Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 3
Open Access

Extraction of Starch from Different Natural Sources and their Characterization by Advanced Spectroscopic Techniques

Preeti Yadav[1], A Elphine Prabahar[2,3], Anurag Verma[2]

[1]Research Scholar, Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad-244001, Uttar Pradesh, India.

[2]Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad-244001, Uttar Pradesh, India.

[3]Chettinad School of Pharmaceutical Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam-603103, Tamil Nadu, India

Cite this paper as: Preeti Yadav, A Elphine Prabahar, Anurag Verma (2024) Extraction of Starch from Different Natural Sources and their Characterization by Advanced Spectroscopic Techniques. *Frontiers in Health Informatics, 13* (3),10457-10466

ABSTARCT

*The naturally occurring polymer serves as the principal polysaccharide source for human energy production. The aim of this research was to extract starch polymer as a whole from lentil **(Lens culinaris)** and barnyard millet **(Echinochloa esculenta)**, by using an alkali pretreatment with sodium hydroxide at 25 ºC. Extracted starches were characterized by spectroscopic techniques as Nuclear magnetic resonance (NMR), Mass Spectroscopy (MS). The extraction process was optimized to ensure high yield and purity by employing methods such as water soaking, sedimentation, and filtration. The extracted starch samples were subjected to structural and molecular analysis using Nuclear Magnetic Resonance (NMR) spectroscopy and mass spectrometry (MS). NMR provided insights into the molecular arrangement and amylose-to-amylopectin ratio, while MS revealed the molecular weight distribution and fragmentation patterns, further elucidating the composition of the starch. The NMR confirmed the presence of hydroxyl and carbonyl group. This obtained starch is suitable for various industrial applications, including food, pharmaceutical and biodegradable materials. The findings contribute to the understanding of lentil and barnyard millet starches potential as alternative starch sources with tailored properties for specific applications. This comprehensive characterization underscores the importance of integrating extraction processes with modern analytical techniques to better understand and exploit the potential of natural starches.*

Key words: *Natural polymer, Starch, Lentil, barnyard millet, Spectroscopic techniques*

INTRODUCTION

A variety of monosaccharides are combined to form polysaccharides, which have immunological properties that have anticancer, antiviral, anti-infective, antioxidant, antimutation, or hematopoietic activity [1]. The starch obtained from various natural sources has been utilised as an excipient in a variety of preparations, such as binding agent, gelling properties, thickening agent, and stabilising. in spite, the native starch is characterized by a variety of properties, including a low process tolerance, high retrogradation, a higher viscosity, and weak shear [2] . The research community has been particularly interested in the development of biodegradable polymers in non-food industries, which has led to the birth of unique biodegradable products. In the food making industry, starch is the quite often used as a primary component of human manufactured food products, and it is held accountable for majority of functional and nutritional properties [3].

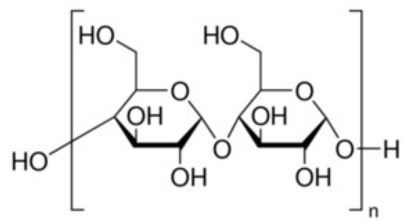


Figure 1 *Structure of Starch*

With the study of comprehensive structural, modification, and chemical based characters of unique polysaccharides', study has improved on the research of their natural origin. The maximum of polysaccharides are now known to be non-toxic and do not have many negative side effects. Because polysaccharides offer the cure of many ailments linked to damage due to oxidation, they can put to use by extending the expiry life of food commodities. Polysaccharides are often used in many industries like food and pharmaceutical industries in the manufacturing of natural food and food additives having fewer side effects[4].

Starch[5] is the main storage agent in form of carbohydrate in plants. It is in plants in the form of as insoluble, half crystalline granules in storage cell tissues such as grains, tubers and roots and to a lower degree vegetative tissues of herbs. Starch is made up of mainly two polymers of D-glucose: first is amylose, a primary unbranched α [1→4] which is associated to glucan, and secondly amylopectin, which is made up of long chains of α [1→4] linked glucoses kept in a main branched structure with α [1→6] forming branching links[5].

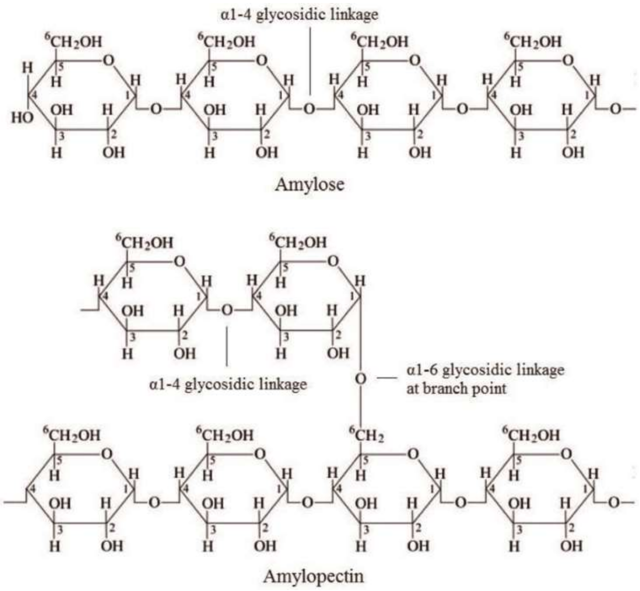


Figure 2 Structure of Amylose and Amylopectin

Lentils *(Lens culinaris)* commonly known as masoor dal, are cultivated on a diverse range of soil types, including silt loam and sand, on an annual basis in semi-arid regions across the globe. China ranks among the foremost nations in lentil crop production. In 2014, lentil production reached 4.82 million tonnes on a global scale. The respective appropriations for China and Asia are 2.59% and 44.3%.[6] Lentils are an abundant source of resistant starch; the concentration of RS in various cultivars ranges from 11.4% to 14.9% [7] It goes by the names split peas, masur, and crimson dal. It is a fine source of nutritional fibers, vitamins, minerals, and complex carbs. Lentils contain 62–64% carbohydrates, the majority of which is starch, and 21–28% protein[89]

In comparison to rice, corn and sorghum, barnyard millet is distinguished by its greater levels of protein, fibre, carbohydrates, and with minute quantities of minerals having including calcium, iron, and phosphorous as its components. However, there is a lack of research on barnyard millet starch, especially surrounding using it as pharmaceutical excipients. [10] Originating in mild and temperate regions of the globe, barnyard millet (Echinochloa esculenta[11]) is a unique millet variety that has been cultivated extensively throughout Asia, specifically in India[12], China, and other south east Asian countries. It's coming at fourth position in most widely cultivated minor millet, ensuring nutritional security for a great number of impoverished individuals throughout the world. On a global scale, India has emerged as the leading producer of barnyard millet, surpassing all other nations in terms of both area and production (0.147 mt), averaging 1034 kg/ha over the past three years[13]

Consequently, the primary aim of this research is to examine the effect of alkali-treated lentil and barnyard millet starch having pharmaceutical applications, with a particular emphasis on its physicochemical parameters. The primary aim of this research is to enhance the comprehension and utilisation of starch in pharmaceutical applications by broadening the knowledge domain and drawing upon prior research.

MATERIALS AND METHOD

The seeds of masoor dal (*lens culinaris*) & Barnyard millet (*Echinochloa esculenta*, commonly known as sanwa rice) were purchased from Patanjali store in buddhi Vihar, Moradabad, India.
All the chemical were analytical grade. Deionised water was utilized in experiment. Sodium hydroxide (NaOH), hydrochloric acid (HCl), ethanol (99.9%) was used. Seed authentication was performed by CSIR-National Institute of Science Communication and Policy Research. Under this Authentication No.- NIScPR/RHMD/Consult/2022/4299-100.The authentication has been done on the basis of macroscopic studies of provided sample followed by comparing the sample with authentic sample kept in the RHMD. The NMR and MS were performed at CDRI-Central Drug research Institute, Lucknow.

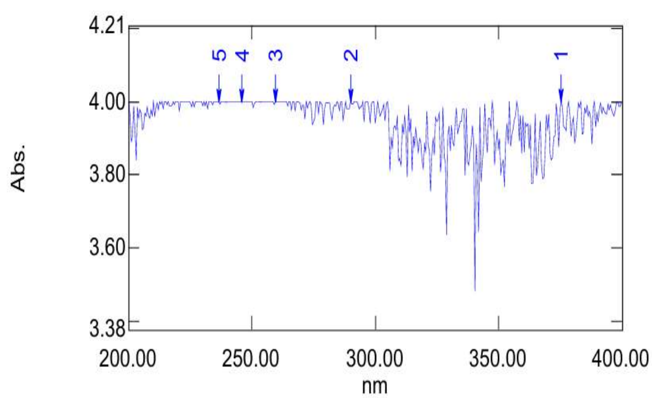
ALKALI EXTRACTION METHOD

With some modifications, starch was extracted using the alkaline extraction method given by (Verma et al., 2018), involved many steps. In this process, Lentil and millet were soaked (25gm) in 100 ml of 0.3% w/v NaOH solution, it was agitated for 30 minutes and stored at 4°C for 24 hours. The yellowish uppermost layer was eliminated. Following the grinding of lentil and rice in to a fine paste separately. Then suspend the lentil and rice paste in a hydrochloric acid (HCl) solution. This suspension incubated at a moderate temperature (40°C) for several hours. The resulting suspension was centrifuged to separate the starch from the other components. After rinsing the sediment with a water to remove any residual hydrochloric acid or other impurities, dry in a convection oven at 35–40 C, the resulting slurry of starch was passed through a sieve with care using a mortar and pestle before being stored in an airtight container for future experiments.

RESULT AND DISCUSSIONS

UV absorption spectra

Absorption of UV light between the range of 300-350 nm indicates the presence of phenolic structures, flavonoids and functional groups.



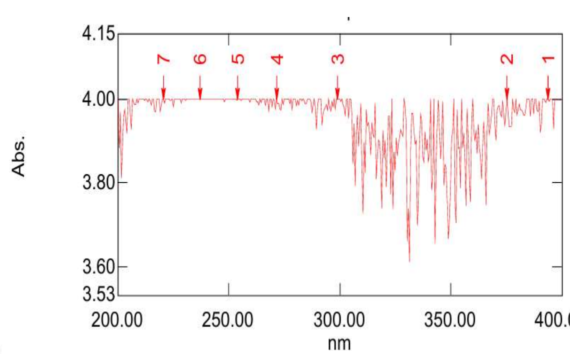


Figure 3 UV Absorption spectra of *Lens culinaris* and *Echinochloa esculenta*

1H-NMR Spectra

1H-NMR spectroscopy can be used as an easy tool for analysis of chemically modified starches both for phosphorylated and carboxymethylated.

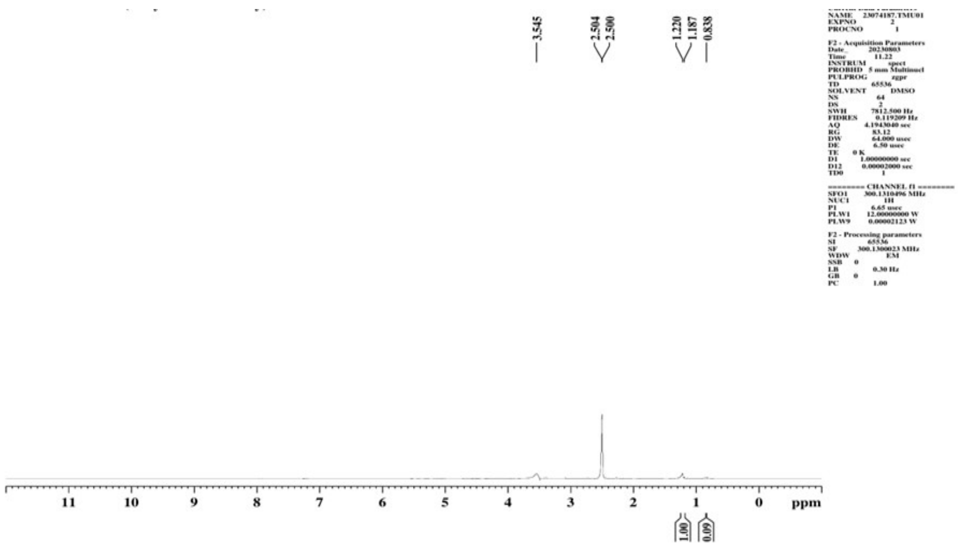


Figure 4 NMR of *Lens culinaris*

There are observed peaks at chemical shift 1.22ppm, 2.54ppm and 3.55ppm with possible peaks 0.88ppm. The presence of a peak near 3.55 ppm indicates the functional group (-OH) or (-O-) group. Carbonyl group present near 2.54 ppm peak.

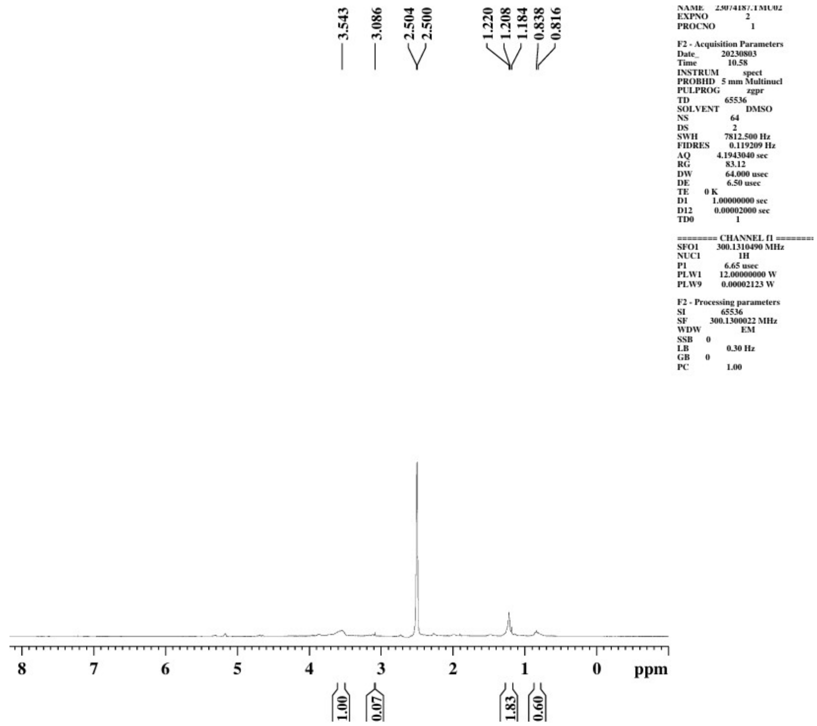


Figure 5 NMR of *Echinochloa esculenta*

There are observed peaks at chemical shift 2.5 ppm, 1.20 ppm and 1.18 ppm with possible peaks 0.86 ppm. The presence of a peak near 2.504 ppm indicates the functional group (-OH) or (-O-) group. Carbonyl group present near 2.50 ppm peak.

Mass Spectra

Each peak represents an ionized molecule or fragment. Peaks can be matched with theoretical or known compounds in Lens culinaris to identify proteins, lipids, or metabolites. High m/z values (above 1000) are likely associated with proteins or large peptides. Peaks in the lower m/z range (below 600) might correspond to small metabolites or specific amino acids. Clusters and specific spacing between peaks could provide insights into fragmentation pathways or isotopic distributions.

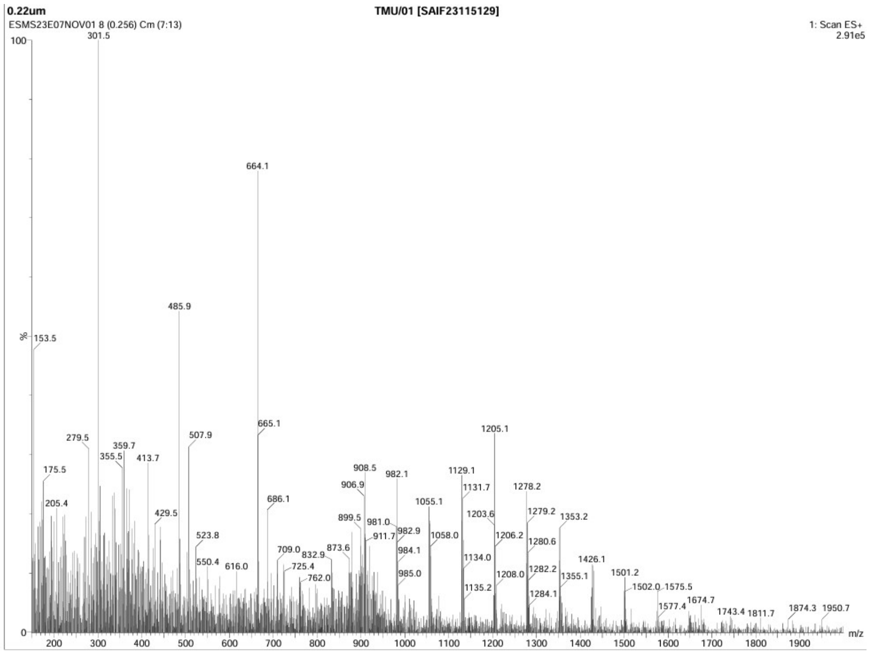


Figure 6 Mass spectra of *Lens culinaris*

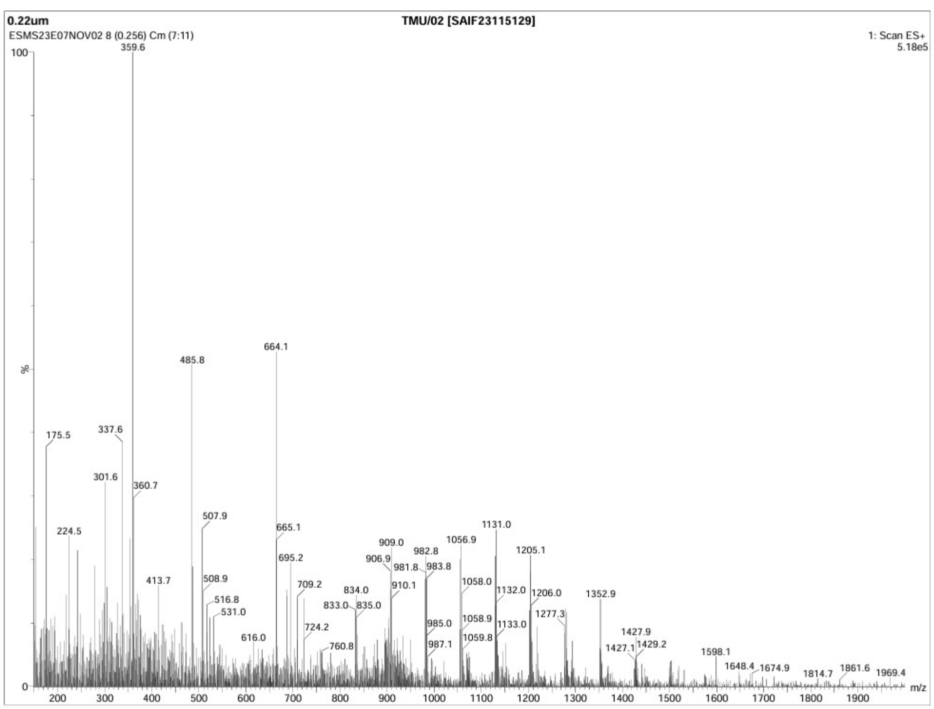


Figure 7 Mass spectra of *Echinochloa esculenta*

Peaks at m/z 337.6, 359.6, 413.7, and 664.1 are notable. These may correspond to specific metabolites or molecular fragments present in the sample.
Larger peaks at m/z 1131.0, 1277.3, and 1427.9 likely indicate higher-mass compounds or complex structures, potentially representing intact molecules or larger fragments.

The extraction of starch from *lens culinaris* and *Echinochloa esculenta* sources has done by a similar process i.e. alkali extraction. The extraction process involves soaking, grinding and sieve to separate the starch granules from other components like protein and fibres.

1. Yield of Starch from Natural Sources

Starch was extracted from *lens culinaris* and *Echinochloa esculents*. The yield of starch was 55-60% and 70-75% respectively, it varied depending on the source, reflecting differences in starch content and extraction efficiency. In Corn, yield was approximately 60-70% of the dry weight, potato around 75-85%, rice about 50-65%, Cassava ranging from 80-90%.
The differences in yield are being related to the starch concentration in the plant material and the ability of the extraction process as a whole, including disruption of the cell matrix and removal of non-starch components.

2. Mass Spectroscopy Analysis

Mass spectroscopy was employed to confirm the molecular weight distribution and fragmentation pattern of starch molecules. The analysis revealed a range of molecular ion peaks corresponding to amylose and amylopectin fragments. For instance:
Amylose: Peaks in the range of m/z 180-900, indicating glucose units and small oligosaccharides. Amylopectin: Peaks beyond m/z 1000, corresponding to larger branched structures. The fragmentation primarily involved cleavage of glycosidic bonds, resulting in characteristic fragments such as glucose (m/z 180), maltose (m/z 342), and higher oligomers.
The mass spectra confirmed the presence of both constituents like amylose and amylopectin, with alterations variations in their concentrations varying on the source used.

3. NMR Spectroscopy Analysis

Nuclear Magnetic Resonance (NMR) spectroscopy provided detailed structural insights into the starch samples, focusing on the ${}_1H^1$ spectra. Peaks in the region of 4.5-5.5 ppm were observed, corresponding to anomeric protons of the glucose units. Broader signals indicated the semi-crystalline nature of starch, with differences in peak intensity reflecting amylose-to-amylopectin ratios.

4. Crystallinity and Functional Properties

The semi-crystalline nature of starch was further corroborated by the NMR linewidths and fragmentation patterns observed in mass spectroscopy. Differences in crystallinity could influence functional properties such as gelatinization and retrogradation, which are critical for industrial applications.

CONCLUSION

The current investigation has concentrated on the characterization of *lens culinaris* and *Echinochloa esculenta* starch in order to investigate its pharmaceutical applications. The NMR spectra have also confirmed the effective substitution of hydroxyl groups of starch molecules with carboxyl group (CO). Mass spectroscopy and NMR spectroscopy proved to be effective tools for distinguishing the structural and molecular features of starch, offering valuable insights for tailoring starch applications in food, pharmaceutical, and industrial sectors. Additionally, the findings suggested applications of the functional properties of these starches, thereby increasing their value beyond conventional applications and rendering them appropriate for specific applications. Their properties are characteristics, and extraction yields provide them competitive with other well-known starch sources. The way forward is to now chemically modify them with different groups such as hydroxylation, carboxymethylation and succinylation. The chemically modified starches will have another impact on their properties which still has to be studied upon.

REFERENCES

(1) Safdar, B.; Pang, Z.; Liu, X.; Jatoi, M. A.; Mehmood, A.; Rashid, M. T.; Ali, N.; Naveed, M. Flaxseed Gum: Extraction, Bioactive Composition, Structural Characterization, and Its Potential Antioxidant Activity. *J Food Biochem* **2019**, *43* (11). https://doi.org/10.1111/jfbc.13014.

(2) Malik, M. K.; Kumar, V.; Singh, J.; Bhatt, P.; Dixit, R.; Kumar, S. Phosphorylation of Alkali Extracted Mandua Starch by STPP/STMP for Improving Digestion Resistibility. *ACS Omega* **2023**, *8* (13), 11750–11767. https://doi.org/10.1021/acsomega.2c05783.

(3) Pérez-Pacheco, E.; Moo-Huchin, V. M.; Estrada-León, R. J.; Ortiz-Fernández, A.; May-Hernández, L. H.; Ríos-Soberanis, C. R.; Betancur-Ancona, D. Isolation and Characterization of Starch Obtained from Brosimum Alicastrum Swarts Seeds. *Carbohydr Polym* **2014**, *101*, 920–927. https://doi.org/10.1016/j.carbpol.2013.10.012.

(4) Kaseem, M.; Hamad, K.; Deri, F. Thermoplastic Starch Blends: A Review of Recent Works. *Polymer Science Series A* **2012**, *54* (2), 165–176. https://doi.org/10.1134/S0965545X1202006X.

(5) Copeland, L.; Blazek, J.; Salman, H.; Tang, M. C. Form and Functionality of Starch. *Food Hydrocoll* **2009**, *23* (6), 1527–1534. https://doi.org/10.1016/j.foodhyd.2008.09.016.

(6) Ma, Z.; Yin, X.; Hu, X.; Li, X.; Liu, L.; Boye, J. I. Structural Characterization of Resistant Starch Isolated from Laird Lentils (Lens Culinaris) Seeds Subjected to Different Processing Treatments. *Food Chem* **2018**, *263*, 163–170. https://doi.org/10.1016/j.foodchem.2018.04.122.

(7) Perera, A.; Meda, V.; Tyler, R. T. Resistant Starch: A Review of Analytical Protocols for Determining Resistant Starch and of Factors Affecting the Resistant Starch Content of Foods. *Food Research International* **2010**, *43* (8), 1959–1974. https://doi.org/10.1016/j.foodres.2010.06.003.

(8) Bhatty, R. S. Composition and Quality of Lentil (Lens Culinaris Medik): A Review. *Canadian Institute of Food Science and Technology Journal* **1988**, *21* (2), 144–160. https://doi.org/10.1016/S0315-5463(88)70770-1.

(9) Khazaei, H.; Subedi, M.; Nickerson, M.; Martínez-Villaluenga, C.; Frias, J.; Vandenberg, A. Seed Protein of Lentils: Current Status, Progress, and Food Applications. *Foods* **2019**, *8* (9), 391. https://doi.org/10.3390/foods8090391.

(10) Bhatt, P.; Kumar, V.; Rastogi, H.; Malik, M. K.; Dixit, R.; Garg, S.; Kapoor, G.; Singh, S. Functional and Tableting Properties of Alkali-Isolated and Phosphorylated Barnyard Millet (Echinochloa Esculenta) Starch. *ACS Omega* **2023**, *8* (33), 30294–30305. https://doi.org/10.1021/acsomega.3c03158.

(11) Sood, S.; Khulbe, R. K.; R., A. K.; Agrawal, P. K.; Upadhyaya, H. D. Barnyard Millet Global Core Collection Evaluation in the Submontane Himalayan Region of India Using Multivariate Analysis. *Crop J* **2015**, *3* (6), 517–525. https://doi.org/10.1016/j.cj.2015.07.005.

(12) Renganathan, V. G.; Vanniarajan, C.; Karthikeyan, A.; Ramalingam, J. Barnyard Millet for Food and Nutritional Security: Current Status and Future Research Direction. *Front Genet* **2020**, *11*. https://doi.org/10.3389/fgene.2020.00500.