

## Valorization of Bitter Gourd (*Momordica charantia*) Seeds as a Functional Food Ingredient: Physicochemical and Structural Characterization

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Article history: submitted: June 12, 2026; accepted: July 30, 2026; available online: August 10, 2026

**Abstract.** Bitter gourd (*Momordica charantia*) seeds are commonly discarded as agro-industrial by-products despite their rich nutritional composition and potential as functional food ingredients. The underutilization of these seeds not only contributes to environmental burdens but also represents a missed opportunity for sustainable waste valorization and resource recovery. Although increasing attention has been paid to the utilization of food processing by-products, limited information is available on the effects of lyophilization on the physicochemical, functional, structural, thermal, and morphological properties of bitter gourd seed powder. Therefore, this study aimed to compare untreated bitter gourd seed powder (BGSP) with lyophilized bitter gourd seed powder (LBGSP) to determine the effectiveness of lyophilization in preserving and enhancing the quality attributes of the resulting powder for potential functional food applications. Functional properties, including the water absorption index, oil absorption index, wettability, and swelling index, were determined using standardized analytical methods. Structural and morphological characteristics were investigated using Fourier-Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), Field-Emission Scanning Electron Microscopy coupled with Energy-Dispersive X-ray Spectroscopy (FESEM-EDX), Dynamic Light Scattering (DLS), and zeta potential analyses. Compared with untreated BGSP, LBGSP exhibited superior functional performance, improved preservation of proteins, polysaccharides, and other functional groups, a more porous and less aggregated microstructure, greater retention of essential mineral elements, enhanced colloidal stability, and reduced particle aggregation. Furthermore, thermal analysis revealed a substantially higher residual mass for LBGSP (23.1%) than for BGSP (11.42%), indicating improved thermal stability. These improvements were primarily attributed to the low-temperature vacuum dehydration process during lyophilization, which effectively minimized thermal degradation while preserving structural integrity and biofunctional components. Overall, the findings demonstrate that lyophilization is an effective processing strategy for improving the functional and structural quality of bitter gourd seed powder, thereby supporting its development as a sustainable functional food ingredient while promoting the valorization of agro-industrial waste.

**Keywords:** bitter gourd seed; functional food ingredient; lyophilization; microstructure; waste valorization

### 1. Introduction

Bitter gourd (*Momordica charantia*) belongs to the family Cucurbitaceae. It is widely cultivated and consumed throughout Asia and different parts of the world owing to its nutritional and medicinal properties ([Dhillon et al., 2020](#)). In addition to commercial cultivation, bitter gourd is commonly grown in household and kitchen gardens, making its actual consumption considerably higher than reported in official statistics. The processing of agricultural commodities generates substantial quantities of by-products, particularly seeds and peels, which are frequently discarded as waste. Improper disposal of these materials contributes to environmental pollution and

resource loss. In recent years, the recovery and utilization of agricultural by-products have gained increasing attention as sustainable approaches for the production of functional food ingredients and nutraceuticals ([Al-Saeed et al., 2023](#); [Ghazwani et al., 2023](#); [Hassan et al., 2024](#); [Mohamed & Hassan, 2023](#); [Mohammad et al., 2023](#); [Salsabil et al., 2022](#); [Shalaby et al., 2022](#); [Sultan, 2023](#)).

Bitter gourd seeds are generally discarded during processing and consumption. It has been estimated that a significant proportion of seeds generated during agricultural and food-processing operations remain underutilized, leading to the accumulation of agro-industrial waste. The valorization of these discarded seeds



aligns with the United Nations Sustainable Development Goal 12 (Responsible Consumption and Production), which emphasizes efficient resource utilization and waste reduction ([Food and Agriculture Organization, 2024](#); [Abbas et al., 2024](#)). Despite being treated as waste, bitter gourd seeds possess remarkable nutritional and phytochemical potential. They contain substantial amounts of carbohydrates, proteins, lipids, dietary fiber, vitamins, minerals, bioactive compounds, and essential fatty acids, contributing to their nutritional value ([Machewad et al., 2021](#); [Oyeleke et al., 2023](#)).

However, some studies have reported potential adverse effects of bitter gourd seeds under specific conditions ([Chung et al., 2022](#)). Therefore, comprehensive characterization of bitter gourd seed-derived powders is essential for establishing their properties and supporting their safe and effective utilization in food systems. The conversion of bitter gourd seeds into stable powders offers a sustainable approach to valorizing this underutilized by-product while providing ingredients with desirable functional properties, including water- and oil-absorption capacity, thermal stability, and particle characteristics suitable for incorporation into bakery products, beverages, snack foods, and nutritional supplements. It was hypothesized that bitter gourd seeds possess physicochemical, structural, and functional characteristics suitable for valorization into functional food ingredients. Accordingly, this study aimed to develop untreated and lyophilized bitter gourd seed powders and comprehensively evaluate their physicochemical, functional, structural, morphological, elemental, and thermal characteristics to determine the effect of lyophilization on their potential application as functional food ingredients.

## 2. Materials and Methods

### 2.1 Raw material collection, Authentication, and Processing

Fresh bitter gourds were harvested from

a farm in Coimbatore, Tamil Nadu, in January 2026. The bitter gourds were collected from the same cultivation area during a single harvest period to minimize variability due to environmental and seasonal factors. Only mature and healthy fruits of comparable size were selected. Seeds were manually separated and processed under identical laboratory conditions. The seeds were manually separated from the pulp and washed thoroughly. The washed seeds were air-dried until all external moisture had disappeared. The dried seeds were ground into a fine powder, sieved through a fine-mesh sieve, and used for further analysis. The bitter gourd seed powder was subjected to standard aqueous extraction under optimized conditions. The resulting extract was filtered, lyophilized, pulverized to obtain the lyophilized powder, and stored in an airtight container for further analysis. The untreated bitter gourd seed powder was coded as sample-I/BGSP, and the lyophilized powder was coded as sample-II/LBGSP in all analyses. The untreated and lyophilized powders were prepared using the same batch of seeds to minimize batch-to-batch variation. All analytical measurements were carried out under standardized operating conditions using calibrated instruments.

The Institutional Human Ethics Clearance (IHEC2026/FSMD17/FHP) was obtained from Avinashilingam Institute for Home Science and Higher Education for Women. The plant identification was done at the Botanical survey of India, Tamil Nadu Agricultural University, Coimbatore (BSI/SRC/5/23/2025-26/Tech/1161) prior to the conduct of the study. In the present study analysis such as FTIR, FE-SEM, EDX, XRD, UV Spectrophotometry, DLS, and Zeta potential were carried out at Prof. C.N.R. Rao Research Center of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

### 2.2 Chemical Characterization of the Bitter Gourd Seed Powder: Determination of the Presence of Protein using UV spectrophotometry

UV spectrophotometry analysis was carried out to identify the absorption characteristics and presence of protein in *Momordica charantia*. For the analysis, 50mg of the samples were diluted in 1ml of distilled water separately. The samples were filtered separately using whatman filter paper to acquire clear solutions. The filtered extracts were transferred into quartz cuvettes and analyzed using a UV-Visible spectrophotometer in the wavelength range of 200-800 nm (Mabasa *et al.*, 2021).

### 2.3 Functional Properties of Bitter Gourd Seed Powders

#### 2.3.1 Water Absorption Index

The water absorption index (Rodríguez-Miranda *et al.*, 2012) of the developed powders was determined based on their water holding capacity. Briefly, 1g of the samples I and II was separately mixed with 10 ml of distilled water in pre-weighed centrifuge tubes and vortexed thoroughly to ensure uniform dispersion. The mixtures were then centrifuged at 1000 rpm for 15 minutes. After centrifugation, the supernatant was carefully decanted without disturbing the sediment, and the WAI was calculated.

$$\text{Water absorption index} = \frac{\text{Gel weight}(g)}{\text{Sample weight}(g)}$$

#### 2.3.2 Oil Absorption Index

The Oil absorption index of the processed powders was analyzed according to the procedure by Rodríguez-Miranda *et al.* (2012). For the analysis, 1g of the sample was mixed in 10ml of corn oil in centrifuge tubes and vortexed thoroughly to obtain a uniform slurry. The mixtures were allowed to stand for 30 minutes with intermittent stirring to facilitate oil absorption, followed by centrifugation at 1000 rpm for 15 minutes. The volume of the separated supernatant oil was measured, and the OAI was calculated.

$$\text{Oil absorption index} = \frac{\text{Weight of oil retained}(g)}{\text{Weight of dry sample}(g)}$$

#### 2.3.3 Swelling Capacity

The swelling capacity of the powders

was analyzed according to the procedure by Robertson *et al.* (2000) with slight modifications. One gram of the sample was transferred into a graduated cylinder, and 10ml of distilled water was added. The suspension was allowed to hydrate at room temperature, after which the final volume occupied by the swollen sample was recorded. Swelling capacity was expressed as the volume of hydrated sample per gram of dry sample (ml/g).

$$\text{Swelling index} = \frac{\text{Weight of swollen sample}(g)}{\text{Sample weight}(g)} * 100$$

#### 2.3.4 Wettability test

The wettability of the developed powders was determined according to the method described by Fitzpatrick *et al.* (2017). For the analysis, 1g of each sample was gently placed on the surface of distilled water in separate beakers without agitation. The time required for the entire powder to become completely wetted and sink below the water surface was recorded using a stopwatch. Wettability was expressed as the wetting time (s), with shorter wetting times indicating better wettability.

### 2.4 Structural Characterization of the Bitter Gourd Seed

#### 2.4.1 Determination of Functional groups in the Bitter Gourd Seed using FTIR

The functional groups present in BGSP and LBGSP were determined using Fourier Transform Infrared Spectroscopy (FTIR). The samples were analyzed using an FTIR spectrometer, and the spectra were recorded in the range of 4000-400  $\text{cm}^{-1}$  at room temperature. The obtained spectra were examined to identify the characteristic functional groups present in the samples by comparing the absorption peaks with standard reference data reported in the literature (Silverstein *et al.*, 2014).

#### 2.4.2 Determination of the nature of BGSP and LBGSP using XRD

The crystalline nature and structural characteristics of BGSP and LBGSP were analyzed using X-Ray Diffraction (XRD). The powdered samples were subjected to XRD analysis, and the diffraction patterns

were recorded over a  $2\theta$  range of  $5^{\circ}$ – $80^{\circ}$  under ambient conditions. The obtained diffraction peaks were analyzed to determine the crystalline or amorphous nature of the samples by comparing the patterns with standard reference data ([Cullity and Stock, 2014](#)).

## 2.5 Morphological Characterization of the Bitter Gourd Seed Powders: Determination of the morphology of the BGSP and LBGSP using FESEM and EDX

The surface morphology and microstructural characteristics of BGSP and LBGSP were examined using Field Emission Scanning Electron Microscopy (FESEM). The samples were analyzed under appropriate operating conditions, and micrographs were obtained to evaluate their surface features, particle shape, and structural characteristics. Energy Dispersive X-ray (EDX) analysis coupled with FESEM was also performed to determine the elemental composition of the samples ([Goldstein et al., 2018](#)).

## 2.6 Thermal Characterization of the Bitter Gourd Seed Powders: Determination of thermal stability of BGSP and LBGSP

The thermal stability and decomposition behavior of BGSP and LBGSP were evaluated using Thermogravimetric Analysis (TGA). The samples were analyzed under controlled heating conditions, and the change in sample weight was recorded as a function of temperature. The obtained thermograms were used to assess moisture loss, degradation patterns, and the thermal stability of the samples.

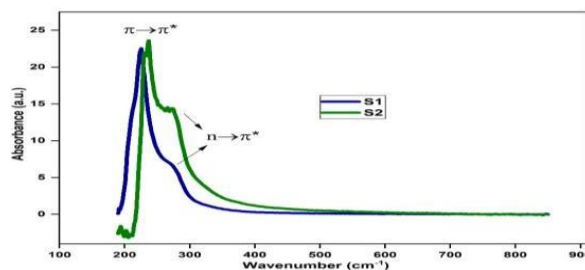
## 2.7 Colloidal Characterization of the Bitter Gourd Seed Powders: Determination of Particle Size and Zeta Potential of the Bitter Gourd Seed Powder using Dynamic Light Scattering

The particle size of the bitter gourd seed was determined using DLS. For this, 40mg of bitter gourd seed powder was diluted in 2ml of distilled water, and the particle size was observed using Dynamic Light Scattering. The particle size and distribution span were computed on the basis of a volume median

diameter. The zeta potential helped to determine the surface charge and colloidal stability of the suspension.

## 3. Results and Discussion

### 3.1 Determination of the presence of protein using UV spectrophotometry



**Figure 1.** UV curves of BGSP (S1) and LBGSP (S2)

The UV–Vis spectra of bitter gourd seed powder (BGSP, S1) and lyophilized bitter gourd seed powder (LBGSP, S2) exhibited distinct absorption profiles, indicating differences in their chemical characteristics following processing ([Figure 1](#)). BGSP showed a maximum absorption peak at 225 nm with a weaker band around 260 nm, whereas LBGSP exhibited a red shift of the primary absorption peak to 237 nm together with a higher absorbance intensity and a more prominent band at 275 nm. These absorption bands are generally associated with  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  electronic transitions of protein- and lipid-related constituents ([Mabasa et al., 2021](#)). The higher absorbance observed in LBGSP suggests that the aqueous extraction and lyophilization process influenced the profile of UV-absorbing constituents in the powder.

Lyophilization removes water by sublimation under reduced pressure and low-temperature conditions, thereby minimizing thermal degradation of thermolabile constituents during drying ([Snowman, 1997](#); [ElKassas et al., 2021](#)). Consequently, the observed spectral differences may be associated with changes in the chemical profile resulting from the aqueous extraction and lyophilization process. Although UV-Vis spectroscopy does not permit direct

identification of specific biomolecules, the results indicate that the processing method influenced the UV-absorbing constituents of the powder, which may be advantageous for developing minimally processed functional food ingredients.

### 3.2 Water Absorption Index of the Bitter Gourd Seed Powders

[Table 1](#) presents the water absorption index (WAI) of BGSP and LBGSP. LBGSP exhibited a higher WAI (6.74) than BGSP (0.29) and the reference value reported by [Rodríguez-Sandoval et al. \(2012\)](#), indicating an improved capacity for water uptake following processing. This observation may be attributed to the structural modifications induced by aqueous extraction and lyophilization, which are likely to increase particle porosity and expose hydrophilic sites capable of binding water. Previous studies have demonstrated that conventional hot-air drying promotes particle shrinkage and structural collapse, thereby reducing porosity and limiting rehydration properties ([Aravindakshan et al., 2021](#)).

**Table 1.** Water Absorption Index of the bitter gourd seed powders

| Variations | Analyzed value | Reference value                                     |
|------------|----------------|-----------------------------------------------------|
| BGSP       | 0.29           | 1.94 ± 0.02                                         |
| LBGSP      | 6.74           | ( <a href="#">Rodríguez-Sandoval et al., 2012</a> ) |

In contrast, freeze-drying preserves a porous, predominantly amorphous structure through sublimation at low temperatures, thereby facilitating water penetration and improving hydration characteristics ([Zhang et al., 2023](#)). Enhanced water absorption is a desirable functional attribute for food ingredients intended for bakery products, instant mixes, soups, and other formulations where moisture retention and reconstitution are critical quality parameters.

### 3.3 Oil Absorption Index of the Bitter Gourd Seed Powders

[Table 2](#) indicates that both variations have a good oil absorption index. The oil absorption of LBGSP was better than that of BGSP (2.74) and the reference value (1.27 ± 0.02) reported by [Rodríguez-Sandoval et al. \(2012\)](#). The improved oil absorption may be associated with structural modifications resulting from aqueous extraction and lyophilization, which are likely to increase particle porosity and enhance the accessibility of hydrophobic binding sites. Comparable findings were reported by [Farooq et al. \(2025\)](#), who observed higher oil absorption in freeze-dried chickpea flour than in untreated flour and attributed this improvement to the porous microstructure generated during freeze-drying and the increased exposure of hydrophobic interaction sites. From a technological perspective, improved oil absorption enhances flavor retention, mouthfeel, and texture, thereby supporting the potential application of LBGSP as a functional ingredient in bakery products, snack formulations, and other lipid-containing food systems.

**Table 2.** Oil Absorption Index of the bitter gourd seed powders

| Variations | Analyzed value | Reference value                                     |
|------------|----------------|-----------------------------------------------------|
| BGSP       | 2.74           | 1.27 ± 0.02                                         |
| LBGSP      | 3.36           | ( <a href="#">Rodríguez-Sandoval et al., 2012</a> ) |

### 3.4 Swelling Capacity of the Bitter Gourd Seed Powders

[Table 3](#) presents the swelling capacity of BGSP and LBGSP. LBGSP exhibited a swelling capacity of 7 mL/g, which was higher than that of BGSP (1 mL/g) and comparable to the reference range of 7–11 mL/g reported by [Robertson et al. \(2000\)](#). The higher swelling capacity of LBGSP indicates an improved ability to absorb water and expand upon hydration. This behavior may be attributed to the structural modifications induced by aqueous extraction and lyophilization, which are likely to preserve a porous matrix and increase the accessibility of hydrophilic components. [Bhatta et al.](#)

(2020) reported that freeze-drying produces highly porous powders with superior quality retention and solubility compared with conventional drying methods, thereby facilitating improved hydration characteristics. Enhanced swelling capacity is a desirable functional property for food ingredients intended for bakery products, instant mixes, and nutritional formulations, where water uptake contributes to improved texture and product stability.

**Table 3.** Swelling Index of the bitter gourd seed powders

| Variations | Analysed value | Reference value                               |
|------------|----------------|-----------------------------------------------|
| BGSP       | 1 ml           | 7-11 ml/g<br>(Robertson <i>et al.</i> , 2000) |
| LBGSP      | 7 ml           |                                               |

### 3.5 Wettability of the Bitter Gourd Seed Powders

Table 4 presents the wettability of BGSP and LBGSP. LBGSP exhibited a shorter wetting time (3 min) than BGSP (>25 min), indicating improved wettability following aqueous extraction and lyophilization. According to Fitzpatrick *et al.* (2017), powders requiring more than 5 min for complete wetting are classified as having poor wettability. Accordingly, LBGSP demonstrated satisfactory wettability, whereas BGSP exhibited poor wettability. The improved wettability of LBGSP may be attributed to structural modifications induced by the processing method, which are likely to facilitate water penetration and particle dispersion during reconstitution. Similar observations have been reported for freeze-dried milk powders, which exhibit higher wettability, dispersibility, and reconstitution properties than conventionally dried powders (Alibekov *et al.*, 2025). Furthermore, freeze-drying has been shown to better preserve the physical and chemical stability of powders during storage compared with conventional

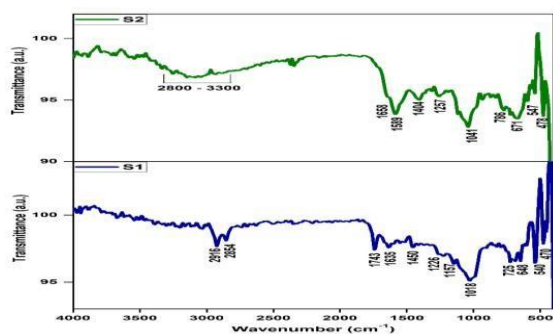
drying techniques (Cais-Sokolińska *et al.*, 2023; Deshwal *et al.*, 2020; Harizi *et al.*, 2023). Improved wettability is an important functional attribute for powdered food ingredients, as it facilitates rapid reconstitution and enhances their suitability for applications in instant beverages, nutritional supplements, and convenience food formulations.

**Table 4.** Wettability of the bitter gourd seed powders

| Variations | Analysed value | Reference value                                                        |
|------------|----------------|------------------------------------------------------------------------|
| BGSP       | >25 mins       | <1 minute - easy wetting powder                                        |
| LBGSP      | 3 mins         | >5 minutes - Poor wetting powder<br>(Fitzpatrick <i>et al.</i> , 2017) |

### 3.6 Determination of Functional groups in the BGSP and LBGSP using FTIR

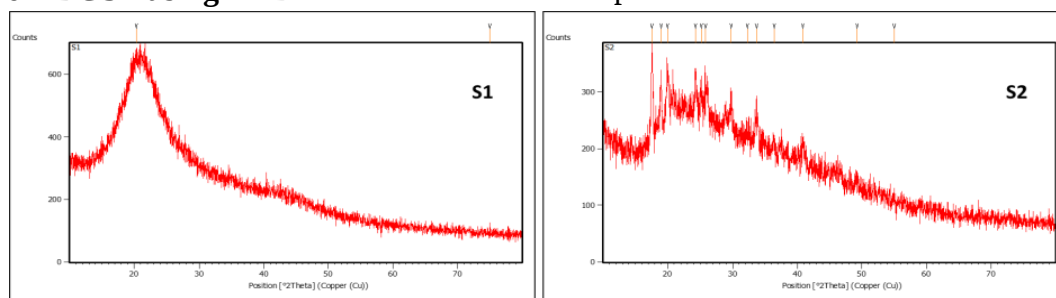
The FTIR spectra of bitter gourd seed powder (BGSP, S1) and lyophilized bitter gourd seed powder (LBGSP, S2) (Figure 2) exhibited characteristic absorption bands corresponding to the major functional groups present in the samples, with noticeable differences in band intensity and spectral profile following processing. BGSP displayed prominent absorption peaks at 2916, 2854, and 1743  $\text{cm}^{-1}$ , corresponding to aliphatic C–H stretching and ester carbonyl (C=O) vibrations commonly associated with lipid constituents. In contrast, LBGSP exhibited a broader absorption band within the 2800–3300  $\text{cm}^{-1}$  region, attributed to O–H stretching vibrations of hydroxyl-containing compounds, together with variations in the amide and carbohydrate regions. These spectral differences indicate that the aqueous extraction and lyophilization process influenced the molecular environment and relative abundance of functional groups present in the powder.



**Figure 2.** FTIR Spectrum of the bitter gourd seed powders

FTIR spectroscopy is widely employed to characterize molecular functional groups in food materials by detecting characteristic vibrational frequencies. Similar observations have been reported by [Qiu et al. \(2020\)](#), who demonstrated that infrared freeze-drying better retained the molecular structure and nutritional quality of edible *Rosa rugosa* flowers than conventional drying, as reflected by the FTIR spectral profile. The broader O–H absorption band and variations observed in the amide and carbohydrate regions of LBGSP are therefore consistent with processing-induced modifications. Such molecular characteristics may contribute to the powder's functional performance in food formulations by influencing its hydration behavior and interactions with other food components.

### 3.7 Determination of the nature of BGSP and ABGSP using XRD



**Figure 3.** XRD Spectra of bitter gourd seed powder (S1) and lyophilized bitter gourd seed powder (S2)

### 3.8 Determination of the morphology of the BGSP and ABGSP using FESEM

Compared with BGSP (S1), LBGSP (S2)

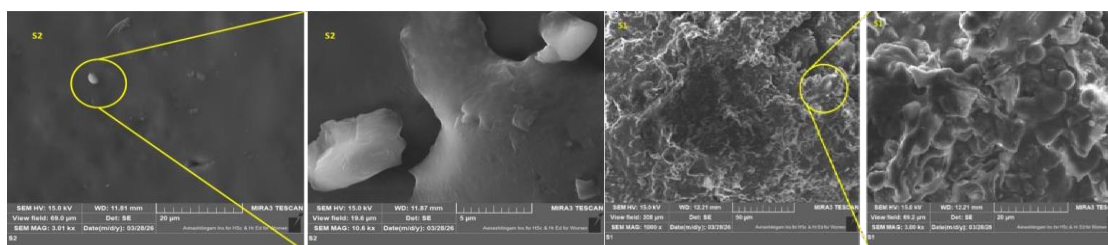
The XRD patterns of bitter gourd seed powder (BGSP, S1) and lyophilized bitter gourd seed powder (LBGSP, S2) exhibited distinct diffraction profiles, indicating differences in their structural organization following processing ([Figure 3](#)). BGSP displayed a broad diffraction peak centered around 20.33°, characteristic of a predominantly amorphous structure. In contrast, LBGSP exhibited several sharper diffraction peaks, suggesting a relatively higher degree of structural order. These observations indicate that the aqueous extraction and lyophilization process influenced the crystalline characteristics of the powder.

Lyophilization removes water by sublimation under low-temperature conditions, thereby minimizing structural collapse that may occur during dehydration. The relatively sharper diffraction peaks observed in LBGSP may therefore be associated with reduced disruption of the powder matrix during processing. Differences in crystallinity can influence the functional behavior of food powders, including hydration, stability, and interactions with other food components. The XRD findings are consistent with the improved functional properties observed for LBGSP, particularly its higher water absorption, swelling capacity, and thermal stability, suggesting that the processing method altered the structural organization of the powder.

exhibited a smoother, more uniform surface morphology ([Figure 4](#)). The observed differences may be attributed to the aqueous extraction and lyophilization process, in

which water is removed by sublimation under reduced pressure and at low temperatures, thereby minimizing structural collapse during dehydration. Consequently, LBGSP displayed a more homogeneous particle morphology with reduced aggregation. Similar observations have been reported for freeze-dried food materials, where low-

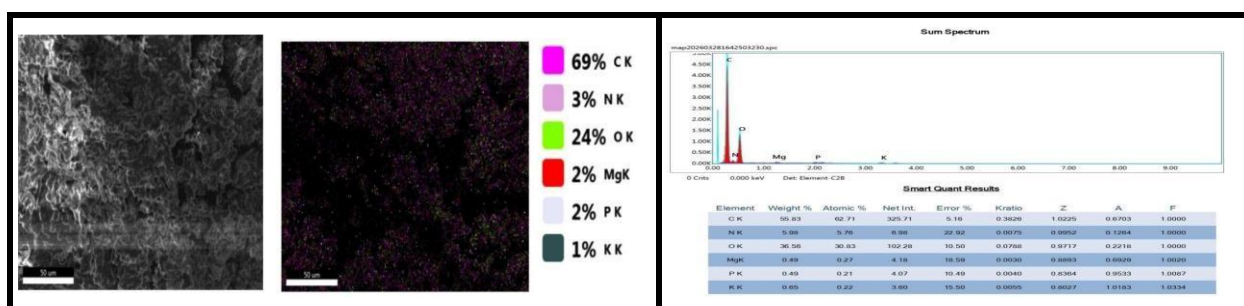
temperature dehydration better retains the physical structure than conventional drying methods (Gade *et al.*, 2018). The porous and uniform morphology observed in LBGSP may contribute to its improved hydration-related functional properties, including water absorption, swelling capacity, and wettability.



**Figure 4.** FESEM images of bitter gourd seed powder (S1) and lyophilized bitter gourd seed powder (S2)

EDX analysis of BGSP (Figure 5) showed carbon (55.83 wt%) and oxygen (36.56 wt%) as the predominant elements, together with nitrogen and trace amounts of magnesium, phosphorus, and potassium, indicating that the powder is primarily composed of organic constituents. In LBGSP (Figure 6), oxygen and carbon remained the major elements, while relatively higher proportions of nitrogen and several mineral elements, including potassium, phosphorus,

magnesium, zinc, calcium, and molybdenum, were detected. The differences in elemental composition between BGSP and LBGSP suggest that aqueous extraction and lyophilization influenced the elemental profile of the powder. The presence of essential mineral elements may be advantageous for the utilization of LBGSP as a functional food ingredient, although further quantitative nutritional analysis would be required to determine their bioavailability.



**Figure 5.** Energy Dispersive X-ray (EDX) analysis of Bitter Gourd Seed Powder (BGSP)

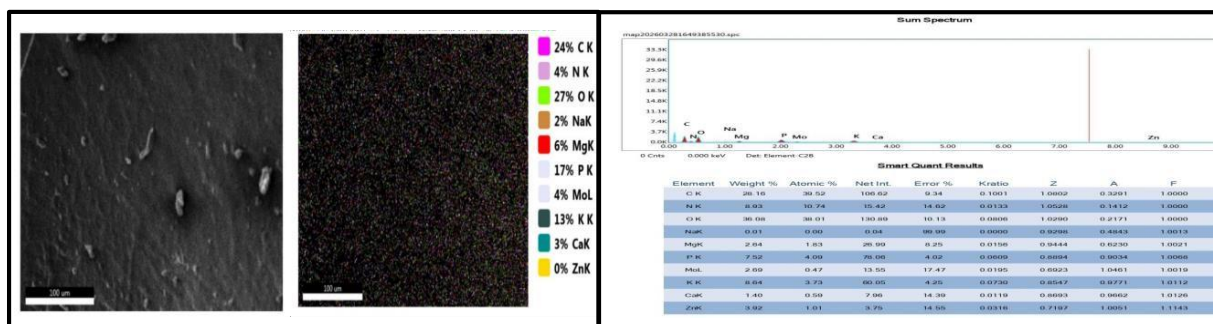


Figure 6. Energy Dispersive X-ray (EDX) analysis of lyophilized bitter gourd seed powder

### 3.9 Determination of thermal stability of BGSP and ABGSP

The thermogravimetric (TGA) curves of BGSP (S1) and LBGSP (S2) demonstrated distinct thermal degradation profile [Figure 7](#), indicating that the processing method influenced the thermal behavior of the powders. LBGSP exhibited a slightly higher initial weight loss around 105°C, which may be associated with the removal of residual moisture retained following lyophilization. The principal decomposition stage occurred at approximately 358°C for LBGSP and

389°C for BGSP, indicating differences in thermal decomposition behavior between the two powders. Notably, LBGSP exhibited a higher residual mass (23.1%) than BGSP (11.42%), suggesting greater char formation during thermal degradation. Similar observations have been reported for freeze-dried food materials, where low-temperature dehydration influences the thermal behavior of the dried matrix. Greater residual mass may be advantageous in food-processing applications that require improved thermal stability during manufacturing.

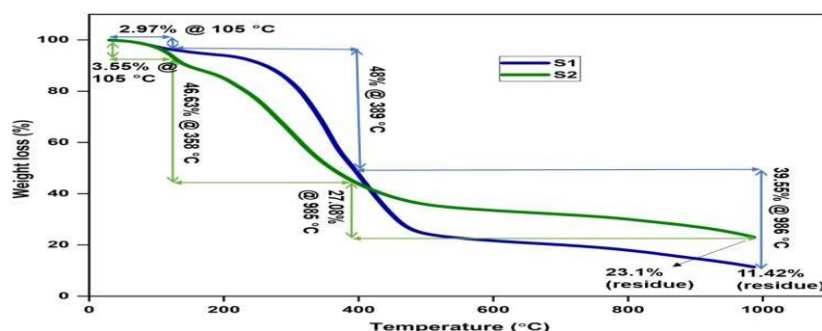
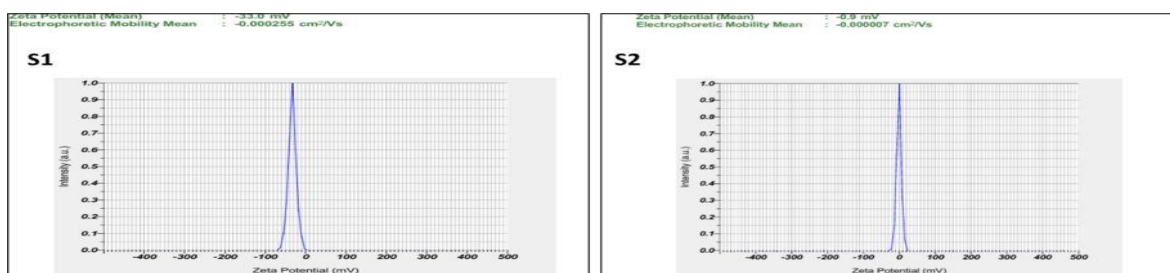


Figure 7. TGA curves of bitter gourd seed powder (S1) and lyophilized bitter gourd seed powder (S2)

### 3.10 Determination of Particle size and zeta potential of the Bitter Gourd Seed Powder using Dynamic Light Scattering

Zeta potential analysis revealed differences in the colloidal behavior of BGSP (S1) and LBGSP (S2) dispersions [Figure 8](#). BGSP exhibited a zeta potential of  $-33$  mV, indicating moderate electrostatic stability, whereas LBGSP showed a near-neutral zeta potential ( $-0.9$  mV), suggesting reduced electrostatic stabilization. These differences indicate that the aqueous extraction and

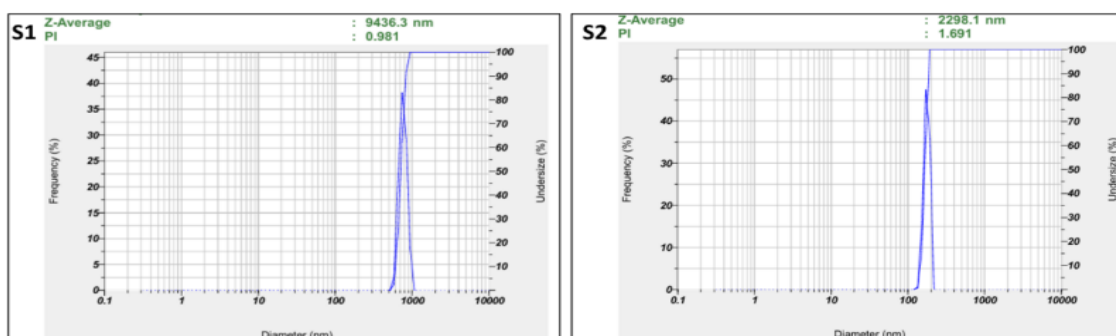
lyophilization process influenced the surface characteristics and dispersion behavior of the powder particles. Similar observations have been reported by [Onugwu et al. \(2023\)](#), who demonstrated that processing methods can alter the surface charge and colloidal stability of plant-derived particles. The reduced electrostatic stability of LBGSP suggests that factors other than surface charge, such as particle morphology and hydration characteristics, may contribute to its functional behavior.



**Figure 8.** Zeta potential curves of bitter gourd seed powder (S1) and lyophilized bitter gourd seed powder (S2)

Dynamic Light Scattering (DLS) analysis showed differences in the particle size distribution profiles of BGSP and LBGSP (Figure 9). The BGSP sample exhibited a Z-average particle size of 9436.3 nm with a polydispersity index (PDI) of 0.981, indicating a highly polydisperse particle population. The LBGSP sample exhibited a Z-average particle size of 2298.1 nm with a PDI of 1.691, also indicating a highly heterogeneous particle size distribution. Although the measured Z-average particle size of LBGSP was smaller

than that of BGSP, the substantially higher PDI suggests a broader particle size distribution, and therefore, the Z-average values should be interpreted with caution. These observations indicate that the aqueous extraction and lyophilization process altered the particle dispersion characteristics; however, because the measurements were obtained from a single representative sample without experimental replication, no statistical comparison can be made between the two samples.



**Figure 9.** DLS curves of bitter gourd seed powder (S1) and lyophilized bitter gourd seed powder (S2)

#### 4. Limitations and Future Directions

The present study was limited to the laboratory-scale characterization of untreated and lyophilized bitter gourd seed powders. Storage stability under different packaging and environmental conditions was not evaluated, and the powders were not tested in actual food formulations. In addition, sensory acceptability, digestibility, bioavailability, and the economic and environmental feasibility of large-scale production were beyond the scope of this study.

Future research should investigate

incorporating bitter gourd seed powder into various food products to assess its functional performance, sensory acceptance, and storage stability. Further studies should also evaluate the digestibility, bioavailability, and health-promoting potential of its bioactive compounds, as well as conduct life-cycle assessment and techno-economic analysis to support its sustainable industrial application.

#### 5. Conclusion

The present study demonstrated a comprehensive characterization of untreated

bitter gourd seed powder (BGSP) and lyophilized bitter gourd seed powder (LBGSP). The physicochemical, functional, structural, morphological, elemental, thermal, and colloidal analyses indicated differences in the characteristics of the two powders following aqueous extraction and lyophilization. LBGSP generally exhibited higher water and oil absorption indices, swelling capacity, improved wettability, distinct FTIR and XRD profiles, a more uniform surface morphology, higher residual mass during thermal analysis, and reduced particle aggregation compared with BGSP. These findings suggest that the processing method influenced the functional and structural properties of bitter gourd seed powder.

The results highlight the potential of bitter gourd seeds as a value-added food ingredient rather than an underutilized agro-industrial by-product. However, further studies on storage stability, food-application trials, sensory evaluation, digestibility, bioavailability, and techno-economic assessment are required before considering large-scale industrial utilization.

#### **Declaration of Generative AI and AI-Assisted Technologies in the Writing Process**

During the preparation of this work, the author(s) used ChatGPT to proofread a few sections. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

#### **Authorship Contribution Statement**

R. Radha: research conception, designing methodology, supervision, project administration, organizing resources like lab facilities, manuscript editing, language and grammar editing (contribution percentage 35%); Bhawaani, S.S.: ethical approval, laboratory analysis work, data curation, interpretation of results, writing first draft manuscript (contribution percentage 35%); Tinu, D.: experimental design development, review of literature, interpretation of results, reference management, review and editing of manuscript

draft (contribution percentage 30%).

#### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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