

Research

Impact of sprouting process on pectin content of ragi seed (*Eleusine coracana* L. Gaertn): isolation and characterization

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Abstract

The study aimed isolation and characterization of pectin from Ragi seeds. The isolation was carried from both sprouted and non-sprouted Ragi seeds. Sprouting of Ragi seeds significantly enhanced pectin yield producing 15–18% more pectin as compared to non-sprouted seeds (4–8%) yield. Isolated pectin was then tested for physico-chemical characters like hygroscopicity, DE, molecular weight, swelling ability, FTIR, DSC, XRD etc. The results indicated non-hygroscopic nature of pectin ideal for use in dry food products and pharmaceutical powders to avoid degradation. The 61% degree of methyl esterification is a mark of good gelling activity for jams, jellies and as an emulsifier. Further 827.6 Da of molecular weight was determined using mass spectrometry. The O–H, C–O, and C–H stretching peak of pectin and the presence of uronic acid was identified by FTIR. Glass transition temperature (T_g) was noted to be 46.81 °C and crystallization temperature (T_c) were observed with an onset of 241.27 °C and end set of 247.29 °C indicating stabilization of structure in the form of crystalline domain. The moderate T_g reflects thermal stability under processing and storage conditions, critical for maintaining functionality in high-temperature food and drug manufacturing processes. XRD and DSC indicate the amorphous nature of pectin. These findings suggest that sprouted Ragi seeds are a promising source of pectin making it suitable for multiple applications like dispersion in sauces and syrups, confectionaries and also as controlled drug release in pharmaceutical formulations like tablets or hydrogels.

Keywords Finger millet seeds · Sprouting · Pectin · Characterization · Food · Pharmaceutical industries

1 Introduction

Pectin is a group of skeletal hetero-polysaccharides present in the middle lamella and primary cell walls of higher plants. Pectin also contributes to the development, morphology, and defense of plants. Pectin contains a backbone of 1,4-linked d-galacturonic acids, an anionic polysaccharide. Chemically, pectin is a neutral methoxy ester of pectic acid. Pectin plays

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a role in intercellular bonding and the cell wall stiffness. Additionally, these substances offer turgidity and robustness, which are essential for the proliferation of plant cell. Besides this, pectin utilized as a swelling, stabilizing, emulsifying, and gel-forming ingredient in food and pharma industries [1]. Pectin also used as dietary fibre and identified as a possible prebiotic with potential therapeutic advantages for gastrointestinal bacteria regulation. Pectin has also been employed for medical and pharmaceutical applications, including as drug delivery, and as a carrier for bioactive release or controlled substances [2].

Eleusine coracana L. Gaertn, commonly referred to as ragi seed in the Indian subcontinent, is a member of the Paniceae tribe and is a species of the genus *Eleusine*. Ragi seed contained proteins (6–13%), fiber (18%), polyphenols (0.3–3%) and calcium (0.38%) [3]. Ragi seeds also contained a blend of starch and non-starch polysaccharides (pectin). The germination process significantly reduced antinutrients such as tannin, phytic acid, and oxalic acid, and blocked trypsin activity while increasing the quantity of protein, minerals, and dietary fibers. Sprouts are "the product derived from the seed germination process and their propagation in water or another medium, harvested before the formation of true leaves and which is intended to be eaten whole, including the seed," according to Regulation (EC) No 208/2013 [4]. Ragi, or finger millet, is a nutritional powerhouse with remarkable health benefits. Known for its high calcium content, ragi supports bone health and prevents osteoporosis, making this food ideal for people of all ages. The low glycemic index ensures a slow release of glucose, thus facilitating blood sugar regulation and making it highly useful for diabetic patients. Ragi is also very rich in iron, which fights anemia and its high dietary fiber content aids digestion, prevents constipation, and helps manage weight. Antioxidants like polyphenols and phytochemicals make ragi effective in reducing oxidative stress, lowering cholesterol levels, and preventing chronic diseases such as cardiovascular issues and certain cancers [3]. Additionally, its gluten-free nature makes it suitable for individuals with gluten intolerance. Ragi's versatile profile, from enhancing metabolism to boosting immunity, underscores its role as a superfood with outstanding benefits for overall health and well-being.

During germination, various indigenous enzyme activity increases. In this process, pectin methyl esterase hydrolyzes the methyl ester groups in the cell wall of pectin. Further, the polygalacturonic chain of pectin is hydrolyzed by pectinases thereby altering the pectin matrix and creating a leaching-out effect. Considering these chemical modifications during germination, we hypothesized that sprouting of ragi seeds could enhance the extraction yield of pectin from the seed. Therefore, this study investigated the effect of sprouting on pectin extraction from sprouted ragi seeds. The characteristics of isolated pectin were confirmed by physicochemical analyses, FTIR, and thermal analyses.

2 Material and methods

2.1 Raw materials

The finger millets (Ragi seeds) were purchased from the local market in Nashik, Maharashtra, India and verified by the Department of Botany, Botanical Survey of India, Pune, Maharashtra, India (Report No: BSI/WRC/100-1/Tech./2020). According to the authentication findings, the grain specimen corresponded to *Eleusine coracana* (Ragi) [Family-Poaceae] seeds. The millets were washed and cleaned using tap water for any foreign particles. The air-dried millets were subjected to a sprouting process.

To perform chemical analyses analytical reagent grade chemicals and reagents were used until and unless mentioned elsewhere.

2.2 Ragi seed sprouting

The seeds (250 g) were steeped in enough water to adequately cover them for eight hr at room temperature. After 8 h, the remaining water from the seeds was filtered out, and the seeds were partially dried [5]. Unbroken and undamaged steeped seeds were chosen, and they were germinated in two replications for 24 h at room temperature in an Amiraj sprout machine (Amiraj Industries, product dimension: 18.5 cm × 18.5 cm × 10 cm). Before sprouting, the dried seeds were weighed. After 24 h, sprout properties were assessed [6]. Measurement of Ragi seed sprout characteristics was performed by analyzing the hypocotyl length. The initial root's origination points and the cotyledons were used to measure the length. A total of 20 samples were chosen at random to measure the hypocotyl length. Three levels of sprouts were

identified: high-quality (HQ), average-quality (AQ), and low-quality (LQ). The total fresh weight of both high-quality and average-quality sprouts was divided by the samples' dry weight to determine the sprouting yield in percentage.

2.3 Isolation of pectin from sprouted ragi seed

The sprouted seeds were ground to obtain the ragi powder. The sprouted ragi powder was then passed through sieve number 100 to obtain uniform particles. A 20 g sprouted seed powder was added to 200 mL of distilled water. Water-soluble polysaccharides and hot water-soluble polysaccharides were isolated from the solution. For the acid extraction of pectin, 100 mL of a 0.5% solution of EDTA was applied to the residue that was free of normal water and hot water-soluble polysaccharides [7]. EDTA was selected as a chelating agent and pH-adjusting agent (pH 4–5). After that, the mixture was incubated for 30 min at 85 °C. The solution was then centrifuged at 500 rpm for 5 min to segregate residue and supernatant. The supernatant was collected and concentrated to a volume of 20 mL using a water bath. The concentrated solution was then treated with ethanol in a ratio of 1:3 (concentrated supernatant: ethanol) to obtain precipitate [8]. After filtration, the precipitate was dried in a hot-air oven (DDIR 101 Universal hot air oven) at 40 °C. Figure 1 depicts the extraction and isolation of pectin from sprouted ragi seeds. The percentage yield was calculated using Eq. 1.

$$\text{Pectin} = \frac{\text{Pectin weight in dry form (g)}}{\text{Amount of powder used for extraction (g)}} \times 100 \quad (1)$$

2.4 Physicochemical characterization

2.4.1 Gelling test for pectin

Two gelling tests were conducted to confirm the gelling ability of isolated pectin. First, 1 g of pectin and 10 g of water were combined and heated to boiling [9]. After cooling, gel formation was observed. The second test involved combining 5 mL of a 1% pectin solution with 1 mL of a 2% KOH/NaOH solution. After 15 min at room temperature, HCl was added to the mixture and shaken to observe any gelling [10].

2.4.2 Hygroscopicity

Pectin (100 mg) was weighed on tared glass petri plates which were then placed into desiccators at ambient temperature conditions. Pectin was retrieved from desiccators after being stored for 24 h, and each sample's final mass was calculated using a calibrated analytical balance. (Make and model: Arkeys scientific instrument ABT 100, 5-NM). The percentage weight gain of the samples was determined using Eq. 2. The specimens are then classified according to their degree of hygroscopicity using the European Pharmacopoeia classification system [11].

$$\text{Increased mass (\%)} = \frac{M3 - M2}{M2 - M1} \times 100 \quad (2)$$

Where, M1 = Mass of empty Petri-plate, M2 = Mass of Petri-plate and sample (Initial), M3 = Mass of Petri-plate and sample (After 24 h).

The outcome was explained as follows:

Deliquescent: A liquid is formed after absorbing enough water,

Very hygroscopic: Mass gain equal to or greater than 15%,

Hygroscopic: The mass increase is less than 15% and equal to or larger than 2%;

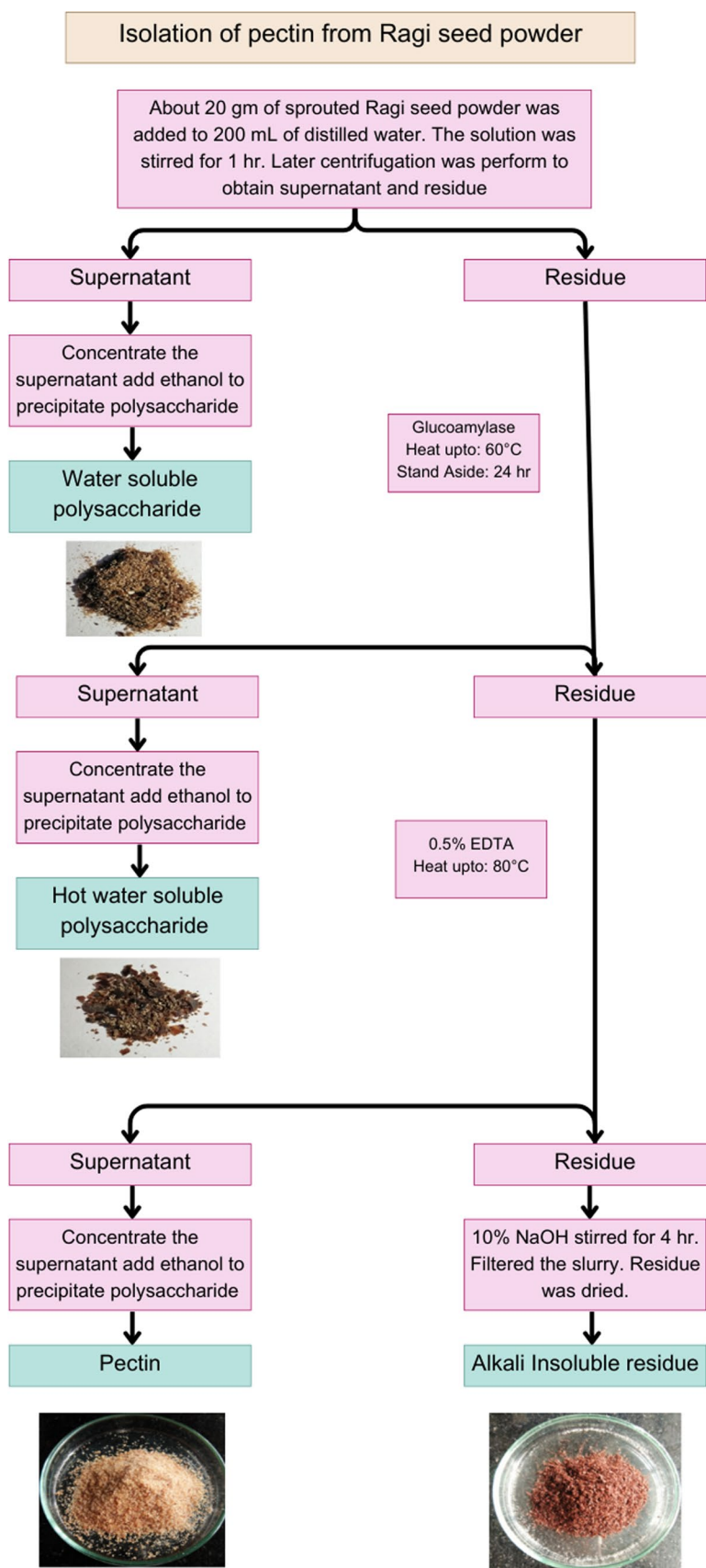
Slightly hygroscopic: This means that the mass increase is less than 2% and equal to or greater than 0.2%

Non-hygroscopic: 0% change in mass is observed.

2.4.3 Water absorption index

A 0.5 g isolated pectin (dry weight) was added to 75 mL of distilled water. After stirring for an hour, the residual gel was centrifuged at 3000 rpm for 10 min and weighed. The weight discrepancies between the supernatant and

Fig. 1 Sophisticated process for the isolation of pectin from sprouted ragi seeds



distilled water were initially used to measure the amount of bound water. Then the amount of water absorbed by the sample was determined using Eq. 3. [12].

$$\text{Water absorption index} = \frac{\text{Bound water (g)}}{\text{Weight of sample (g)}} \quad (3)$$

2.4.4 Swelling index

The extracted pectin (1 g) was added to 50 mL of water and boiled at 90 °C in a water bath for 1 h while constantly stirring. The suspension was chilled, and the weight of the sediments was calculated after centrifugation for 10 min at 3000 rpm [13]. The swelling index of the sample was calculated using Eq. 4.

$$\text{Swelling Index} = \frac{\text{Weight of swollen sediment}}{\text{Weight of dry isolate}} \quad (4)$$

2.4.5 Degree of methyl esterification

The degree of methyl esterification (DM) of isolated pectin was conducted using the titration method as described by Yu et al. [14] Initially, 500 mg of pectin was treated with an HCl–ethanol solution to eliminate organic acid impurities. Subsequently, the refined pectin was titrated with 0.1 M NaOH, using phenolphthalein as an indicator. The required volume of NaOH during the titration was recorded as V1. Later, the saponification of pectin was conducted by the addition of 20 mL of 0.5 M NaOH. After the reaction, 20 mL of 0.5 M HCl was added and titrated with 0.1 M NaOH (V2). The degree of methyl esterification was calculated using Eq. 5.

$$DM = \frac{V2}{V1 + V2} \times 100\% \quad (5)$$

2.4.6 Weight-average molecular weights

The molecular weight of isolated pectin was determined using Waters, Q-TOF Micromass 152 mass spectrometer operated in electrospray positive (ES+) ionization. A pectin sample of 20 µL was injected at 0.574 18 mL/min flow rate and a response spectrum was obtained.

2.5 Structural characterization

2.5.1 Fourier-transform infrared spectroscopy analysis

The FTIR spectra of pectin were captured using a Fourier Transform Infra-Red Spectrophotometer (SHIMADZU, Japan). The sample was previously blended and completely combined with KBr at a ratio of 1:5. The scans were obtained with a resolution ranging from 4000 to 400 cm⁻¹ after the potassium bromide plates had been fabricated.

2.5.2 X-ray diffraction analysis

XRD analysis of isolated pectin was conducted using an X'pert-PRO High-Resolution Powder Diffractometer (PANalyticals, Almelo, Netherlands) fitted with a diverging beam and a scintillation counter detector. A Cu Kα⁻¹ radiation source with a wavelength of λ 1.5418- and 10-mm slits covering a range of 5–80° 2θ was used in this beam. At a temperature of 25 °C, X-ray diffraction data were gathered and scanned for 20 s at a time, using a step size of 0.0170 2θ.

2.5.3 Thermal analysis (Differential scanning calorimetry, DSC)

A differential scanning calorimeter (PerkinElmer 4000) was used to measure the thermal property of isolated pectin as described by Zhu et al. [15] with a slight modification. An aluminum crucible was filled with 1 mg of pectin sample and then it was instantly sealed. The PerkinElmer 4000 equipment was utilized, and PYRIS Version 11.1.0.0488, 2009, PerkinElmer, Inc. software was used for examination. A flow rate of 20 mL/min was maintained while nitrogen was being purged. The scanning range was kept between 30–350 °C with a rate of 10 °C/min.

2.5.4 Statistical analysis

All experiments were conducted in triplicate and the results were presented as mean $\pm$ SD. The data on the XLSTAT program (Microsoft Excel) were analyzed using a one-way analysis of variance (ANOVA). At a difference threshold of 0.05, the post hoc Tukey's and Duncan's Multiple Range tests were used to compare means.

3 Results and discussion

3.1 Ragi seed sprouting characteristics and pectin extraction

As illustrated in Fig. 2, sprouted seeds were divided into three categories based on their quality. HQ sprouts had vigorous, long hypocotyls (1.5 cm), AQ sprouts had short hypocotyls (1 cm), and LQ sprouts had 0.4 cm hypocotyls. There were 59.4%, 30.3%, and 10.3% of high-, average-, and low-quality sprouts, respectively. High and average sprout was selected for further experiments. Hence, the sprout yield of 89.7% and the sprout hypocotyl length of 1.5 ± 0.1 cm were considered. Light and phytohormones are examples of internal and external stimuli that have a substantial impact on hypocotyl elongation and conversion to plant. The seedling depends on the parent sporophyte's stores until it can support itself nutritionally. Some seeds use their cotyledons, which subsequently grow in the light to become the plant, to absorb nutrients from reserves [16]. If grown for an extended period, the sprout starts to consume the nutrients that were stored in the grain, causing the grain's nutrient content to decrease). Therefore, it is crucial to remove the sprouts after the sprouting process is complete.

The pectin was extracted from sprouted ragi seed powder and the percentage yield was reported in the range of 15 – 18% (Table 1). On the other hand, pectin extracted from non-sprouted ragi seed was found to be 4 – 8%. This indicated that the sprouting process increases the leaching effect of pectin through various biochemical reactions.

Fig. 2 **A** High-quality (HQ) sprouts with 1.5 ± 0.1 cm long hypocotyls, **B** Average-quality (AQ) sprouts with 1 ± 0.1 cm long hypocotyls, **C** Low-quality (LQ) sprouts with 0.4 ± 0.1 cm long hypocotyls, **D** Group of decayed seeds and seedlings



Table 1 Sprout yield, pectin yield, water absorption, and swelling index of pectin

Parameter	Results (Mean ± SD)
Sprout Yield (%)	89.63 ± 0.12
Pectin Yield (%)	17.16 ± 0.58
Hygroscopicity	0
Water Absorption (g/g)	11.82 ± 0.01
Swelling Index (g/g)	8.16 ± 0.01
Degree of Methyl Esterification (%)	61.5 ± 0.2

3.2 Physicochemical characterization

3.2.1 Hygroscopicity

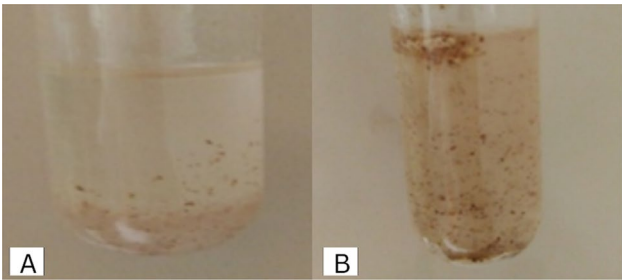
The capacity of a material to adsorb moisture from its surroundings is known as hygroscopicity. It is commonly known that any food ingredient's hygroscopic nature has an impact on how the substance behaves at multiple phases of the production process, including manufacturing, packaging, warehousing, as well as shipping. It also significantly affects the effectiveness, equilibrium, and appeal of the item. Therefore, it is crucial to identify this parameter in every isolated sample. The hygroscopicity value of isolated ragi seed pectin was 0%. According to EP, compounds representing 0% hygroscopic value are considered non-hygroscopic [11]. The non-hygroscopic nature of pectin suggests ease of handling of the component in various environmental circumstances throughout the process and storage.

3.2.2 Gelling properties and water absorption Index

Positive gelling results for pectin were found in both tests. In the first experiment, isolated pectin showed stiff gel formation in the solution. The second test confirmed the initial brownish translucent gel formation followed by thick, gelatinous, colorless precipitate upon heating (Fig. 3). It indicates the gelling capacity of the isolated pectin and the texture of dosage systems. The pectin KOH test, demonstrating gel formation upon cooling, holds significance for formulation and development, as like as carbopol [17]. The results aid in optimizing formulations for controlled release or targeted delivery systems where pH sensitivity is crucial. The ability of pectin to form a gel under acidic conditions has implications for its role in physiological processes. By forming a gel in acidic conditions, pectin-based polymers could potentially aid in gastrointestinal protection or drug delivery systems designed to release active ingredients in specific regions of the digestive tract [18].

A water absorption index of isolated pectin was determined as 11.83 g/g. This number suggests that isolated pectin can be added to aqueous formulations, particularly those that handle dough and seek an increase in unit yield. To obtain high-quality gel, a material water-holding capacity must be high. One of the physical characteristics of soluble dietary fiber that influences nutrient digestion and absorption is water-holding capacity. This can form a gelatinous suspension and can prevent the stomach from absorbing lipids and glucose [19]. Because of this, isolated ragi pectin tends to have numerous possibilities as an excipient in nutraceuticals.

Fig. 3 **A** Test 1 with stiff gel at the bottom, **B** Test 2 thick, gelatinous, colorless precipitate



3.2.3 Swelling index

The swelling index of pectin isolated from sprouted ragi seed was 8.17 g/g. Different polymers require a fluid carrier (solvent) to allow the chemical changes into the network which is known as fluid-induced swelling. The gel is commonly referred to as a hydrogel when the fluid is made up of water. To increase the swelling qualities of pectin-based hydrogel preparation and turn them into super absorbents, pectin's swelling property plays a crucial role. The swelling index of a polymer is used in many drug delivery systems to understand how the medication is released into the body in bioavailability and biotransformation studies [20]. Pectin polysaccharide is utilized because of its capacity to create reservoirs or structural frameworks as well as its potential mucous adhesive and swelling properties. This could help to effectively control the pace at which drugs are delivered. The swelling index of *Ficus carica* fruit was found to be 450 depicting *Ficus carica* pectin is an ideal super disintegrate [10].

3.2.4 Degree of methyl esterification and molecular weight

The degree of methyl esterification (DM) is defined as the molar percentage of carboxyl groups esterified with the methyl group. The DM of isolated pectin was calculated as $61.5 \pm 0.2\%$, which indicates the high methoxy content of the sample. The DM influences the gelling, stabilization, and thickening of pectin. Furthermore, DM specifically governs the conditions and mechanisms underlying gel formation [14]. The *Citrus sinensis* shows DE 78.3% whereas *Citrus nobilis* shows DE 6.95% [8]. The degree of esterification of *Ficus carica* fruit range was between 55.6—65.7% [10].

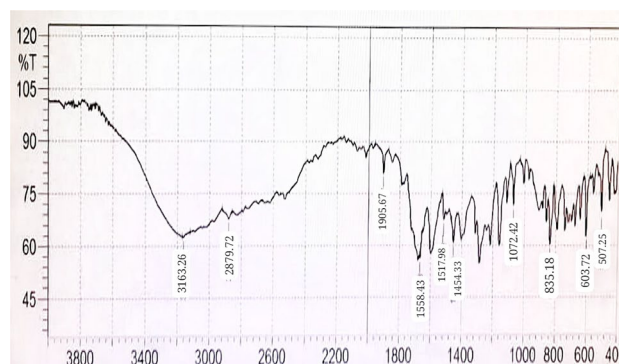
The molecular weight of pectin can be estimated by identifying the ion with the highest m/z value. In this case, the highest m/z value was noted at 827.6. This represents the molecular ion $[M+H]^+$, where M is the molecular weight of the pectin. Therefore, the molecular weight of pectin isolated from ragi seeds was 827.6D.

3.3 Structural characterization

3.3.1 FTIR

The pectin FTIR spectra exhibit a prominent absorption peak at 3163 cm^{-1} that appears like stretching of O–H because of the hydrogen-linked hydroxyl groups. The C–H stretching vibrations of CH_2 groups produce medium-wavelength waves around 2879 cm^{-1} (Fig. 4). It is well known that frequencies ranging between 3200 and 2600 cm^{-1} are connected to (C–H) stretching vibrations [21]. Broadband (O–H) stretching vibrations overlap the bands, making it difficult to ascribe specific (C–H) stretching modes to methyl and methylene groups or the pyranose ring of pectin [22]. In general, stretched (CO) and (O–H) vibrations fall as a result of hydrogen bonding interactions. This demonstrates that intramolecular and intermolecular hydrogen bonding stabilizes pectin structures [22]. The bending modes of H–C–H, and C–H were responsible for the peaks at 1658 cm^{-1} and 1517 cm^{-1} . The presence of O–H, C–O, and C–H represents the presence of the carboxyl group in pectin [15]. In the research described here, the strong intensity characteristic band at about 1072 cm^{-1} in the FT-IR spectra of pectin was primarily linked to the C–O stretching vibrations (Fig. 4). Furthermore, the C–O–C bridge vibrations that are typical of polysaccharides are principally responsible for the moderately powerful bands in the "fingerprint" region below 835 cm^{-1} [23]. Hence the presence of spectrum related to particular stretching confirmed the polysaccharide nature of the compound.

Fig. 4 FTIR spectra of isolated pectin



The FTIR spectra in the region of 1200 to 850 cm^{-1} indicated the presence of pectic polysaccharides [24]. Further, Coimbra et al. [25] reported that the peak between 1110 and 1018 cm^{-1} indicates the presence of uronic acid. Similarly, the FTIR spectra of isolated pectin showed an intense peak at 1072 cm^{-1} (Fig. 4) which represents the presence of uronic acid in the sample. In the context of pectin, uronic acid units are integral components of its backbone, contributing to its anionic nature. The interpretation of the presence of uronic acid in isolated ragi seed pectin is, therefore, essential for understanding and harnessing its functional characteristics in practical applications.

3.3.2 X-ray diffraction analysis

The X-ray diffraction (XRD) analysis of the ragi pectin elucidates its crystalline structure through the identification of distinct peaks as observed in Fig. 5A. The first peak, observed at 13.28° 2-theta, exhibits a relatively narrow Full Width at Half Maximum (FWHM) of 4.116°, indicative of a well-defined crystalline structure. It was an indication of temperature-specific structure stabilization through the exothermic crystallization domain observed after heating the substance beyond the glass transition temperature. It is also supported by exothermic down in the DSC thermogram as shown in Fig. 5B. The intense intensity of 585.67 counts and sharp peak shape, characterized as a Split pseudo-Voigt, suggest a pronounced crystalline presence. The second peak, positioned at 19.90° 2-theta, displays a broader FWHM of 3.432° but with a substantially higher intensity of 2123.68 counts. This peak, exhibits a Split pseudo-Voigt peak shape, indicating potential structural complexities. The Pseudo-Voigt function serves as an approximation to the Voigt function, which, in turn, results from the convolution of a Gaussian function with a Lorentzian function. The third peak at 35.45° 2-theta reveals characteristics similar to the first two peaks, with an FWHM of 4.116° and an intensity of 66.84 counts. The observed peaks collectively signified the amorphous nature of the ragi pectin [26, 27].

3.3.3 DSC

Differential scanning calorimetry was used to assess the thermodynamic characteristics of sprouted ragi seed pectin. DSC is a method for examining how polymers react to heating [28]. The heat absorption peak and the heat release peak are the two principal peaks on the DSC diagram. The melting temperature, which is indicated by the endotherm peak looking upward, is the heat absorption peak [29] and the surface area of the peak represents the melting temperature change. The degradation temperature is the heat release peak's exothermic downward peak, and the peak's area represents the degradation's temperature change. DSC thermogram of an unknown sample may show peaks representing either or all transitions like glass transition (T_g), crystallization (T_c), and melting (T_m) based on the crystalline, amorphous, or semi-crystalline nature of the sample. The Glass Transition Temperature is the temperature point when an amorphous polymer becomes "viscous liquid or rubbery" [30]. Not all polymers experience all three transitions when heated. Amorphous domains are frequently present in crystalline polymers, and they will also have a glass transition [29].

In the DSC thermogram depicted in Fig. 5B for the sprouted ragi seed pectin the glass transition temperature reported was 46.81 °C with an onset of 38.63 °C and end set of 53.97 °C. The glass transition temperature is the point at which a heated amorphous polymer transforms into a "viscous liquid or rubbery" state. Usually, the heat capacity of polymers, C_p , exceeds T_g . Chains of polymers can move around a lot above the glass transition temperature. The chains possess sufficient energy to form crystals and crystallize at temperatures higher than T_g . Due to the exothermic nature of crystallization, heat was discharged into the environment [28]. Further, exothermic down in the DSC thermogram was observed with an onset of 241.27 °C and an end set of 247.29 °C. It suggests that a molecular structure that was very mobile at glass transition temperature has stabilized. At this temperature, molecules arrange into a structure and work to stabilize themselves by transferring heat. As the melting endotherm representing the jump above cannot be observed in the thermogram, it indicates the presence of an amorphous component. Also, it demonstrates that the structure of the pectin sample under examination lacks crystalline functions. The structure of the polymer determines the precise temperatures at which these transitions occur in the polymer chains [26, 28]. All these findings related to the thermal behavior of sprouted ragi seed pectin would help formulation and development scientists to make appropriate judgments related to processing parameters for pectin during its utilization in dosage form designs like films, gels, tablets, etc.

3.4 Statistical analysis

All experiments were done in triplicates and the results are given as mean $\pm$ standard deviation (SD) in Table 1. Statistical differences between experimental groups were established at $p < 0.05$. Variability in parameters such as sprout yield,

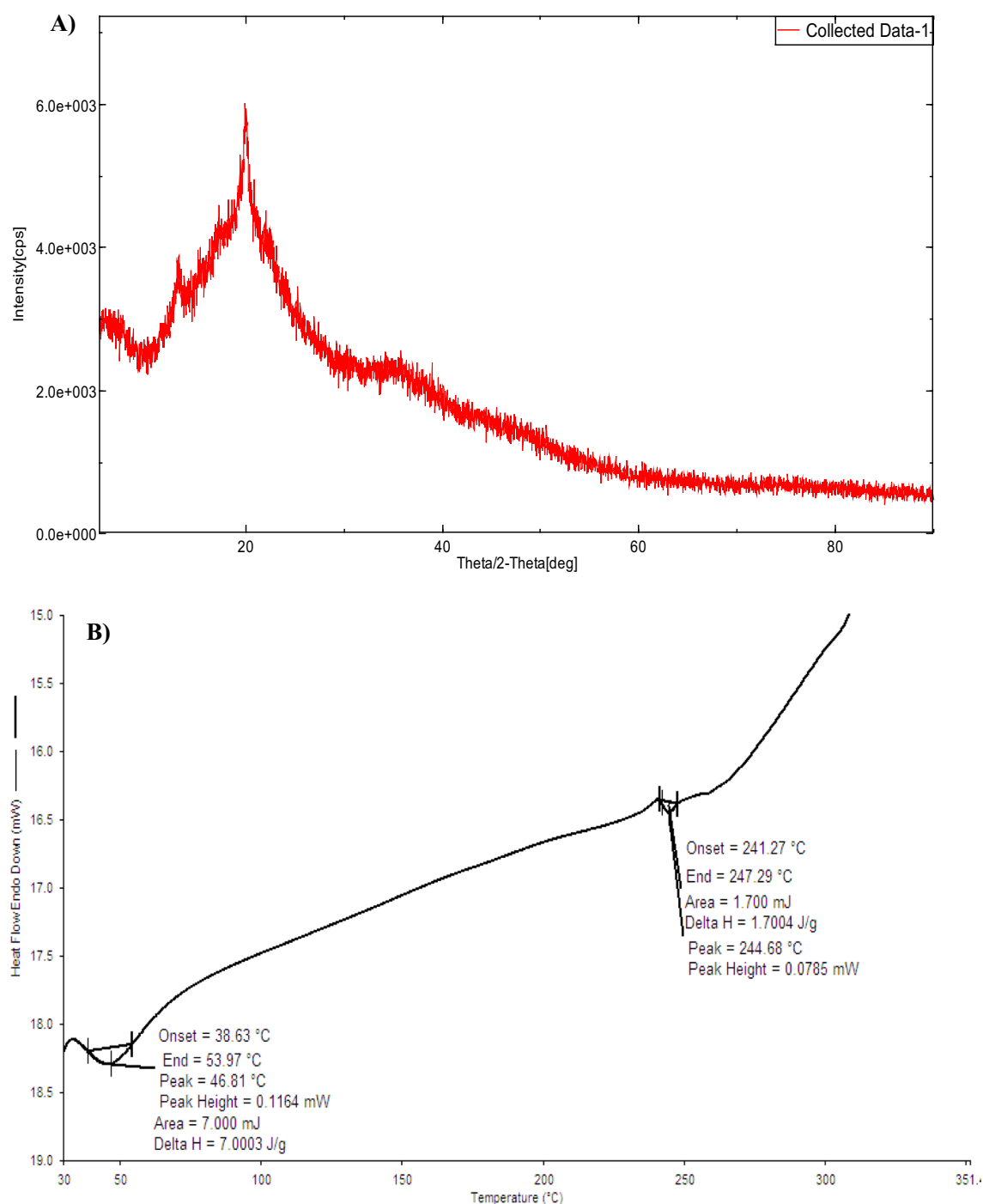


Fig. 5 X-ray diffraction pattern (A) and DSC thermogram (B) of pectin isolated from sprouted ragi seed

pectin yield, water absorption, swelling index, and degree of methyl esterification was analyzed and significant differences were found across all tested parameters, which confirmed the reproducibility and reliability of the results.

Other relevant experimental analyses including seed soaking optimization, sprout weight, and SEM have been provided in supplementary material 1.

4 Conclusions

This research work aligns with SDG 12, Responsible Consumption and Production by highlighting the proper utilization of Ragi as a staple crop for producing value-added products. This study identifies sprouted Ragi as a high-quality pectin source as it aligns with the principles of circular economy and resource optimization. The findings depict the sprout yield as 89.7% and pectin yield as 15–18% in comparison to 4–8% pectin yield in non-sprouted Ragi seeds. The isolated pectin had shown multiple properties like non-hygroscopic nature, water-repellent ability, and excellent gelling capabilities with a water absorption ratio of 11.83 g/g. FTIR proved the polysaccharide structure and uronic acid's presence. The degree of methylation $\geq 60\%$ categorized it as a high-methoxy pectin indicating its excellent gelling abilities. The molecular weight of 827.6 Da and amorphous nature, as confirmed by XRD, SEM, and IR analyses made it suitable for pharmaceutical, nutraceutical, and food applications. Furthermore, the emphasis on pectin extraction from sprouted seeds aligns with SDG 2 (Zero Hunger) by encouraging innovative uses of local crops to enhance food security and nutrition. The adoption of sprouting as a natural, cost-effective method aligns with SDG 9 (Industry, Innovation, and Infrastructure) by promoting sustainable biotechnological applications in food and pharma industries. Future research could be directed toward optimizing germination and extraction processes to maximize pectin yield and quality as well as its role in developing vegan food and pharmaceutical formulations. Further research on the impact of ragi pectin on soil biodiversity and agroecological sustainability, as well as its applications in bioactive product development, can further strengthen its contribution toward achieving SDG 15 (Life on Land) and fostering a sustainable future.

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Author contribution Gitanjali Sambhajirao Deokar: Data curation, Methodology, Formal analysis, Writing- Original draft preparation, Project administration. Nilesh Nirmal: Conceptualization, Methodology, Investigation, Formal analysis, Writing- Original draft preparation, Writing-review and editing, Supervision, Validation, Project administration. Vaishnavi Avdhut Pathak: Data curation, Formal analysis, Writing- Original draft preparation. Sanjay Jayprakash Kshirsagar: Writing—review and editing. Pavan Bhausaheb Udawant: Investigation, Writing- Original draft preparation. Pankaj Koirala: Validation and visualization. All authors have read and agreed to the published version of the manuscript.

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Declarations

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