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**Sivagangai Kalaiyarasan**
Department of Food Science and Technology, Pondicherry University, Puducherry, India

**Sundaramoorthy Haripriya**
Department of Food Science and Technology, Pondicherry University, Puducherry, India

**Corresponding Author:**
**Sivagangai Kalaiyarasan**
Department of Food Science and Technology, Pondicherry University, Puducherry, India

# Proximate composition, functional properties and antioxidant activity of raw and defatted watermelon (*Citrullus lanatus*) seed flour

Sivagangai Kalaiyarasan[1], Sundaramoorthy Haripriya[2]

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**Abstract**

Watermelon seeds are commonly discarded even though they contain substantial oil and protein. The present work compared the proximate composition, selected functional properties and antioxidant indices of raw and petroleum-ether-defatted flours prepared from seeds of mature fruits collected in Villupuram district, Tamil Nadu. After washing and oven-drying at 45 °C the seeds were milled; one portion was extracted with petroleum ether in a Soxhlet apparatus. Moisture, ash, fat, protein and carbohydrate were determined by AOAC (2012) methods. Bulk and tapped densities, water- and oil-holding capacities, emulsifying capacity and foaming capacity were measured by published gravimetric and volumetric procedures. Methanolic extracts were assayed for total phenolics (Folin–Ciocalteu), total flavonoids (aluminium-chloride), total antioxidant capacity and DPPH radical-scavenging activity. Raw flour contained 3.43 % moisture, 2.11 % ash, 49.78 % fat, 37.29 % protein and 7.12 % carbohydrate. Defatting reduced fat to 2.17 % and moisture to 2.74 % while concentrating protein to 65.82 %, ash to 7.24 % and carbohydrate to 21.81 % ($p < 0.05$). All functional parameters rose significantly: bulk density from 0.43 to 0.58 g $ml^{-1}$, tapped density from 0.64 to 0.92 g $ml^{-1}$, water absorption from 2.51 to 3.31 g $g^{-1}$, oil absorption from 1.24 to 2.35 ml $g^{-1}$, emulsion capacity from 48.24 to 56.83 % and foaming capacity from 36.28 to 43.32 %. Antioxidant values declined after solvent extraction: total phenolics from 1.86 to 1.63 mg GAE $g^{-1}$, flavonoids from 3.24 to 2.81 mg CE $g^{-1}$, total antioxidant capacity from 41.56 to 16.88 mg AAE $g^{-1}$ and DPPH scavenging from 46.26 to 20.12 %. The large increase in protein together with improved hydration, emulsifying and foaming behaviour indicates that the defatted flour is a concentrated, multifunctional plant-protein ingredient suitable for bakery goods, emulsions and meat analogues, despite a reduction in antioxidant activity after solvent extraction.

**Keywords:** Watermelon seed flour, Defatting, Functional properties, Antioxidant, Protein concentrate, Proximate composition

## Introduction

Watermelon (*Citrullus lanatus*) is widely cultivated for its juicy fruit, yet the seeds that accompany it are frequently discarded as waste. These seeds, however, represent a valuable yet under-utilised by-product with considerable nutritional and functional potential. They are rich in oil and protein, placing them among the oilseed crops that can contribute meaningfully to food security and the development of plant-based ingredients (El-Adawy *et al.*, 2001; Jyothi Lakshmi *et al.*, 2011) [1, 2].

Whole watermelon seed meals typically contain 40–50 % fat and 27–40 % protein, values that vary with cultivar and growing conditions (El-Adawy *et al.*, 2001; Jyothi Lakshmi *et al.*, 2011) [1, 2]. The high lipid content limits storage stability and restricts direct incorporation into many food systems. Defatting removes most of the oil, concentrates the protein and minerals, and alters the functional behaviour of the resulting flour. Protein levels in defatted material can exceed 60 %, making the flour attractive for fortification and for the formulation of high-protein products (Jyothi Lakshmi *et al.*, 2011; Wani *et al.*, 2011) [2, 3].

Beyond composition, the functional properties of seed flours—water and oil absorption, emulsifying capacity and foaming ability—determine their technological usefulness in bakery goods, emulsions and meat analogues. Watermelon seed proteins, particularly the albumin and globulin fractions, display favourable surface activity that supports these applications (Wani *et al.*, 2011) [3, 4]. In addition, the seeds contain phenolic compounds and other antioxidants that contribute measurable radical-scavenging activity, although levels may decline after solvent extraction (Seidu *et al.*, 2016; Jaroszewska *et al.*, 2023) [5, 6].

Despite growing interest, comparative data on the effects of defatting on the proximate profile, functional performance and antioxidant capacity of watermelon seed flour remain limited. The present study therefore



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examines these characteristics in both raw and defatted flour in order to clarify the nutritional and technological value of the material and to support its wider use as a functional food ingredient.

## Materials and Methods

### *Sampling*

Matured watermelon fruits (fresh condition) were purchased from watermelon farmland at Ranganavaram village, Villupuram district, Tamil Nadu. The fruits were sliced open using a clean stainless steel laboratory knife. The seeds were separated from the fruits, washed with distilled water and oven dried (at 45 °C) for 12 h. Immature seeds and extraneous materials were removed, and the cleaned, dried seeds were powdered and sieved through a 100 µm mesh to obtain a fine, uniform powder. The resulting watermelon seed flour was packed in an airtight container and stored in a desiccator (containing silica gel) until further analysis. All chemicals used were of analytical grade.

### *Preparation of defatted watermelon seed flour*

The flour sample was divided into two parts: one part was defatted using a Soxhlet extractor with petroleum ether as the extracting solvent (Fasasi *et al.*, 2004; Rana *et al.*, 2024) [7, 8] prior to chemical analysis, while the other was not.

### *Chemical composition*

Moisture, ash, fat, protein and carbohydrate contents were determined by the methods of the Association of Official Analytical Chemists (AOAC) [9]. Protein was calculated as nitrogen × 6.25.

### *Functional characteristics*

#### *Bulk and tapped density*

The bulk density of watermelon seed flours was analyzed following the method of (Smita *et al.*) [10]. Bulk density was analyzed by recording the volume occupied by watermelon seed flours in a pre-weighed 10-mL graduated cylinder up to the 10 mL mark. During filling, the cylinder was tapped 100 times and weighed again, and the bulk and tapped density of processed watermelon seeds is expressed as g/ml (weight of the cylinder/volume).

#### *Water-holding capacity*

The water-holding capacity (WHC) of processed watermelon seed was analyzed following the procedure of (Meera *et al.*) [11]. In this assay, 2.0 g of the sample was dispersed in 20 mL of deionized water, stirred for 20 min at 30 °C, and centrifuged at 3000 RPM for 15 min. The WHC is expressed as mL of water absorbed/g of sample.

#### *Oil-holding capacity*

The oil-holding capacity (OHC) of processed watermelon seed flours was analyzed following the procedure described by (Meera *et al.*) [11]. The accurately weighed (1.0 g) sample was dispersed in 10 mL of vegetable oil, stirred well for 5 min, and centrifuged at 3000 RPM for 15 min. The OHC was displayed as the weight difference.

#### *Emulsifying capacity*

The emulsifying capacity (EC) of processed watermelon seed flours was determined according to the procedure of (Meera *et al.*) [11]. In this assay, 0.5 g of the sample was added to 50 mL of 0.1 M NaCl and was stirred well, and 10 mL of vegetable oil was added. The suspension was homogenized for 5 min, centrifuged at 5000 RPM for 10 min, and then poured into a 50-mL graduated measuring cylinder and allowed to stand for a few minutes until the emulsified layer was stable. The EC was calculated using the following equation.

$$\mathrm{EC}\left(\mathrm{mL}/100\ \mathrm{g}\right) = \left(\text{Height of emulsified layer}/\text{Height of total volume}\right) \times 100.$$

#### *Foam capacity*

The foaming capacity (FC) and foam stability (FS) of processed watermelon seed flour were analyzed according to the method of (Meera *et al.*) [11]. In this assay, 1.0 g of processed watermelon seed flour was added to 50 ml of distilled water in a 100-mL graduated cylinder. The mixture was stirred for 3 min, and the generated foam volume was noted and was considered as FC. Furthermore, the foam volume noted after 15, 20 and 30 min was considered the percentage of FS.

### *Total antioxidant activity*

### *Extraction of watermelon seed flour*

The watermelon seed flour extract for antioxidant analysis was prepared according to the method described by (Mir *et al.*) [12] with little modifications. One gram of each watermelon seed flour was weighed accurately and extracted for 8 h with 25 ml HPLC grade methanol (Himedia) in the dark at ambient temperature (28 ± 1 °C) using a magnetic stirrer (IKA C-MAG HS 4 Stirrer, India). The mixture was centrifuged at 3000×g for 15 min and the supernatant extract was stored in sealed polypropylene tubes at 4 °C until further analysis.

#### *Total phenolic content*

Total phenolic content of samples was determined using the Folin-Ciocalteu spectrophotometric method (Reddy *et al.*, 2016) [13].

#### *Total flavonoid content*

Total flavonoid content of the watermelon seed flour was determined by the method of (Kamatchi *et al.*) [14]. Briefly, 250 µl of extract and standard catechin was diluted with 1.25 ml of distilled water. To it, 75 µl of 5% sodium nitrite was added and incubated at room temperature for 6 min. After incubation, 150 µl of 10% aluminium chloride was added and again kept for incubation for 5 min, and 0.5 ml of 1 M sodium hydroxide was added; the mixture was vortexed and the absorbance was taken immediately at 510 nm against a blank.

#### *DPPH radical scavenging activity*

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of watermelon seed flour was determined using the method described by (Reddy *et al.*) [13]. Watermelon seed flour extract (100 µl) was mixed with methanol (3.9 ml) and 1.0 ml of DPPH solution (concentration: 1.0 mM in methanol).

$$\text{DPPH radical scavenging}\ (\%) = \left(1 - \frac{\text{Absorbance at 517 nm of sample}}{\text{Absorbance at 517 nm of control}}\right) \times 100$$

## Results and Discussion

The proximate composition of raw and defatted watermelon (*Citrullus lanatus*) seed flour is presented in Table 1. The raw flour contained 3.43 ± 0.04 % moisture, 2.11 ± 0.02 % ash, 49.78 ± 0.02 % fat, 37.29 ± 0.04 % protein, and 7.12 ± 0.03 % carbohydrate. Defatting significantly reduced moisture to 2.74 ± 0.02 % and fat to 2.17 ± 0.03 %, while significantly increasing ash (7.24 ± 0.03 %), protein (65.82 ± 0.04 %), and carbohydrate (21.81 ± 0.02 %) contents ($p < 0.05$).

The high fat content of the raw flour (~50%) is characteristic of watermelon seeds, which are known oilseeds. This value is consistent with reports of 46.83 % fat in whole watermelon seed meal by (Jyothi



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Lakshmi and Kaul) [2] and falls within the broader range of 40–50 % or higher reported for various cultivars (El-Adawy *et al.*, 2001) [1]. Protein content of the raw flour (37.29 %) also aligns approximately with the reported range (27–40 %) for whole seeds (El-Adawy *et al.*, 2001; Jyothi Lakshmi *et al.*, 2011) [1, 2]. Oil seed flours with less than 10 % moisture content have enhanced storage stability.

Removal of the lipid fraction enriched the non-lipid components. Protein nearly doubled to 65.82 %, closely matching the 61.29 % reported for defatted watermelon seed flour by (Jyothi Lakshmi and Kaul) [2] and comparable to values of 50–71 % in other studies depending on residual oil and processing (Wani *et al.*, 2011) [3]. Ash and carbohydrate contents also increased substantially, consistent with the concentration of mineral matter and non-lipid organic components after oil removal. The observed changes confirm that defatted watermelon seed flour is a protein-rich ingredient suitable for food fortification and formulation of high-protein products.

The functional properties of raw and defatted flour are presented in Table 2. Defatting significantly improved all measured functional parameters ($p < 0.05$). Bulk density increased from 0.43 ± 0.02 to 0.58 ± 0.03 g/mL and tapped density from 0.64 ± 0.03 to 0.92 ± 0.02 g/mL. Water absorption capacity rose from 2.51 ± 0.03 to 3.31 ± 0.02 g/g, oil absorption capacity from 1.24 ± 0.02 to 2.35 ± 0.03 mL/g, emulsion capacity from 48.24 ± 0.03 % to 56.83 ± 0.04 %, and foaming capacity from 36.28 ± 0.02 % to 43.32 ± 0.03 %.

These enhancements are attributable to the exposure of hydrophilic and hydrophobic sites after lipid removal owing to the higher protein content. Water and oil absorption capacities of the defatted flour are as reported for watermelon seed flours and protein fractions (El-Adawy *et al.*, 2001; Wani *et al.*, 2011) [1, 3, 4]. Enhanced emulsifying and foaming properties of the defatted watermelon seeds are consistent with the functional behaviour of watermelon seed proteins, particularly albumins and globulins, which exhibit good surface activity (Wani *et al.*, 2011) [3]. Changes in particle packing after oil extraction are reflected through higher bulk and tapped densities in the defatted flour. Overall, the functional properties of the defatted flour indicate its potential as a multifunctional ingredient in bakery products, meat analogues, emulsions, and foamed systems.

The antioxidant properties are presented in Table 3. Raw flour exhibited significantly higher ($p < 0.05$) total phenolic content (1.86 ± 0.04 mg GAE/g), total flavonoid content (3.24 ± 0.02 mg CE/g), total antioxidant capacity (41.56 ± 0.03 mg AAE/g), and DPPH radical scavenging activity (46.26 ± 0.03%) compared with the defatted flour (1.63 ± 0.03 mg GAE/g, 2.81 ± 0.03 mg CE/g, 16.88 ± 0.02 mg AAE/g, and 20.12 ± 0.02%, respectively).

The phenolic contents observed are within the lower-to-moderate range reported for watermelon seed materials (approximately 1.6–8.4 mg GAE/g depending on extraction method and cultivar) (Seidu *et al.*, 2016; Jaroszewska *et al.*, 2023) [5, 6]. Moderate DPPH scavenging activity is also consistent with previous findings for watermelon seed extracts. The partial removal of lipid-soluble antioxidants (*e.g.*, tocopherols) and some phenolic compounds during solvent extraction could be the main reason for the decrease in antioxidant activity. Similar declines or variable antioxidant retention after processing have been noted in watermelon seed products (Jaroszewska *et al.*, 2023) [6]. Despite the decrease, the defatted flour retained measurable phenolic and flavonoid contents and residual radical-scavenging capacity, indicating that it still contributes bioactive compounds when used as a food ingredient.

## Conclusion

The defatted watermelon seed flour markedly improved protein concentration and functional properties while reducing residual oil and moderately lowering antioxidant activity. The resulting defatted flour represents a valuable, protein-rich functional ingredient with potential applications in the development of nutritious and technologically versatile food products.

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**Table 1.** Proximate composition of raw and defatted watermelon seed flour

| Parameter (%) | Moisture | Ash | Fat | Protein | Carbohydrate |
|---|---|---|---|---|---|
| Raw | 3.43 ± 0.04$^a$ | 2.11 ± 0.02$^b$ | 49.78 ± 0.02$^a$ | 37.29 ± 0.04$^b$ | 7.12 ± 0.03$^b$ |
| Defatted raw | 2.74 ± 0.02$^b$ | 7.24 ± 0.03$^a$ | 2.17 ± 0.03$^b$ | 65.82 ± 0.04$^a$ | 21.81 ± 0.02$^a$ |

Note: Values are mean ± standard deviation of three replicates. Values in a column bearing different superscripts differ significantly at $p < 0.05$.

**Table 2.** Functional properties of raw and defatted watermelon seed flour

| Parameter | Bulk density (g/ml) | Tapped density (g/ml) | Water absorption capacity (g/g) | Oil absorption capacity (ml/g) | Emulsion capacity (%) | Foaming capacity (%) |
|---|---|---|---|---|---|---|
| Raw | 0.43 ± 0.02$^b$ | 0.64 ± 0.03$^b$ | 2.51 ± 0.03$^b$ | 1.24 ± 0.02$^b$ | 48.24 ± 0.03$^b$ | 36.28 ± 0.02$^b$ |
| Defatted raw | 0.58 ± 0.03$^a$ | 0.92 ± 0.02$^a$ | 3.31 ± 0.02$^a$ | 2.35 ± 0.03$^a$ | 56.83 ± 0.04$^a$ | 43.32 ± 0.03$^a$ |

Note: Values are mean ± standard deviation of three replicates. Values in a column bearing different superscripts differ significantly at $p < 0.05$.

**Table 3.** Antioxidant properties of raw and defatted watermelon seed flour

| Parameter | Total phenolic content (mg GAE/g) | Total flavonoid content (mg CE/g) | Total antioxidant capacity (mg AAE/g) | DPPH radical scavenging (%) |
|---|---|---|---|---|
| Raw | 1.86 ± 0.04$^a$ | 3.24 ± 0.02$^a$ | 41.56 ± 0.03$^a$ | 46.26 ± 0.03$^a$ |
| Defatted raw | 1.63 ± 0.03$^b$ | 2.81 ± 0.03$^b$ | 16.88 ± 0.02$^b$ | 20.12 ± 0.02$^b$ |

Note: Values are mean ± standard deviation of three replicates. Values in a column bearing different superscripts differ significantly at $p < 0.05$. GAE, gallic acid equivalent; CE, catechin equivalent; AAE, ascorbic acid equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

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