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Effect of Pumpkin (*Cucurbita pepo*) Seeds Flour Enrichment and Cooking Process on Protein, *In Vitro* Protein Digestibility and Antinutritional Properties of Maize (*Zea mays* L.) Flour Fritters (*Zitumbuwa*)

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Abstract

Protein-energy malnutrition (PEM) is one of the leading causes of death for under-five children in developing countries like Malawi. This is because these children consume more products made from whole maize flour, like fritters (*zitumbuwa*), with poor protein content. Pumpkin seeds are locally available and contain high amounts of protein and amino acids like lysine. This makes pumpkin seed flour a good choice to improve the protein quality of fritters when combined with maize flour. This study aimed at evaluating the effect of pumpkin seed flour (0%, 15%, 30%, and 45%) and cooking method (baking and frying) on protein content, *in vitro* protein digestibility, and antinutritional content (phytate, tannins, and total phenolic) of the fritters. The pumpkin seed flour (PSF) and maize flour fritters were prepared by frying at 180˚C for 10 min while baking was at 150˚C for 2 h. Pumpkin seed flour significantly increased the protein content of the baked and fried fritters, ranging from 8.5% to 19.6% and 9.1% to 21.9%, respectively. Antinutritional content also increases as the level of pumpkin seed flour increases in fritters. The phytate, tannin, and total phenolic content of the raw maize and pumpkin seed flour ranged from 76.4 mg/100 g to 164 mg/100 g, 4.1 mg/100 g to 88 mg/100 g, and 31.9 mg/100 g to 69.4 mg/100 g, respectively. Cooking reduced antinutritional properties significantly ($p < 0.05$). Frying and baking significantly increased *in vitro* protein digestibility, which ranged from 80.7% to 90.4% and 82.9% to 93.1%, respectively. This study found that there was a weak, significant negative correlation between *in vitro* protein digestibility and antinutritional properties. The high protein content of the enriched fritters may indicate the potential of using locally available, underutilized pumpkin seed flour to serve as a protein supplement when developing different cereal food products, which in turn contribute to food security.

Keywords

Protein Energy Malnutrition (PEM), Fritters, Food Security, Pumpkins, Enrichment

1. Introduction

Malnutrition is a universal public health problem in both children and adults globally with greater concern among under-five children. One form of malnutrition that is becoming a main public health concern especially in children in most developing countries including Malawi is Protein energy malnutrition (PEM) [1]. This is mostly due to poverty in the African region, because populations depend on cereals such as maize for both their protein and energy needs due to their inability to purchase the more nutritious animal source foods [1]. Statistics show that in Malawi 37% and 3% of under-five children are stunted and wasted respectively [2]. In Malawi, strategies such as food fortification and dietary diversity are being used in order to address protein energy malnutrition. However, food fortification is expensive, and fortified foods are not always accessible to those who are vulnerable to malnutrition. The Government of Malawi has formulated the 2018-2022 National Nutrition Policy and Strategic Plan, which promotes the use and consumption of cheap, nutritious, safe, and locally available raw materials such as pumpkins in order to reduce malnutrition.

Whole maize flour fritters (*zitumbuwa*) are the most popular in Malawi and are mostly consumed by both adults and children at breakfast and midmorning. In Malawi, almost 30% of school-going children consume maize flour fritters [3]. Maize flour used to prepare fritters constitutes 75% carbohydrates but is low in protein content (9% - 12%), especially some essential amino acids such as lysine and tryptophan, which are vital for human growth [4]. In Malawi, production of pumpkins reached 428754000.79 metric tonnes in 2024, and its consumption increased from 20% to 40% [5] [6]. Approximately 32.47% protein is present in common pumpkin seeds [7]. Therefore, this study focuses on the use of pumpkin seed flour, which has a high quantity of proteins, when preparing fritters in order to contribute to food security while at the same time eliminating protein-energy malnutrition. The objective of using the frying and baking method was to find another alternative way to prepare fritters since the snack is only prepared using the frying method in Malawi. This is because consumption of highly oily foods contributes to obesity and other cardiovascular diseases [8] [9].

Estimates of protein quality are crucial, especially when developing any type of food product. The *in vitro* protein digestibility (IVPD) provides information on the stability of proteins and how they endure digestive processes. In vitro protein digestibility is categorized into two approaches, which are the multiple-enzyme approach and the single-enzyme approach. Comparing two methods, the advantage of multiple enzymes is that it lessens the negative effects of enzyme inhibitors, but it is very expensive and time-consuming, while the single-enzyme approach is cheap, easier, and faster, although it is a bond-specific enzyme, it might not hydrolyze all peptide bonds for proteins with various amounts of amino acids [10] [11]. Cooking was also used as another strategy to reduce antinutritional factors like phytate, tannins, and total phenols found in pumpkin seeds in order to improve protein digestibility of the fritters.

2. Materials and Methods

2.1. Materials and Study Site

The study was conducted at Department of Dairy and Food Science and Technology, Egerton University Kenya. Dried pumpkin seeds (*Curcubita pepo*), dried maize grain, salt, sugar and oil were purchased from Nakuru market, Kenya. The fritters were processed at the food pilot plant at the Department of Dairy and Food Science and Technology, Egerton University. Determination of protein content, *in vitro* protein digestibility and anti-nutrients were carried out in the departments of Dairy and Food Science and Technology and Animal Science laboratories at Egerton University.

2.2. Flours and Fritters Preparation

Preparation of pumpkin seeds flour

Firstly, the seeds were sorted to remove some foreign matter and then cleaned using tap water to avoid contamination. The cleaned seeds were oven-dried at 60˚C for 3 h, then pumpkin seeds were milled into flour using ARMCO blender (model ABL-742RX, China), fitted with 500-μm sieve to give whole grain flour, and then stored under refrigeration (4˚C - 5˚C) to avoid oxidation of the flour until further analyses.

Preparation of maize flour

Dry maize was sorted to remove some foreign matter, then ground into fine flour using the commercial hammer mill to pass through a 0.5 mm mesh sieve and packed into tight container and stored at room temperature until needed for further analysis.

Preparation of Fritters Using Frying and Baking Method

Composite maize and pumpkin seeds fritters were prepared by mixing water (250 mL) into 500 g of each of the flour blends, 50 g of sugar and 5 g of salt. The mixture was continuously stirred until a stiff dough was formed and it was hand-kneaded for 5 min. The dough was moulded into small rounded portions and for the deep-frying method, the shaped dough was fried using WNGREAT Double Tank 6 L + 6 L Stainless Steel Deep Fryer (Model OG-DF12) in hot top fry vegetable oil at 180˚C for 5 - 10 min using a deep fryer until the fritters were golden brown (Figure 1). The second mixture followed the procedure mentioned above but frying was replaced by baking at 150˚C for 2 h. Then fried and baked fritters were transferred into a tray lined with paper and left to cool at 37 ℃ for 1h.

Figure 1. Baked and fried maize and pumpkin seeds flour fritters. where M stands for maize flour while P stands for pumpkin seeds flour.

2.3. Determination of Crude Protein Content

Determination of protein content in the raw flour and fritters involved use of Kjeldahl procedure AOAC [12], method 979.09 using a nitrogen conversion factor of 6.25.

2.4. Determination of *in vitro* Protein Digestibility (IVPD) of the Composite Flour and Fritters

Determination of *in vitro* protein digestibility of the composite flour and fritters was done as described by [13]. The process utilized single enzyme pepsin for protein digestibility. Sample (0.05 g) was weighed into a 50-mL centrifuge tube. Exactly 25 mL of 0.1 NHCl solution containing 0.033 g/100 g sodium azide and 0.025 g pepsin from porcine gastric mucosa powder (Sigma P7000-100G; activity C 250 units/mg solid, Sigma-Aldrich Co, USA) was added. The content was mixed for about 2 min at speed of 2500 g using vortex (model XH-D, China) and the incubated in water bath (Model-155 Precision, Scientific, Group, Chicago, USA) maintained at 37˚C for 3 h. This was followed by filtration using Whatman no. 1 filter paper and then supernatant was discarded. The residue was then dried at 100˚C for 3 h in an oven and the nitrogen content was determined according [12], Method number 920.87. Protein digestibility was calculated by getting the difference between the total amount of protein and the residual amount of protein after pepsin digestion divided by the total amount protein and expressed as a percentage.

$$\text{Protein digestibility }\% = \frac{\text{Nitrogen released by enzymes}}{\text{total nitrogen content of undigested sample}} \times 100 \quad (1)$$

2.5. Determination of Condensed Tannin Content in Composite Flour and Fritters

Modified vanillin-HCl in methanol method was used in determining condensed tannins [14] as modified by [15]. Ground samples (0.25 g) were weighed into a 100 ml test tube, and then 10-mL of 4% HCI in methanol (v/v) was added and the content shaken for 20 min using Ratek Orbital Incubator (Boronia, Victoria, Australia). Samples were centrifuged at 4000 ×g for 10 min at room temperature using Hermle centrifuge (Model Z362K, Hermle, Fisher, Germany). Sample extracts (1-mL), were then mixed with 5-mL of Vanillin-HCI reagent. The specific reagent (Vanillin-HCI) for the determination was prepared just before use by mixing equal volumes of 1% Vanillin in methanol (w/v) and 8% conc. HCI in methanol (v/v). Absorbance was read at 500 nm using UV/VIS Spectrophotometer (model V-200-RS, Shimadzu, Japan) exactly after 20 min. Sample blanks in which 4% HCI in methanol replaced vanillin reagent were included. Catechin was used as standard. Condensed tannin was expressed as mg catechin equivalent per 100 mg sample.

2.6. Determination of Extractable Phenolic Content in Composite Flour and Fritters

Total phenolic content of the samples was determined using Folin-Denis Method as explained by [15] with slight modification. This method is based on the non-stoichiometric oxidation of the molecules containing a phenolic hydroxyl group where tannic acid was used as standard. Briefly, Phenolic compounds were extracted from a sample (150 mg) by using 10 ml of ethanol (70:30 v/v) and pure distilled water. The mixtures were homogenized using fisher Scientific HVY DTY vortex mixer (Model SN 111220001, U.S.A) at 2500 g for 90 min. Samples were centrifuged at 3000 g for 15 min at room temperature using Hermle centrifuge (Model Z362K, Hermle, Fisher, Germany). This was followed by transferring of 1 ml of extract to a 100 ml volumetric flask, then 5 ml of Folin-Denis reagent was added followed by adding 10 ml of saturated carbonate solution then distilled water was added up to 100 ml of volumetric flask. The mixtures were left to stand for 30 minutes and absorbance read at 760 nm using a UV/VIS Spectrophotometer (model V-200-RS, Shimadzu, Japan) at 760 nm. Tannic acid was used as standard. Extractable phenol content was expressed as mg tannic acid equivalent per 100 mg dry sample.

2.7. Determination of Phytate Content in Composite Flours and Fritters

Determination of phytic acid content Phytic acid was analyzed by Davis and Reid method as described by [16] with minor modification. 2 g of the sample was dissolved in 30 ml of 2% hydrochloric acid for 3 h with continuous shaking. This was followed by filtration using Whatman no. 1 filter paper. 25 ml of the filtrate was mixed with 5 ml of ammonium thiocyanate solution which was titrated against ferric chloride solution containing 1.95 mg Fe/ml to brownish yellow color that lasted for 5 min. Phytate content was calculated as follows:

$$\text{Phytate phosphorus} = \text{iron equivalent} \times 1.95 \text{ mg of titre.} \quad (2)$$

$$\text{Phytate} = \text{phytate phosphorus} \times 3.65 \text{ g}$$

2.8. Statistical Analysis

Data obtained was analyzed using SPSS software Version 21. Test of significance was done by performing an analysis of variance (ANOVA) at 5% significance level. The means separation was done using Tukey's Honestly Significant Difference (HSD) method. The results are expressed as mean ± standard deviation from three replication measurements.

3. Results and Discussion

3.1. Effect of Pumpkin Seeds Flour Substitution Level and Cooking Process on Protein Content and *in vitro* Protein Digestibility of the Flour Blends and Fritters

Table 1 shows crude protein content of pumpkin seeds flour which is 35.9% and this explains why addition of pumpkin seeds flour increased crude protein content significantly ($P < 0.05$) in composite flour and fritters. The highest crude protein was recorded in the sample with a ratio of 55:45% for both baked and fried where 100% maize flour (control) was lowest as indicated in Figure 2.

Table 1. Showing protein content (%), *in vitro*-protein digestibility (%), Phytic acid (mg/100 g), tannins (mg/100 g) and total Phenolic (mg/100 g) of pumpkin seeds flour.

Protein content (%)	In vitro-protein digestibility (%)	Phytic acid (mg/100 g)	Tannins (mg/100 g)	Total phenols (mg/100)
35.9 ± 0.06	59.8 ± 1.2	208.5 ± 3.78^{e}	242.5 ± 2.11^{e}	89.9 ± 1.71^{e}

Mean ± standard deviation (n = 3).

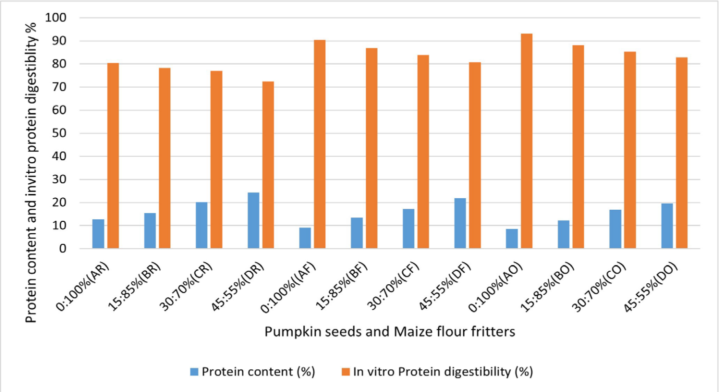

Figure 2. Graph showing protein content and *in vitro* protein digestibility of the flour, fried and baked fritters. where: PSF = Pumpkin seed flour, M = Maize flour, R = Raw four, F = Fried fritters, O = Oven Baked fritters. A (Control) = 0PSF:100%M; B = 15PSF:85%M; C = 30PSF:70%M; D = 45PSF:55M%.

As indicated in **Figure 2**, pumpkin seed flour significantly increased protein content in both raw flour and fritters. For instance, after adding 15%, 30%, and 45% pumpkin seed flour to maize flour, protein content increased by 2.81%, 7.57%, and 11.7%, respectively. This is because pumpkin seed flour used in this study contains a high protein content (**Table 1**). Based on findings in **Figure 2**, enriched fritters at each level have more than 10% protein content except for the control, which is 100% maize flour, and this supports why pumpkin seed flour was used in this study to improve the protein content of maize flour fritters. The protein content found after adding pumpkin seed flour in this study is within the recommended minimum value by the Food and Agriculture Organization/World Health Organization, which emphasized that the protein content of any food should be more than 10% [17]. These findings are similar to those reported by various researchers who found that as the level of pumpkin seed flour increases, the level of protein in bread, cookies, and cakes also increases [18]-[20]. **Figure 2** also shows the results obtained from the analysis of the effect of frying and baking on the protein content of the fritters. According to [21], the processing method utilized may cause nutrients in food to be lost or gained. The results demonstrate that cooking reduced the protein content of the fritters when compared to raw flour blends (**Figure 2**). Protein content significantly reduced ($p < 0.05$) after baking and frying the fritters. For example, at levels of 0%, 15%, 30%, and 45%, the protein content of the fritters reduced by 3.55%, 2.09%, 3.16%, and 2.46% after frying and by 4.16%, 3.21%, 3.38%, and 4.77% after baking. The findings are consistent with those reported by [22], who found that the protein content of pumpkin seed flour decreased from 65.05% to 60.17% after roasting. Another study also observed a decrease in protein content after roasting pumpkin seeds from 36.27% to 33.75% [23]. There was a higher loss of protein in baked fritters than in fried fritters; this is because of baking fritters for a long time at a high temperature (150˚C for 2 h). The decrease in protein content after baking and frying may be attributed to the Maillard reaction, which is an interaction between the carbonyl group of a reducing sugar and the free amino acid or protein, and also due to protein denaturation, which resulted to the breakdown of chemical bonds that maintain protein structures, and this makes the protein non-functional [23]. In contrast, other studies found that cakes made from roasted pumpkin seed flour contained more protein than raw pumpkin seed flour, and this might be because roasting did not create any changes in nitrogen content [24].

3.2. Effect of Pumpkin Seeds Flour Blends and Processing Method on *in vitro* Protein Digestibility (IVPD) of the Raw Maize and Pumpkin Seeds Flour and Fritters

Determination of *in vitro* protein digestibility (IVPD) is very important when evaluating the nutritional quality of the fritters. This is because the quality of the protein increases with protein digestibility. Results in **Figure 2** show that the addition of pumpkin seed flour to maize flour when preparing fritters decreased protein digestibility of the raw flour blends and fritters. For example, after adding pumpkin seed flour to maize flour by 15%, 30%, and 45%, IVPD decreased by 2.09%, 3.43%, and 7.96%. **Table 1** shows the in vitro protein digestibility of pumpkin seed flour. The *in vitro* protein digestibility was found to be 59.8%. The IVPD value was lower than 90%, which was reported by [25], and higher than 45.84%, which was reported by [26]. These differences may be due to variation of the varieties and the content of the antinutritional factors in the seeds that hinder protein digestibility [27]. In contrast with what was reported by other researchers who found that pumpkin seed flour bread had higher digestibility than 100% [28]. Cooking, such as baking, roasting and frying plays an important role in improving the palatability of the pumpkin seeds, reducing antinutrients and toxicants, improving the nutritional quality, and enhancing the digestibility and bioavailability of essential nutrients [29]. The current study found that baking and frying significantly ($p < 0.05$) increased IVPD of the fritters. As indicated in **Figure 2**, IVPD of baked fritters ranged from 82.9% to 93.1%, while fried fritters ranged from 80.7% to 90.4%. *In vitro* protein digestibility (IVPD) of the fritters significantly increased after baking and frying; the highest increase was recorded in 100%. Oven-baked maize flour fritters 93.1% and the lowest in 55:45% fried maize and pumpkin seed flour fritters 84.7%. These results are in agreement with what was discovered by other researcher: after microwaving the pumpkin seeds, IVPD increased from 85.5% to 96% [30]. This indicates that when this product is consumed, there will be more available protein for nourishing the body. Baked fritters had a higher IVPD than fried fritters; this is due to the baking of fritters at a high temperature for a long time (150˚C for 2 h). This is because heat treatment causes hydrogen bond cleavage, which makes more sites available for a proteolytic attack, and also subsequent heat causes denaturation of protease inhibitors such as trypsin-chymotrypsin inhibitors, which affects protein globular structure, hence hindering the action of digestive enzymes in the small intestine and the other heat-labile substances, like phytate, during cooking [30] [31]. This observation is different from a study by [32], who reported a decrease in protein digestibility after cooking sorghum and okara instant porridge and further explained that cooking may have contributed to the formation of complex disulfide bonds between organic γ- and β-kafirins, which are difficult to be digested by pepsin.

3.3. Effect of Pumpkin Seeds Flour Blends and Processing Method on Phytic Acid Content of the Baked and Fried Fritters

Phytic acid is the primary storage compound of phosphorus in oilseeds. The addition of pumpkin seed flour and processing methods (frying and baking) had a significant ($p < 0.05$) effect on the phytate content of the flour blends and fritters. The increase was in the range of 76.4 mg/100 g - 164.4 mg/100 g for the flour blends. The highest increase was recorded in the 55%:45% maize and pumpkin seed flour ratio, and the lowest in the control, which is 100% maize flour. This is attributed to the fact that pumpkin seed flour had a high content of phytic acid, 208 mg/100 g, as indicated in **Table 1**. This value is higher than that reported by 35.56 mg/100 g and lower than 216.8 mg/100 g [22] [23]. For instance, maize flour had 76.4 mg/100 g of phytic acid, and after adding 45% pumpkin seed flour, it increased up to 164.4 mg/100 g. The increase in the level of phytic acid as the level of pumpkin seed flour increases in both flour and fritters could be due to the high level of phytic acid in pumpkin seed flour [33]. One of the primary challenges in utilizing PSP for novel food applications is the presence of anti-nutritional factors, such as phytates. This is because phytates bind to essential minerals, e.g., zinc and iron, making them less bioavailable and potentially leading to deficiencies in these nutrients [23]. Different strategies have been used when preparing food incorporated with pumpkin seeds in order to reduce the phytic acid [30]. In this study, frying and baking proved to reduce phytic levels in formulated fritters, as indicated in **Table 2** phytate content ranged from 57.2 to 145 mg/100 g for the fried fritters and 40.1 to 107 mg/100 g for the baked fritters. The present results show that at level 100% maize/pumpkin seed flour (control), frying and baking reduced the level of phytic acid by 22.8% and 47.5%, while at a ratio of 55:45%, it reduced it by 11.5% and 34.7%. The high decrease of phytic acid, especially in baked fritters, might be due to cooking the fritters for a long time (150˚C for 2 h), which may lead to leaching into the cooking medium, degradation by heat, or formation of insoluble complexes between phytate and other components, such as protein and minerals [23]. Similar results were observed by various researchers while working with pumpkin seeds, indicating that roasting pumpkin seeds reduced phytate content from 35.56 mg/100 g to 14.56 mg/100 g and from 216 mg/100 g to 208 mg/100 g [22] [23]. The difference in how much phytic acid is removed after processing may vary due to different preparation methods, varieties, and growing conditions of the pumpkin seeds. The analysis of variance showed that both cooking method and pumpkin seed flour ratio had significant effects, and there is also an interaction between formulation ratios and cooking method on phytate content at $p < 0.001$.

Table 2. Effect of pumpkin seeds flour substitution level and cooking process on Phytic acid, condensed Tannins, and Total phenolic content (mg/100 g) on dry basis(db.) of the composite flour blends and fritters.

Pumpkin seeds flour: Maize flour blends and fritters	Processing	Phytate (mg/100 g)	Tannins (mg/100 g)	Phenols (mg/100 g)
A (0:100%)	Raw	76.4 ± 3.85f	4.1 ± 0.46h	31.97 ± 0.85d
B (15:85%)	Raw	105.5 ± 3.97d	21.4 ± 0.85d	42.4 ± 0.46c
C (30:70%)	Raw	135.7 ± 3.17c	59.8 ± 0.80b	54.1 ± 0.52b
D (45:55%)	Raw	164.4 ± 1.3a	88.0 ± 0.80a	69.4 ± 0.46a
E (0:100%)	Fried	57.2 ± 1.93g	2.1 ± 0.00i	10.9 ± 0.46j
F (15:85%)	Fried	84.4 ± 1.93ef	12.2 ± 0.46g	17.4 ± 0.52h
G (30:70%)	Fried	113.0 ± 4.75d	17.7 ± 0.85e	24.6 ± 0.80f
H (45:55%)	Fried	145.5 ± 1.25b	24.5 ± 0.52c	30.7 ± 0.52d

Continued

I (0:100%)	Baked	40.1 ± 3.75h	1.7 ± 0.46i	8.7 ± 0.80k
J (15:85%)	Baked	62.2 ± 1.93g	10.5 ± 0.46g	15.6 ± 0.46i
K (30:70%)	Baked	87.4 ± 1.54e	14.7 ± 0.40f	20.9 ± 0.81g
L (45:55%)	Baked	107.3 ± 2.55d	20.5 ± 0.80d	27.6 ± 0.46e

Key Values are means ± Standard deviations. of triplicate measurements (n = 3). Different superscript letters along the column are mean separation showing significant difference at ($p < 0.05$); column with the same letter are not significantly different ($p > 0.05$).

3.4. Effect of Pumpkin Seeds Flour Blends and Processing Method on Condensed Tannins of the Baked and Fried Fritters

Table 1 shows the tannin content of raw flour blends and fritters increased with the increase in pumpkin seed flour. The tannin content of the raw flour blends and fritters was found to increase with the addition of the pumpkin seed flour. The tannin content for control maize flour was 4.1 mg/100 g, and it increased to 88 mg/100 g for flour with 45% pumpkin seed flour. Similar findings were reported by [27] after adding pumpkin seed flour to wheat flour, tannin content increased in cookies. Condensed tannins are known to have a detrimental effect on the physiology of the gastrointestinal tract by limiting the bioavailability of starch, protein, and amino acids and altering metabolic processes. [34] [35]. This study used baking and frying in order to reduce tannin levels in fritters. The findings indicate that the tannin content of the fritters was significantly ($p < 0.05$) reduced by the cooking methods; fried values ranged from 2.1 to 24.5 mg/100 g, whereas baked values ranged from 1.7 to 20.5 mg/100 g. As indicated in Table 2, sample E55:45% (maize: pumpkin seed flour tannin level was 88 mg/100 g), but after frying and baking, it decreased by 72.2% and 76.7%, respectively. These results are in line with what was reported by [35] who found that after roasting pumpkin seeds tannins level reduced from 221 mg/100 g - 125 mg/100 g. The results in the current study show that baking greatly reduced the tannin content of pumpkin seed flour compared to frying. This is because the fritters were baked at a higher temperature for a long time (150˚C for 2 h) than the fried fritters, which were cooked for 10 minutes at 180˚C. These findings collaborate with what was reported by [35], who found that tannin levels significantly reduced from 221.01 mg/100 g to 99.25 mg/100 g as boiling time increased. The lower tannin content in fritters is safe for consumption. This is because it did not reach its lethal point, which is 90 mg/100 g [36]. The analysis of variance showed that both cooking method and pumpkin seed flour ratio had significant effects, and there is also an interaction between formulation ratios and cooking method on condensed tannins.

3.5. Effect of Pumpkin Seeds Flour (PSF) Blends and Processing Method on Total Phenolic Content of the Baked and Fried Fritters

Total phenolics increased in both flour blends and fritters as the ratio of PSF increased; this is due to the high amount of total phenolics (89.9 mg/100 g) in pumpkin seed flour, as indicated in **Table 1**. Total phenolic found in this study is higher than 22.92 mg/100 g reported by [37]. The level of total phenols in fried and baked fritters ranged from 10.9 mg/100 g to 30 mg/100 g and 8.7 mg/100 g to 27.7 mg/100 g, respectively. 100% maize flour fritters recorded the lowest, and 45% pumpkin seed flour fritters recorded the highest. Other researchers also found that the level of total phenols in cookies increased with the increase of pumpkin seed flour [27]. Comparing two methods, baked fritters contained lower phenolic content than fried fritters since they were cooked for a long time (150˚C for 2 h). Phenolic concentration decreased by cooking the fritters; for instance, at 45% pumpkin seed flour, it was 69.4 mg/100 g; after frying and baking, it was reduced by 55.8% and 60.2%, respectively. According to various researchers, the breakdown of certain phenolic compounds, which leads to their reaction with other molecules and subsequent degradation, as well as leaching out effects during hydration, are the causes of the decrease in total phenolic content [10] [38]. However, prior studies also examined the effects of thermal treatment and found that phenolic concentrations increased after cooking, and this could be because cooking deactivates the enzyme polyphenol oxidase, which prevents polyphenolic degradation [39] [40]. According to [41], the effect of thermal processing on total phenolics sometimes depend on the variety and amount of heat; this is because the study found that after roasting pumpkin seeds at 90˚C, the total phenolic content of *Cucurbita maxima* remained unaffected while *Cucurbita pepo* had a significant decrease in total phenolic ($p < 0.05$). Two-way analysis of variance showed that the addition of pumpkin seed flour and processing of fritters significantly ($p < 0.001$) affected the amounts of total phenolics in the fritters, and there was interaction between pumpkin seed flour and the cooking process.

Table 3. Correlation between *in vitro* protein digestibility (IVPD) and antinutritional properties of the fritters.

	Phytate	Tannins	Total Phenolics	Protein Digestibility
Phytate	1			
Tannins	0.834**	1		
Total Phenolics	0.841**	0.918**	1	
Protein Digestibility	−0.855**	−0.793**	−0.934**	1

**Correlation is significant at the 0.01 level (2-tailed).

The coefficients of correlation between in vitro protein digestibility and antinutritional properties are shown in **Table 3**. There was a significant negative correlation between *in vitro* protein digestibility and phytate ($r = -0.855$), tannins ($r = -0.793$), and total phenolics ($r = -0.939$). Phytate was significantly ($p < 0.01$) positively correlated with tannins ($r = 0.834$) and total phenolics ($r = 0.841$). Total phenolics positively correlated with tannins (r = 0.918).

4. Conclusion

The protein content of the raw flour and fritters significantly increases with an increase in pumpkin seed flour ratios. Baking and frying methods significantly reduced phytic acid, tannins, and total phenols content found in pumpkin seed flour, and this led to an increase in *in vitro* protein digestibility. Based on these observations, compositing maize flour with pumpkin seed flour can be used in formulating various snacks in order to reduce protein-energy malnutrition in developing countries.

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Conflicts of Interest

Regarding this paper's publication, the authors declare that they have no conflicts of interest.

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