GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Analysis of *Lepidium sativum* L. (Garden cress) seed mucilage: A comprehensive characterization using XRD, DSC, and PSA techniques

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GSC Biological and Pharmaceutical Sciences, 2025, 30(03), 174-181

Publication history: Received on 29 January 2025; revised on 10 March 2025; accepted on 12 March 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.30.3.0102

Abstract

Objective: The current study aims to evaluate the particle size and the properties of mucilage particles, such as crystallinity and thermal stability, of *Lepidium sativum* seed mucilage using particle size analysis (PSA), X-ray diffraction (XRD), and differential scanning calorimetry (DSC) characterization.

Method: The mucilage extracted from *Lepidium sativum* seeds was obtained by utilizing distilled water as a solvent, followed by precipitation with 95% ethanol to isolate the mucilage effectively. To assess the size distribution and average particle size of the extracted mucilage, a Particle Size Analysis (PSA) was conducted. Crystallinity and crystal characteristics were investigated using X-ray Diffraction (XRD), which provided insights into the nature of the mucilage and detailed the size of its crystallites. Additionally, thermal stability was analyzed through Differential Scanning Calorimetry (DSC).

Results: The particle size distribution analysis results illustrated a range of particle sizes: 25% of the particles measured at 169.68 µm. In contrast, the median size (50th percentile) was determined to be 292.50 µm, and 75% of the particles reached a size of 408.64 µm. The average particle size was calculated to be 259.89 ± 0.24 nm, indicating a relatively fine particle size distribution. The average size of the crystallites calculated from the XRD data was found to be 47.47 nm, confirming the presence of crystalline regions within the mucilage. The DSC analysis provided a detailed thermogram, revealing two distinct thermal events: an endothermic peak recorded at 68.24 ºC, and an exothermic peak at 268.86 ºC. Furthermore, the latent heat of fusion was quantified at -161.86 J/g for the endothermic transition and 38.17 J/g for the exothermic transition.

Conclusion: The Particle Size Analysis (PSA) and X-ray Diffraction (XRD) revealed that *L. sativum* mucilage has an average particle size of 259.89 nm and a partially crystalline structure. Differential Scanning Calorimetry (DSC) showed a melting point of 68.24 ºC and decomposition at 268.86 ºC, indicating thermal stability with latent heats of -161.86 J/g and 38.17 J/g, respectively.

Keywords: *Lepidium sativum*; Garden cress; Mucilage; DSC; XRD; PSA; Characterization

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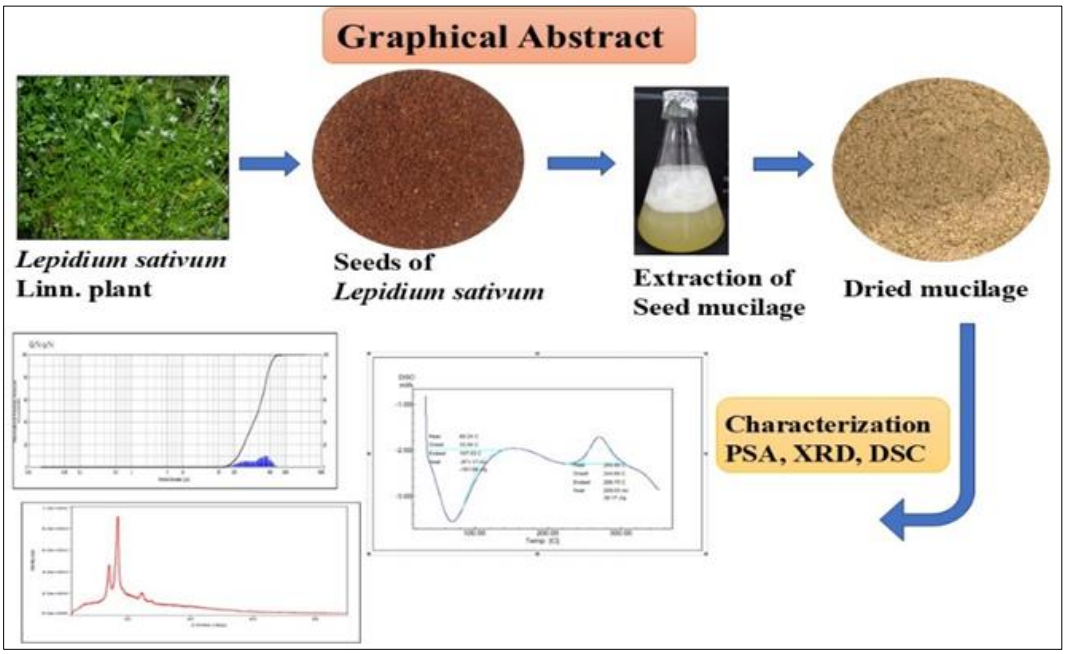
Graphical abstract



1. Introduction

Lepidium sativum (*L. sativum*), commonly known as garden cress, contains seed mucilage. This natural polysaccharide has attracted attention in both the pharmaceutical and food industries due to its distinctive properties such as biodegradability, biocompatibility, and low cost [1-3]. The gum is extracted from the seeds of the *L. sativum* plant and has been studied for its potential as a pharmaceutical excipient and a multifunctional polymer**.** Historically, the seeds of *L. sativum* have been employed in medicinal practices to remedy a range of health concerns, including asthma, hypertension, and hyperglycemia [4-6]. These seeds are rich in mucilaginous compounds, rendering them an exceptional source of high molecular weight hydrocolloids [7-9]. Recent scholarly investigations have focused on the physicochemical and functional attributes of *L. sativum* mucilage, emphasizing optimizing extraction methodologies and analyzing its properties [10-13]. Research indicates that *L. sativum* seed mucilage is predominantly constituted of carbohydrates, comprising 77% of its composition, and possesses a weight-average molecular weight of 540 kDa, alongside a radius of gyration measuring 75 nm [14-16]. The presence of carboxyl groups imparts the material with traits characteristic of a standard polyelectrolyte, evidenced by a reduction in intrinsic viscosity corresponding to an increase in NaCl concentration [17-19]. A 1% *L. sativum* mucilage solution in steady shear assessments reveals shear-thinning characteristics, while dynamic analyses showcase its weak gel-like properties. The rheological characteristics of *L. sativum* mucilage are modulated by various factors, including shear rate, temperature, duration, pH, and biopolymer concentration, as well as the concentration and type of salts and sugars present [20].

The characterization of materials using various analytical techniques is a crucial step in understanding their physical and chemical properties. This research paper aims to provide a comprehensive analysis of the *L. sativum* seed gum using X-ray diffraction, particle size analysis, and differential scanning calorimetry.

X-ray diffraction is a widely used analytical technique for determining the crystallographic structure of materials [21-22]. It provides detailed information on the atomic arrangement, phase composition, and crystal parameters of the samples. Recent advancements have demonstrated the potential to enhance the analysis of XRD data by enabling fast and interpretable classification of small XRD datasets [23]. Particle size analysis is an effective method for determining the size distribution of particles within a material. This information is vital for understanding the physical and chemical properties of a material, as well as its performance in various applications. Differential scanning calorimetry (DSC) is a thermal analysis technique that measures the heat flow into or out of a material over time or as a function of temperature. This technique provides valuable insights into phase transitions, crystallinity, and thermal stability [24].

2. Material and methods

2.1. Extraction of *L. sativum* seed mucilage

L. sativum seeds (100 g) were cleaned to remove surface contaminants, followed by the addition of 900 mL of distilled water. This mixture was continuously stirred for 5 hours at a speed of 300 rpm, while maintaining a temperature of 60°C, in accordance with the protocol established by Cui (2000)[25]. The resulting mucilage solution was filtered and then precipitated by adding 95% ethanol, equal to twice the volume of the mucilage solution. The precipitated mucilage was subsequently centrifuged at 3000 rpm for 10 minutes. Finally, the isolated mucilage was dried at 60°C in a hot air oven overnight.

2.2. Differential Scanning Calorimetry (DSC)

The samples were analyzed using a Shimadzu DSC-60Plus for differential scanning calorimetry (DSC). The analysis was conducted over a temperature range of 0 to 450 °C, with a heating rate of 10 °C/min. This was done in a controlled nitrogen atmosphere to maintain inert conditions during the measurements.

2.3. X-ray Diffraction Analysis (XRD)

X-ray Diffraction (XRD) analysis was conducted using a Rigaku Mini Flex 600 diffractometer to assess the crystalline characteristics of the mucilage particles. The average crystalline size was calculated using the Debye-Scherrer equation, as outlined by Debye et al. (2010).

2.4. Particle Size Analysis (PSA)

Particle Size Analysis (PSA) was performed with a Shimadzu SALD-2300 instrument, which effectively measures both the size distribution and the average particle size within the sample.

3. Results and discussion

3.1. Differential scanning calorimetry (DSC)

The evaluation of polysaccharide thermal stability is crucial for assessing its suitability in food products that undergo thermal processing. [26]. This study used differential scanning calorimetry (DSC), a technique known for its accuracy and sensitivity, to investigate the temperature-dependent physical and chemical changes of a specific polysaccharide—L. sativum mucilage. The DSC thermogram for this mucilage, shown in Fig. 1, clearly presents two peaks: an endothermic peak at 68.24 ºC and an exothermic peak at 268.86 ºC. The latent heat of fusion values associated with these peaks is -161.86 J/g for the endothermic peak and 38.17 J/g for the exothermic peak.

Thanzami et al. (2015) identified a DSC thermogram for Albizia stipulata Boiv. Gum exudates feature a broad endothermic peak at 115.37 °C [27]. Kamboj et al. (2014) reported that pure corn fiber gum (CFG) showed two endothermic transitions at 89.16 °C and 231.15 °C and one exothermic transition at 280.31 °C. The initial transition likely represents moisture content, while the second peak may indicate the melting of the CFG sample. Endothermic peaks generally indicate phase transitions within a system, such as melting or evaporation, highlighting significant thermal events [28]. The specific shape of the melting endotherm provides critical insight into the purity of the polysaccharide: a sharp and symmetric curve typically denotes high purity, whereas a broad and asymmetric curve suggests potential impurities or multiple thermal processes. Razmkhah et al., (2016) further identified an endothermic peak within the range of 70-90ºC in both crude and protein-free purified samples of *L. sativum* mucilage. In addition, their analysis of the thermal behavior revealed a single exothermic peak in the crude sample, whereas the purified samples exhibited two distinct peaks occurring between 255-315 ºC, which were attributed to thermal degradation or destruction [12]. The thermal properties of galactomannans, including *L. sativum* mucilage, are significantly affected by their unique polysaccharide structure. Key factors such as the mannose-to-galactose (M/G) ratio, the molecular weight of the polysaccharide, and the degree of branching play pivotal roles in defining their thermal stability (Karazhiyan et al., 2009) [16]. Specifically, *L. sativum mucilage* is classified as a galactomannan with a notably high M/G ratio of 8.2 and a considerable molecular weight of 540 kDa, as reported by Shahbazizadeh et al. (2021), Razavi et al. (2015), and Karazhiyan et al. (2009) [29,16,15]. The structural characteristics collectively contribute to stable thermal behavior, highlighting the mucilage's potential for various applications in food technology.

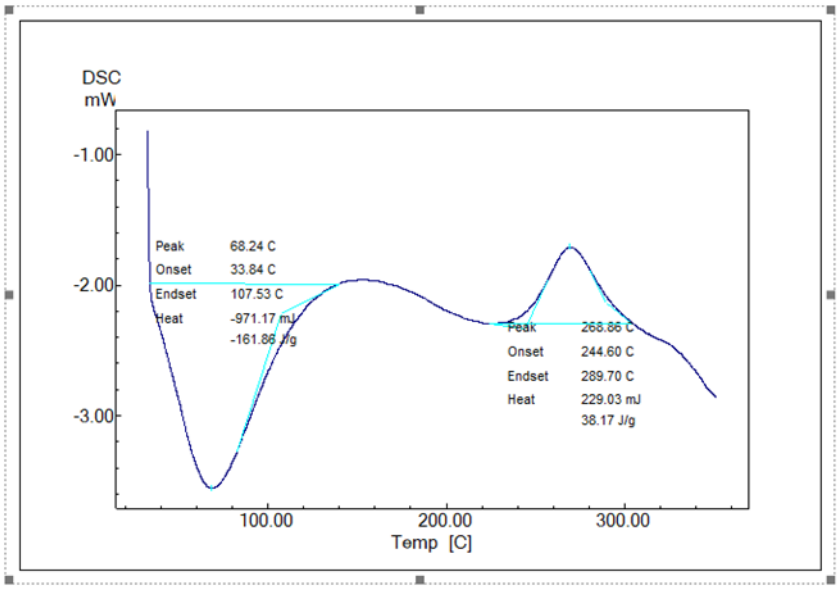


Figure 1 DSC thermogram of Lepidium. sativum seed mucilage

3.2. X-ray Diffraction Analysis (XRD)

X-ray diffraction (XRD) is one of the most precise and established analytical methods for determining and quantifying molecular structures, particularly crystallinity. It is utilized to examine the degree of crystallinity or the amorphous characteristics of materials. The XRD pattern of *L. sativum* mucilage exhibited sharp, well-defined peaks at 20° 2θ, indicating a crystalline structure. The absence of significant broad peaks suggests that the material is not entirely amorphous. Narrow peaks, characterized by a small Full Width at Half Maximum (FWHM), signify larger crystalline domains, while broad peaks indicate smaller crystalline grains or partial amorphous content. Using the Debye-Scherer equation (Debye et al., 2010), the average crystallite size was calculated to be 47.47 nm [30].

Mudgil et al.. (2012) demonstrated that guar gum and partially hydrolyzed guar gum displayed an amorphous structure with crystalline regions at angles (2θ) of 20.2° and 72.5° [31]. Similarly, Hussain et al. (2023) reported that crude guar gum (CGG), purified guar gum (PGG), and acid-hydrolyzed guar gum (AHGG) exhibited amorphous structures with low crystallinity (2θ = 20.2°), while enhanced hydrolyzed guar gum (EHGG) showed slightly higher crystallinity at angles of 20.4°, 40.2°, and 49.5°. Basic hydrolysis significantly improved the crystallinity of basic hydrolyzed guar gum (BHGG), with peaks ranging from 20.5° to 42.8°; a characteristic guar gum peak at 19.94° suggests weak crystallization or an amorphous nature. [32]. Additionally, Falsafi et al. (2020) studied nano-encapsulated food materials and observed that pure Cydonia oblonga mucilage (COM) exhibited a partially crystalline pattern. This pattern showed a diffuse diffraction halo centered at 19.08°, indicating the amorphous component, alongside two sharp peaks at Bragg angles (2θ) of 22.02° and 44.07°, reflecting the crystalline fractions. The presence of such sporadic crystalline lattices may result from extensive hydrogen bonding between the hydroxyl groups of adjacent polymer chains [33].

Crystalline structures have sharp melting points due to the simultaneous breaking of intermolecular interactions when heated. In contrast, amorphous solids lack long-range order, leading to poorly defined XRD patterns and varying intermolecular forces, which cause different regions to soften at different temperatures [34]. As a result, the analysis conducted using Differential Scanning Calorimetry (DSC) supports the findings from the X-ray Diffraction (XRD) results. The DSC analysis revealed two distinct thermal events: the first transition, occurring around 68°C, suggests water interaction, typical for semi-crystalline polysaccharides. The second transition, occurring around 268°C, confirms structural decomposition, indicating the presence of organized crystalline regions within the material.

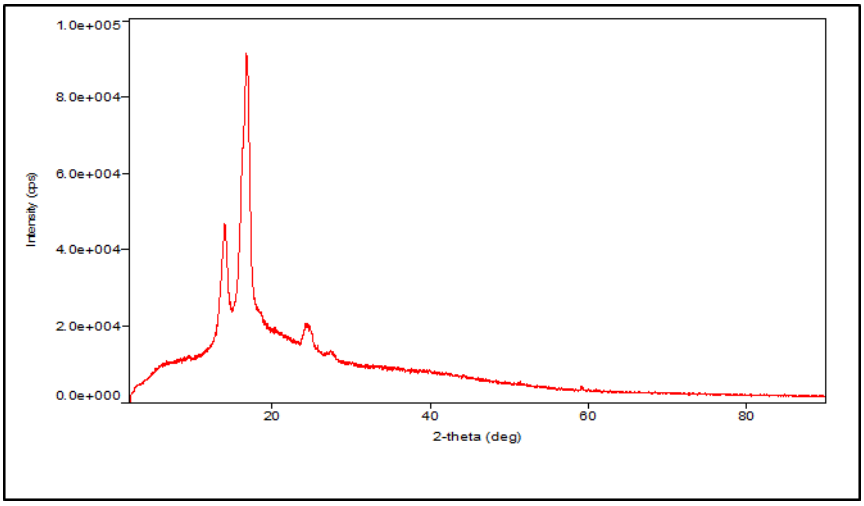


Figure 2 XRD pattern of Lepidium sativum seed mucilage

3.3. Particle Size Analysis (PSA)

A comprehensive particle size analysis was conducted to assess the diameter of pulverized *L. sativum* mucilage, an important biopolymer. The findings revealed an average particle size of 259.89 ± 0.24 nm, indicating a high level of precision in the measurements. As illustrated in Figure 4.5 and summarized in Table 4.10, the particle size distribution was mapped, showing that 25% of the particles are of size 169.68 µm, the median size was 292.50 µm (50%), and 75% of the particles reached a size of 408.64 µm. The analysis demonstrated a diverse range of particle sizes, predominantly between 100 and 500 µm, with a significant concentration in the 300-400 µm range.

In contrast, previous studies have reported varying results regarding the particle sizes of dried mucilage. For instance, Archana et al. (2023) documented a much broader average particle size ranging from 500 to 1000 µm based on microscopic examination [35]. Similarly, Berhe et al. (2023) reported a mean particle size of 182 ± 1.2 µm using sieve testing methods [36]. Previous research by Patel et al. (2010) indicated a particle size of 189.57 µm [37], while Kumar et al. (2012) observed more detailed dimensions, noting a particle length of 1.3674 mm and a breadth of 0.875 mm [38].

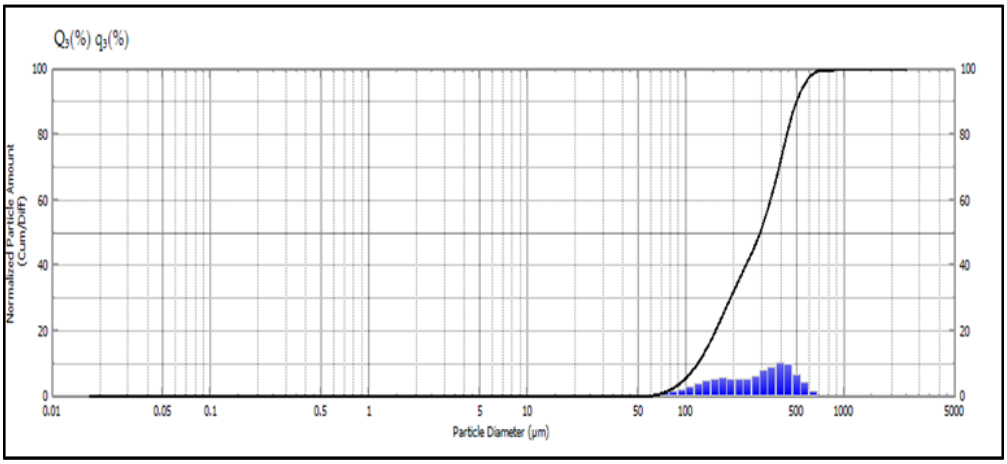


Figure 3 PSA of Lepidium sativum seed mucilage

The polydisperse nature of polysaccharides, such as mucilage, often results in variations in size distribution, primarily due to phenomena like particle aggregation. Larger particles typically suggest hydrocolloid crosslinking, which requires a longer duration for adequate hydration or swelling. In contrast, smaller particles, resulting from mechanical breakdown or enhanced solubility, exhibit rapid swelling behavior, which may influence their functional applications. Additionally, differential scanning calorimetry (DSC) analysis indicated that the mucilage undergoes thermal degradation, classifying the particles as semi-crystalline in nature. This semi-crystalline nature contributes to their strong thermal resistance, enhancing polymeric interactions and promoting effective cross-linking. This assertion is

further supported by X-ray diffraction (XRD) pattern analysis, which suggests that the material has a defined crystalline structure, indicative of its stability and potential applications in various fields.

4. Conclusion

In conclusion, the comprehensive characterization of *Lepidium sativum* seed mucilage using Differential Scanning Calorimetry (DSC), X-ray Diffraction (XRD), and Particle Size Analysis (PSA) provides important insights into *L. sativum* mucilage's thermal stability, crystalline structure, and morphology. DSC analysis showed an endothermic peak at 68.24 ºC for water evaporation and an exothermic peak at 26gar8.86 ºC for thermal degradation, suggesting its potential application in food thermal processing. XRD confirmed the mucilage's crystalline nature with a crystallite size of 47.47 nm. The sharp peaks indicate a predominantly crystalline structure, which enhances its functional properties. This study highlights the potential of *L. sativum* mucilage as a functional ingredient in food technology and encourages further research to explore its applications.

Compliance with ethical standards

Acknowledgments

The authors would like to express their deep gratitude to the Central Instrumentation Facility (CIF) at Jiwaji University in Gwalior for providing essential facilities for this research. They also extend their heartfelt thanks to the late Dr. G.B.K.S. Prasad, Coordinator of the Centre for Ayurvedic Translational Research at Jiwaji University, for his invaluable guidance and support throughout the research process.

Disclosure of conflict of interest

All authors declare no conflict of interest.

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