# Qualitative and Quantitative Phytochemical Evaluation of Leaf Protein Concentrates and Bagasse of Scent Leaf (*Ocimum gratissimum*) Collected in Edo State, Nigeria

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**Abstract**
In light of the growing demand for plant-based protein substitutes, it is critical to comprehend how processing techniques impact the phytochemical composition of leaf protein concentrates (LPCs) in order to maximize their functional and nutritional advantages. This study evaluated the qualitative and quantitative phytochemical properties of scent leaf (*Ocimum gratissimum*) meal (SLM), wet-milled scent leaf protein concentrate (Wet-SLPC), dry-milled scent leaf protein concentrate (Dry-SLPC), and scent leaf bagasse (SLB). Qualitative screening revealed the consistent presence of key phytochemicals, such as alkaloids, flavonoids, saponins, tannins, phenols, glycosides, terpenoids, and others, across all samples, regardless of processing. However, anthocyanins and phlobatannins were absent in all treatments. Quantitative analysis showed that SLM contained significantly higher levels ($p < 0.05$) of most phytochemicals compared to SLPCs and SLB. Notably, flavonoids, alkaloids, saponins, and tannins decreased markedly in the processed fractions, likely due to aqueous extraction and thermal degradation. In contrast, glycosides, phytates, terpenoids, and phenols remained relatively stable. These findings highlight the influence of processing on phytochemical retention, with potential implications for the nutritional, medicinal, and functional food applications of LPCs. Wet- and dry-milled SLPCs retained sufficient levels of beneficial bioactives while exhibiting reduced concentrations of antinutritional factors such as tannins and saponins. This study offers a promising approach to producing protein concentrates with balanced phytochemical profiles, supporting their use as nutritionally enhanced and functionally safe plant-based ingredients in human and animal diets.
***Keywords:*** Phytochemicals, scent leaf meal, leaf bagasse, leaf protein concentrates, *Ocimum gratissimum*, plant-based proteins

## Introduction

*Ocimum gratissimum*, commonly known as scent leaf, is a tropical herb extensively used in West Africa for culinary and medicinal purposes due to its rich phytochemical composition (Moneme *et al*., 2024). The leaves contain diverse bioactive compounds, including alkaloids, flavonoids, tannins, saponins, phenols, and terpenoids, which contribute to its antimicrobial, antioxidant, and anti-inflammatory properties (Okoye *et al*., 2023). These phytochemicals have been implicated in health-promoting effects such as immune enhancement, cardiovascular protection, and management of metabolic disorders (Fawehinmi *et al*., 2022; Nalado and Tijjani, 2023).

In recent years, there has been growing interest in the production of leaf protein concentrates (LPCs) from plants as alternative protein sources to support food and nutrition security (Akaeze *et al*., 2014; Agbonghae and Nwokoro, 2023). LPCs from leafy vegetables retain not only essential amino acids but also significant levels of phytochemicals, making them valuable in functional food formulations (Enenya *et al*., 2022). However, the impact of processing methods, such as wet and dry milling, on the stability and bioavailability of these phytochemicals remains underexplored.

Phytochemicals are naturally occurring compounds in plants. They represent a broad class of substances believed to play a key role in the health benefits associated with diets rich in fruits, vegetables, whole grains, legumes, and plant-based beverages such as tea and wine (Plamada and Vodnar 2022). Anti-allergic, anti-inflammatory, antioxidant, and wound-healing capabilities are only a few of the many qualities and medicinal applications of phytochemicals (Martel *et al*., 2020). Certain phytochemicals are anti-nutritional substances produced in natural foods and/or feedstuffs by a variety of processes and regular metabolisms of species. They may obstruct nutrient absorption and digestive functions (Salehi *et al*., 2018). Despite the positive effects of phytochemicals, it is important to note that both people and animals may suffer negative consequences from excessive ingestion (Aravind *et al*., 2021).

Therefore, understanding how processing influences the qualitative and quantitative distribution of phytochemicals in LPCs is essential to optimize their nutritional potential. Previous studies have shown that certain phytochemicals, especially phenolic compounds, can degrade or leach during thermal or aqueous processing (DeBenedictis *et al*., 2023). Moreover, some compounds, like glycosides and alkaloids, may exhibit relative stability due to their structural characteristics (Nishad, 2022).

This study evaluated the qualitative and quantitative phytochemical properties of scent leaf meal (SLM), wet-milled leaf protein concentrate (Wet-SLPC), dry-milled leaf protein concentrate (Dry-SLPC), and the resulting bagasse (SLB). The findings will help determine the suitability of different processing methods for retaining health-beneficial phytochemicals in LPCs derived from scent leaf.

## Materials and Methods

### Experimental Location

The study was conducted at the University of Benin in Benin City, Nigeria, which is situated in the rain forest zone at latitude 6°24′17″N and longitude 5°36′39″E. The average temperature, annual rainfall, relative humidity, and daily sunshine were 27.6°C, 2162 mm, 72.5%, and 6.68 hours, respectively (NAA, 2018; Google Earth, 2024).

### Sample Collection and Preparation

Fresh scent leaves were harvested from mature plants cultivated in different parts of Edo State, Nigeria. The leaves were washed with water to remove dirt and debris and then spread on clean flat surface at ambient temperature (25–30 °C) to partially air-dry for three hours to lose moisture.

### Scent Leaf Meal (SLM)

The partially air-dried scent leaves were further air-dried at ambient temperature (25–30 °C) for 5–7 days until a constant weight was achieved. The dried leaves were ground into a fine powder using a laboratory mill and stored in airtight containers. The powdered dried scent leaves served as the scent leaf meal (SLM) and were used directly for phytochemical analyses.

### Scent Leaf Protein Concentrates (SLPCs)

SLPCs were prepared using both wet and dry milling methods as described in a previous study (Agbonghae and Nwokoro, 2023). For wet milling method, partially air-dried fresh scent leaves were homogenized with distilled water in a 1:5 (w/v) ratio using a blender. The homogenate was filtered through muslin cloth to obtain the leaf juice, which was then heated to coagulate the proteins. The coagulated proteins were separated via sieving and collected as the wet-SLPC. For dry milling method, scent leaf meal was mixed with distilled water in a 1:10 (w/v) ratio and stirred for two hours at room temperature. The mixture was filtered, and the filtrate was subjected to the same heating and sieving process as in wet milling method to obtain the dry-SLPC.

### Scent Leaf Bagasse (SLB)

The residual fibrous material obtained after juice extraction during SLPC preparation was collected, dried at 40 °C to constant weight, ground into powder, and stored as scent leaf bagasse (SLB).

### Qualitative Phytochemical Analysis

Various bioactive chemicals were identified using standard phytochemical screening methodologies, in accordance with the guidelines provided by Harborne (1998), Evans (2022), Ajuru *et al*. (2017), and Nalado and Tijjani (2023). Mayer's reagent and dilute hydrochloric acid were added to 1 mL of the extract to identify alkaloids; the presence of alkaloids was indicated by a white precipitate. A yellow coloring was used to establish the presence of coumarins when 1.5 mL of extract was combined with alcoholic sodium hydroxide. When a combination of 1.3 mL of extract and 0.5 g of magnesium turnings was heated, flavonoids were detected by a shift in color from orange to red. To identify glycosides, a paste was made from 2.5 mL of extract, anthrone, and a drop of strong sulfuric acid. The mixture was then gently heated until it became dark green. When 2.5 mL of extract were shaken with water, a persistent froth was indicative of saponins. After treating 1.6 mL of extract with a basic lead acetate solution, tannins were visible as a white precipitate. Using well-established qualitative techniques from the previously stated sources, other phytochemicals, including anthocyanins, oxalates, phenols, phlobatannins, phytates, steroids, terpenoids, and triterpenes, were screened.

### Quantitative Phytochemical Analysis

Quantitative determinations of phytochemicals were conducted using established spectrophotometric methods, as outlined by Fawehinmi *et al*. (2022) and Nalado and Tijjani, (2023):

For alkaloids, 200 mL of 20% acetic acid in ethanol and 5 g of the scent leaf sample were added to a 250 mL beaker, and the combination was let to stand for four hours. The extract was filtered and then concentrated in a water bath to 25% of its original volume. Drops of concentrated ammonium hydroxide were added to the extract until the preparation was complete. The precipitate was filtered out and weighed once the entire solution had settled.

For flavonoids, 100 g of the plant material was repeatedly extracted using 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered using Whatman filter paper No. 42 (125mm). The filtrate was transferred to a crucible, dried over a water bath, and then weighed.

For saponin, 20 g of ground scent leaf samples were made using 200 mL of 20% ethanol at 55°C for four hours while stirring. After being concentrated to 40 mL at 90°C, the mixed filtrates were moved to a separator funnel and purified using diethyl ether. Afterward, 60 mL of n-butanol was added after the ether layer was discarded, and then 10 mL of 5% sodium chloride was used twice. The saponin concentration was determined as a percentage after the final extract was evaporated and oven-dried at 40°C to constant weight.

For tannin, 500 mg of scent leaf samples were put into a 100 mL plastic container and shaken with 50 mL of distilled water for one hour. This was filtered into a 50 mL volumetric flask and then adjusted to the appropriate amount. Afterward, 3 mL of 0.1M FeCl3 in 0.1N HCl and 0.008M potassium ferrocyanide were added to 5 mL of the filtrate, which had been pipetted into a tube. In 10 min, a spectrophotometer was used to measure the absorbance at 120 nm wavelengths. In addition to creating and reading the color, a blank sample was prepared at the same wavelength. A standard made of tannin acid was evaluated at 100 parts per million (ppm).

For phenols, scent leaf sample was boiled for 15 min with 50 mL of ether to extract phenolics. Thereafter, 10 mL of distilled water, 2 mL of ammonium hydroxide, and 5 mL of concentrated amyl alcohol were combined with a 5 mL aliquot of the extract. A spectrophotometer

was used to detect absorbance at 505 nm following 30 min of color development.

Phytates and Oxalates were determined using the Wade reagent technique, which reads the pink complex at 500 nm. Following acid digestion, potassium permanganate titration was used to determine the oxalates. Glycosides, coumarins, terpenoids, triterpenes, and steroids were all quantified using conventional procedures as described by Abayomi (1993) and Harborne (1998).

**Statistical Analysis**

Data obtained from quantitative analyses were subjected to one-way analysis of variance (ANOVA) to determine significant differences among the samples. Means were separated using Duncan's Multiple Range Test at a 5% significance level. Statistical analysis was conducted using SPSS software version 25. The data are presented as mean ± standard deviation.

**Results and Discussions**

Qualitative and quantitative phytochemical analyses of scent leaf and its derivatives—such as SLM, Wet-SLPC, Dry-SLPC, and SLB—provide important insights into the bioactive properties of plant-based products. These findings are particularly relevant for functional food development, nutritional enhancement, medicinal applications, and livestock nutrition in Nigeria.

**Qualitative Phytochemical Analysis**

A wide range of bioactive compounds were found in all samples when phytochemicals in scent leaf derivatives were qualitatively screened (Table 1). In particular, all the samples contained alkaloids, coumarins, flavonoids, glycosides, oxalates, phenols, phytates, saponins, steroids, tannins, terpenoids, and triterpenes. This consistent presence suggests that, regardless of the processing technique, the essential phytochemicals in scent leaves are preserved (Ajuru *et al*., 2017; Fawehinmi *et al*., 2022; Enenya *et al*., 2022; Nalado and Tijjani, 2023).

Conversely, none of the samples contained anthocyanins or phlobatannins. Their absence raises the possibility that these chemicals are either degraded during processing or are only found in trace concentrations in the raw leaf material (Onyeike *et al*., 2008; DeBenedictis *et al*., 2023). Although their quantitative amounts may fluctuate, the wide range of reliably discovered phytochemicals across all sample types suggests that processing scent leaves into protein concentrates or bagasse does not remove their basic qualitative phytochemical content.

**Quantitative Phytochemical Analysis**

The effect of processing on the stability of bioactive compounds is demonstrated by the significant decrease of certain phytochemicals in SLPCs and SLB. Quantitative analysis (Table 2) showed significant variations ($p<0.05$) in the concentration of several phytochemicals across the different samples. Alkaloid content was highest in SLM ($4.55 \pm 0.06$ mg/100 g), followed by Wet-SLPC and Dry-SLPC ($3.52 \pm 0.03$ and $3.53 \pm 0.03$ mg/100 g, respectively), and lowest in SLB ($2.35 \pm 0.05$ mg/100 g). Alkaloids are known for their therapeutic potential, including antimicrobial and analgesic activities, thus their retention in moderate quantities in the protein concentrates is beneficial. Their water solubility and heat sensitivity during protein extraction may be the cause of the dramatic drop in alkaloids (DeBenedictis *et al*., 2023; Kohre *et al*., 2024).

Flavonoid concentration also followed a similar trend, being significantly higher ($p<0.05$) in SLM ($3.44 \pm 0.19$ mg/100 g) compared to all other samples (~0.60 mg/100 g). This may be attributed to their water solubility and thermal instability, as previously reported by Oboh and Rocha (2007) and further supported by DeBenedictis *et al*. (2023). Given their strong antioxidant and anti-inflammatory properties, the reduction in flavonoids suggests that while protein content may be enriched, the antioxidant capacity of the concentrates could be compromised (Ahmed and Sarkar, 2022).

Glycoside, phytate, terpenoid, and triterpene levels did not differ significantly across the samples ($p>0.05$), indicating stability of these compounds during protein concentrate extraction. This stability aligns with the findings of Nishad (2022), who noted that terpenoids and triterpenes tend to resist degradation during moderate processing Saponin content decreased in the concentrates ($0.05 \pm 0.01$ mg/100 g) and SLB ($0.11 \pm 0.01$ mg/100 g) compared to the meal ($1.21 \pm 0.05$ mg/100 g), with statistically significant differences ($p<0.05$). The significant decrease in saponins, which are known to have immunomodulatory and cholesterol-lowering properties, suggests that aqueous extraction results in its significant loss. A similar trend was observed for tannins, which dropped significantly ($p<0.05$) from

**Table 1: Qualitative analysis of phytochemical properties of leaf meal, leaf protein concentrates, and bagasse of scent leaf**

| Phytochemicals | SLM | Wet-SLPC | Dry-SLPC | SLB |
|---|---|---|---|---|
| Alkaloids | + | + | + | + |
| Anthocyanins | - | - | - | - |
| Coumarins | + | + | + | + |
| Flavonoid | + | + | + | + |
| Glycosides | + | + | + | + |
| Oxalate | + | + | + | + |
| Phenols | + | + | + | + |
| Phlobatannins | - | - | - | - |
| Phytate | + | + | + | + |
| Saponin | + | + | + | + |
| Steriod | + | + | + | + |
| Tannin | + | + | + | + |
| Terpenoids | + | + | + | + |
| Triterpenes | + | + | + | + |

SLM = Scent leaf meal; Wet-SLPC = Scent leaf protein concentrates from wet milling; Dry-SLPC = Scent leaf protein concentrates from dry milling; SLB = Scent leaf bagasse; + = Present; - = Absent

1.78 ± 0.04 mg/100 g in SLM to between 0.13 and 0.19 mg/100 g in the processed fractions. Perhaps as a result of interacting with proteins during coagulation, tannins, which contribute to antibacterial and astringent properties, significantly reduced (Dentinho and Bessa, 2016). These reductions in saponin and tannin are advantageous from a nutritional perspective, as both compounds are considered antinutritional factors when consumed in high amounts (Odukoya *et al*., 2021). The low levels observed in Wet- and Dry-SLPC indicate that protein concentrates derived from scent leaf may be more suitable for animal and human consumption, reducing the risk of adverse effects such as protein precipitation or gastrointestinal irritation

Steroid content remained relatively high in both the leaf meal and concentrates (~37 mg/100 g) but dropped sharply in the bagasse (22.48 ± 1.81 mg/100 g), with the difference being significant ($p<0.05$). These compounds are essential in modulating physiological processes and exhibit strong bioactivity, including antimicrobial and cardioprotective effects (Ajuru *et al* 2017; Nalado and Tijjani, 2023). The concentration of these bioactives in the protein-rich fractions underscores the dual nutritional and pharmacological potential of the extracts

Coumarins, oxalates, and phenols exhibited only slight reductions across the processed scent leaf samples, and these changes were not significant ($p>0.05$), indicating a degree of stability during both wet and dry milling. This moderate effect of processing suggests that these compounds may be relatively resistant to degradation under mild thermal and mechanical conditions. Similar findings have been reported by DeBenedictis *et al*. (2023), who observed minimal losses of phenolic compounds in leafy vegetables subjected to moderate drying techniques. Additionally, the chemical structures of oxalates and coumarins may contribute to their stability, as noted by Nishad (2022), who emphasized that certain phytochemicals retain their integrity due to low volatility and reduced solubility in aqueous processing media.

Overall, the results suggest that while most phytochemicals are retained in the protein concentrates, flavonoids, saponins, and tannins are significantly reduced during processing. This indicates selective concentration or loss of certain phytochemicals during the protein extraction procedures.

## Conclusions and Recommendations

This study demonstrated that processing scent leaf into protein concentrates significantly influences its phytochemical profile. While essential bioactive compounds such as alkaloids, flavonoids, saponins, and tannins were present in all samples, their concentrations declined in Wet- and Dry-SLPCs and SLB compared to the unprocessed SLM. The marked reduction of flavonoids and alkaloids—compounds with strong therapeutic benefits—suggests some loss of medicinal efficacy during processing. However, the decline in antinutritional factors like saponins and tannins could improve digestibility and reduce potential toxicity, thereby enhancing the nutritional suitability of SLPCs for functional food and feed applications. The stability of glycosides, phenols, terpenoids, and steroids under both wet and dry milling processes indicates that moderate processing preserves many of the health-promoting constituents of scent leaf. The wet and dry milling techniques employed in this study offer a promising approach to producing protein concentrates with balanced phytochemical profiles, supporting their use as nutritionally enhanced and functionally safe plant-based ingredients in human and animal diets.

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**Conflict of Interest**

No conflict of interest associated with this work

**Contribution of Authors**

We declare that this work was done by Osagie Wisdom Agbonghae (OWA) and Emmanuel Obinna Nwachukwu (EON) and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. OWA conceived and designed the study, EON collected the data, OWA analyzed the data. EON wrote the first draft of manuscript and OWA revised and wrote the final draft of the manuscript. All authors read and approved the manuscript for publication.

**Availability of Data and Materials**

Datasets used and/or analyzed during the current study are available from the corresponding author.

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**Table 2: Quantitative analysis of phytochemical properties of leaf meal, leaf protein concentrates, and bagasse of scent leaf**

| Phytochemicals | SLM (mg/100g) | Wet-SLPC (mg/100g) | Dry-SLPC (mg/100g) | SLB (mg/100g) | SEM |
|---|---|---|---|---|---|
| Alkaloids | 4.55±0.06$^{a}$ | 3.52±0.03$^{b}$ | 3.53±0.03$^{b}$ | 2.35±0.05$^{c}$ | 1.14 |
| Coumarins | 1.59±0.04 | 1.33±0.06 | 1.33±0.05 | 1.12±0.04 | 0.07 |
| Flavonoid | 3.44±0.19$^{a}$ | 0.59±0.07$^{b}$ | 0.60±0.06$^{b}$ | 0.66±0.09$^{b}$ | 0.03 |
| Glycosides | 42.73±1.28 | 40.92±1.19 | 41.09±1.20 | 41.66±1.27 | 3.29 |
| Oxalate | 0.31±0.02 | 0.21±0.02 | 0.22±0.02 | 0.28±0.03 | 0.01 |
| Phenols | 0.59±0.03 | 0.49±0.04 | 0.48±0.03 | 0.53±0.04 | 0.02 |
| Phytate | 5.22±1.10 | 5.20±0.09 | 5.19±1.00 | 5.28±1.09 | 2.11 |
| Saponin | 1.21±0.05$^{a}$ | 0.05±0.01$^{c}$ | 0.05±0.01$^{c}$ | 0.11±0.01$^{b}$ | 0.01 |
| Steriod | 37.49±2.11$^{a}$ | 37.01±1.83$^{a}$ | 37.02±1.83$^{a}$ | 22.48±1.81$^{b}$ | 2.18 |
| Tannin | 1.78±0.04$^{a}$ | 0.13±0.01$^{b}$ | 0.13±0.01$^{b}$ | 0.19±0.02$^{b}$ | 0.03 |
| Terpenoids | 5.84±1.10 | 5.57±0.09 | 5.54±1.00 | 5.51±1.12 | 1.02 |
| Triterpenes | 2.96±0.08 | 2.67±0.05 | 2.68±0.05 | 2.76±0.08 | 0.07 |

SLM = Scent leaf meal; Wet-SLPC = Scent leaf protein concentrates from wet milling; Dry-SLPC = Scent leaf protein concentrates from dry milling; SLB = Scent leaf bagasse; SEM = Standard Error of Mean.
$^{abc}$ = means on the same row with different superscript are significantly different ($p<0.05$).