

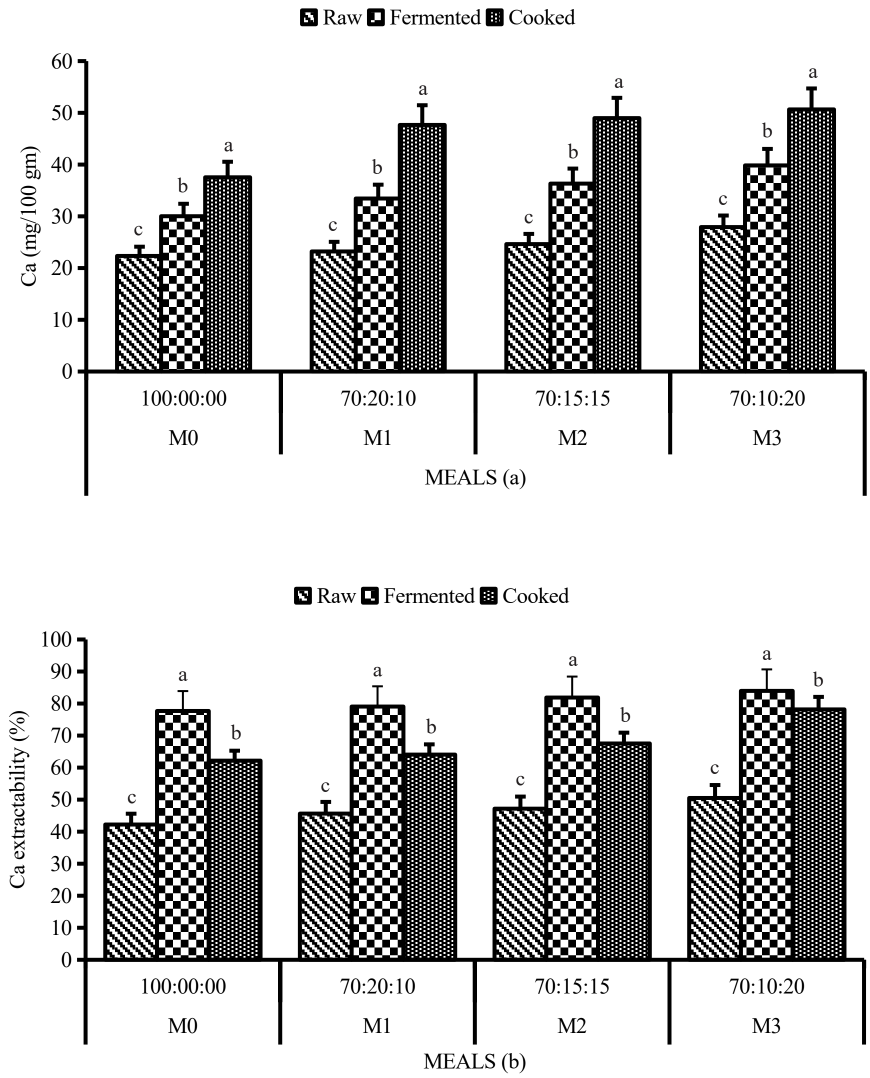
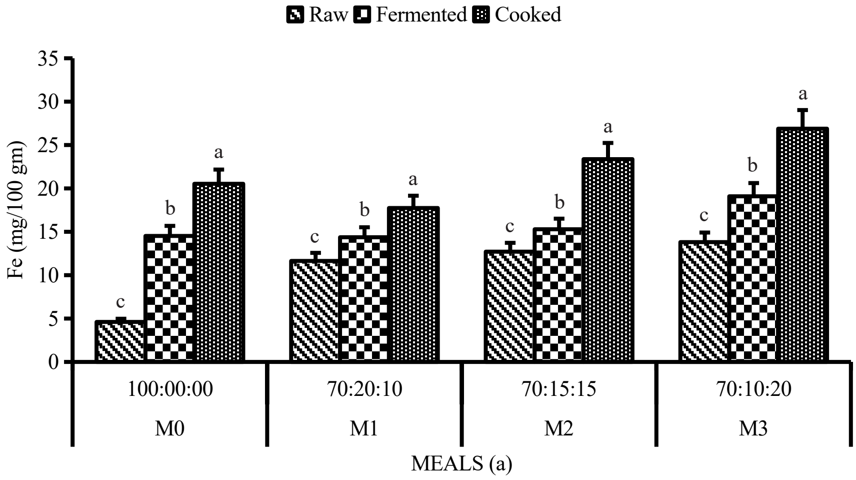


Figure 2. Total (mg/100 g, a) and extractable (%, b) calcium of ready-to-use fermented and/or cooked meal (M, Sorghum: Wheat: Pumpkin). Superscript a, b or c in a column indicate significant difference at $P \leq 0.05$ (Duncan's Multiple Range Test)

Following fermentation and cooking, a further increase in mineral content was observed. According to Adebiyi et al. [44], fermented pearl millet showed an increase in mineral elements like Ca, Na, Cu, Fe, Zn, and K but a decrease in total ash content. Ash was reduced by the leaching of soluble salts, while better mineral extractability and availability as a result of fermentation led to an increase in mineral elements. Fermented rice has been found to have higher mineral and ash concentrations, with the increased ash content being linked to higher mineral solubility and bioavailability [45]. Phytase hydrolyzes phytate and generates more P from phenolics, which may be the cause of fermented meals' higher P content [46]. To increase the bioavailability of critical minerals, the phytate analysis involves the removal of phosphate groups from the phytate's inositol ring. This reduces the strength of mineral binding to phytate. As the ratio of the ingredients increased, so did the mineral level of prepared meals, especially defatted pumpkin seed four, which has a high mineral content [31].



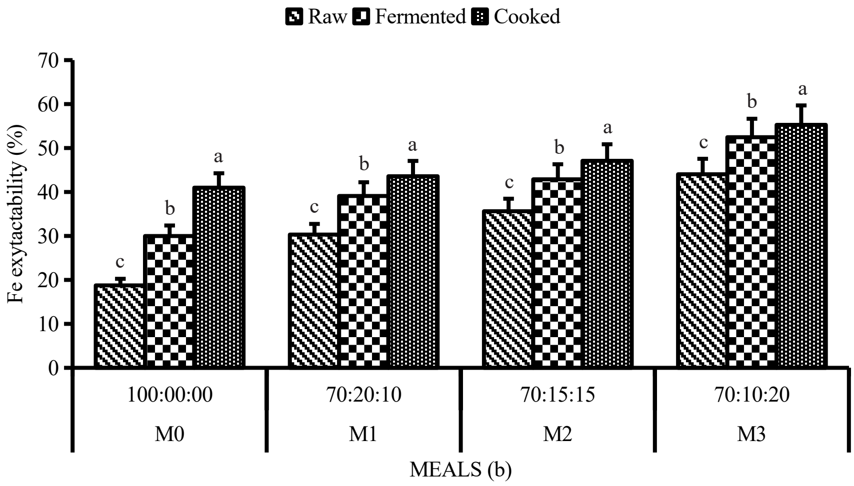
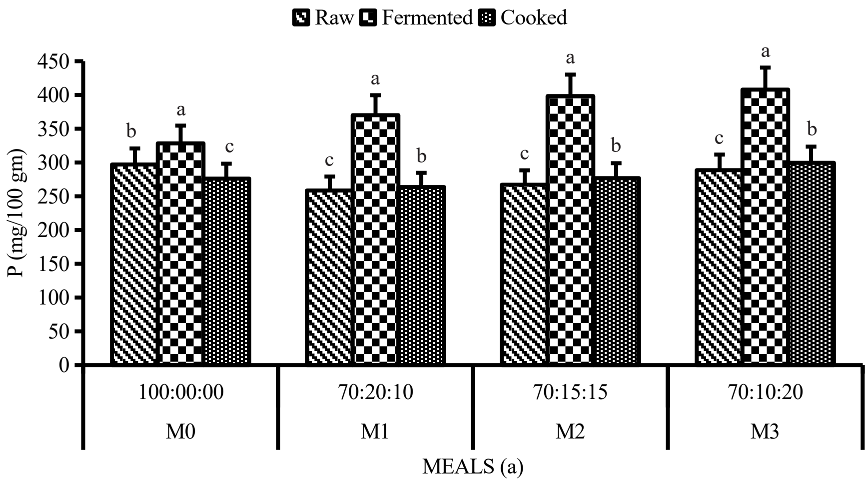


Figure 3. Total (mg/100 g, a) and extractable (%, b) iron of ready-to-use fermented and/or cooked meal (M, Sorghum: Wheat: Pumpkin). Superscript a, b or c in a column indicate significant difference at P ≤ 0.05 (Duncan's Multiple Range Test)



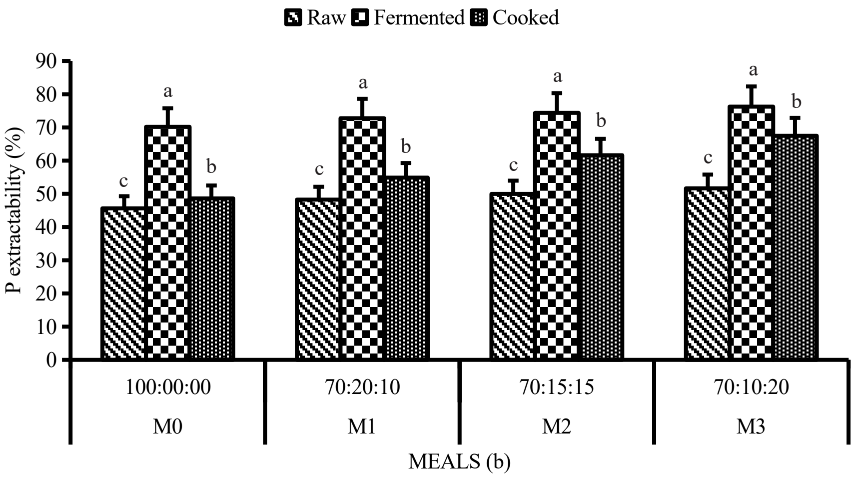


Figure 4. Total (mg/100 g, a) and extractable (%, b) phosphorus of ready-to-use fermented and/or cooked meal (M, Sorghum: Wheat: Pumpkin). Superscript a, b or c in a column indicate significant difference at $P \leq 0.05$ (Duncan's Multiple Range Test)

The highest extractable mineral in raw meals, according to the extractability study, was Ca, which was followed by P and Fe. The process of fermentation significantly ($P \leq 0.05$) improved the mineral extractability of meals. According to Sokrab et al. [47], fermenting both high- and low-phytate maize increased mineral extractability. Makawi et al. [36] suggest that the enhanced mineral extractability of meals may be related to the enzymatic degradation of phytate and tannin by phytase and tannase during the fermentation process. Moreover, the breakdown of carbohydrates and protein by bacteria during fermentation may result in the loss of dry matter, which could account for the increase in mineral content [48]. Fermentation enhances the bioavailability of calcium, phosphorus, and iron, most likely due to the breakdown of tannin and phytates, which bind to minerals and restrict their availability. Minerals extractability in cooked meals improved gradually and significantly ($P \leq 0.05$) as ingredient levels increased. This increase may be attributed to the components' high mineral extractability.

3.4 *Sensory attributes of meals formulate*

Table 3 shows the sensory qualities of fermented and cooked meals with varying ratios of DPSPF and wheat flour. When compared to sorghum meal, wheat- and DPSP-formulated meals, all sensory qualities were superior and significantly different from the control meal (M0). This could be because the combination of wheat and pumpkin significantly improved such properties. According to the current study, adding wheat and pumpkin to sorghum could improve meals without affecting their organoleptic properties. Overall acceptability results suggest that the panelists preferred the flour blend formulations M1, M2, and M3, whereas the control sample (M0) was the least appreciated. These findings suggest that adding a large proportion of pumpkin seed pulp flour to fermented sorghum flour improves the sensory rating of the resulting flour blend compositions. The findings are consistent with Kiharason et al. [49], who reported that including pumpkin flour in wheat at or above 40% resulted in a bitter taste and pungent odor, resulting in a general dislike of bakery products, whereas substituting pumpkin flour at 20% and 30% produced consumer-acceptable food products. In contrast, Adeyeye et al. [50] investigated the effect of fermentation on the nutritional composition, anti-nutritional factors, and acceptability of cookies made from fermented sorghum and soybean flour blends, finding that fermentation affected all sensory properties as well as overall acceptability of the cookie samples.

Table 3. Sensory data of meals formulated from sorghum, wheat and DPSP flour blends

Meal	Appearance	Aroma	Taste	Flavor	Texture	Overall acceptability
M0 (100% sorghum)	6.37 ± 1.32^b	6.15 ± 1.21c	6.52 ± 1.12^b	6.35 ± 0.75^b	6.06 ± 1.16^b	6.29 ± 1.02^b
M1 (S:W:P, 70:20:10)	7.19 ± 0.85^a	7.17 ± 1.03^b	7.65 ± 0.76^a	7.49 ± 0.84^a	7.59 ± 0.66^a	7.42 ± 0.88^a
M2 (S:W:P, 70:15:15)	7.07 ± 1.02^a	7.16 ± 0.95^b	7.34 ± 0.73^a	7.39 ± 0.89^a	7.38 ± 1.31^a	7.27 ± 1.02^a
M3 (S:W:P, 70:10:20)	7.56 ± 0.81^a	8.16 ± 0.87^a	7.61 ± 1.02^a	7.52 ± 0.83^a	7.49 ± 1.01^a	7.67 ± 0.77^a

Values are mean ± SD. Means not sharing a common superscript(s) a, b, or c in a column are significantly different at $P \leq 0.05$ as assessed by Duncan's Multiple Range Test. S:W:P = percent of sorghum, wheat, and pumpkin flour in the meal

4. Conclusion

The results of this study showed the nutritional effect of incorporating DPSP and wheat flour into sorghum flour following fermentation and cooking. The protein, iron, calcium, phosphorus, and total energy content of the flour blend formulations increased with the addition of DPSP and wheat flour. Following processing, the resulting flour blend formulations had high nutrition quality due to the removal of antinutrients and were effective in satisfying preschool children's daily protein, iron, and calcium nutrient requirements. This demonstrates that such a formulation can be utilized as a fortifying agent to improve the nutrient quality of low-nutrition cereal diets, thereby addressing protein, calcium, and iron deficiency in children. Sensory investigations revealed that blend formulations considerably improved the sensory qualities of flour blend formulations; thus, such formulations, particularly those containing 20% DPSP and 10% wheat flour (M3), have the potential to improve the acceptability of sorghum-based goods. Further research is needed to determine the efficacy of such blend formulations in enhancing the vitamin status of preschool children. Furthermore, *in vivo* studies on absorption are required to determine the real amounts of nutrients absorbed in the body after consuming meals containing a sorghum-pumpkin-wheat flour combination.

Authors' contributions

All persons designated as authors contributed equally to this paper. Their substantial contributions include:
1) Conception, design, analysis, and interpretation of data.
2) Drafting the article and revising it critically for important intellectual content.
3) Final approval of the version to be published.

Conflict of interest

The authors report there are no competing interests to declare.

References

[1] Cuesta LL, Rearte A, Rodríguez S, Salinas R, Sosaa C, Rasse S. Anthropometric and biochemical assessment of nutritional status and dietary intake in school children aged 6-14 years, Province of Buenos Aires, Argentina. *Arch Argent Pediatr*. 2018; 116(1): e34-e46. Available from: https://doi.org/10.5546/aap.2018.eng.e34.

[2] Tamilselvan T, Kushwaha A. Effect of traditional processing methods on the nutritional composition of sorghum (*Sorghum bicolour* L. Moench) flour. *European Journal of Nutrition & Food Safety.* 2020; 12(7): 69-77. Available from: https://doi.org/10.9734/ejnfs/2020/v12i730252.

[3] Nath H, Samtiya M, Dhewa T. Beneficial attributes and adverse effects of major plant-based foods anti-nutrients on health: A review. *Human Nutrition & Metabolism*. 2022; 28: 200147. Available from: https://doi.org/10.1016/j.hnm.2022.200147.

[4] Hossain A, Skalicky M, Brestic M, Maitra S, Ashraful Alam M, Syed MA, et al. Consequences and mitigation strategies of abiotic stresses in wheat (*Triticum aestivum* L.) under the changing climate. *Agronomy*. 2021; 11(2): 241. Available from: https://doi.org/10.3390/agronomy11020241.

[5] Shajan AE, Dash KK, Bashir O, Shams R. Comprehensive comparative insights on physico-chemical characteristics, bioactive components, and therapeutic potential of pumpkin fruit. *Future Foods*. 2024; 9: 100312. Available from: https://doi.org/10.1016/j.fufo.2024.100312.

[6] El Salhy M., Ystad SO, Mazzawi T, Gundersen D. Dietary fiber in irritable bowel syndrome. *International Journal of Molecular Medicine*. 2017; 40(3): 607-613. Available from: https://doi.org/10.3892/ijmm.2017.3072.

[7] Almaiman SA, Rahman IA, Gassem M, Dalal A, Alhuthayli HF, Mohammed MA, et al. Biochemical changes during traditional fermentation of saudi sorghum (*Sorghum bicolor* L.) cultivars flour into khamir (local gluten free bread). *Journal of Oleo Science*. 2021; 70(3): 409-415. Available from: https://doi.org/10.5650/jos.ess20311.

[8] Samtiya M, Aluko RE, Puniya AK, Dhewa T. Enhancing micronutrients bioavailability through fermentation of plant-based foods: A concise review. *Fermentation*. 2021; 7(2): 63. Available from: https://doi.org/10.3390/fermentation7020063.

[9] Rashwan AK, Yones HA, Karim N, Taha EM, Chen W. Potential processing technologies for developing sorghum based food products: An update and comprehensive review. *Trends in Food Science and Technology*. 2021; 110: 168-182. Available from: https://doi.org/10.1016/j.tifs.2021.01.087.

[10] Ogodo AC, Ugbogu OC, Onyeagba RA, Orji FA. Dynamics of functional properties of sorghum flours fermented with lactic acid bacteria (consortium isolated from cereals). *International Food Research Journal*. 2017; 24(6): 2666-2671.

[11] Tsafrakidou P, Michaelidou AM, Biliaderis CG. Fermented cereal based products: Nutritional aspects, possible impact on gut microbiota and health implications. *Foods*. 2020; 9(6): 734. Available from: https://doi.org/10.3390/FOODS9060734.

[12] Yousaf L, Hou D, Liaqat H, Shen Q. Millet: A review of its nutritional and functional changes during processing. *Food Research International*. 2021; 142: 110197. Available from: https://doi.org/10.1016/j.foodres.2021.110197.

[13] Ertop MH, Bektaş M. Enhancement of bioavailable micronutrients and reduction of antinutrients in foods with some processes. *Food and Health*. 2018; 4(3): 159-165. Available from: https://doi.org/10.3153/FH18016.

[14] Codex Alimentarius. *Guidelines on formulated complementary foods for older infants and young children, CAC/GL 8-1991*. World Health Organization, Food and Agriculture Organization of the United Nations; 2013. Available from: http://www.fao.org/input/download/standards/298/CXG_008e.pdf.

[15] Oyet GI, Chibor B. Nutrient composition and physical characteristics of biscuits produced from composite blends of wheat, coconut and defatted fluted pumpkin seed flour. *Journal of Nutrition Food Science and Technology*. 2020; 1(1): 1-8.

[16] Chibor BS, Kiin-Kabari DB, Ejiofor J. Physicochemical properties and fatty acid profile of shea butter and fluted pumpkin seed oil, a suitable blend in bakery fat production. *International Journal of Nutrition and Food Sciences*. 2017; 6(3): 122-128. Available from: https://doi.org/10.11648/j.ijnfs.20170603.12.

[17] Bolarinwa IF, Abioye AO, Adeyanju JA, Kareem ZO. Production and quality evaluation of biscuits produced from malted sorghum-soy flour blends. *Journal of Advances in Food Science and Technology*. 2016; 3: 107-113.

[18] AOAC. *Official Methods of Analysis.* 18th ed. Arlington, VA, USA: Association of Official Analytical Chemists; 2005.

[19] Osborne DR, Voogt P. Calculation of caloric value. In: *Analysis of Nutrients in Foods*. New York: Academic Press; 1978. p.23-34.

[20] Egan H, Kirk RS, Sawyer R. *Pearson's Chemical Analysis of Foods.* 8th ed. Churchill Livingstone, London, New York; 1981.

[21] Chapman HD, Pratt FP. *Determination of Minerals by Titration Method: Methods of Analysis for Soils, Plants and Water*. 2nd ed. USA: Agriculture Division, California University; 1982. p.169-170.

[22] Chapman HD, Pratt FP. *Ammonium Vandate-molybdate Method for Determination of Phosphorus: Methods of Analysis for Soils, Plants and Water*. 1st ed. USA: Agriculture Division, California University; 1961. p.184-203.

[23] Kumar A, Chauhan BM. Effects of phytic acid on protein digestibility (in vitro) and HCl-extractability of minerals in pearl millet sprouts. *Cereal Chemistry*. 1993; 70: 504-509.

[24] Price ML, Van Scoyoc S, Butler LG. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry*. 1978; 26(5): 1214-1218. Available from: https://doi.

org/10.1021/jf60219a031.
[25] Wheeler EL, Ferrel RE. A method for phytic acid determination in wheat and wheat fractions. *Cereal Chemistry*. 1971; 28: 313-320.
[26] Monjula S, John E. Biochemical changes and in-vitro protein digestibility of the endosperm of germinating dolichos lablab. *Journal of the Science of Food and Agriculture*. 1991; 55(4): 529-538. Available from: https://doi.org/10.1002/jsfa.2740550405.
[27] Tasie MM, Gebreyes BG. Characterization of nutritional, antinutritional, and mineral contents of thirty-five sorghum varieties grown in Ethiopia. *International Journal of Food Science*. 2020; 2020(1): 8243617. Available from: https://doi.org/10.1155/2020/8243617.
[28] Abah CR, Ishiwu CN, Obiegbuna JE, Oladejo AA. Sorghum grains: nutritional composition, functional properties and its food applications. *European Journal of Nutrition & Food Safety*. 2020; 12(5): 101-111.
[29] Javid Iqbal M, Shams N, Fatima K. Nutritional quality of wheat. In: *Wheat-Recent Advances*. IntechOpen; 2022.
[30] El Khatib S, Muhieddine M. Nutritional profile and medicinal properties of pumpkin fruit pulp. In: *The Health Benefits of Foods-Current Knowledge and Further Development*. IntechOpen; 2020.
[31] Hamed SY, El Hassan NM, Eltayebl MM, Babiker EE. Nutritional evaluation and physiochemical properties of processed pumpkin (*Telfairia occidentalis* Hook) seed flour. *Pakistan Journal of Nutrition*. 2008; 7(2): 330-334.
[32] Ike CC, Emeka-Ike PC, Ogwuegbu HO. Nutritional and microbiological qualities of pumpkin (*Cucurbita pepo*) seed composite flours. *GSC Biological and Pharmaceutical Sciences*. 2020; 12(3): 51-60. Available from: https://doi.org/10.30574/gscbps.2020.12.3.0222.
[33] Mbijiwe J, Ndung'u Z, Kinyuru J. Enrichment of fermented sorghum flour with pumpkin pulp and seed for production of a vitamin A and iron enhanced supplementary food. *Journal of Food Research*. 2021; 10(6): 36-55. Available from: https://doi.org/10.5539/jfr.v10n6p36.
[34] Kårlund A, Gómez-Gallego C, Korhonen J, Palo-Oja OM, El-Nezami H, Kolehmainen M. Harnessing microbes for sustainable development: Food fermentation as a tool for improving the nutritional quality of alternative protein sources. *Nutrients*. 2020; 12(4): 1020. Available from: https://doi.org/10.3390/nu12041020.
[35] Adebo JA, Njobeh PB, Gbashi S, Oyedeji AB, Ogundele OM, Oyeyinka SA, et al. Fermentation of cereals and legumes: Impact on nutritional constituents and nutrient bioavailability. *Fermentation*. 2022; 8(2): 63. Available from: https://doi.org/10.3390/fermentation8020063.
[36] Makawi AB, Mustafa AI, Adiamo OQ, Ahmed IAM. Quality attributes of Kisra prepared from sorghum flour fermented with baobab fruit pulp flour as a starter. *Journal of Food Science and Technology*. 2019; 56(8): 3754-3763. Available from: https://doi.org/10.1007/s13197-019-03848-w.
[37] Babalola RO, Giwa OE. Effect of fermentation on nutritional and anti-nutritional properties of fermenting Soybeans and the antagonistic effect of the fermenting organism on selected pathogens. *International Research Journal of Microbiology*. 2012; 3(10): 333-338.
[38] Olagunju OF, Ezekiel OO, Ogunshe AO, Oyeyinka SA, Ijabadeniyi OA. Effects of fermentation on proximate composition, mineral profile and antinutrients of tamarind (*Tamarindus indica* L.) seed in the production of daddawa-type condiment. *LWT-Food Science and Technology*. 2018; 90: 455-459. Available from: https://doi.org/10.1016/j.lwt.2017.12.064.
[39] Annor GA, Tyl C, Marcone M, Ragaee S, Marti A. Why do millets have slower starch and protein digestibility than other cereals? *Trends in Food Science & Technology*. 2017; 66: 73-83. Available from: https://doi.org/10.1016/j.tifs.2017.05.012.
[40] Suarti B, Budijanto S. Bio-active compounds, their antioxidant activities, and the physicochemical and pasting properties of both pigmented and non-pigmented fermented de-husked rice flour. *AIMS Agriculture & Food*. 2021; 6(1): 49-54. Available from: https://doi.org/10.3934/agrfood.2021004.
[41] Joye I. Protein digestibility of cereal products. *Foods*. 2019; 8(6): 199. Available from: https://doi.org/10.3390/foods8060199.
[42] Ogodo AC, Ugbogu OC, Onyeagba RA, Okereke HC. Microbiological quality, proximate composition and in vitro starch/protein digestibility of Sorghum bicolor flour fermented with lactic acid bacteria consortia. *Chemical and Biological Technologies in Agriculture*. 2019; 6(1): 1-9. Available from: https://doi.org/10.1186/s40538-019-0145-4.
[43] Liomba CM, Kunyanga CN, Adhiambo A. Formulation of a complementary flour with high nutrient density and micronutrient content. *International Journal of Food and Nutrition Research*. 2018; 2: 1-12. Available from: https://doi.org/10.28933/ijfnr-2018-06-1801.
[44] Adebiyi JA, Obadina AO, Adebo OA, Kayitesi E. Comparison of nutritional quality and sensory acceptability of biscuits obtained from native, fermented, and malted pearl millet (*Pennisetum glaucum*) flour. *Food Chemistry*.

2017; 232: 210-217. Available from: https://doi.org/10.1016/j.foodchem.2017.04.020.
[45] Ilowefah M, Bakar J, Ghazali HM, Mediani A, Muhammad K. Physicochemical and functional properties of yeast fermented brown rice flour. *Journal of Food Science and Technology*. 2015; 52: 5534-5545. Available from: https://doi.org/10.1007/s13197-014-1661-7.
[46] Humer E, Schedle K. Fermentation of food and feed: A technology for efficient utilization of macro and trace elements in monogastric. *Journal of Trace Elements in Medicine and Biology*. 2016; 37: 69-77. Available from: https://doi.org/10.1016/j.jtemb.2016.03.007.
[47] Sokrab AM, Mohamed AIA, Babiker EE. Effect of fermentation on antinutrients, and total and extractable minerals of high and low phytate corn genotypes. *Journal of Food Science and Technology*. 2014; 51(10): 2608-2615. Available from: https://doi.org/10.1007/s13197-012-0787-8.
[48] Day CN, Morawicki RO. Effects of fermentation by yeast and amylolytic lactic acid bacteria on grain sorghum protein content and digestibility. *Journal of Food Quality*. 2018; 2018(1): 1-8. Available from: https://doi.org/10.1155/2018/3964392.
[49] Kiharason JW, Isutsa DK, Ngoda PN. Nutritive value of bakery products from wheat and pumpkin composite flour. *Global Journal of Bio-Secience and Biotechnology.* 2017; 6(1): 96-102.
[50] Adeyeye SAO, Adebayo-Oyetoro AO, Fayemi OE, Tiamiyu HK, Oke EK, Soretire AA. Effect of co-fermentation on nutritional composition, anti-nutritional factors and acceptability of cookies from fermented sorghum (*Sorghum bicolor*) and soybeans (*Glycine max*) flour blends. *Journal of Culinary Science & Technology*. 2019; 17(1): 59-74. Available from: https://doi.org/10.1080/15428052.2017.1404536.