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Chemical, microbiological and organoleptic evaluation of Nigerian Pepper Soup Spice blends from calabash nutmeg (*Monodora Myristica*), scent leaf (*Ocimum Gratissimum*) and grain of selim (*Xylopia Aethiopica*)

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Abstract
Spices are widely used by different cultures for some specific reasons. Packed with antioxidants, anti-inflammatory compounds, and immune-boosting properties, these flavourful ingredients offer a natural path to improved well-being. In this study spice blends were formulated from Calabash Nutmeg (*Monodora myristica*), Scent Leaf (*Ocimum gratissimum*), and Grain of Selim (*Xylopia aethiopica*) in varying proportions. Proximate, mineral content, vitamins composition, phytochemical properties, microbiological safety and sensory attributes were carried out on the control and the blends. The proximate content of the spice blends and the control varied from 4.00% to 12.30% moisture, 3.50% to 6.50% ash, 13.10% to 23.50% fat, 3.40% to 14.10% crude fibre, 12.78% to 17.78% sample (103) was significantly different ($p<0.05$) from other samples with highest protein value and 40.53% to 54.65% carbohydrate. The mineral composition ranged from 62.41±0.25 to 91.31±0.00, 212.51±0.53 to 265.28±5.88, 101.73±0.71 to 118.85±0.40, 22.38±0.38 to 41.99±0.18 and 74.84±0.56 to 86.41±0.25 for calcium, potassium, phosphorus, sodium and magnesium accordingly. The vitamin content ranged from 0.23±0.02 to 0.70±0.03mg/100g, 0.88±0.02 to 2.24±0.04mg/100g, 0.49±0.32 to 1.70±0.04mg/100g, 29.63±0.57 to 44.89±0.62 mg/100g and 8.14±0.00 to 14.39±0.39 mg/100g for vitamin B1, B2, B3, C and A respectively. Phytochemical analysis showed the availability of bioactive substances like tannin, flavonoids, oxalate and phytate which ranged from 0.76 to 4.39 mg/100g, 3.38 to 5.83 mg/100g, 0.41 to 2.03 mg/100g and 2.67 to 4.29 mg/100g respectively. Total heterothrophic bacteria of the spice blends varied from 6.020 to 6.9771 log_{10}cfu /g but was not significantly different from each other ($p>0.05$). Microbial analysis showed no growth for all the indicator organisms in the spices. Sensory evaluation on a 9-point hedonic scale showed that the blends were well accepted in terms of colour 4.53 to 7.33, appearance 4.93 to 8.07, taste 5.13 to 8.13, aroma 4.53 to 8.53 and mouthfeel 4.73 to 7.20. Overall acceptability score showed that sample 105 (8.07) was rated better than the other samples. However, there was no significant difference ($p>0.05$) in the control and sample 105. Therefore, blends of *Monodora myristica*, *Ocimum gratissimum*, and *Xylopia aethiopica* can be developed into nutritionally rich pepper soup spice blends with excellent consumer acceptability.

Keywords: Chemical, organoleptic, calabash nutmeg, scent leaf, grain of selim

Introduction

Spices are provided by nature and are important component of wholesome meals. These spices are intriguing from a scientific and health standpoint due to their abundance of bioactive compounds (Ahmad Wani *et al*., 2022) [4]. Spice-derived extracts have numerous health advantages and are abundant in antioxidants, which reduce reactive oxygen species (Ahmad Wani *et al*., 2022) [4]. In addition to being essential flavour enhancers, spices are also significant sources of bioactive substances with possible health advantages, making them essential part of culinary traditions (Al-Habsi *et al*., 2025) [5]. Pepper soup is a traditional dish in West African cuisine, liked for its fragrant flavour and its healing potentials especially in convalescent care (Keswet & Abia, 2020) [24]. Many of the native spices are underutilised in contemporary food processing despite their rich ethnobotanical significance. (Djiazet *et al*., 2022) [13].

Three prominent spices commonly incorporated in pepper soup preparation includes Calabash Nutmeg (*Monodora myristica*), Scent Leaf (*Ocimum gratissimum*), and Grain of Selim (*Xylopia aethiopica*). These spices are not only appreciated for their unique sensory properties but are also widely recognized in traditional medicine for their pharmacological activities (Asfaw *et al*., 2023) [9]. *Monodora myristica* (Calabash nutmeg), has been shown to have antioxidant, antimicrobial, anti-inflammatory properties. In terms of nutrition, it has notable amounts of fibre, protein, fat, calcium, magnesium, and essential oils (Mudau *et al*., 2022) [32]. *Ocimum gratissimum* (scentleaf) is rich in essential oils and is known for its antibacterial, antifungal, and hypoglycemic effects. It also provides a notable amount of vitamins A and C, calcium, iron, potassium, and dietary fiber, making it beneficial addition to a functional (Nxusani *et al*., 2023) [34]. *Xylopia aethiopica* (grain of selim), often used to relieve respiratory and gastrointestinal ailments, contains compounds with analgesic and antimicrobial activities. Its nutritional content includes carbohydrates, proteins, fats, dietary fiber, and minerals like magnesium, calcium, and potassium (Matlala *et al*., 2024) [30]. In people with history of cancer, spice therapy may help prevent the disease from developing. They can also be used to prevent cancer from returning, in addition to lessening the negative effects of radiation and chemotherapy on cancer patients (Ugbogu *et al*., 2021) [41]. For individuals with advanced cancer, they have been utilised to be an alternative treatment when traditional treatments are no longer effective (Al-Habsi *et al*., 2025) [5]. Spices serve as aroma carriers generated from all other parts of the plant, including resins, roots, stigmas, flower buds, seeds, arils, and bark. They are typically, but not often, dried form is used and preserved (Inada *et al*., 2023) [21]

Despite the widespread traditional use of *Monodora myristica*, *Ocimum gratissimum* and *xylopia aethiopica* in west Africa, pepper soup spice for both flavor enhancement and medicinal benefits, there are no standardized formulations for these spice blends, moreso these spice mixtures have not received much scientific attention despite their lengthy history of use. Their nutritional, mineral, vitamin, phytochemical, microbiological and sensory characteristics remain largely uncharacterised (Omotayo *et al*., 2025) [36], limiting their scientific validation and industrial application. As consumer demand for natural and health-promoting food products continues to grow, there is a pressing need to investigate the possibility of indigenous spices as functional components in commercial food formulations. Moreso with scientific validation, this research promotes the development of standardized, locally sourced spice products that align with contemporary health and dietary needs, while also supporting food security and the sustainable utilization of indigenous plant sources. Therefore, this study addresses these gaps by systematically formulating, analyzing and sensory evaluating spice blends to develop a nutritionally superior, microbiologically safe and consumer-acceptable pepper soup spice.

Materials and Method

1. Sample collection

The study was conducted in Ozoro, Isoko North local government of Delta state, south-south Nigeria. Calabash Nutmeg (Monodora myristica), Scent Leaf (Ocimum gratissimum), and Grain of Selim (Xylopia aethiopica) were bought from Ozoro main market Delta state Nigeria and were properly stored under optimal conditions to prevent deterioration, microbial contamination, or loss of bioactive compounds. Analytical grade reagents and chemicals used were obtained from the chemical store of the department of Food Science Technology, Southern Delta University Ozoro.

Table 1: Formulation of spice blends

Sample	Calabash nutmeg	Grain of selim	Scentleaf
103	61.9	38.1	_
104	35.4	21.6	43.1
105	43.1	21.6	35.4
106	28.3	22.2	49.5
107	49.5	22.2	28.3
108	23.9	20.2	55.9
109	Control		

(Pearson's method of formulation) (Zinzendoff Okwonu *et al*., 2020) [45]

Calabash Nutmeg
↓
Sorting
↓
Washing
↓
Drying (60^0c for 5hrs)
↓
Manual Dehulling
↓
Grinding
↓
Sieving (250µ sieve)
↓
Packaging in Airtight Containers
↓
Storage
↓
Calabash nutmeg powder

Fig 1: Calabash Nutmeg Sample Preparation (Dawodu *et al*., 2023)

Fresh Scentleaf

↓

Sorting

↓

Washing (with clean water)

↓

Drying

↓

Grinding

↓

Sieving (250µ sieve)

↓

Packaging

↓

Scentleaf powder

↓

Storage (25^0C)

Fig 2: Scent Leaf Sample Preparation (Dawodu *et al*., 2023)

Grain of Selim

↓

Sorting

↓

Washing (with clean water)

↓

Drying (60^0c)

↓

Grinding

↓

Sieving (250 µm sieve)

↓

Grain of selim powder

↓

Packaged

↓

Storage ($25\ ^0C$)

Fig 3: Grain of Selim Sample Preparation (Dawodu *et al*., 2023)

2. Protein determination

Weighing 0.5g of the sample into a Kjeldahl Digestion flask, 3g of sodium sulphate (Na2SO4), which acts as a catalyst, and 0.3g of copper sulphate (CuSO4) were added. After adding 12 ml of concentrated H2SO4, the KDN-04C Digest Furnace was set up and left to digest for one hour at 420^oc (forming a transparent solution) (Kovalev *et al*., 2018) [26]. 20 ml of the digest was measured into a distillation flask after the diluent was digested with distilled water to make 100 ml using a measuring cylinder. The Foss, Kjeltec 2100 Distillation unit was filled with 20 millilitres of 45% NaOH. Ten millilitres of boric acid indicator were measured and added to the receiving flask, where it was allowed to distil for five minutes. The distilled sample was titrated with 0.1N HCL solution until a pink colour solution was seen (end point).

3. Mineral determination (A. O. A. C.2006)

A porcelain crucible that had been cleaned and dried was filled with 1g of the sample.
In a muffle furnace (Model SXL), the sample was fired for two hours at 5500C and then allowed to cool. The crucible's ash was mixed with 5 ml of strong hydrochloric acid and diluted with 20 ml of deionised water. On a hot plate, the crucible's contents were heated until they boiled down to half of their volume, or roughly 10 ml. On cooling, it was filtered through Whatman No. 1 filter paper into a 100 ml volumetric flask, and the volume was adjusted with de-ionized water. The Buck Scientific 210VP Atomic Absorption Spectrophotometer was used for the elemental assay.
The main switch was turned on, and the acetylene gas was applied. The air-acetylene knob was turned on, the fuel supply was turned on, the flame lit, and the lamp of the element to be analysed was placed in the lamp position. The blank was used to zero the apparatus, each metal's standard was run, and the sample digest was aspirated.

The content of the element was calculated using the formula below

$$\text{Metal (\%)} = \frac{\text{Concentration (ppm) x solution volumes}}{10^4 \text{ x sample weight.}}$$

Metal (mg/100g sample) = metal (

4. Phytochemical determination of flour blends

4.1 Tannin

Folin Denis colometric method was used to determine the tannin (Omotayo *et al*., 2025) [36]. A 50 ml volumetric flask was filled with five (5) grammes of each sample, then the flask was topped up with distilled water. After 30 minutes of shaking at room temperature, the mixture was filtered. A tannic acid solution was prepared. Two millilitres of the standard solution and a corresponding volume of distilled water were added to separate 50 millilitre volumetric flasks as a standard and reagent blank. Then, two millilitres of each filtrate were put into the labelled flasks.
Each flask's contents were combined with 35 ml of distilled water, then 1 ml of the Folin Denis reagent, 2.5 ml of saturated Na2CO3, and the remaining 50 ml of distilled water were added. The flasks were then allowed to sit at room temperature for 90 minutes. In a spectrophotometer, their absorbance was measured at 760 nm while the reagent blank was set to zero (Ishiwu *et al*., 2013) [22].
The tannin concentration was determined as indicated below:
% Tannin = 100 x Au x C x Vt
W As Va
Where:
W = Weight of sample
Au = Absorbance of test sample
As = Absorbance of standard tannin solution
C = Concentration of standard tannin Solution
Vt = Total volume of extract
Va = Volume of extract analysed

4.2 Phytate

The described bi-pyridine colorimetric technique was applied (Marolt & Kolar, 2020) . Each sample weighed 2 g and was placed in a test tube. Distilled water (about 10 ml) was added. Two ml of 0.2M HCl (aq) were used to extract the sample, and 0.5 ml of the extract were pipetted into a test tube with a glass stopper. The tube was filled with 1 ml of the solution, and a stopper was placed over it. After 30 minutes of heating in a boiling water bath, the tube was chilled in ice water for 15 minutes to bring it to room temperature. After thoroughly shaking the sample in the tube by hand, it was centrifuged for half an hour. 1.5 ml of the solution was added to another test tube containing 1 ml of the supernatant. Measured was the absorbance at 420 nm in comparison to distilled water (Marolt & Kolar, 2020).
% Phytate = Au x C x 100 x Vf
As W Va
Au is the test sample's absorbance.
As = Standard Solution Absorbance
C is the standard solution's concentration.
W is the sample weight.
Vf is the extract's total volume; Va is the extract's utilised volume.

5. Enumeration of Microbial Population

Twenty-five (25 g) of cut meat was immersed in 22 ml buffered Peptone Water (BPW) in a sterile conical flask to serve as stock. After the preparations of the stock, a ten-fold serial dilution was carried out using sterilized normal saline as the diluent. In this method, one ml of the stock was withdrawn and moved into a test tube containing sterile 9mL normal saline to give a dilution of 1:100. The dilution was repeated in a step wise fashion by transferring 1ml from the 1:100 dilution to another test tube containing 9mL sterile normal saline. This was done until dilutions of 10^{-6} was obtained. This technique was repeated for all the samples.
Aliquots of 10^{-2} and 10^{-4} dilutions of the diluted samples were plated out on well labelled freshly prepared pre-dried plates containing *Salmonella-Shigella agar* (SSA) (for Salmonella and Shigella), Eosine methylene blue agar (for E. coli and coliforms), mannitol salt agar (for *Staphylococcus* sp) nutrient agar (NA) and sabouraud dextrose agar (for fungi) in duplicates. The plates were incubated for 24 to 48 hours at 37°C.
For bacteria and 25°C for 3-7 days for fungi (Rubab & Yousuf, 2022) [39].
The bacterial populations were enumerated using the following formulae; spice samples

$$\frac{CFU}{mL} \, or \, g = \frac{\text{Number of colonies}}{\text{Dillution}\times\text{volume plated}}$$

6. Statistical analysis

The analysis was carried out in triplicate (n = 3). Statistical analysis was performed using Stastical Package for Service Solution (SPSS), version 27 all results were expressed as mean ± standard deviation. The statistical analysis was interpretated by applying analysis of variance (ANOVA) . Using Tukey's multiple comparison tests, differences between the means were tested at 95% confidence level, and the significance level was set at $P < 0.05$.

Results and Discussion

Table 2 displayed the nutrient composition of seven pepper soup spice formulations, incorporating varying proportions of calabash nutmeg, scent leaf, and grain of Selim. The moisture, ash, fat, fibre, protein, and carbohydrate contents were among the components examined. Significant differences ($p>0.05$) were seen in all metrics evaluated, showing that they blends are excellent sources of these nutrients.

Table 2: Nutrient composition of spice blends

Sample	Moisture (%)	Ash (%)	Fat (%)	Fibre (%)	Protein (%)	Carbohydrate (%)
103	$4.00^{f}±0.28$	$3.50^{g}±0.00$	$23.50^{a}±0.14$	$10.70^{c}±0.14$	$17.78^{a}±0.04$	$40.53^{c}±0.04$
104	$12.30^{a}±0.14$	$4.00^{f}±0.00$	$13.10^{g}±0.14$	$14.10^{a}±0.14$	$12.78^{f}±0.18$	$43.73^{c}±0.32$
105	$8.80^{b}±0.14$	$4.60^{e}±0.14$	$14.60^{e}±0.14$	$13.70^{a}±0.00$	$13.15^{ef}±0.00$	$45.15^{b}±0.14$
106	$8.20^{bc}±0.00$	$5.00^{d}±0.00$	$16.60^{d}±0.14$	$12.65^{b}±0.21$	$13.50^{e}±0.00$	$44.05^{c}±0.35$
107	$7.60^{cd}±0.00$	$5.50^{c}±0.00$	$18.90^{c}±0.42$	$10.63^{c}±0.18$	$14.78^{d}±0.11$	$42.60^{d}±0.35$
108	$7.10^{d}±0.42$	$6.00^{b}±0.00$	$20.30^{b}±0.14$	$9.00^{d}±0.00$	$15.25^{c}±0.00$	$42.35^{d}±0.28$
109	$5.40^{e}±0.00$	$6.50^{a}±0.00$	$13.85^{eg}±0.07$	$3.40^{e}±0.00$	$16.20^{b}±0.14$	$54.65^{a}±0.21$

Values are means ± Standard Deviaton of triplicate determination
Means with the same superscripts in the same column are not significantly different ($p > 0.05$)

Key: 103 = 61.9 calabash nutmeg 38.1grain of selim; 104= 35.4 calabash nutmeg 21.6 Grain of selim 43.1 scentleaf ; 105= 43.1 calabash nutmeg 21.6 Grain of selim 35.4 scentleaf ; 106 = 28.3 calabash nutmeg 22.2 grain of selim 49.5 scentleaf; 107= 49.5 calabash nutmeg 22.2 Grain of selim 28.3 scentleaf; 108 = 23.9 calabash nutmeg 20.2 Grain of selim 55.9, Scentleaf; 109 control

Moisture content of the samples ranged from 4.00% (Sample 103) to 12.30% (Sample 104). Low moisture content suggests better shelf stability and lower susceptibility to microbial spoilage, which is desirable in dried spice formulations (Nakra *et al*., 2025) [33].

Ash, which represents the total mineral content, ranged from 3.50% (103) to 6.50% (109). These values differed as earlier report by (Abukawsar *et al*., 2018) [1]. Ash content gives a rough mineral content of foods (Harris & Marshall, 2017) [19]

Fat content had the highest significant difference in sample 103 (23.50%) and lowest in sample 104 (13.10%). The fat content was higher than reported by (Al Dhaheri *et al*., 2023) [6] Calabash nutmeg, a known oilseed spice, likely contributed significantly to the fat levels. High fat content enhances flavor retention and palatability but may also influence oxidative stability over time (Wang *et al*., 2023) [44]. The fat content in the spce blends was significantly higher than the control sample

Crude fibre content ranged from 3.40% to 14.10%. High fibre values, especially in samples 104 and 105, suggest a greater contribution from scent leaf, which is rich in dietary fibre. Dietary fibre promotes gastrointestinal health and has been linked to reduced risk of chronic diseases (He *et al*., 2022). The fibre contents were higher than the control sample (109).

Protein levels ranged from 12.78% in sample 104 (lowest) to (17.78%) in sample 103 (highest). The protein content in these samples was discovered to be greater than earlier research by (BAMIGBOYE *et al*., 2020) and (CHINEDU NDEFO *et al*., 2024) [40] . The protein content reflects the combined contribution of all three spices, with calabash nutmeg particularly notable for its protein-rich seed composition. Spice blends contribute meaningfully to dietary protein intake, particularly in protein-deficient regions. In order to stop muscle loss, 0.4 g of protein per kilogramme of body weight per meal is advised (Peters *et al*., 2023) [37]. Carbohydrate content was highest in sample 109 (54.65%) and lowest in sample 103 (40.53%). Carbohydrates contribute to the energy value of the spice blend, which is vital for metabolic activities, especially when used in convalescent diets or for flavor enhancement in nutrient-dense meals (Petersen *et al*., 2024) [38]. Strategic blending can yield products with enhanced dietary fibre, protein, or energy value, supporting the traditional medicinal and culinary uses of these spices (Eneojo & Martins, 2024) [15]. The nutritional composition was higher than that observed in the spice study of (Evuen *et al*., 2022) [16].

Table 3: Mineral content of spice blends

Sample	Calcium (mg/100g)	Potassium (mg/100g)	Phosphorus (mg/100g)	Sodium (mg/100g)	Magnesium (mg/100g)
103	$65.98^{f}±0.64$	$253.39^{b}±1.49$	$118.85^{a}±0.40$	$24.56^{f}±0.00$	$86.41^{a}±0.25$
104	$91.31^{a}±0.00$	$212.51^{e}±0.53$	$101.73^{f}±0.71$	$41.99^{a}±0.18$	$74.84^{e}±0.56$
105	$87.38^{b}±0.37$	$215.99^{e}±0.18$	$104.78^{e}±0.47$	$39.34^{b}±0.00$	$75.12^{e}±0.00$
106	$84.17^{c}±0.39$	$219.84d^{e}±0.55$	$107.55^{d}±0.00$	$36.43^{c}±0.43$	$76.75^{d}±0.40$
107	$81.49^{d}±0.25$	$224.41^{cd}±0.00$	$109.29^{c}±0.18$	$34.04^{d}±0.71$	$78.49^{c}±0.00$
108	$77.49^{e}±0.37$	$229.07^{c}±0.23$	$111.22^{b}±0.00$	$31.59^{e}±0.36$	$80.42^{b}±0.00$
109	$62.41^{g}±0.25$	$265.28^{a}±5.88$	$117.71^{a}±0.39$	$22.38^{f}±0.38$	$85.45^{a}±0.00$

Values are means ± Standard Deviaton of triplicate determination
Means with the same superscripts in the same column are not significantly different ($p > 0.05$)

Key: 103 = 61.9 calabash nutmeg 38.1, grain of selim; 104= 35.4 calabash nutmeg 21.6 Grain of selim 43.1 scentleaf; 105= 43.1 calabash nutmeg 21.6 Grain of selim 35.4 scentleaf; 106 = 28.3 calabash nutmeg 22.2 grain of selim 49.5 scentleaf; 107= 49.5 calabash nutmeg 22.2 Grain of selim 28.3 scentleaf; 108 = 23.9 calabash nutmeg 20.2 Grain of selim 55.9, Scentleaf; 109 control in this study, it was found that calcium composition of sample 104 was significantly (p≤0.05) greater than the rest of the samples. The values for calcium ranged from 62.41±0.25 to 91.31±0.00. There was significant differences between the blended samples and the control (sample 109). The values as shown are lower than the stated values by (Adebayo *et al*., 2025) [2] and, but significantly higher as observed in (Evuen *et al*., 2022) [16]. Calcium is an essential mineral component in the body which helps in regulating muscular contractions, strong teeth formation also development of strong bones and is needed for the prevention of osteoporosis, arthritis, rickets and tooth decay (E.O *et al*., 2024) [40]. Potassium content was relatively high in all the samples it ranged from 212.51±0.53 to 265.28±5.88. Potassium functions in the body to regulate processes such as nerve transmission, muscle contraction and control fluid balance and enhances the metabolism of protein and carbohydrate (Lindinger & Cairns, 2021) [28]. However, the values observed in this study were significantly higher that as observed by (Evuen *et al*., 2022) [16] in their study for suya spice but lower than the values reported by (BAMIGBOYE *et al*., 2020) in their study for spices. The
Phosphorus content ranged from 101.73f±0.71 to 118.85a±0.40, the sodium content ranged from 24.56±0.00 to 41.99±0.18 and magnesium content ranged from 74.84±0.56 to 86.41±0.25. The lowest mineral content of the blends was sodium while the highest was seen in potassium.

Table 4: Vitamins content of spice blends

Sample	B1 (mg /100g)	B2 (mg /100g)	B3 (mg /100g)	C (mg /100g)	A (µg /100g
103	0.23^{f}±0.02	0.88^{f}±0.02	0.49^{c}±0.32	39.83^{b}±0.42	14.39^{a}±0.39
104	0.32^{e}±0.00	1.18^{e}±0.01	0.86^{bc}±0.01	29.63^{g}±0.57	7.01^{g}±0.35
105	$0.36d^{e}$±0,01	1.21^{de}±0.00	0.94^{bc}±0.01	31.32^{f}±0.29	8.14^{f}±0.00
106	0.40^{d}±0.01	1.28^{cd}±0.02	1.05^{b}±0.03	33.65^{e}±0.00	9.65^{e}±0.30
107	0.45^{bc}±0.00	1.34^{c}±0.01	1.10^{b}±0.00	35.19^{d}±0.00	10.95^{d}±0.11
108	0.49^{b}±0.01	1.42^{b}±0.00	1.18^{b}±0.01	37.62^{c}±0.23	12.10^{c}±0.29
109	0.70^{a}±0.03	2.24^{a}±0.04	1.70^{a}±0.04	44.89^{a}±0.62	13.35^{b}±0.00

Values are means ± Standard Deviaton of triplicate determination
Means with the same superscripts in the same column are not significantly different (p > 0.05)

Key: 103 = 61.9 calabash nutmeg 38.1, grain of selim; 104= 35.4 calabash nutmeg 21.6 Grain of selim 43.1 scentleaf; 105= 43.1 calabash nutmeg 21.6 Grain of selim 35.4 scentleaf; 106 = 28.3 calabash nutmeg 22.2 grain of selim 49.5 scentleaf; 107= 49.5 calabash nutmeg 22.2 Grain of selim 28.3 scentleaf; 108 = 23.9 calabash nutmeg 20.2 Grain of selim 55.9, Scentleaf; 109 control.

Table 3 showed the vitamin content of the different spice blends, the values ranged from 0.23±0.02 to 0.70±0.03 for vitamin B1, there was a notable variation between the blended samples as well as the control sample (p<0.05). Vitamin B2 values ranged from 0.88±0.02 to 2.24±0.04, sample 104 was not significantly different from sample 105 (p>0.05), likewise sample 106 and 107. The values obtained in the vitamin B1 and B2 of the spice blends were comparable with those reported by (CHINEDU NDEFO *et al*., 2024) [40]. Vitamin B3 values ranged from 0.49c±0.32 to 1.70±0.04 sample 104 – 108 were not significantly different from each other. These values for the B vitamins are lower when compared with the report of (Wahab Gbolahan Ayoade *et al*., 2023) [43]. Vitamin c ranged from 29.63±0.57 to 44.89±0.62. The values of the vitamin c followed the same trend with the report of (Uzoamaka & Olanrewaju, 2023) [42] in their study of some indeginious Nigerian spices, and also comparable with the values obtained by (Giwa *et al*., 2025) [18](Uzoamaka & Olanrewaju, 2023) [42].

Vitamin A is a fat-soluble vitamin that is essential for healthy vision, the integrity of epithelial cells (skin and mucous membranes), reproduction, embryonic development, growth, and immunological response (Amimo *et al*., 2022) [8]. Vitamin A is also referred to as retinol, retinal, retinoic acid, and beta-carotene (plant form) (Wahab Gbolahan Ayoade *et al*., 2023) [43]. Values for vitamin A ranged from 8.14±0.00 to 14.39±0.39 with sample 103 having the highest value. The content of vitamin C was comparable with the report of (Giwa *et al*., 2025) [18].

Table 5: Phytochemical composition on spice blends.

Sample	Tannin (mg /100g)	Flavonoid (mg /100g)	Oxalate (mg /100g)	Phytate (mg /100g)
103	0.76^{e}±0.00	3.74^{d}±0.11	1.86^{a}±0.11	2.67^{g}±0.34
104	4.39^{a}±0.24	5.83^{a}±0.09	0.41^{c}±0.03	4.29^{a}±0.00
105	4.04^{b}±0.11	5.73^{b}±0.07	0.61^{de}±0.00	4.08^{b}±0.04
106	3.65^{bc}±0.13	5.27^{b}±0.07	0.76^{cd}±0.02	3.91^{c}±0.23
107	3.39^{cd}±0.07	4.87^{b}±0.00	0.87^{bc}±0.03	3.72^{d}±0.00
108	3.19^{d}±0.00	4.39^{c}±0.49	1.08^{b}±0.04	3.39^{e}±0.4
109	0.82^{c}±0.00	3.38^{d}±0.19	2.03^{a}±0.09	2.85^{f}±0.08

Values are means ± Standard Deviaton of triplicate determination
Means with the same superscripts in the same column are not significantly different (p > 0.05).

Key: 103 = 61.9 calabash nutmeg 38.1, grain of selim; 104= 35.4 calabash nutmeg 21.6 Grain of selim 43.1 scentleaf; 105= 43.1 calabash nutmeg 21.6 Grain of selim 35.4 scentleaf; 106 = 28.3 calabash nutmeg 22.2 grain of selim 49.5 scentleaf; 107= 49.5 calabash nutmeg 22.2 Grain of selim 28.3 scentleaf; 108 = 23.9 calabash nutmeg 20.2 Grain of selim 55.9, Scentleaf; 109 control.

Table 5 shows the levels of four phytochemicals - tannin, flavonoid, oxalate, and phytate. These bioactive compounds are renowned for their well-being promoting properties, antioxidant, anti-inflammatory, antimicrobial and antinutritional effects (Sipra *et al*., 2024) [31]. The variation in their concentrations across samples is statistically significant ($p < 0.05$), indicating that the composition and ratio of the spice ingredients are essential in modulating their phytochemical contents.

Tannin levels varied from 0.76 mg/100g (Sample 103) to 4.39 mg/100g (Sample 104), and was not significantly different from sample 106 and 107 ($p>0.05$). The tannin content was significantly higher than was obtained in the study of (Evuen *et al*., 2022) [16] Tannins exhibit strong antioxidant and antimicrobial activities, Spices have ten times the antioxidant capacity of fruits and vegetables (Karakol & Kapi, 2021) [23] (Lazaridis *et al*., 2024) [27].

Flavonoids are well-known antioxidants that can capture free radicals and prevent radical chain reactions. They are found in practically every part of the plant, including the stems, roots, leaves, fruits, and seeds (Zheng *et al*., 2022), were highest in Sample 104 (5.83 mg/100g) and lowest in Sample 109 (3.38 mg/100g). These values followed the same trend as reported by (Ali *et al*., 2021) [7] .The higher flavonoid levels in Samples 104 - 106 point to a rich contribution from scent leaf (*Ocimum gratissimum*), which is renowned for its flavonoid richness. The flavonoid content was comparable with a previous study by (Ogunka-Nnoka & Mepba, 2008) [35]. These blends offer substantial protective effects against oxidative stress, cardiovascular diseases, and inflammation (Al-Habsi *et al*., 2025) [5].

Dietary oxalate can be found in grains, fruits, nuts, and vegetables. In healthy people, the diet provides around half of the urine oxalate, while the other half comes from endogenous synthesis (Mitchell, 2019). Oxalate was lowest in Sample 104 (0.4 1 mg /100g) and highest in Sample 109 (2.03 mg/100g). These values when compared with the report of (Ghosh Das & Savage, 2012) [17] in some spices was found to be lower. This implies that Sample 104 is safer for individuals prone to renal disorders or those requiring calcium supplementation, whereas Sample 109, despite its strong nutritional profile (as seen in vitamins and minerals), may require moderation in thermal processing to reduce oxalate content.

Phytate values ranged from 2.67 mg /100g (Sample 103) to 4.29 mg /100g (Sample 104). For literature comparison these values are higher than that stated by (Mawouma *et al*., 2024) [31]. Phytates are considered antinutritional because of their ability to chelate vital minerals like iron, zinc and calcium (Amos *et al*., 2020) [10] . However, at moderate levels, they also possess antioxidant and anticancer potential. The highest phytate level in Sample 104 could potentially reduce mineral bioavailability, though this may be offset by the overall mineral richness of the blend. Lower phytate levels in Samples 103 and 109 suggest improved mineral absorption and better nutritional efficiency. There is a wide range in the quantity of phytate found in grains, nuts, legumes, and seeds; the amounts that researchers detect when they examine a particular product likely rely on factors including growing circumstances, harvesting practices, processing processes, testing procedures, and even the food's age (Amos *et al*., 2020) [3].

Table 6: Microbiological analysis of spice samples

Sample	THB log_{10} cfu /g	TSA log_{10} cfu /g	TSH log_{10} cfu /g	TFC log_{10} cfu /g	TCC log_{10} cfu/g	TSC log_{10} cfu /g	FC log_{10} cfu /g
103	$6.7782^a±0.25$	NG	NG	NG	NG	NG	NG
104	$6.0207^a±0.29$	NG	NG	NG	NG	NG	NG
105	$6.5000^a±0.71$	NG	NG	NG	NG	NG	NG
106	$6.8997^a±0.77$	NG	NG	NG	NG	NG	NG
107	$6.5603^a±0.68$	NG	NG	NG	NG	NG	NG
108	$6.9771^a±0.03$	NG	NG	NG	NG	NG	NG
109	$6.6901^a±0.30$	NG	NG	NG	NG	NG	NG

Values are means ± Standard Deviaton of triplicate determination

Means with the same superscripts in the same column are not significantly different ($p > 0.05$)

Key: 103 = 61.9 calabash nutmeg 38.1, grain of selim; 104= 35.4 calabash nutmeg 21.6 Grain of selim 43.1 scentleaf; 105= 43.1 calabash nutmeg 21.6 Grain of selim 35.4 scentleaf; 106 = 28.3 calabash nutmeg 22.2 grain of selim 49.5 scentleaf; 107= 49.5 calabash nutmeg 22.2 Grain of selim 28.3 scentleaf; 108 = 23.9 calabash nutmeg 20.2 Grain of selim 55.9, Scentleaf; 109 control

THB: Total heterotrophic bacteria; TSA: Total Salmonella count; TSH: total Shigella count; TFC: total faecal coliform; TCC: total coliform count; TSC: total staphylococcal count

Total Heterotrophic Bacteria (THB) counts across the samples ranged from 6.5000±0.71 to 6.9771±0.03 log_{10}cfu/ml. The percentage of bacterial contamination of the spices was observed to be low. The observed differences in the total heterothrophic bacteria of samples were not significantly different from each other ($p > 0.05$), as indicated by their shared superscript. This data showed that the spices could have antimicrobial properties (Khatri *et al*., 2023) [25]. There was no growth (NG) for Total Salmonella Count (TSA), Total Shigella Count (TSH), Total Faecal Coliform (TFC), Fungal count (FC), total coliform count (TCC), and total staphylococcal count (TSC). In addition to being considered a sign of faecal contamination, coliform, Shigella, and Salmonella are more likely to be a sign of inadequate personal hygiene and sanitary habits.

The absence of Salmonella, Shigella, coliforms, and fungal contaminants in all samples showed that the production process was hygienic and complied with food safety standards (Tusa *et al*., 2024) [40].

Table 7: Sensory Evaluation

Sample	Colour	Appearance	Taste	Aroma	Mouthfeel	Overall Acceptability
103	6.67^{a}b±0.82	$7.53^{a}\pm0.64$	$7.40^{ab}\pm0.91$	$7.53^{ab}\pm1.06$	$6.86^{ab}\pm0.74$	$7.53^{ab}\pm0.64$
104	$5.40^{d}\pm0.51$	$5.80^{bc}\pm0.56$	$5.73^{c}\pm0.79$	$5.53^{c}\pm0.92$	$6.33^{ab}\pm1.23$	$6.07^{cd}\pm0.70$
105	$7.33^{a}\pm1.29$	$8.07^{a}\pm1.09$	$8.13^{a}\pm1.13$	$8.53^{a}\pm0.99$	$7.33^{a}\pm1.63$	$8.07^{a}\pm1.39$
106	$5.87^{bc}\pm1.64$	$5.47^{c}\pm1.88$	$5.13^{c}\pm2.03$	$5.13^{c}\pm2.07$	$5.73^{bc}\pm1.44$	$5.00^{d}\pm1.93$
107	$6.07^{bc}\pm0.88$	$7.00^{ab}\pm1.00$	$6.33^{bc}\pm0.62$	$6.53^{bc}\pm0.99$	$6.07^{ab}\pm0.78$	$6.67^{bc}\pm0.72$
108	$4.53^{d}\pm0.83$	$4.93^{c}\pm0.79$	$5.13^{c}\pm1.30$	$4.53^{c}\pm0.83$	$4.73^{c}\pm0.96$	$4.93^{d}\pm0.88$
109	$7.33^{a}\pm1.18$	$7.33^{a}\pm1.17$	$7.20^{ab}\pm1.26$	7.20 ± 1.26	$7.20^{a}\pm1.26$	$7.20^{ab}\pm1.26$

Values are means ± Standard Deviaton of triplicate determination

Means with the same superscripts in the same column are not significantly different ($p > 0.05$)

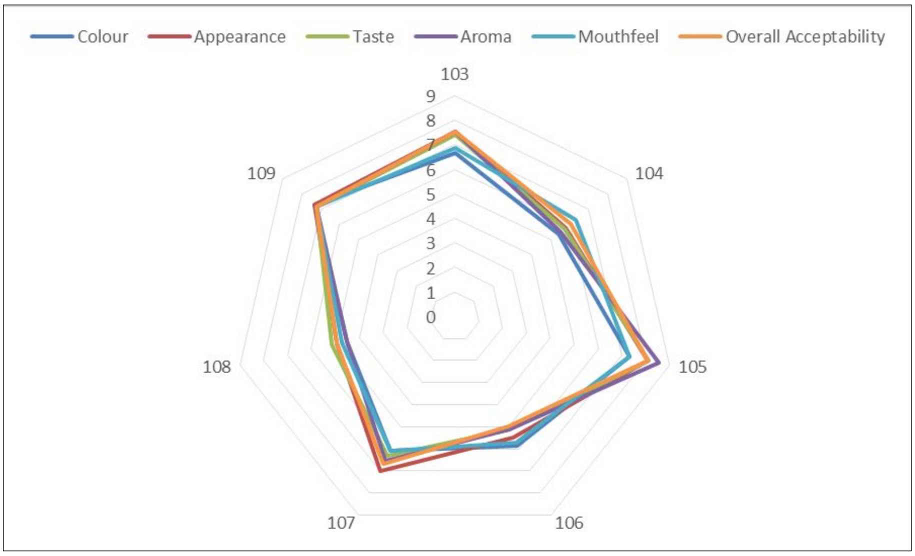

Fig 4: Sensory properties of the spice blends

The sensory attributes of the six pepper soup spice formulations, using 9-point Hedonic scale shows that sample 105 recorded the highest score in colour (7.33) and appearance (8.07), indicating a good appealing visual presentation. Similarly, Sample 109 was also rated high in colour (7.33) and appearance (7.33), suggesting that spice formulations with balanced compositions or a dominant proportion of *Monodora myristica* or *Xylopia aethiopica* may contribute to more attractive sensory characteristics.

Taste and aroma are core sensory attributes determining the spice's palatability (Dawodu *et al*., 2023) [12]. Sample 105 received the highest ratings in taste (8.13) and aroma (8.53), clearly indicating its superior organoleptic quality. Mouthfeel refers to the texture and physical sensation in the mouth during tasting (Agorastos, 2020) [3]. Sample 105 with a score of 7.33, indicating a pleasant and acceptable texture.

Overall acceptability reflects the integrated perception of all sensory parameters (Huey *et al*., 2024) [20]. Sample 105 was the most preferred sample (8.07), followed by Sample 103 (7.53) and Sample 109 (7.20), highlighting these blends as most favorable for consumer use.

The sensory scores show significant variation ($p < 0.05$) across samples, indicating the importance of the blend ratio and ingredient synergy

Conclusion

This research revealed the proximate, mineral, vitamins, bioactive, microbiological and sensory constituents of spice blends from Calabash Nutmeg (*Monodora myristica*), Scent Leaf (*Ocimum gratissimum*), and Grain of Selim (*Xylopia aethiopica*). The spices were very rich in nutrients, vitamins, minerals and phytochemicals that, whether taken whole or as a supplement, are necessary for regular physiological function.

According to the study's findings, all spice blends contains protein, fat, fibre, carbohydrates, vitamins, minerals and phytochemicals—all of which the body needs to improve immunity and as good antioxidants. This suggests that the spices could be used as a good raw material to make some medications and dietary supplements. Moreso the absence of Salmonella, Shigella, coliforms, and fungal growth shows that the blends are very safe for consumption. Spices are considered as insignificant sources of nutrients, but the combination of these three spices provides a significant amount of vitamins and mineral. Therefore by adding these spices to the daily diet, you can increase your intake of vital vitamins, minerals, and antioxidants, as well as other nutrients that can support overall nutritional health.

Conflict of interest: There is no conflict of interest

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