

Effect of Butternut Squash (*Cucurbita Moschata*) Flour Substitution on the Nutritional, Functional, and Sensory Properties of Wheat Bread

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Abstract

A growing trend in the production of new and innovative bakery products is to use underutilised plant materials as functional ingredients in the bakery industry. *Cucurbita moschata* (butternut squash) has the potential to be used as a composite flour; however, its effects on the nutritional, functional and sensory properties of wheat-based bread have yet to be determined. The proximate composition, functional properties and colour characteristics of *Cucurbita moschata* flour and the sensory evaluation of wheat-composite bread were studied. *Cucurbita moschata* flour was produced by drying, milling and sieving the squash. This flour was blended with wheat flour at 20%, 30%, 40% and 50% substitution levels to produce the bread. Proximate composition, functional properties and colour of *Cucurbita moschata* flour were determined by standard methods. Sensory analysis of the bread samples was conducted using a 7-point hedonic scale with 50 untrained panellists. The percentage compositions of moisture, crude fat, crude fibre, ash, crude protein and carbohydrate in the *Cucurbita moschata* flour were: 8.23 ± 0.07 , 1.53 ± 0.09 , 5.54 ± 0.03 , 6.90 ± 0.05 , 11.90 ± 0.04 and 65.90 ± 0.03 , respectively. The energy content was 324.40 ± 0.05 kcal/100 g. The water absorption, oil absorption, swelling power, and solubility of the flour in g/g were, respectively, 2.59 ± 0.02 , 1.26 ± 0.09 , 3.43 ± 0.12 , and 17.24 ± 0.08 . Proximate analysis was done on selected bread samples and the results indicated that the addition of 20% *C. moschata* flour modified the moisture, fibre, ash, protein, fat, and carbohydrate contents of the bread, when compared with the control. Sensory evaluation showed important differences among formulations, with 20% composite bread having the highest mean sensory score. The results also showed the impact of the incorporation of *C. moschata* flour into wheat bread formulae on the compositional, functional, and sensory characteristics of the composite bread.

Keywords: *Cucurbita Moschata*; Bread Production; Proximate Composition; Functional Properties; Sensory Evaluation

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INTRODUCTION

The increase in demand for healthful, sustainable, and locally-adapted food systems has led to an increase in research and development of composite flours to replace wheat products. Wheat flour is critical in bread production, however, countries with a high dependence on imported wheat face issues with this. This has suggested switching to more locally available cereal-based foods to improve diet quality and increase resilience in food systems (Aderinola et al., 2026). Composite flour technology (CFT) has attracted interest as a method for incorporating underutilised crops in more mainstream food products by improving their dietary fibre, minerals and functional properties to help improve nutritional quality (Kabir et al., 2026). The possibility of substitution is however impacted by the effect of the alternative flour on gluten formation, dough rheology and consumer acceptability of the final product (Dhenge et al., 2026). *Cucurbita moschata* (butternut squash) has been described as a potential functional food ingredient due to its nutritive value and bioactive components. Recent work has demonstrated that *C. moschata* may be a source of dietary fibre, carotenoids, minerals, and antioxidative compounds that contribute to the nutritional aspects of processed foods (Alqahtani, 2025). Aside from its edible importance, the value-addition of this crop is its processing into flour with increased storage and usage in certain mainstream food items, though it is not as much adopted in bakery products when compared to other types of pumpkins (Chikpah et al., 2025).

Studies on pumpkin composite flours for bread-making have reported benefits and challenges. Alija et al. (2025) found that incorporating pumpkin flour changes the water absorption behaviour of wheat composite flour, pasting properties, and bioactive potential in the flour as compared to control flour. These changes are technologically important, as the functional properties of flour are related to dough development, water absorption, and baking quality. Similarly, Liu et al. (2026) stated that pumpkin supplementation increases the nutritional and antioxidant characteristics of the composite bread. They also have an influence on the texture and the structural properties of the loaf. Therefore, the ratio of substitution must be optimised. The effective nutritional enhancement versus sensory appeal trade-off remains a nontrivial problem in pumpkin-based bakery food formulation. Early investigations have shown that, while pumpkin addition can improve dietary fibre, minerals and antioxidant compounds, excessive substitution can adversely affect product color, flavour, crumb structure and overall acceptability (Chikpah et al., 2025). Similarly, Alqahtani (2026) indicated enrichment of bread with seeds of pumpkin permitted the improvement of its nutritional value, but the obtained composite bread remained acceptable in terms of sensory properties. It can be concluded that success in producing composite bread depends on the balance between nutritional properties and sensory qualities of the product.

Despite a growing body of research on the potential of pumpkin-based foodstuffs, few studies have directly investigated the flesh flour of *C. moschata*. Results from studies on *C. maxima*, *C. pepo*, or *C. moschata* seed and peel fractions may be less relevant due to species and processing differences (Alqahtani, 2026). Furthermore, most studies have addressed lower substitution rates, whereas studies on higher substitution rates are scarce (Amponsah et al., 2026). Only a few studies have focused on the relationship between the functional qualities of flour and the quality of composite bread. Determining the water absorption, swelling capacity and solubility will help in creating composite bread (Alija et al., 2025). Despite the local presence of *C. moschata* in Ghana, there are limited food applications, warranting research efforts to develop functional foods that reduce wheat in the local diet. This study, therefore,

aims at the use of *C. moschata* flour in bread making by evaluating the chemical characteristics, functional properties, organoleptic characteristics at various levels of substitution, given that previous studies focused on other pumpkin species or other levels of substitution. Therefore, the present study was designed to investigate the effect of different levels of *Cucurbita moschata* flour substitution on the nutritional profile, physicochemical properties and sensory quality of composite wheat bread and to assess the feasibility of *Cucurbita moschata* flour in the production of composite bread as a sustainable ingredient.

MATERIALS AND METHODS

Study Design

The study used a completely randomised design (CRD) to investigate the effect of substituting wheat flour with *Cucurbita moschata* (butternut squash) flour on the nutritional, functional, physicochemical and sensory characteristics of samples of composite bread produced. Five bread samples, A1(0% substitution; 100% wheat flour), B2 (20% substitution), C3 (30% substitution), D4 (40% substitution) and E5(50% substitution), were produced based on different levels of substitution with butternut squash flour. The independent variable was the replacement level of flour with flour from *Cucurbita moschata*. The responses were proximate composition, functional properties, color, and sensory acceptability of bread. Each product was composed of three experimental replicates and was baked in three different batches. Each batch was baked separately. For lab-based studies, all samples were analysed in triplicate and results are expressed as mean $\pm$ standard deviation. The unit of replication in the study was one batch of bread with a certain formulation. Replications of independent bread making were used for statistical comparisons between treatments, with analytical replicates strengthening the reliability of the measurements.

Source of Raw Materials

Cucurbita moschata (butternut squash) was sourced from Kumasi Central Market in the Ashanti Region of Ghana. Wheat flour, egg, iodated salt (Annapurna), granulated sugar, Saf-instant yeast, margarine (Cook brand) and evaporated milk were purchased from supermarkets at Kumasi Asafo Market. All raw materials were stored until processing and analysis under suitable conditions.

Preparation of *Cucurbita moschata* Flour

Cucurbita moschata flour was prepared using the method reported by Chikpah et al. (2025) with slight modifications to the processing conditions to meet the current study's conditions. *Cucurbita moschata* fruits were harvested and washed under tap water to remove dirt and surface contaminants. The fruits were peeled, sliced uniformly into thin chips, to compose chips of uniform size to support the moisture content reduction. Chips were soaked in water for 90 min and dried. After pretreatment, the slices were dried in a Bosch food dehydrator (model BS-6612) at 60 °C for 96 hrs until a constant weight was obtained. The dried samples were powdered into flour using an electric grinder and passed through a 63 μ m sieve to obtain a fine flour particle size. *Cucurbita moschata* flour was immediately placed into sealed polythene containers and stored in a cool dry area until analysis and use in bread formulations.

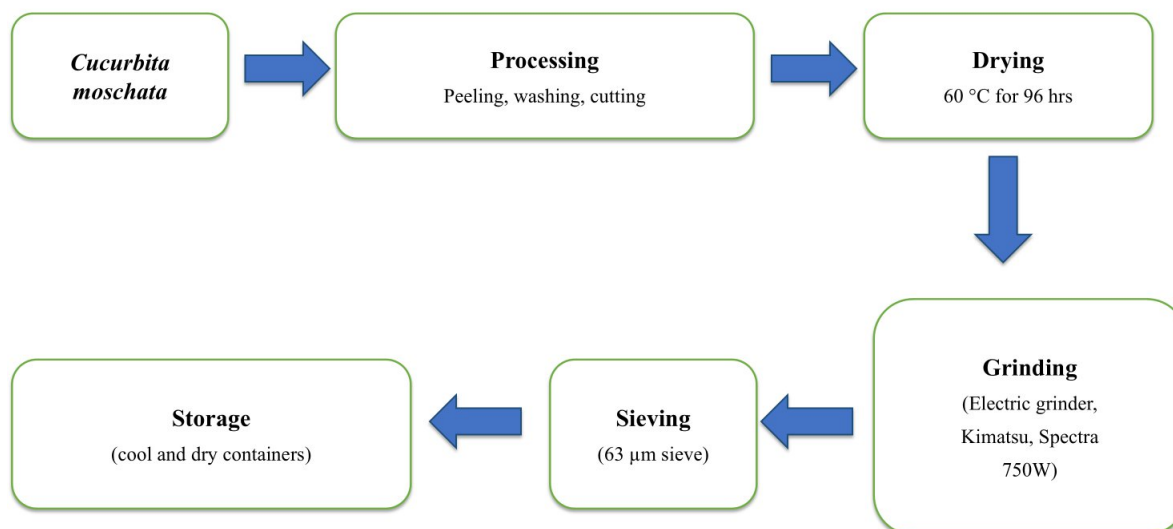


Figure 1: Flow chart of the method of preparation of *Cucurbita moschata* flour

Product Formulation and Blending

Composite flour formulations based on wheat and *Cucurbita moschata* were obtained by replacing a certain percentage of wheat flour with *C. moschata* flour. The flour replacement fractions were calculated on a weight-for-weight (w/w) basis for 100 g of total flour, based on a weight replacement of wheat flour by 100 g of *C. moschata* flour. Thus, the total weight of wheat and *C. moschata* flour remains constant, only in relation to the flour replacement level.

In control formulation A1, 100 g of wheat flour and no *C. moschata* flour were used, while in composite formulations, such as formulations A2 to A5, the wheat flour was substituted with 20, 30, 40, and 50% of *C. moschata* flour. The blends B2, C3, D4 and E5 were, respectively, the mixtures of wheat flour and *C. moschata* flour in 80:20, 70:30, 60:40 and 50:50 (wheat/cucurbit flour). This means that B2 was composed of 80 g of wheat flour and 20 g of *C. moschata* flour, C3 was composed of 70 g of wheat flour and 30 g of *C. moschata* flour, and so on. E5 was mixed with equal amounts of wheat and *C. moschata* flour. Other ingredients (margarine, 10 g; yeast, 5 g; evaporated milk, 63 mL; granulated sugar, 1 g; and salt, 1 g per 100 g total flour formulation) were held constant. Each flour component was separately weighed before mixing into the homogeneous flour composition, and other ingredients were subsequently added to the mixture.

Table 1: Formulation of wheat and *Cucurbita moschata* composite bread on a 100 g of total flour basis

Ingredient	Treatment Code				
	A1	B2	C3	D4	E5
Butternut squash (%)	0	20	30	40	50
Wheat flour (%)	100(control)	80	70	60	50
Margarine (g)	10	10	10	10	10
Yeast (g)	5	5	5	5	5
Milk (ml)	63	63	63	63	63

Sugar (g)	1	1	1	1	1
Salt (g)	1	1	1	1	1

Preparation of Wheat-Cucurbita moschata Composite Bread

Bread was prepared using the method of Chikpah et al. (2025), with modifications in formulation and processing conditions to those used in this study. Wheat flour and Cucurbita moschata flour were passed through a 63 µm sieve to achieve uniform particle size before mixing the ingredients. The two flours were then blended at the treatment ratios shown in Table 1. Salt, sugar, margarine, evaporated milk and yeast were each measured and added to their respective flour mixtures, with the yeast added to a warm mixture of milk and water. The dough was kneaded several times, until it formed a smooth and homogeneous mass, placed in a different mixing bowl, covered with a clean cloth, and allowed to undergo the first proofing stage at room temperature for 20 min. The dough was then knocked back and re-kneaded to redistribute the gas evenly and create a homogeneous structure. Approximately 450 g of dough from each formulation was placed in an oiled 18 cm x 9 cm x 5 cm baking tin and then proofed for a second time for 30 minutes in a proofer. Just before baking, the surface of each piece of dough was brushed with beaten egg for surface coloration. The pieces of dough were baked in a preheated oven (230°C) for a period of 25 min. The loaves were removed from the loaf tins, cooled at room temperature, weighed and underwent laboratory and sensory analysis.

Functional Properties of Cucurbita Moschata Flour

The water absorption capacity (WAC), oil absorption capacity (OAC), swelling power and solubility index of the Cucurbita moschata flour were determined. These parameters are essential for the production of composite breads and are generally indicative of the handling characteristics of the dough, with respect to the incorporation of water and oil into the flour ingredients. They may also affect the final quality attributes of the bread. The analysis was performed according to the standard analytical procedures prescribed by AOAC (2019). The data were expressed as the mean ± standard deviation of triplicate determinations.

Water Absorption Capacity (WAC)

Water absorption was determined by mixing a known weight of dehydrated Cucurbita moschata flour and distilled water to obtain an appropriate hydration level, and then centrifuging to remove the unabsorbed water. Retention was calculated by subtracting the mass of the original (dry) weight from that of the hydrated sample, and the WAC was expressed in grams of water absorbed per gram of flour (g/g).

Oil Absorption Capacity (OAC)

Oil absorption capacity (OAC) was determined by mixing a known amount of the sample flour with vegetable oil, centrifuging, removing the excess oil, and calculating the difference between the initial weight of the flour sample and the oil-bound flour residue at the end of the test. The OAC was expressed as grams of oil absorbed per gram (g/g) of flour.

Swelling Power and Solubility Index

Swelling power and solubility index were determined by suspending flour in distilled water and heating it. Heating allowed the starch granules to hydrate and swell, and the swollen

sediment and soluble fractions were separated by centrifugation and weighed. Swelling power was defined as the ratio of the weight of swollen flour granules to the mass of the dry flour before swelling. Solubility index was defined as the ratio of the solids that were soluble in the supernatant to the mass of the original dry sample. All functional properties were measured three times and expressed as means $\pm$ standard deviation.

Proximate Composition of the Flour of *Cucurbita Moschata* and Selected Bread Samples

The proximate composition of *Cucurbita moschata* flour and the experimental bread samples was determined using standard methods of AOAC (2019) for moisture content, crude protein, crude fat, crude fibre, ash content and carbohydrate content. The proximate composition of only the control bread (A1; 100% wheat flour) and 20% *Cucurbita moschata* composite bread (B2; 80% wheat flour and 20% *C. moschata* flour) samples was determined. The proximate composition of the other bread samples was not determined. These arguments are consistent with the methods and outcomes in Table 5. Moisture content was determined by drying the samples at 105 degrees C until constant weight. Ash was determined by incinerating the samples in a muffle furnace. Crude protein (as nitrogen) was determined by the Kjeldahl method. Crude fat was determined by solvent extraction and crude fibre by digesting the food in acid and alkali. Carbohydrate was determined by difference as follows:

$$\text{Carbohydrate (\%)} = 100 (\text{Moisture} + \text{Protein} + \text{Fat} + \text{Fibre} + \text{Ash})$$

All values were determined in triplicate and reported as mean $\pm$ standard deviation (SD).

Sensory Evaluation of Bread Samples

Sensory evaluation of the five treatment breads (A1, B2, C3, D4 and E5) was achieved through a panel of 50 untrained panellists (students and staff) from Kumasi Technical University who were informed on the topic and assured of anonymity and confidentiality of their responses. Evaluations were conducted in a well-ventilated classroom in the university under ambient conditions thus normal room temperature and lighting. No formal sensory training was given because this was an assessment of consumer acceptability. To avoid any identification and expectation bias the bread samples were assigned random numbers. Each sample was presented separately under the same testing conditions. The panellists evaluated colour, texture, flavour, aftertaste and overall acceptability using a 7-point hedonic scale of 1 = Dislike extremely, 2 = Dislike very much, 3 = Dislike moderately, 4 = Neither like nor dislike, 5 = Like moderately, 6 = Like very much, 7 = Like extremely. To reduce carry-over effects, mouth rinsing between samples was conducted with potable drinking water. The five bread formulations were all rated in a single sensory session by the same 50 panellists. Those sensory responses were then analysed accounting for the fact that all formulations were assessed multiple times by each panellist.

Data Analysis

All quantitative measurements were expressed as mean $\pm$ standard deviation (SD). Physicochemical and proximate measurements were performed in triplicate ($n = 3$), considered analytical replicates, and expressed accordingly. Where comparisons between bread formulations were required, treatment effects were considered important when $p < 0.05$. The sensory qualities of the five bread formulations were rated by the same set of 50 panellists, making the sensory data repeated measures rather than independent. The ratings of color,

texture, flavour, aftertaste and overall acceptability were analysed using a linear mixed-effects (LME) model. The bread formulation was treated as a fixed effect and the panellist was treated as a random effect (to account for repeated measures from the same panellist). Differences between formulations were determined by pairwise comparisons (Tukey-adjusted multiple comparisons) where the overall effect of formulation was meaningful, with an importance level of 5%. Therefore, the effective number of panellists per formulation was 50 ($n = 50$) for the sensory analyses. Notation ($n = 3$) was used for any laboratory analysis performed in triplicate but was never used to describe sensory analyses.

RESULTS AND DISCUSSION

Proximate Composition of *Cucurbita Moschata* Flour

The proximate composition of *Cucurbita moschata* flour is shown in Table 2. The *Cucurbita moschata* flour contained $8.23 \pm 0.07\%$ moisture, $1.53 \pm 0.09\%$ crude fat, $5.54 \pm 0.03\%$ crude fibre, $6.90 \pm 0.05\%$ ash, $11.90 \pm 0.04\%$ crude protein and $65.90 \pm 0.03\%$ carbohydrate and a calorific value of 324.40 ± 0.05 kcal/100 g. The carbohydrate was found to be the major component of the *Cucurbita moschata* flour followed by crude protein, moisture, ash and crude fibre while crude fat was the subdominant component. This pattern suggests that butternut squash flour is a carbohydrate based ingredient with a variable but meaningful protein, fibre and mineral content which may be relevant when incorporated into wheat based products. The moisture content observed was 8.23%. This indicated an important water loss during drying and is comparable to the moisture content of 7.06% of the *C. moschata* pulp flour obtained by Bemfeito et al. (2020). Bemfeito et al. (2020) provided proximate composition of 1.88% fat, 11.08% protein, 6.37% ash, 64.07% carbohydrate, and 317.53 kcal/100 g which are very close to the present work values of 1.53% fat, 11.90% protein, 6.90% ash, 65.90% carbohydrate and 324.40 kcal/100 g. Proximity between these values further lends plausibility to the proximate profile determined in the present work. Minor variations can be attributed to differences in cultivar, maturity of harvest, drying technique, particle size and growing locale. A crude fat value of 1.53% was obtained, which was in agreement with the 1.16% crude fat obtained by Konadu et al. (2021) for butternut squash pulp in Ghana. However, carbohydrate content of 72.70% was higher than the value of 65.90% in this study. The fat content may not be too different because both studies investigated the fleshy part of *C. moschata*, which is much lower in fat compared to the seeds. The difference in carbohydrate may have been from moisture and other proximate fractions, since carbohydrate is calculated by difference, it depends on the amounts of moisture, protein, fat, fibre and ash contained in the sample (Konadu et al., 2021).

For comparison, spanned ranges of moisture, ash, crude fat, protein, fibre and carbohydrate content of pumpkin flours made with varying pre-drying treatments reported by Bekele and Admassu (2022) were 7.57 to 8.23, 5.73 to 6.57, 1.17 to 1.85, 8.72 to 11.32, 10.92 to 13.11, 61.47 to 64.23%, respectively. Our moisture and fat content measurements fall within this range whereas our protein and ash contents are slightly higher. Meanwhile, the crude fibre content was 5.54%, which appears to be lower than the literature values, indicating that pre-drying treatment, drying conditions and analytical techniques can have a pronounced effect on the proximate composition of pumpkin flour (Bekele & Admassu, 2022). The contents of crude fibre and ash (5.54% and 6.90%) are important factors in its addition to composite food products. While fibre will add to the dietary fibre contents of the end-product, its addition may

interfere with hydration through competition with starch and gluten-forming proteins for water molecules in the dough. Thus, the relatively high level of ash is not indicative of high levels of any particular mineral, though minerals were only measured as total ash. The differences in fibre, protein, fat and ash levels between studies might be strongly influenced by flour that is made from pulp, peel, seeds or combinations thereof. For instance, Soares et al. (2023) showed that pulp-, peel- and seed-derived *C. moschata* flours had strong compositional differences and seed-derived flours had higher concentrations of some micronutrients than pulp flour.

In terms of bread-making, this high carbohydrate content with moderate levels of fibre, protein and ash makes *C. moschata* flour a useful candidate as a partial replacement for wheat flour. Studies in the literature show that the inclusion of pumpkin flour in wheat bread products can change the structure and nutritional functionality of the product, for example through the addition of fibre and other non-wheat ingredients. For example, Ge et al. (2021) noted that pumpkin flour affected the structure and digestibility of wheat bread, but this had no detrimental effect on the sensory attributes. Thus, the proximate composition data support the use of butternut squash flour in composite flour bread, but alone do not indicate a preferred level of incorporation. (Ge et al., 2021). Overall differences in composition between this study and previous work could also arise from variability in *C. moschata*. Alqahtani (2026) also found variation in the content of nutrients and bioactive compounds in various *C. moschata* cultivars, suggesting that both cultivar and environment can have a meaningful impact on those compounds of interest. Therefore, not all differences between studies can be attributed to their analytical methods, as some studies involved different cultivars, regions, maturity stages, and processing methods (Alqahtani, 2026). From the overall proximate composition, the high carbohydrate content, noticeable protein content, the fibre and ash content and the relative low fat and moisture content suggest that butternut squash flour has a potential in bread production as envisaged in the present study. The scientific implications of this paper are that the flour is likely to be suitable for use as a composite bakery ingredient whilst the practical implications are that the fibre, protein and inorganic fractions could be expected to influence the nutritional and technological properties of wheat bread. However, the level of substitution would be determined, not only by the proximate composition of the components used, but also by the functional properties of the flour and the sensory properties of the bread.

Table 2: Proximate composition on the Butternut squash flour

Components (%)	Composition (%)
Moisture	8.23±0.07
Crude fat	1.53±0.09
Crude Fibre	5.54±0.03
Ash	6.90±0.05
Crude Protein	11.90±0.04
Carbohydrate	65.9±0.03
Energy (Kcal)	324.4±0.05

Results presented as mean ± standard deviation, (n=3).

Functional Properties of Cucurbita Moschata Flour

The functional properties of the *Cucurbita moschata* flour are given in Table 3. The water absorption capacity, oil absorption capacity, swelling power and solubility of the flour in the present study were found to be 2.59 ± 0.02 g/g, 1.26 ± 0.09 g/g, 3.43 ± 0.12 g/g and $17.24 \pm 0.08\%$ respectively. The properties also reflect the capacity of the flour to absorb water and oil, its swelling power during hydration, ability to leach out the soluble substances, and suggest that *C. moschata* flour may have some desirable functional properties which may improve the dough handling, moisture absorption, textural properties, and the quality of the composite bread. WAC of *C. moschata* flour was 2.59 g/g, indicating a good water absorption capacity in relation to the weight of the flour. Water absorption is an important functional property of bakery products, as it impacts dough consistency, mixing time, crumb softness, and product yield. The high WAC observed in this study can be due to the presence of hydrophilic components such as dietary fibre, proteins and non-starch polysaccharides associated with the flour. Plant materials with high fibre content have a higher WAC because of the presence of hydroxyl groups in their structures which have an affinity for water. The recorded WAC is similar to other studies with flour from pumpkin. According to Bemfeito et al. (2020), the high WHC of *C. moschata* pulp flour is justified by the presence of fibre and other hydrophilic constituents in the product. Likewise, Bekele and Admassu (2022) found the hydration properties of pumpkins' flour to be affected by several processing factors, such as the cultivar, drying method, particle size and the proportions of the remaining flour's fibre and protein fractions, which may account for the deviation between the study's findings and the current study.

WAC is important in composite bread making. A greater water absorption would likely yield a softer bread crumb (due to its increased water retention capacity) at the cost of a lesser gluten development, as the amount of water available for the hydration of the wheat gluten proteins would be lower. Therefore, this optimal concentration of *C. moschata* flour should correspond to the best nutritional improvement of the bread and the quality. The OAC of the flour was 1.26 g/g, which was lower than the WAC value, indicating that the flour had a higher tendency to absorb water than oil. The OAC is affected by the non-polar side chains of the amino acids present in the proteins and the structure of flour components capable of interacting with lipids. *C. moschata* flour has a moderate oil-holding capacity which can be used to deliver flavours and mouthfeel in products and has lower oil-holding capacity than traditional oil-rich ingredients. The reduction of OAC identified in this study is corroborated by the findings of Soares et al. (2023) who observed that *C. moschata* flour materials from seed had higher OAC than *C. moschata* flour materials from flesh due to seed material having a higher lipid and protein content. The flour-based material from the flesh may have lower lipid-binding properties, which could explain the low OAC found in the present study, as our flour was from the butternut squash flesh rather than from its seeds. The flour's swelling power was 3.43 g/g. The swelling power of a food ingredient containing starch is its ability to absorb water and swell during the cooking process. It is an important parameter in bakery systems, affecting viscosity development, dough structure and crumb characteristics. The swelling ability suggests the starch fraction of *C. moschata* flour may play a role in the formation of the structural elements of bread.

Different studies have found changes in starch-water interaction and behaviour in composite flour systems with the addition of pumpkin flour. Ge et al. (2021) reported that the rheological behaviour and structural characteristics of wheat-based bread changed, due to changes in the

starch behaviour and non-wheat ingredients in the composite flour system. The discrepancy between the present value and those previously measured may be due to differences in starch composition, flour particle size, the drying process, or the processing history. The solubility of the flour of *C. moschata* is 17.24%, which indicates that some of its components are soluble in water. It is a measure of the soluble sugars, proteins, and hydrolyzed starches that are leached into the dough and may affect product properties. This solubility figure indicates that flour also contains soluble matter which may affect the behaviour of the resulting bread. Per Alqahtani (2026), the contents of the soluble compounds and bioactive components of *C. moschata* are influenced by cultivar and agronomic conditions. The reason that the solubility value was slightly different from previous studies may be due to varietal differences, growth conditions, and processing methods. Overall, the functional properties indicated that *C. moschata* flour could potentially be used as a functional ingredient in bread composites. This is due to its water absorption, swelling power and solubility properties. These properties suggest that *C. moschata* flour may be used in wheat bread to provide moisture retention and nutritional functional value. To use *C. moschata* flour, this result indicates that the maximum amount of *C. moschata* flour must be controlled, since its high fibre-water binding capacity may affect dough development as well as sensory attributes of the products. Thus, the data confirm the results of proximate composition and the possibility of partial substitution of wheat flour.

Table 3: Results of functional properties of the Butternut squash flour

Functional Properties	Composition (g/g)
Water Absorption Capacity	2.59±0.02
Oil Absorption Capacity	1.26±0.09
Swelling power	3.43±0.12
Solubility	17.24±0.08

Results presented as mean ± standard deviation, (n=3).

Colour Characteristics of *Cucurbita Moschata* Flour

The colour characteristics of *Cucurbita moschata* flour are presented in Table 4. The flour exhibited an L* value of 55.73 ± 0.02, an a* value of 8.56 ± 0.05, and a b* value of 22.69 ± 0.09. These parameters indicate that the flour possessed moderate lightness with noticeable red and strong yellow colour components. Flour colour is an important quality attribute because it influences the appearance of composite bread and may affect consumer perception and acceptance. The L* value represents lightness on a scale ranging from 0 (black) to 100 (white). The observed L* value of 55.73 indicates that the flour was moderately light rather than completely pale. The reduced lightness compared with refined wheat flour may be due to natural pigments in *C. moschata*, particularly carotenoid compounds responsible for the yellow-orange appearance of pumpkin and squash tissues. These pigments are a key distinguishing characteristic of *Cucurbita*-based ingredients and may influence the final colour of bakery products. The positive a* value (8.56) indicates the presence of redness, while the positive b* value (22.69) demonstrates a stronger yellow colour intensity. The higher b* value compared with a* suggests that yellowness was the dominant colour component of the flour. This colour pattern is consistent with the natural pigmentation of *C. moschata*, which is generally associated with high carotenoid content, particularly β-carotene.

The observed colour values are comparable with findings from previous studies on pumpkin and squash flours. Bemfeito et al. (2020) reported that *C. moschata* pulp flour exhibited noticeable yellow-orange coloration due to the presence of carotenoid pigments. Similarly, Soares et al. (2023) observed distinct colour differences among *C. moschata*-derived flours depending on the plant fraction used, with pumpkin-based materials showing strong yellow-orange characteristics. These similarities confirm that the colour profile observed in the present study is typical of *C. moschata* flour. However, variations in colour parameters among studies are expected due to differences in cultivar, maturity stage, growing conditions, drying temperature, drying duration, and particle size. Processing conditions can influence carotenoid stability because these pigments are sensitive to oxidation and prolonged exposure to heat and oxygen. Bekele and Admassu (2022) demonstrated that pre-drying treatments significantly influenced the quality characteristics of pumpkin flour, including properties associated with colour development. Therefore, differences between the present results and previous reports may reflect differences in processing conditions rather than inconsistencies in the raw material.

The colour characteristics observed in this study have important implications for bread formulation. The strong yellow colour associated with *C. moschata* flour may naturally enhance colour in composite bread and reduce reliance on artificial colouring agents. However, higher substitution levels may produce bread with a colour different from conventional wheat bread, which could affect consumer expectations. Therefore, although *C. moschata* flour may provide nutritional and aesthetic benefits, its incorporation level should be optimized alongside sensory acceptability. From a scientific perspective, the colour results complement the proximate and functional properties by demonstrating that *C. moschata* flour contributes not only nutritional components but also bioactive pigment-related characteristics to composite food systems. Carotenoid-associated colour may also indicate antioxidant-related compounds, although this study did not directly measure pigment concentration or antioxidant activity. Overall, the colour profile of *C. moschata* flour, characterized by moderate lightness and dominant yellowness, confirms its potential as a natural colour-contributing functional ingredient in composite bread production. These findings support the objective of evaluating butternut squash flour as a wheat-flour substitute while highlighting the need to balance its nutritional advantages with consumer expectations for bread appearance.

Table 4: Results of determination of colour of the Butternut squash flour

Sample	L*	a*	b*
Butternut Squash	55.73±0.02	8.56±0.05	22.69±0.09

Results presented as mean ± standard deviation, (n=3).

Proximate Composition of Wheat (Control Sample) and Selected Butternut Squash Composite Bread

The proximate composition analysis of the various slices of bread analysed, including reference wheat bread (CB, 100% wheat flour) and composite bread (AB, 80% wheat flour and 20% *C. moschata* flour), is presented in Table 5. The moisture, crude fibre, ash and protein contents showed important differences ($p < 0.05$) between the experimental and control samples. However, the content of crude fat and carbohydrate in the bread prepared by the substitution of *C. moschata* flour was considerably lower when compared with the control bread. The moisture content was considerably higher in the AB bread than in the control bread (15.79 ± 0.007 and $12.46 \pm 0.54\%$, respectively). One possible explanation is the increase of the water retention capacity of *C. moschata* flour, which is improved by the presence of fibre and

hydrophilic constituents such as polysaccharides in plant-based flours. The hydroxyl groups of polysaccharides are capable of forming hydrogen bonds with water (Bemfeito et al., 2020; Ge et al., 2021). Increased water retention in the pumpkin-based bakery products was attributed to dietary fibre and non-starch polysaccharides in the pumpkin flour. It was concluded that the increased moisture could improve the softness and freshness of bread, potentially having an adverse influence on the shelf life of the products as higher water availability makes the products more susceptible to microbial spoilage.

On the other hand, the crude fat decreased from $11.80 \pm 0.02\%$ in CB to $2.57 \pm 0.40\%$ in AB. This decrease may have occurred due to the presence of *C. moschata* flour, which contains a lower fat content (1.53%) compared to wheat flour. Similar characteristics were observed in *C. moschata* pulp flour due to a lower lipid content compared to pumpkin materials from seeds (Bemfeito et al., 2020). The reduction in fat may suggest that the partial replacing of wheat flour with butternut squash flour influenced the lipid composition of the bread matrix. However, bread lipid composition is impacted by the use of margarine and other ingredients, and therefore this finding should be viewed with caution. The crude fibre content of these samples increased considerably from $0.41 \pm 0.11\%$ for the CB up to $4.45 \pm 0.22\%$ for the AB. This can be attributed to the crude fibre content of the *C. moschata* flour used in the composite flour being 5.54%. This finding is consistent with other studies with wheat-based bakery products containing pumpkin-derived ingredients (Soares et al., 2023; Ge et al., 2021). The nutritional implications of the substitution are the health benefits attributed to dietary fibre, such as improving gut health and appetite and glycaemic response. An area of concern with high levels of substitution is the effect on the properties of the bread such as gluten network formation and dough rheology requiring careful evaluation.

The ash percentage of CB was $2.22 \pm 0.08\%$ and increased considerably ($P < 0.05$) to $10.32 \pm 0.31\%$ in AB. This high ash percentage in the composite bread might be due to the ash percentage of *C. moschata* flour (6.90%). Pumpkin-based ingredient and product inclusion in cereals has likewise increased ash levels, since vegetable flours provide inorganic residues in addition to flour from purified wheat grains (Bemfeito et al., 2020). Ash is a measure of total mineral residue content, but does not account for the concentration or bioavailability of individual minerals. Thus, the increase itself does not prove that enrichment of any particular mineral has occurred, and more mineral analysis would be required. The protein content of AB treatments was higher than the CB treatments ($11.20 \pm 0.16\%$ vs $10.49 \pm 0.21\%$, respectively) due to the *C. moschata* flour (11.90% crude protein) used in the AB treatment. Similar trends in protein contribution were reported in composites with pumpkin flour (Bekele & Admassu, 2022). The utilisation of pumpkin or squash flour depends on the level of substitution and the nutritional composition of the raw materials. The slight increase in our study indicates that the substitution of *C. moschata* flour for wheat flour changes the protein composition of the bread; wheat flour is still the major contributor to gluten-forming proteins.

The proportion of carbohydrates decreased from $73.52 \pm 0.09\%$ in CB to $55.67 \pm 0.31\%$ in AB, due to the substitution of wheat flour, composed mainly of starch, for the pasta pumpkin (*C. moschata*) flour, which has a higher content of fibre, ash, and non-starch compounds. Similar decreases in carbohydrate concentrations were reported for partially replacing wheat flour with pumpkin flour, due to dilution of wheat starch components and increasing of the contribution of the fibre and mineral fractions (Ge et al., 2021). As carbohydrate content was calculated by difference, all reductions in moisture, protein, fat, fibre and ash contents would

thus result in a corresponding increase in carbohydrate content. Differences between the CB and AB also showed the contribution of the 20% *C. moschata* flour to the composition of wheat breads with respect to the amount of the dietary fibre, ash, moisture and protein that increased and the amount of fat and carbohydrate that reduced; thus, the aim of the study to investigate the effect of butternut squash flour as a functional ingredient for bread making was attained. Practically, this could mean an opportunity to increase the dietary fibre content and diversify the cereal foods produced with local vegetable resources by using flour from *C. moschata*. These results should be interpreted in light of the current study in mind. As proximate composition analyses were only performed for control bread and the 20% substitution, conclusions cannot be made for the 30%, 40% and 50% incorporated breads. Although a shift in product composition was observed in the composited breads, no conclusive evidence for commercial application and better nutrition was observed. However, additional studies on mineral profiling, shelf-life, and consumer acceptability for different levels of substitution would still be needed.

Table 5: Proximate composition of wheat (control sample) and selected butternut squash composite bread

Parameters	Bread Samples	
	CB	AB
Moisture	12.46±0.54 ^b	15.79±0.007 ^a
Crude Fat	11.8±0.02 ^b	2.574±0.403 ^a
Crude Fibre	0.41±0.11 ^b	4.454±0.22 ^a
Crude Ash	2.22±0.08 ^b	10.32±0.31 ^a
Crude Protein	10.49±0.21 ^a	11.20±0.16 ^b
Carbohydrate	73.52±0.09 ^b	55.67±0.31 ^a

Results presented as mean ± standard deviation. Values are statistically significant at:

^a $p < 0.001$, ^b $p < 0.05$ when AB was compared to CB using Duncan multiple comparison test.

AB stands for the composite bread which contains butternut squash bread and wheat bread in a ratio of 20:80 respectively. CB also stands for the reference bread which contains 100% wheat flour.

Sensory Evaluation of Composite Wheat and Cucurbita moschata Bread Samples

The sensory properties of bread made with wheat flour and Cucurbita moschata composite flour are shown in Table 6. Sensory properties of bread were colour, texture, flavour, aftertaste and overall acceptance using 7-point hedonic scale where higher points mean consumers' acceptance. The sensory evaluation to measure consumer acceptance were done by 50 food panellists. All breads (A1, B2, C3, D4 and E5) were evaluated by the same 50 panellists, and results are shown as mean ± standard deviation (n = 50). The sensory evaluation of composite bread samples showed that all bread samples were acceptable. However, the composite bread sample containing 20% Cucurbita moschata composite bread (B2) has the overall highest mean score of 6.16 ± 1.12, 6.54 ± 0.78, 6.28 ± 2.37, 6.44 ± 2.58, and 6.16 ± 1.97 for colour, texture, flavour, aftertaste and overall acceptability respectively. These previous findings showed that moderate incorporation of *C. moschata* flour would improve consumer acceptability compared to control bread (A1) which has the colour, texture, favour, aftertaste and acceptability scores of 5.70 ± 0.87, 5.92 ± 0.97, 5.40 ± 1.43, 5.16 ± 1.36 and 5.38 ± 1.42

respectively. Furthermore, the higher sensory scores of B2 may be due to the presence of *C. moschata* flour on the composite bread which might have increased its colour, aroma formation, moisture retention and texture. The functional properties of the flour with higher water absorption capacity might have also contributed positively toward higher moisture retention and softness of the composite bread. A similar result was observed for pumpkin-enriched baked products, where low to moderate levels of pumpkin flour improved some sensory attributes due to natural pigments, and fibre and flavour compounds (Bemfeito et al., 2020; Ge et al., 2021).

The acceptance of control bread (A1) was also high, indicating that the consumers are used to consuming the conventional wheat bread, but they accepted the replacement of wheat flour with *C. moschata* flour at 20% level (B2) and found it acceptable to consume it. Thus, the moderate incorporation of vegetable flour in bread could improve its properties without compromising consumer acceptance. The sensory characteristics that showed the most gradual decline with respect to the increase of substitution from A1 (0% substitution level) to C3 (30% substitution level) were colour (4.58 ± 1.10), texture (3.96 ± 1.52), flavour (4.18 ± 1.91), aftertaste (4.04 ± 2.32) and overall acceptance (4.04 ± 2.00). The lowest levels of acceptance for color and texture were found in 40% and 50% substitution, respectively D4 and E5. Above 20% substitution the addition of *C. moschata* flour may sensibly deviate from consumer preferences. This decreased acceptability at higher substitution levels could be the result of the increased fibre content or dilution of the wheat gluten. The increased amount of non-wheat flour may interfere with the gluten network and affect bread volume, crumb structure and texture. The yellow-orange tint that *C. moschata* pigments give flour may also affect sensory acceptance, especially if the color is very different from that of traditional wheat bread. This decrease in sensory acceptability from high substitution levels has also been reported for wheat-pumpkin composite breads when too much pumpkin is used (Ge et al., 2021). However, these raw values should be considered in light of the fact that samples were all rated by the same 50 panellists, thus, differences should be interpreted with respect to the repeated-measures analysis statistics, not as simple mean differences. The panellist variable is included in the model by including the formulation as a fixed effect and the panellist as a random effect.

Some sensory attribute standard deviations were also high, including B2 flavour (6.28 ± 2.37), B2 aftertaste (6.44 ± 2.58), D4 overall acceptance (3.06 ± 2.51) and E5 colour (2.14 ± 2.31), suggesting that some panellists were less accepting than others. Variability is to be expected for consumer sensory evaluations since consumers' flavour, texture and appearance assessments depend on their preferences, dietary and sensory experiences and how familiar they are with composite food products. These variations illustrate the importance of supplementing mean values with measures of variability in addition to average acceptance scores. The sensory results suggest that substitution of wheat flour with *Cucurbita moschata* flour can produce a composite bread which is acceptable to consumers, with the 20% substitution level being the most acceptable of all formulations tested. This in no way suggests that B2 is always the most acceptable formulation or that any larger levels of substitution may not be acceptable. These results show that using moderate levels of *C. moschata* flour in bread-making is feasible, although compromise is needed between nutritional and sensory characteristics of the resulting bread.

Table 6: Mean responses on the sensory attributes of wheat and composite bread samples assessed by 50 panellists

Bread Sample	Color	Texture	Flavor	Aftertaste	Overall Acceptance
A1	5.70 ± 0.87	5.92 ± 0.97	5.40 ± 1.43	5.16 ± 1.36	5.38 ± 1.42
B2	6.16 ± 1.12	6.54 ± 0.78	6.28 ± 2.37	6.44 ± 2.58	6.16 ± 1.97
C3	4.58 ± 1.10	3.96 ± 1.52	4.18 ± 1.91	4.04 ± 2.32	4.04 ± 2.00
D4	2.20 ± 1.80	2.48 ± 1.64	3.08 ± 2.36	2.92 ± 2.32	3.06 ± 2.51
E5	2.14 ± 2.31	2.36 ± 1.79	2.86 ± 1.84	2.92 ± 1.87	2.60 ± 1.80

Values are means ± SD for 50 panellists (n = 50). Means were compared using a linear mixed-effects model with bread formulation as the fixed effect and panellist as the random effect. Differences were considered meaningful when $p < 0.05$.

Limitation of the Study

One limitation of this study is that no demographic data was collected on sensory panellists. Even though, the sensory panel evaluated the acceptability of the composite bread samples, no inquiries were made about their age, gender, level of education, dietary preference and pattern of consumption. Therefore, as demographic data were unavailable for those who participated in the research, the role that population characteristics may have played in influencing consumer preferences can only be speculated and sensory results need to be interpreted accordingly. Further profiling of sensory panellists should include demographic information to correlate the panellists' profiles to the consumers' preferences and to find potential market segments that may be more receptive to a butternut squash composite bread product.

CONCLUSION

The effect of replacing wheat flour with *Cucurbita moschata* (butternut squash) flour on the proximate and functional physicochemical, nutritional and sensory quality of wheat-based bread was studied. The study showed that *C. moschata* flour was found to be a good source of carbohydrate, protein, fibre and ash with a fairly low moisture and fat content. Functional properties such as water absorption capacity, oil absorption capacity, swelling power and solubility of the flour indicate that the flour may play an important role in the behaviour of composite flour systems. The proximate compositions of the wheat breads were affected by the substitution of wheat flour with 20% of *C. moschata* flour. The composite bread was higher in moisture, crude fibre, ash and protein, but lower in crude fat and carbohydrate than the control bread. Thus, bread made with a portion of wheat flour replaced by *C. moschata* flour will have a different nutritional composition, but not necessarily any nutritional advantage because not all nutritional parameters have been measured and bioavailability has not been determined for each nutrient. The sensory analysis conducted by 50 panellists revealed different acceptability levels. Despite these variations, formulation B2 (20% of *C. moschata* flour) presented the highest average sensorial score, becoming the most accepted variation among the different formulations in the present study. These values can only be taken as an indication of consumer preference on the tested formulations and not as an upper limit for a proper substitution. The results of this study do not cover all the nutritional, technological, economic and shelf life parameters that should be considered for an ideal formulation to be

proposed for commercial application. Overall, it can be concluded that the incorporation of *C. moschata* flour to wheat flour is sufficient to produce acceptable composite bread products and to modify the final composition and functional properties. However, further research into the shelf life, microbial stability, mineral and bioactive composition, rheological properties, and sensory analysis, and involving different consumer groups, is needed to assess the commercial viability and to explore other applications.

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