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Proximate composition, phytochemical screening, and *in vitro* activity of musk melon (*Cucumis melo*) seed powder to incorporate into chicken nuggets

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Abstract

The current investigation was conducted to assess the proximate composition, phytochemical constituents, total phenolic content, total flavonoid content, and *in vitro* antioxidant qualities of musk melon seed powder (MMSP) and its incorporation in meat nugget. According to the proximate analysis, MMSP has ash (2.35%), fat (38.38%) and protein (29.13%). Phytochemical screening revealed the existence of bioactive substances that support its functional characteristics. The results showed that the total flavonoid content was 83.68 ± 0.69 mg QE/100 g and the total phenolic content was 168.12 ± 0.12 mg GAE/100 g. The *in vitro* antioxidant activity showed a ferric reducing antioxidant power (FRAP) value of 26.25 and a notable DPPH radical scavenging activity of 61.28 percent. The amounts of flavonoids and phenolics, as well as strong antioxidant activity determined in the present study indicate that MMSP may be a useful natural source of functional chemicals. As a result, it can be incorporated into meat products to develop functional food products with improved nutritional and health benefits.

Keywords: Musk melon seed powder (MMSP), proximate composition, phytochemical properties, total phenolic content, total flavonoid content, and *in-vitro* antioxidant activity

Introduction

The use of plant-based by-products as functional food ingredients has gained popularity recently because of their nutritional and health-promoting qualities. Fruit seeds, which are frequently seen as agro-industrial waste, are becoming more widely acknowledged as important sources of dietary fibre, proteins, lipids, and bioactive substances (Sharma *et al.*, 2021; Patel and Singh, 2022) ^[17, 12]. Among them, the rich nutritional profile and potential functional uses of musk melon (*Cucumis melo*) seeds have drawn attention.

Musk melon seed powder (MMSP) is a prospective component for nutritional enrichment because it is known to contain significant amounts of protein, fat, and important minerals (Kumar *et al.*, 2020) ^[7]. MMSP is rich in phytochemicals, including flavonoids and phenolics, which are linked to anti-inflammatory, antibacterial, and antioxidant qualities, in addition to its proximate makeup (Rani *et al.*, 2023) ^[14]. These bioactive substances are essential for scavenging free radicals and lowering oxidative stress, which helps prevent several chronic illnesses (Das and Roy, 2021) ^[3].

A common method for determining the antioxidant capacity of plant-based materials is to evaluate their total phenolic and flavonoid concentrations. Additionally, the capacity of natural substances to scavenge free radicals is frequently assessed using *in vitro* antioxidant tests, including ferric reducing antioxidant power (FRAP) and DPPH radical scavenging activity (Singh *et al.*, 2022) ^[12]. These techniques offer a trustworthy assessment of dietary items' antioxidant capacity. The use of musk melon seeds is still restricted, despite their nutritional and practical value. To investigate musk melon seed powder's potential as a functional food ingredient, the current study was conducted to assess its proximate composition, phytochemical characteristics, total phenolic content, total flavonoid content, and *in vitro* antioxidant activity.

Materials and Methods

Fresh musk melons were purchased from the local markets in Bengaluru and the musk melon seeds were collected. The seeds were kept in a hot air oven for drying at a time temperature combination of 50-55 °C for 24 hr after thorough cleaning with clean water. Post drying, the seeds were ground to a fine powder using an electrical grinder and packaged in high-density polyethylene (HDPE) pouches and stored at room temperature until further use. The seed powder was used for the assessment of proximate composition, screening for phytochemicals (total phenols and total flavonoids), and *in vitro* antioxidant property.

The proximate analysis: Proximate compositions such as crude protein, ether extract and ash were estimated as per the procedure outlined by AOAC (2005) [2].

Moisture Content: Moisture content was determined by oven drying method at 105 °C until constant weight. The loss in weight was recorded after drying.

$$\text{Moisture (\%)} = (W_1 - W_2)/W_1 \times 100$$

Crude Protein: Crude protein was estimated by the Kjeldahl method involving digestion, distillation, and titration. Nitrogen content was calculated and multiplied by 6.25.

$$N (\%) = (0.014 \times N \times V \times V_m \times 100)/(V_a \times W)$$

$$\text{Crude Protein (\%)} = N \times 6.25$$

Ether Extract (Fat): The ether extract was determined using Soxhlet extraction with petroleum ether (40-60 °C) for 6-8 hours. The extracted fat was obtained by weight difference.

$$\text{Ether Extract (\%)} = (W_2 - W_1)/W \times 100$$

Ash Content: Ash content was determined by incineration of the sample in a muffle furnace at 550 °C until white ash was obtained.

$$\text{Ash (\%)} = (W_2 - W_1)/W \times 100$$

The phytochemical screening: Total phenolic content (TPC) of musk melon seed powder (MMSP) was determined using the Folin-Ciocalteu method (Singleton and Orthofer, 1999) [19]. Briefly, 1 mL of sample extract was mixed with 1.5 mL of Folin-Ciocalteu reagent and incubated for 5 min at room temperature. Subsequently, 4 mL of 20% sodium carbonate solution was added, and the volume was adjusted to 25 mL with distilled water. The mixture was incubated for 30 min, and absorbance was measured at 765 nm using a UV-Vis spectrophotometer. Results were expressed as mg gallic acid equivalents (GAE) per 100 g using a standard calibration curve.

Total flavonoid content (TFC) was estimated by the aluminium chloride colorimetric method (Zhishen *et al.*, 1999) [23]. One millilitre of sample extract was mixed with distilled water, followed by addition of 5% sodium nitrite. After 6 min, aluminium chloride (10%) and NaOH (1 M) were added. The final volume was adjusted with distilled water, and absorbance was measured at 510 nm. Results

were expressed as mg quercetin equivalents (QE) per g of dry weight.

The assay of *In-vitro* Antioxidant activity

The antioxidant activity of MMSP was evaluated using the DPPH assay (Vinay *et al.*, 2012) [22]. Sample extract (0.5 mL) at different concentrations was mixed with 2 mL of DPPH solution (0.5 mM) and incubated in the dark for 30 min. Absorbance was recorded at 517 nm, and radical scavenging activity (%) was calculated relative to control. FRAP assay was performed following the method of Descalzo *et al.* (2007) [4]. The FRAP reagent was prepared using TPTZ, FeCl₃, and acetate buffer (pH 3.6). Sample extract was mixed with FRAP reagent and incubated for 4 min at room temperature. Absorbance was measured at 593 nm, and results were expressed as mmol Fe²⁺ equivalents per kg using a standard curve.

Statistical Analysis

The data generated with respect to proximate composition, phytochemical properties, and antioxidant properties of musk melon seed powder was expressed as Mean ± SE by using one way analysis of variance (ANOVA).

Results and Discussions

The mean ± S.E. values of proximate composition, pH, total phenolic content, total flavonoid content, DPPH scavenging activity and Ferric reducing antioxidant power (FRAP) of musk melon seed powder are presented in Table 1.

Table 1: Mean ± S.E. proximate composition, phytochemical content, *in vitro* antioxidant activity of Musk melon seed powder (MMSP)

Parameters	MMSP (n = 3)
Moisture (%)	4.06±0.15
Crude Protein (%)	29.13±0.07
Crude Fat (%)	38.38±0.19
Crude fibre (%)	1.43±0.05
Ash (%)	2.35±0.26
Total phenolic content (mg GAE/100 g)	168.12±0.12
Total Flavonoids content (mg QE/100 g)	83.68±0.69
Ferric reducing antioxidant power (mM FRAP/g)	26.25±0.98
DPPH radical scavenging activity (%)	61.28±5.12

In the present study the moisture, crude protein, crude fat, crude fiber and ash content were 4.06±0.15, 29.13±0.07, 38.38±0.19, 1.43±0.05 and 2.35±0.26 percent, respectively. The moisture content of the product generally exhibits a direct relation with the ability in preserving the nutritional value and quality of the end product. In similar lines, Nishat *et al.* (2024) [11] reported a percent moisture content of 3.23, ash of 4.86, crude protein of 31.37, crude fat of 35.70, crude fiber of 6.67 and carbohydrate content of 24.40 in musk melon seeds. The proximate composition of this study also corroborates with the findings Amin *et al.* (2018) [1] and Gade *et al.* (2022) [5] with respect to chemical composition of musk melon seeds.

In similar to the findings of this study Umar *et al.* (2013) [21] reported that the moisture content of *Cucumis melo* seeds was 3.73 percent, while Petkova and Antova (2015) [13] reported that the moisture content in different varieties of melon seeds ranged between 5.8 and 6.0 percent and the variation can be due to varietal difference and climatic changes in different areas of cultivation.

The crude protein content of MMSP in this study was 29.13 ± 0.07 percent. Similar findings have been reported by Saeed *et al.* (2024) ^[16] who reported that the CP content of *Cucumis melo* (musk melon) seed was 20.2 ± 0.06 percent. In previous literature, it was reported that the *C. melo* seeds contains an erudite meditation of essential and non-essential amino acids which have a leading structural and functional role in the human bodies. Tabiri *et al.* (2016) ^[20] reported that the protein content in the different varieties of musk melon varieties ranged between 16-18 percent. However, Petkova and Antova (2015) ^[13] reported higher protein content in musk melon seeds (34-39%) as compared to the present study.

The crude fat content of MMSP was 38.38 ± 0.19 percent, and similar findings have been reported by Mallek-Ayadi *et al.* (2018) ^[8] and Saeed *et al.* (2024) ^[16], who have reported that the musk melon seeds had 30.7 and 22.28 percent lipid content, respectively. The ash content of MMSP in the present study was 2.35 ± 0.26 percent. Ash content is generally associated with the inorganic residue that is present in organic compounds and can be recovered after the complete incineration of organic compounds at high temperature ($>550^\circ\text{C}$). These residual inorganic compounds are minerals, including Na, K, Mg, Ca, etc., which have the major role in the regulation of the human body, including homeostasis and blood flow regulation. Comparative study showed that ash content in *C. melo* seed was 2.2 ± 0.02 (Saeed *et al.*, 2024) ^[16], and a study by Jacob *et al.* (2015) on *C. melo* seed reported similar findings, which are closely related to the current study (2.7%).

Phenolic components are bioactive polyphenols present in food sample and generally associated as plant secondary metabolites which have tremendous therapeutic and functional properties in human body. In the present study, the total phenolic content and the flavonoid content of MMSP was 168.12 ± 0.12 mg GAE/100 g and 83.68 ± 0.69 mg QE/100 g, respectively. These results are closely corroborated with the findings of Rolim *et al.* (2018) ^[15], who reported that different extracts of melon seed showed total phenolic contents which ranged from 69.77-111.65 mg GAE/100 g.

In similar lines, Gade *et al.* (2022) ^[5] determined the contents of amino acids, fatty acids, vitamins, and minerals present in muskmelon seeds (variety, Hara Madhu). The results showed that the muskmelon seeds contained high levels of protein, fat, vitamins, and minerals with a total phenolic content (TPC) of 176 mg GAE/100 g and total flavonoid content (TFC) of 159 mg QE/100 g. Flavonoids are a complex class of polyphenols with electron-donating ability, scavenging potential, biosynthesis of α -tocopherol, and metal chelation. After the evaluation of its tremendous antioxidant potential and its ability to combat oxidative damage in the body it is characterized as vitamin P. In the study by Saeed *et al.* (2024) ^[16] the total flavonoid content in musk melon seed powder were 87.4 ± 1.3 mg CE/100 g, like that of the present study.

The DPPH assay has a fundamental potential to detect bioactive components at low concentrations and has been widely used for assessing antioxidant activities. *in vitro* antioxidant assay based on DPPH radical scavenging activity (%) of MSP revealed values of 61.28 ± 5.12 . Similar to our findings Saeed *et al.* (2024) ^[16] reported that the musk melon powder had DPPH scavenging activity of 46.8 ± 0.04 percent. The results of the current study were corroborated

with the findings of Neglo *et al.* (2021) ^[10], the antioxidant activity of watermelon seeds with DPPH assay and reported value was 41.1 percent. Further, Tabiri *et al.* (2016) ^[20] reported the scavenging potential using DPPH assay of different varieties of watermelon seeds and reported the results within the range of 59.88 to 94.46 percent.

The Ferric reducing antioxidant power (mM FRAP/g) of MSP in this study was 26.25 ± 0.98 . In similar lines, Saeed *et al.* (2024) ^[16] reported that the FRAP activity of musk melon seed was 27.8 mg RE/100 g and Naz *et al.* (2013) ^[9] in watermelon juice extract showed the FRAP value of 21.67 mM FRAP/g.

Conclusion

Musk melon seed powder (MMSP) has a good nutritional and functional profile, according to the findings of present study. Its potential for improved nutritional value and shelf stability was indicated by the proximate composition, which showed significant quantities of protein and fat with minimal moisture content. Its abundance of bioactive substances is shown by the significant levels of total phenolic and flavonoid contents. Additionally, the strong free radical scavenging and reduction potential is demonstrated by the considerable *in vitro* antioxidant activity, as shown by FRAP values and DPPH radical scavenging activity. The results of this study indicate that the nutritional and antioxidant properties of musk melon seeds are consistent with earlier observations. Therefore, MMSP can be used as a potential natural source of phytochemicals and nutrients that may be used in the development of functional foods.

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