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Research Article

Extraction methods significantly impact physicochemical, amino acid, phytochemical and textural properties of pumpkin seed protein concentrate in Nigeria

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Abstract: The increasing interest in sustainable plant-based proteins has driven research into underutilized seeds like pumpkin. This study investigated the colour, functional (bulk density, water and oil absorption capacities, and swelling power), amino acid, phytochemical, and textural properties of defatted pumpkin seed protein concentrate obtained using precipitation (PM), salt solubilization (SM), and aqueous alcohol wash (AM) methods. Analyses were conducted using standard methods. Results showed that protein concentrate varied significantly with the extraction method. AM produced the lightest isolate (L* = 58.25) with reduced redness and yellowness, while PM exhibited superior functional attributes: bulk density 0.69 g/mL, water absorption 2.84 g/g, oil absorption 2.00 g/g and swelling power 4.50 g/g. Amino acid profiling demonstrated that PM possessed higher levels of essential (leucine 7.03 g/100 g and lysine 6.00 g/100 g) and non-essential (glutamic acid 11.99 g/100 g) amino acids, accompanied by enhanced retention of flavonoids (5.15 mg/g), alkaloids (2.10 mg/g), saponins (3.50 mg/g), and tannins (2.85 mg/g), while textural analysis further revealed increased hardness (7.20 N), chewiness (40.10 N-mm), and resilience (0.50). Thus, precipitation extraction yielded protein concentrate with superior functional, textural, and nutritional qualities, highlighting its potential for use in fortified and functional foods.

Keywords: Pumpkin seed, protein concentrate, extraction methods, physicochemical properties, textural properties

1. Introduction

Proteins are vital macronutrients composed of amino acids that perform critical biological functions, including tissue repair, immune support, and the synthesis of enzymes and hormones (Olatoye et al., 2024). According to the FAO (2013), sufficient intake of recommended protein levels, particularly from animal sources, is important because they provide all essential amino acids required for sustaining metabolic processes, promoting well-being, and preventing nutrient deficiencies, thereby contributing significantly to human development and overall physiological health.

In sub-Saharan Africa, reliance on animal proteins is constrained by cost, supply chains, and environmental conditions. Many communities in this region struggle to afford regular intake of meat, dairy and fish (Olatoye et al., 2018). Plant-based proteins offer a more sustainable and often more accessible alternative in such settings. The World Health Organization has recommended increasing consumption of vegetable protein in regions with low animal protein access, citing its benefits for health and sustainability (FAO/WHO, 2013). Plant proteins are lower in saturated fat, often richer in fibre and phytochemicals, and cause fewer greenhouse emissions. Common sources include legumes (beans, lentils), cereals, nuts, and oilseed crops. Pumpkin seed protein, especially from *Telfairia occidentalis*, has been

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highlighted for its nutritive potential and bioactive compounds (Sa et al., 2022).

Telfairia occidentalis (fluted pumpkin) is a widely cultivated vegetable in West Africa, recognized for both its leafy greens and nutrient-dense seeds. The seeds are reported to contain over 30% protein, high levels of lipids, essential minerals, and vitamins (Fagbemi, 2007), positioning them as a valuable local source of macronutrients. In sub-Saharan Africa, where protein-energy malnutrition (PEM) remains a persistent public health challenge, the incorporation of locally available, nutrient-rich crops like *T. occidentalis* seeds into diets is critical for improving nutritional status and supporting health. Despite their potential, traditional and local extraction methods often produce protein concentrates with low purity, diminished functional or bioactive properties, and limited digestibility. These limitations reduce the efficacy of the protein fraction as a dietary supplement or functional food ingredient, constraining its role in nutritional interventions.

Although numerous studies have reported the chemical properties of *Telfairia occidentalis* (Fagbemi, 2007; Sert et al., 2023), limited attention has been given to the effects of extraction techniques on the physicochemical and nutritional characteristics of its protein concentrate, particularly from seeds cultivated in Nigeria. Fagbemi (2007) further showed that processing fluted pumpkin seeds alters their nutritional profile while retaining high levels of essential nutrients, whereas Sa et al. (2023) demonstrated that cold-pressed pumpkin seed meal contains high-quality protein with substantial *in vitro* digestibility. Despite these findings, evidence remains scarce regarding how processing conditions influence the functional quality of *T. occidentalis* seed protein concentrates in the Nigerian context. The present study therefore aims to characterize the colour, functional (bulk density, water and oil absorption capacities, and swelling power), amino acid, phytochemical and textural properties of protein concentrates derived from *T. occidentalis* seeds cultivated in Ilorin, Nigeria. Findings from this study could support innovative utilization of pumpkin seeds as a sustainable and functional protein source for both food and industrial applications.

2. Materials and Methods

2.1. Pumpkin seed and defatted flour

Dried seeds of the *T. occidentalis (large brown* variety), a major cultivar from Western Nigeria, were used for this study. The seeds were sourced from a local producer in Moro Local Government, Kwara State. About 2 kg sample was milled using a kitchen blender (Sokany, SK 999, China), then defatted with petroleum ether in a Soxhlet apparatus for 6 hours (Akinoso et al., 2025). The resulting residue was dried (60 ◦C, 48 h) in the oven (Model QUB 305010 G, Gallenkamp, UK) and stored (2-8 ◦C) in a laboratory freezer (Model BPR-5V118, Biobase 250 L, China) until required for analysis.

2.2. Pumpkin seed protein concentrate

2.2.1. Precipitation

Defatted meal was dispersed in distilled water (1:20 w/v) and the pH adjusted to 9.0 with NaOH. The mixture was stirred and centrifuged (Hettich EBA 270, Germany) to obtain the soluble protein fraction. The supernatant was acidified to pH 4.5 using HCl acid, followed by centrifugation at 16,000×g for 20 min (Sert et al., 2023).

2.2.2. Salt solubilization

Defatted meal was extracted with 0.5 M NaCl at a 1:10 (w/v) ratio, followed by stirring and centrifugation (16,000×g, 20 min). The resulting supernatant was dialyzed against distilled water to remove salts and subsequently freeze-dried using a Model FD18T 28 L (China) freeze dryer (Sa et al., 2023). Defatted meal was mixed with 0.5 M NaCl solution at a 1:10 (w/v) ratio, stirred and centrifuged. The supernatant was dialysed against distilled water to remove salts, and then freeze-dried (Model FD18T 28 L, China) to obtain a dry powder for further analyses (Sa et al., 2023).

2.2.3. Aqueous alcohol wash

Defatted meal was extracted with 60% ethanol at a 1:5 (w/v) ratio to remove non-protein components. The mixture was centrifuged for 20 min, at 16,000×g and the alcohol distilled off using a rotary evaporator (Buchi Rotavapor, China) at 40–50 °C. The resulting extract was freeze-dried (Model FD18T 28 L, China) to obtain a dry powder for further analyses (Sert et al., 2023).

2.3. Physical and functional properties

2.3.1. Colour

The colour of the sample was measured using a Hunter Lab colourimeter (CR- 400, Minolta, Tokyo, Japan). The instrument provides value for L* (lightness), a* (red-green coordinate), and b* (yellow-blue coordinate). Samples were placed in the sample holder, calibrated against a white standard, and measurements were taken in triplicate (Chang et al., 2002).

2.3.2. Bulk density

Bulk density was measured by filling a pre-weighed 10 mL graduated cylinder with 3 g concentrate flour, tapping to settle. It was calculated as the ratio of mass to final tapped volume (g/cm^3). Measurements were done in triplicate, and the average value was reported (AACC, 2000).

2.3.3. Water absorption capacity (WAC)

WAC was measured using the AACC (2000) method. Exactly 2.5 g of flour was mixed with 37.5 ml water, hydrated, and centrifuged. The supernatant was discarded, and WAC was calculated as grams of water absorbed per gram of sample.

2.3.4. Oil absorption capacity (OAC)

OAC was determined following the AACC (2000) method. One gram of protein concentrate was mixed with 10 mL of refined vegetable oil in a centrifuge tube and allowed to stand for 30 min at room temperature. The mixture was centrifuged, and the supernatant volume was measured. OAC was expressed as mL of oil absorbed per gram of concentrate.

2.3.5. Swelling power

Swelling power was determined using the AACC (2000) method 56-21.01. Exactly, 0.20 g sample was mixed with distilled water to a total weight of 20 g, heated at 60 °C for 30 min, centrifuged at 1700 g for 5 min, and expressed as sediment weight per dry sample weight (g/g).

2.4. Amino acid

Amino acid composition of the protein concentrate was determined using the Technicon Sequential Multiple (TSM) Amino Acid Analyser (Technicon Instrument Corporation, New York, USA). Samples were first hydrolysed with 6 N HCl at 110 °C for 24 h under nitrogen atmosphere. The hydrolysates were filtered, evaporated to dryness, reconstituted in buffer, and injected into the analyzer for separation and quantification of individual amino acids (AOAC, 2005)

2.5. Phytochemical

2.5.1. Total flavonoid

Total flavonoid content of the sample was determined according to the method of Chang *et al.* (2002) using the $AlCl_3$ colorimetric assay. An aliquot (1 ml) of the extract was mixed with 10% $AlCl_3$ solution and incubated (Model HNY-211B, Beijing, China) at 30 ± 2 °C for 30 min. Absorbance was measured at 415 nm using a UV-Visible spectrophotometer (Model Metash UV-2600, China), and results were expressed as mg quercetin equivalents per gram of sample.

2.5.2. Alkaloid

Alkaloid content of the sample was determined according to the AOAC (2005) method. Exactly, 1 g portion of the sample was weighed and extracted with acidic ethanol at a 1:10 sample-to-solvent ratio. The extract was filtered through Whatman No. 1 filter paper. The filtrate was then treated with $NH_4(OH)$ to precipitate alkaloids. The precipitate was collected, washed, dried, and weighed. Alkaloid content was expressed as a percentage of the dry weight of the sample.

2.5.3. Saponin

Saponin content was determined according to the AOAC (2005) method. A 5 g sample was defatted with petroleum ether and extracted with 20% aqueous ethanol at a 1:5 (w/v) sample-to-solvent ratio, followed by heating. The extract was concentrated, and saponins were precipitated using diethyl ether. The resulting precipitate was dried at 60 °C and weighed. Saponin content was expressed as a percentage of the dry weight of the sample.

2.5.4. Tannin

Tannin content of the sample was measured by extracting 1 g sample with aqueous methanol at a 1:4 (w/v) sample-to-solvent ratio, followed by filtration and reacting the filtrate with Folin–Denis reagent. Absorbance was read at 760 nm using a UV-Visible spectrophotometer, and tannin content was calculated as a percentage of the dry sample weight (AOAC, 2005).

2.6. Textural properties

The textural properties of the protein concentrate were measured using a texture analyzer (Model:TA. XT Plus,UK). Samples were compressed using a 5 mm cylindrical standard probe to assess hardness, springiness, cohesiveness, gumminess, and chewiness. Measurements were performed in triplicate at room temperature (29.0 ± 2ºC) (AACC, 2000).

2.7. Statistical analysis

Statistical analysis of the experimental data was performed using SPSS software (version 16.0, Inc., Tulsa, USA) at a 95% confidence level. Means were

compared using Tukey's test. All results are presented as the mean ± standard deviation of at least triplicate determinations.

3. Results and discussion

3.1. Colour and functional properties

The colour parameters (L*, a*, b*) and functional properties of protein concentrates are presented (Table 1). The L* values indicate a progressive increase (50.05-58.25) in lightness from precipitation method (PM) to aqueous alcohol wash (AM), suggesting that the aqueous alcohol wash produced a lighter-coloured protein concentrate. The decrease in a* values (7.00-5.40) show a reduction in redness, possibly due to the removal of coloured pigments or phenolic compounds during alcohol extraction. Similarly, the decreasing b* values (17.35–14.14) from PM to AM indicated a reduction in yellowness, further confirming the bleaching effect of ethanol in removing residual lipophilic pigments. These findings are consistent with reports by Sa et al. (2023), who noted that processing conditions and solvent type significantly influence protein colour characteristics. For functional properties, the bulk density (BD) values differed significantly ($p < 0.05$), with the PM concentrate exhibiting the highest density (0.69 g/mL), implying tighter molecular packing, lower air entrapment, and improved particle cohesion within the protein matrix. This is consistent with Rangel et al. (2020), who reported that protein concentrates obtained through precipitation showed higher BD and attributed it to increased aggregation of protein particles compared with those obtained using solvent-based extraction methods. Lower BD observed in AM (0.58 g/ml) may indicate partial removal of lipophilic and carbohydrate fractions, resulting in a looser structure (Adedeji & Ngadi, 2019; Lawal & Akinoso, 2019). The Water absorption capacity (WAC) values (2.04–2.84 g/g) indicated that protein concentrates obtained through precipitation method exhibited greater water-binding affinity and this could be due to retention and exposure of hydrophilic amino acid residues during the extraction process (Akinoso et al., 2021). Comparable results were reported by Adeoti and Aworh (2019), who observed that higher WAC in precipitation-derived concentrates improved hydration and textural stability in composite foods. The lowest value (2.04 g/g) recorded for AM could be attributed to protein denaturation or decreased exposure of polar groups after alcohol washing. Oil absorption capacity (OAC) values followed a similar trend, indicating that PM (2.00 g/g) concentrates retained more non-polar sites capable of binding lipids. Similar findings by Rangel *et al*. (2020) reported enhanced oil-binding capacity in protein concentrates extracted under mild alkaline conditions, where hydrophobic interactions remain intact. Swelling power (SP) was highest in PM concentrates (4.5 g/g), reflecting superior water retention and matrix expansion upon heating. These results are consistent with the observations of Lin et al. (2022), who found that extraction methods significantly influenced swelling index and functional performance of legume protein concentrates. The lower swelling power in AM could be linked to partial protein dehydration and structural collapse during alcohol treatment (Sa et al., 2023).

3.2. Amino acid

The total amino acid composition of defatted pumpkin protein concentrates ranged from 31.37 to 36.84% and 38,86 to 44.86% for essential and non-essential amino acids, respectively (Table 2). Essential amino acids such as leucine, lysine, isoleucine, and valine were highest in the PM sample (7.03, 6.00, 4.82, and 5.39 g/100 g, respectively) compared to Salt Solubilisation Method (SM) (6.84, 5.21, 4.59, and 5.01 g/100 g,

Table 1. Colour and functional properties of defatted pumpkin seed protein concentrate

Sample	Colour			Bulk Density (g/ml)	WAC (g/g)	OAC (g/g)	Swelling power (g/g)
	L*	a*	b*				
PM	50.05 ± 0.07^{c}	7.00 ± 0.14^{a}	17.35 ± 0.07^{a}	0.69 ± 0.01^{a}	2.84 ± 0.01^{a}	2.00 ± 0.01^{a}	4.50 ± 0.00^{a}
SM	54.25 ± 0.07^{b}	6.25 ± 0.07^{b}	15.80 ± 0.00^{b}	0.64 ± 0.00^{b}	2.55 ± 0.00^{b}	1.83 ± 0.02^{b}	4.20 ± 0.00^{b}
AM	58.85 ± 0.07^{a}	5.40 ± 0.00^{c}	14.10 ± 0.00^{c}	0.58 ± 0.00^{c}	2.04 ± 0.01^{c}	1.58 ± 0.01^{c}	3.80 ± 0.00^{c}
Mean	54.38	6.22	15.75	0.64	2.48	1.80	4.17

Values (mean ± standard deviation) with different superscripts in the same column differ significantly ($p \leq 0.05$). Abbreviations: PM, Precipitation method; SM, Solubilization method; AM, Aqueous alcohol method; WAC, Water absorption capacity; OAC, Oil absorption capacity.

respectively) and AM (6.33, 4.52, 4.27, and 4.85 g/100 g, respectively). This trend indicates that the precipitation method preserved more amino acids, possibly due to lower protein denaturation during extraction. These values are comparable to those reported by Fagbemi (2007) for pumpkin seed protein concentrate (leucine 7.12 g/100 g; lysine 5.92 g/100 g) and by El-Adawy & Taha (2001), who recorded 6.9 g/100 g leucine and 4.8 g/100 g lysine for *Cucurbita pepo* protein concentrates. Non-essential amino acids such as glutamic acid (11.99 g/100 g), aspartic acid (9.54 g/100 g), and alanine (3.85 g/100 g) were also more abundant in PM, indicating high flavor-enhancing potential since glutamic acid contributes to umami taste. Similar glutamic acid dominance (10.8–12.5 g/100 g) was reported for pumpkin and soybean concentrates (Sa et al., 2023). The observed methionine content (2.10 g/100 g) was higher than that reported by Hamed et al. (2020) (1.76 g/100 g), while tryptophan (1.31 g/100 g) was consistent with FAO (2013) reference values for plant proteins, showing good essential amino acid balance. Significant variations ($p < 0.05$) among methods may be attributed to solvent polarity and protein solubility behavior, which influence amino acid recovery efficiency. The precipitation method yielded concentrates with a more balanced amino acid profile, closely aligning with the FAO/WHO (2013) recommended pattern for human nutrition, especially in lysine and leucine. Amino acids such as arginine (6.86 g/100 g) and histidine (2.52 g/100 g) also enhance cardiovascular and antioxidant benefits, confirming the nutritional potential of pumpkin protein for food fortification.

3.3. Phytochemical

The phytochemical profiles of the protein concentrates are presented in **Table 3**, showing variations in flavonoid, alkaloid, saponin, and tannin contents among the different extraction methods. Flavonoid content ranged from 4.20 to 5.15 mg/g, with PM exhibiting the highest value which is an indication of better phenolic compounds retention. This aligns with findings by Chang et al. (2002), who reported higher flavonoid retention in precipitation-extracted plant proteins. Alkaloid levels decreased from 2.10 mg/g in PM to 1.50 mg/g in AM, suggesting that alcohol washing effectively removes alkaloids. Adeoti & Aworh (2019) observed similar reductions in alkaloid content when using solvent-based

Table 2. Amino profiless of protein concentrate

Samples	PM	SM	AM
Essential (g/100 g)			
Luecine	7.03 ± 0.03a	6.84 ± 0.01b	6.33 ± 0.03c
Lysine	6.00 ± 0.02a	5.21 ± 0.01b	4.52 ± 0.02c
Isoleucine	4.82 ± 0.01a	4.59 ± 0.03b	4.27± 0.01c
Phenylalanine	4.04 ± 0.04a	3.81 ± 0.01b	3.54 ± 0.01c
Valine	5.39 ± 0.00a	5.01 ± 0.01b	4.85 ± 0.00c
Methionine	2.10 ± 0.01a	1.83 ± 0.01b	1.70 ± 0.01c
Histidine	2.52 ± 0.01a	2.25 ± 0.01b	2.00 ± 0.01c
Threonine	3.62 ± 0.01a	3.50 ± 0.01b	3.14 ± 0.01c
Tryptophan	1.31 ± 0.01a	1.17 ± 0.00b	1.02 ± 0.01c
Non-essential (g/100 g)			
Glycine	2.91 ± 0.01a	2.76 ± 0.01b	2.59 ± 0.00c
Serine	3.19 ± 0.01a	2.98 ± 0.01b	2.83 ± 0.01c
Aspartic acid	9.54 ± 0.01a	8.95 ± 0.01b	8.19 ± 0.01c
Glutamic acid	11.99 ± 0.01a	11.21 ± 0.01b	10.57 ± 0.01c
Alanine	3.85 ± 0.00a	3.55 ± 0.01b	3.42 ± 0.01c
Cysteine	1.58 ± 0.01a	1.43 ± 0.00b	1.29 ± 0.01c
Tyrosine	2.81 ± 0.01a	2.58 ± 0.01b	2.28 ± 0.00c
Proline	2.13 ± 0.01a	1.95 ± 0.00b	1.72 ± 0.00c
Arginine	6.86 ± 0.01a	6.42 ± 0.01b	5.97 ± 0.00c

Values (mean ± standard deviation) with different superscripts on the same column differ significantly ($p \leq 0.05$).
Abbreviations: PM, Precipitation method, SM, Solubilization method; AM, Aqueous alcohol method.

Table 3: Phytochemical properties of defatted pumpkin seed protein concentrate

Sample	Flavonoids	Alkaloids	Saponins	Tannins
PM	5.15 ± 0.07a	2.10 ± 0.00a	3.50 ± 0.00a	2.85 ± 0.07a
SM	4.55 ± 0.07b	1.75 ± 0.07b	3.10 ± 0.00b	2.50 ± 0.00b
AM	4.20 ± 0.00c	1.50 ± 0.00c	2.75 ± 0.07c	2.20 ± 0.00c
Mean	4.63	1.78	3.12	2.52

Values (mean ± standard deviation) with different superscripts on the same column differ significantly ($p \leq 0.05$). Abbreviations: PM, Precipitation method, SM, Solubilization method; AM, Aqueous alcohol method.

extraction methods. Saponin content also decreased from 3.50 mg/g (PM) to 2.75 mg/g (AM), reflecting the impact of alcohol washing on saponin removal. Sert et al. (2023) reported that reduced saponin content in concentrates subjected to solvent washes was due to the removal of hydrophilic secondary metabolites. Tannin levels decreased progressively from 2.85 mg/g (PM) to 2.20 mg/g (AM). This reduction is consistent with the study by Lin et al. (2022), who found that alcohol washing decreased tannin-protein precipitation capacity. It is noteworthy that these bioactive compounds play crucial roles in human health. Flavonoids function as potent antioxidants that help protect against oxidative stress and chronic diseases such as cardiovascular disorders and cancer (Scalbert et al., 2005). Alkaloids possess antimicrobial and anti-inflammatory properties, contributing to immune support and metabolic regulation (Cushnie et al., 2014). Saponins are known for their cholesterol-lowering and anticancer effects, while tannins aid in antimicrobial activity and digestive regulation when consumed in moderate amounts (Scalbert et al., 2005). The concentrations observed in this study were within safe and nutritionally acceptable ranges, as none exceeded the tolerable levels reported for plant-based protein products (<10 mg/g for flavonoids, <5 mg/g for saponins, and <3 mg/g for tannins) (FAO/WHO, 2013). Therefore, the phytochemical levels in the concentrates, particularly those obtained through precipitation, indicate potential health-promoting benefits without posing toxicological concerns.

3.3. Texture

The textural behavior of protein concentrate is illustrated in the table. 4. The analysis revealed significant differences ($p < 0.05$) across all parameters, indicating that extraction techniques influenced the protein-protein interactions and mechanical behaviour of the concentrates. Hardness ranged from 5.95 to 7.20 N, with the PM concentrate exhibiting the highest value, suggesting stronger molecular interactions and compact structural organization. This higher hardness implies better structural rigidity, a desirable property in high-protein foods such as meat analogues and textured vegetable proteins, where firmness enhances mouthfeel and mechanical stability. A similar observation was reported by Rangel et al. (2020), who found that soy protein concentrates obtained through alkaline precipitation exhibited higher hardness due to improved protein cross-linking and reduced porosity. Cohesiveness (0.60–0.72) and **springiness** (0.79–0.88) followed the same pattern, with PM showing superior values, indicating better internal bonding and elasticity. High cohesiveness ensures that food products retain their shape during handling and processing, while springiness contributes to product resilience and bite quality. Lin et al. (2022) also observed that minimally disrupted protein networks during extraction enhanced cohesiveness and elasticity in legume-based protein gels. Chewiness, which combines hardness, cohesiveness, and springiness, varied widely from 2.80 to 40.10 N-mm. The PM concentrate displayed the highest chewiness, suggesting greater energy requirement for mastication, an attribute preferred in high-moisture extruded products and plant-based meat analogues. This variation aligns with Adedeji & Ngadi (2019)**,** who noted that extraction and drying conditions significantly affect chewiness and texture retention in legume protein concentrates. Resilience (0.40–0.50) represents the ability of the protein network to recover its structure after deformation, which is crucial for maintaining product consistency during packaging and storage. The higher resilience of PM concentrates implies better elastic recovery and gel strength. Comparable patterns were reported by Lin et al. (2022) in mung bean protein concentrates, where alkaline precipitation yielded stronger and more resilient gels due to enhanced intermolecular cross-linking.

Table 4: Textural properties of protein concentrate

Samples	Hardness (N)	Cohesiveness	Springiness	Chewiness (N-mm)	Resilience
PM	7.20 ± 0.00a	0.72 ± 0.01a	0.88 ± 0.01a	4.10 ± 0.00a	0.50 ± 0.00a
SM	6.75 ± 0.07b	0.68 ± 0.00b	0.85 ± 0.00b	3.50 ± 0.01b	0.45 ± 0.00b
AM	5.95 ± 0.07c	0.60 ± 0.00c	0.79 ± 0.01c	2.80 ± 0.00c	0.40 ± 0.00c
Mean	6.63	0.67	0.84	3.47	0.45

Values (mean ± standard deviation) with different superscripts on the same column differ significantly ($p \leq 0.05$). Abbreviations: PM, Precipitation method, SM, Solubilization method; AM, Aqueous alcohol method.

4. Conclusion

Overall, the extraction technique had a significant influence on the physicochemical, amino acid, phytochemical and textural properties of pumpkin seed protein concentrate in Nigeria. The aqueous alcohol washing (AM) method yielded the lightest and least pigmented concentrate, which is advantageous for food formulations where minimal colour interference and high consumer acceptability are desired. In contrast, the precipitation method produced protein concentrates with superior hydration, lipid-binding, and swelling capacities, confirming its suitability for applications requiring enhanced emulsification, water retention, and texture modification. Moreover, the precipitation method preserved higher levels of bioactive compounds such as flavonoids and saponins, thereby improving the nutraceutical potential of the concentrate, while the alcohol washing step effectively reduced antinutritional factors such as tannins. Textural evaluation revealed that precipitation-derived concentrates exhibited higher hardness, cohesiveness, springiness, chewiness, and resilience, supporting their potential in structured or elastic food systems like meat analogues, protein bars, and high-protein bakery products. Additionally, their amino acid composition closely aligned with FAO/WHO standards for high-quality plant proteins. These findings confirm that precipitation extraction offers the most balanced combination of nutritional, functional, and mechanical qualities, making pumpkin seed protein concentrate a promising sustainable ingredient for health-oriented and functional food formulations.

5. Conflict of Interest Statement

Authors hereby declared no conflict of interest.

6. Authors contributions

K.K Olatoye: Conceptualization; methodology; supervision; project administration; writing– review and editing; visualization. A. I. Lawal: Conceptualization; writing– original draft; reviewing and editing. B. I. Muhammed: Conceptualization; data collection; methodology; writing– review and editing, Kafayat Adebukola Babalola was committed.

7. Ethical approval

Ethics approval was not required for this research

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