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Egyptian *Opuntia ficus-indica* (OFI) Residues: Recovery and Characterization of Fresh Mucilage from Cladodes

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Abstract: The utilization of biopolymers gained recent attention worldwide due to their effective role in producing eco-friendly and economical products. Mucilage from *Opuntia ficus-indica* (OFI, and other succulent plants) has the capacity to absorb huge amounts of water, forming a viscous colloid with interesting rheological properties. It also had the potential ability for use as food additives or food technological products. We, therefore, aimed at extracting and characterizing pure liquid mucilage from the cladodes of OFI (Egyptian variety) using multidimensional approaches including HPLC-RID, FTIR, XRD, NMR, DSC, and TGA assays. The chemical composition, total polyphenols, total flavonoids, total flavonols, and antioxidant capacity by DPPH and ABTS assays were also measured. HPLC-RID analysis showed that the mucilage's sugars are composed of glucose, xylose, rhamnose, galacturonic acid, arabinose, galactose, and fucose with total sugars of 0.375 mg/mL, which were subsequently confirmed by FTIR and NMR results. The specific signals of these sugars were also observed in ^{13}C and ^{1}H NMR spectra and their chemical fingerprint was obtained by FTIR. XRD patterns showed that mucilage has high calcium content, and the glass transition temperature was observed at 85.9 °C. Meanwhile, total phenolic content, flavonoids, and flavonols were about 7.96 mg GAE/g FW, 3.61 mg QE/g FW, and 1.47 mg QE/g FW, respectively. The antioxidant capacity of mucilage was around 26.15 and 22.5 μmol TE/g FW for DPPH and ABTS methods, respectively. It can be concluded that OFI cladode mucilage showed promising properties that would improve and open new opportunities and trends in the food, pharmaceutical, cosmetic, and other industries. The use of pure liquid mucilage could also be economically profitable due to its low cost, availability, and effectiveness for many edible applications.

Keywords: *Opuntia ficus-indica*; biopolymers; mucilage; bioactive compounds; liquid extraction

1. Introduction

The consumer's interest in more natural and healthy products has recently led to research and development for natural compounds with bioactive properties that can replace synthetic compounds [1]. Due to the rising demand for goods that promote health and well-being, one of the food industry's fastest-growing segments is functional foods, for instance. Among synthetic compounds added to food as food supplements or food-improving technological properties, polymers cover a pivotal role. Polymers help to improve the texture and rheological features of food [2]. Recently, plant-derived biopolymers, such as mucilage, have received significant attention due to their effective role in producing eco-friendly and economical products [3]. For example, the worldwide hydrocolloids market has expanded significantly recently due to the rising demand from various industries (oil, paper, pharmaceutical, textiles, and food), reaching $8.5 billion in 2022. Mucilage belongs to the class of

soluble dietary fibers, constituted by large molecules of sugars and uronic acids. Mucilage can be classified as a hydrocolloid as it is a long-chain polymer that dissolves in water to give a thickening or viscosity-producing effect. The mucilage in plants plays various functions, such as H_2O storage, seed germination helper, and thickening of membranes [4]. For human utilization, plant mucilage has been traditionally exploited for food, cosmetic, and pharmaceutical industries due to its non-toxic and biodegradable properties [5]. It is used as a natural gelling agent to increase thickening, texturizing, stabilizing, or emulsifier as an alternative to synthetic polymers and additives [6]. The production of natural edible coatings with a high nutraceutical value, helpful for the preservation of fruit and food, is further made fascinating by the rheological properties of mucilage; its concentrations vary on genotype, cladode age, and environmental factors. However, seeking innovative and low-cost sources for plant mucilage is still demanded.

In this context, *Opuntia ficus-indica* L. (OFI), i.e., cactus pear, prickly pear, Indian fig, or barbary fig) is one of the most promising and economic sources of mucilage [3]. On around 100,000 acres, OFI plants are grown for use as fruits and vegetables in more than 30 countries. Mucilage from OFI (like other succulent plants) has the capacity to absorb huge amounts of water, forming a viscous colloid with interesting rheological properties, and has the potential use as an additive for several industrial products [7].

Meanwhile, OFI, cultivated or wild, can be found in various agro-climatic conditions, especially in warm climates, throughout the world and in the Mediterranean basin. Several parts of the plant have been used for centuries as a medicinal remedy. The anatomy and physiology of OFI make this plant a multipurpose dryland crop destined to become more important in view of an ever-increasing world population and water and land scarcity [8]. OFI is cultivated mainly for fruit production, but also for forage, in lands suffering from aridity during drought periods when there is a shortage of herbaceous plants. It is also cultivated to produce tender pads, called 'nopalito', limited to Mexico and Southern US, where it is traditionally consumed both as fresh and/or processed stuffs [9].

OFI fruits contain polyphenols and betalains, promising protective agents against inflammation, oxidative stress, and metabolic-related diseases [10,11]. Cladodes are not only rich in polyphenols but also contain polysaccharides and soluble fibers which are able to counteract hyperglycemia and related physiological disorders. Recently, the health benefits of OFI have received much attention owing to its content of bioactive compounds [8,12,13].

OFI mucilage is a polymer present in the parenchyma's cells of the tissues of different parts (fruits and cladodes) with the major role to preserve water. The mucilage content in the cladodes is more than in the other plant parts. Young cladodes have a higher content of water, proteins, and phenolic compounds than mature ones, which instead contain more ashes and fibers [14,15]. OFI mucilage is a hetero-polysaccharide of high molecular weight which mainly comprises six sugars (arabinose, galactose, rhamnose, xylose, glucose, and galacturonic acid) [14–16]. It has been found that the chemical composition and properties of OFI mucilage can change as a function of the age of the cladode, growing, climate conditions, and extraction methodologies [17–19]. Yield, functional characteristics, and rheological features of mucilage depend on the extraction protocols. Many factors can affect the extraction process, such as cladodes/water ratios, extraction temperature, extraction time, and types of alcohol used for precipitation [20]. The bioprospecting and potentiality of cactus mucilage have been reviewed recently [21]. Moreover, it has been found that the highest-quality mucilage is derived from cladodes harvested in hot and dry conditions [18], as in Egypt occurs.

The mucilage extracted from several varieties and ecotypes of OFI have been characterized [21,22]. To date, no studies focused on the full profiling and characterization of the fresh liquid mucilage extracted from the Egyptian OFI variety. Moreover, no studies also tried to extract the mucilage from OFI's cladodes without using water in terms of saving water resources and simplifying the extraction methods with high yield and acceptable characterizations. Therefore, we aimed to (**I**) extract a pure liquid mucilage without using water or any solvents from the Egyptian variety of OFI cladodes, and (**II**) study the

characteristics of the extracted mucilage using multidimensional approaches including High-Pressure Liquid Chromatography-Refractive Index Detector (HPLC-RID), Fourier Transform-Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), Nuclear Magnetic Resonance (NMR), Differential Scanning Calorimetry (DSC), and Thermogravimetric Analysis (TGA) techniques. The chemical composition, total polyphenols, total flavonoids, total flavonols, and antioxidant capacity (by DPPH, ABTS, and ORAC assays) were also assessed to study the characteristics of the extracted liquid mucilage.

2. Materials and Methods

2.1. Reagents and Solutions

Sodium carbonate (Na_2CO_3) and methanol (CH_3OH) were obtained from Elnasr. Co. (Egypt). 1, 1-diphenyl-2 picrylhydrazyl radical (DPPH•), 6-hydroxy-2,5,7,8-tetramethylchroman carboxylic acid (Trolox), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one (quercetin), 2, 2′-azobis (2-amidino-propane) dihydrochloride (AAPH), and fluorescein sodium salt were obtained from Sigma Aldrich (Germany). Folin–Ciocalteu reagent was purchased from Alpha Chemika (India). Gallic acid was obtained from Techno Pharmchem (India). All chemicals and reagents were of analytical grade and Mill-Q H_2O was used.

2.2. Raw Materials

Two-year old cladodes of OFI, an Egyptian yellow-orange variety (Shamia), were picked in May 2022 from Elkordy farm, Toukh city (Egypt), around 40–45 days after fruits flowered. These cladodes are either non-flowering or result from the process of thinning the plant as the farmers say. The farm has mixed soil (sand and clay), and the main characteristics of the production area are mild and rainy winters (12–25 °C) with hot and dry summers (22–37 °C) with a relative humidity of 50–70%, 21′13.33° N latitude and 31°12′7.39 E longitude. The cladodes were purchased from a specialized farm in OFI cultivation. Irrigation is carried out at a rate of 6 times and fertilizing once during the year (200–300 g nitrogen fertilizer for each plant mid of February), but no pesticide treatment was conducted at all. The two-year old cladodes were selected as they had already produced fruits and mostly became wastes, meanwhile, the mature cladodes have higher fiber than the younger ones which mostly consisted of water and soluble solids [14,15]. The collected cladodes were transported to the laboratory and immediately prepared for the extraction process.

2.3. Mucilage's Extraction

The extraction of liquid mucilage from fresh cladodes was performed using a modified methodology based on several pre-trials, fitting with the extraction of liquid mucilage [23]. The cladodes were washed with dH_2O, and the spines were removed via a stainless knife. The external layer (chlorenchyma) was peeled to obtain a high purity of mucilage. Subsequently, the internal layers (parenchyma) were cut lengthwise, crushed in an extractor (Sonai.sh-600, Shanghai, China), and centrifuged using a refrigerated centrifuge (Hermle Z36HK Labortechnik GmbH, Wehingen, Germany) at 9000× *g* for 30 min at 4 °C. The supernatant was collected and filtrated through a fine cloth. The purified mucilage was kept at −18 °C until further analysis (around 10 days). Figure 1 summarizes the extraction procedures and analysis methodology of the mucilage from OFI cladode, Egyptian variety.
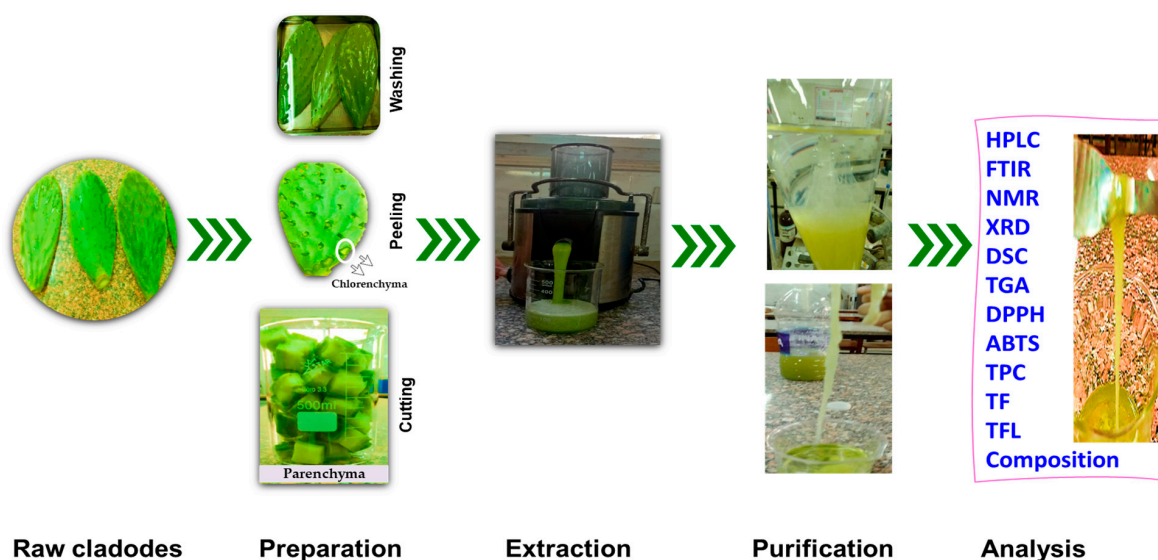
Figure 1. The extraction procedures and analysis of the mucilage from the Egyptian OFI cladode.

2.4. Chemical Characterization

2.4.1. Chemical Composition

The proximate chemical composition of OFI cladode liquid mucilage was measured according to the AOAC [24] and AACC [25] methods. These analyses include moisture, ash, crude fiber, ether extract, and crude protein. The moisture was calculated after drying the OFI liquid mucilage in an oven at 105 °C for 4 h. The ash was calculated after burning in a muffle furnace at 550 °C for 8 h. The ether extract was measured using the procedures of the Soxhlet method. Briefly, 5 g of the sample was mixed with 150 mL of petroleum ether (40–60 °C) with a ratio of 1:30 sample: solvent. It was then kept at room temperature for 30 min for a cold static extraction and continued with Soxhlet extraction for around 15 h. The crude fiber content was quantified by the Weende method through an acid/alkaline hydrolysis of the insoluble residues. Meanwhile, the crude protein content (N × 6.25) was estimated by the macro-Kjeldahl assay through the key three steps of digestion, distillation, and titration. The nitrogen-free extract (NFE) was calculated by difference (% NFE = 100 − [% moisture + % Crude Fiber + % Crude Protein + % Ether Extract + % Ash]).

2.4.2. Sugar Composition

Sugar composition was measured by an HPLC system (Agilent 1.200, USA) consisting of a quaternary pump with a vacuum degasser, a thermostatted column, and an autosampler. In brief, the sample (10 mL) was hydrolyzed with 2 M trifluoroacetic acid (TFA) (200 μL) in a sealed glass tube (13 × 100 mm) with a screw cap filled with pure nitrogen gas at 121 °C for 2 h in an oven. The hydrolyzed sample was evaporated to dryness at 45 °C before the addition of 2-propanol (500 μL) to remove TFA completely. Solid barium carbonate was used to neutralize the sample for 3 h, and it was filtered through Whatman no.1 filter paper, and resin was used to remove the extra amount of barium ions. Water was used to dissolve leftovers (1.0 mL), before filtering through a 0.45 μm filter (Waters Millipore Bedford, MA, USA). The hydrolyzed samples were analyzed through a carbohydrate column (Zorbax NH2, Agilent, USA, 250 mm length, 4.6 mm I.D., 5 μm particle size) equipped with a guard column (12.5 mm length × 4.6 mm I.D., 5 μm particle size). Temperatures for column and RID were set at 30 and 35 °C, respectively. The mobile phase was made-up of acetonitrile and water (75:25, *v/v*), and the flow rate was 1.0 mL/min with a running time of 50 min. Peak detection and integration were conducted using a Breeze Chromatographic System (Waters Company, Milford, MA, USA). A mixed standard stock solution was prepared at a concentration of 0.05 mg/mL for each monosaccharide, xylose, rhamnose, galacturonic acid, glucose, galactose, arabinose, and fucose which were

used based on the previous studies. Each sugar was then individually calculated based on its peak area in comparison with the peak area of its standard sugar, and then the total sugars based on the combination of the seven standards were calculated and compared with the total sugars measured by the phenol-H_2SO_4 method with the aid of glucose as a respective standard.

2.4.3. Antioxidant Polyphenols

The total phenolic compounds (TPC) of OFI liquid mucilage were measured as described by Khalifa, et al. [26]. A 200 μL sample was mixed with 1 mL of 10-fold diluted Folin–Ciocalteu reagent. The reaction was terminated after 5 min by 1 mL Na_2CO_3 (75 g/L), then 1.5 mL dH_2O was added. The mixtures were incubated in the dark for 1 h then the absorbance at 760 nm was measured by a spectrophotometer (Acculab UVS-90, New York, NY, USA). The TPC was expressed as milligrams of gallic acid equivalents (mg of GAE/g fresh weight, FW) using gallic acid as a standard, for Y = 0.013x + 0.103 (R^2 = 0.99).

The total flavonoids (TF) of OFI liquid mucilage were also measured. A 0.5 mL aliquot of $AlCl_3$ solution (2 g $AlCl_3$ in 100 mL of EtOH) was added to 0.5 mL of OFI liquid mucilage and mixed well. The solution was then kept for 1 h at room temperature and the absorbance at 420 nm was measured. The total flavonols (TFL) were also measured. A 0.5 mL aliquot of 2% $AlCl_3$ solution (prepared as above) was added to 0.5 mL of OFI liquid mucilage and 1.5 mL of 5% sodium acetate solution, then mixed well and kept for 2.5 h at room temperature. The absorbance at 440 nm was measured. The final concentration was expressed as quercetin equivalents (mg quercetin equivalent, QE/g FW) with the equation of Y = 0.037x + 0.1363 (R^2 = 0.998) [24].

The antioxidant activity (AOA) of OFI liquid mucilage was determined according to Khalifa et al. [26]. A 100 μL sample aliquot was added to 3.9 mL of DPPH methanolic solution (0.0025 g/100 mL) then the absorbance at 517 nm was measured after the solution had been incubated in the dark for 60 min. The results were expressed as Trolox Equivalents (μmol TE/g FW) using Trolox as a standard, for Y = 0.001x + 0.657 (R^2 = 0.99).

The AOA of OFI liquid mucilage was also determined using ABTS assay. $ABTS^{\bullet+}$ radicals were produced by reacting (in a 1:1 ratio) a 7 mM ABTS solution with a 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. $ABTS^{\bullet+}$ radicals' solution was diluted to an absorbance of 0.70 ± 0.02 at 734 nm and added to the sample, duly diluted, according to Khalifa et al. [27]. The results were expressed as Trolox Equivalents (μmol TE/g FW) using Trolox as a standard.

The Oxygen Radical Absorbance Capacity assay (ORAC) was also measured using the method of Khalifa et al. [28]. Fluorescence of the samples was detected at 37 °C within 45 min every 5 min at λ_{em} = 515 nm and λ_{ex} = 493, using an F-4600 fluorescence spectrophotometer (Hitachi, Japan). Results were expressed via the area under the curve (AUC).

AUC = $(5 + \frac{f5}{f0} + \frac{f10}{f0} + \cdots \frac{f45}{f0}) \times 5$, where f_0 = the initial fluorescence before AAPH addition at times of $f_0, f_5, f_{10}, ..., f_{45}$ fluorescence at time 0, 5, 10,..., 45 min, orderly. The relative capacity was expressed by withholding the AUC of the sample from the AUC of dH_2O as a blank, in the presence of TE as a standard curve of Y = 0.001x + 70.05 (R^2 = 0.99).

2.5. Structural Properties

2.5.1. FTIR Analysis

The FT-IR spectrum of OFI liquid mucilage was acquired by an FTIR Nicolet 470 (Thermo Fisher Scientific, Waltham, MA, USA), following the protocol of Khalifa et al. [28]. The OFI liquid mucilage was lyophilized. The dried sample was ground and mixed with dried KBr (0.99:0.1 ratio) in a dried environment. Then, it was shaped and pressed to clear the lozenge to be scanned immediately. This approach was used to determine the functional groups of OFI in the wavelength range of 400–4000 cm^{-1} and with a resolution of 4 cm^{-1} with a clarity degree of ±2 cm^{-1} at 21 scans per min. The Omnic software was used to adjust FTIR spectra baselines via Fourier self-deconvolution. The whole FTIR spectrum of OFI was portrayed.

2.5.2. XRD Analysis

X-ray diffractometer (Bruker D8 Discover, Germany) was used to measure the XRD of OFI liquid mucilage. Cu-Kα radiation (λ = 1.54060 Angstrom). The relative intensities (I/I_0) of the OFI with Cu-Kα radiation were defined from the chart and JCPDS carts were used to identify the minerals of core materials. The relative intensity data were gathered across a 2θ range of 5–100°.

2.5.3. NMR Analysis

^{1}H NMR and ^{13}C NMR analysis were performed on OFI liquid mucilage using a JEOL ECZ-R 500 MHz NMR spectrometer (JEOL, Tokyo, Japan) with Superconducting Magnet (SCM) 11.74 Tesla (resonance frequency of 500 MHz and 125 MHz, respectively. Analyses were recorded in D$_2$O at 25 °C.

2.6. Thermal Properties

Thermal analysis including TGA and DSC were performed using an instrument (Labsys, evo; Setaram, France) under a nitrogen atmosphere. Samples were heated from 25 to 500 °C with a heating rate of 10 °C/min.

2.7. Statistical Analysis

Statistical tests were dedicated using the SPSS program (ver., 26.0, IBM; Armonk, NY, USA). All analyses were completed in triplicates and means and standard deviations were conveyed and valued by ANOVA with an individual-sample analysis.

3. Results

3.1. Chemical Categorization

3.1.1. Chemical Composition and Antioxidant Polyphenols

The proximate chemical composition of the liquid mucilage extracted from OFI cladodes was measured according to fresh weight and the results are shown in Table 1. The moisture content (92.14%) fell in the range of the results reported in a previous study [23], this high content of moisture is because we characterized the freshly extracted mucilage which was extracted without heat treatment. The crude protein content (0.82%) of mucilage was lower or higher than that reported in previous studies considering that we used fresh mucilage [20,22,23], where they found that the protein content found in the mucilage ranged from 4.01 to 7.3% in OFI measured in the dried samples. The mucilage is only found in mucilage cells, which do not contain proteins in their final form but do contain a significant number of polysaccharides, which explains the low protein content. A negligible content of the lipid (Ether extract) was found with a ratio of 0.07%, agreeing with a related study [29], which found that fat content was about 0.2% in dried mucilage. Long-chain fatty acids (α-linolenic and linoleic acid, among others) were previously noted in OFI, which could increase its thermal stability [23]. However, because they are essential for storing energy, lipids are the fundamental components in plants and are necessary for their survival and growth. They are exclusively kept in certain tissues and cells and are hardly ever stored in leaves, roots, or stems. Ash content (0.31%) was also lower than that recently reported (3.15–5.18%, in dried mucilage) [23]. The degree of mineral interaction in the structure, which contributes to the functional qualities of the polysaccharides, is indicated by the ash content, which has been reported to be a valuable attribute. A higher level of purity is correlated with a lower ash content. The crude fiber content (0.59%) and the NFE were about 6.04%, agreeing with a previous report [23]. It was explained that OFI mucilage is constituted by different H$_2$O-soluble fractions including pectin with Ca-dependent gelling features, a non-gelling mucilage, and a polysaccharide fraction with thickening features demonstrating ≤10% of H$_2$O-soluble substances [30]. Thus, high NFE and low crude fiber content were projected, since the crude fiber analysis only considered the H$_2$O-insoluble compounds, i.e., cellulose, hemicellulose, and lignin [23]. The differences in the chemical composition of OFI mucilage may be due to several factors such as the variety, the season of

collection, the age of the plant and cladode development stage, the growing conditions, the postharvest treatment, and the extraction method. These factors, undoubtedly, manipulate the nutrient content of the OFI from the Egyptian species. Our results disagree with the previous reports, due to the extraction process used: our study is based on liquid extraction and direct analysis, instead, all previous studies used precipitation with alcohol [20] or microwave treatment [19], followed by freeze-drying of extracted mucilage into a powder, and thus the chemical composition shows differences. Overall, the chemical composition analysis showed the applicability of our mucilage in food applications, especially in the fabrication of edible coating since its adequate amounts of the component enhance the strength of the prepared composite.

Table 1. Proximate chemical composition and antioxidant polyphenols of liquid OFI mucilage based on the fresh weight (FW).

Items	Quantity
Moisture (%)	92.17 ± 0.97
Ashes (%)	0.31 ± 0.04
Proteins (%)	0.82 ± 0.08
Lipids (Ether extracts, %)	0.07 ± 0.0
Crude fibers (%)	0.59 ± 0.08
NFE (%)	6.04 ± 0.06
TPC (mg GAE/g)	7.96 ± 0.9
TF (mg QE/g)	3.61 ± 0.85
TFL (mg QE/g)	1.47 ± 0.27
AOA (DPPH$^{\bullet}$, µmol TE/g)	26.15 ± 0.2
AOA (ABTS$^{+\bullet}$, µmol TE/g)	22.5 ± 1.73
AOA (ORAC$_{FC}$, µmol TE/g)	164.8 ± 7.86

All the parts of OFI are rich in polyphenols (flavonoids and phenolic acids) [8,31]. TPC of liquid mucilage extracted from fresh OFI cladodes was measured using Folin–Ciocalteu reagent. The results in Table 1 show that TPC was 7.96 mg GAE/g FW mucilage, and, considering 92% moisture, nearly 99.5 mg GAE/g DW. Our result was like the findings reported by Messina et al. [15], and a bit lower than the value reported by Gheribi et al. [32]. TF and TFL were also measured using the aluminum chloride method. The fresh OFI cladodes contain TF and TFL of 3.61 and 1.47 mg QE/g FW mucilage, respectively (Table 1). Rodrigues et al. [33] narrated that the total flavonoids in *Opuntia* spp. vary from 1.55 to 2.64 mg in 100 g. Variations in TPC, TF, and TFL concentrations of OFI cladodes can occur in connection to age, environment, soil type, and climate. quercetin, (+)-dihydroquercetin, quercetin 3-methyl ether, rutin, and isorhamnetin were previously found as the key flavonoids of OFI cladodes [34]. The potential for polyphenols to act as antioxidants have led to increased interest in them due to the possible health advantages, showing the advantage and multifunction potential application of our extracted mucilage to formulate an edible coating rich in polyphenols. TPC an EtOH-extract from South Korean OFI found to be responsible for the AOA. AOA of liquid mucilage extracted from fresh Egyptian OFI cladodes was, thus, measured by DPPH, ABTS, and ORAC methods. Several bioactive compounds in OFI-mucilage could show a quenching capacity towards the free radicals, namely DPPH-radicals, where reaction kinetics' function could be categorized into fast-kinetics, fast + slow-kinetics, and slow-kinetics based-groups [35,36]. As reported in Table 1, the AOA of fresh OFI cladode was found to be 26.15, 22.5, and 164.8 µmol TE/g FW measured by DPPH, ABTS, and ORAC assays, respectively. The obtained value agreed with the results reported by Messina et al. [15], and by Procacci et al. [37]. As it has been already found in OFI cladode extract [34], OFI mucilage is also a good source of antioxidant functional compounds. It is known that carbohydrates also exhibit antioxidant activity, due to their reducing groups, able to counteract free radicals, where a positive correlation between carbohydrates and/or polyphenols with AOA of the mucilage was found [15].

Again, these results confirmed the multifunction of our extracted mucilage with potential applications in foods.

3.1.2. Sugar's Composition

Liquid mucilage was run on HPLC-RID to determine the monosaccharide composition. A mixed standard of monosaccharides was used for reference (Figure 2). Results showed that xylose is the major sugar among seven sugars, i.e., glucose, xylose, rhamnose, galacturonic acid, arabinose, galactose, and fucose presented in liquid mucilage followed by galacturonic acid (Figure 2). The total amounts of each sugar were 0.083, 0.055, 0.103, 0.040, 0.037, 0.031, and 0.023 mg/mL for galacturonic acid, glucose, xylose, galactose, rhamnose, arabinose, and fucose, respectively (Table 2). The total sugars calculated by HPLC-RID were found to be 0.375 mg/mL, where the result of the phenol-H_2SO_4 method was around 0.488 mg/mL, mostly due to the accuracy of the HPLC-RID method equated with the spectroscopies-based method. According to McGarvie and Parolis [30], a hypothetical model of the mucilage from OFI is represented by alternating rhamnose and galacturonic acid, attached to the side chains formed of three galactose residues. Galacturonic acid was found to be the second major sugar in our fresh mucilage. Branching with arabinose and xylose sugars on the galactose side chains is also hypothesized. Previous studies on OFI cladode reported major sugars galactose and arabinose, and other minor sugars (xylose, rhamnose, and glucose) [22,31,32], where adequate amounts of galactose and arabinose were also found in our fresh mucilage. Glucose which was the third key sugar in our Egyptian fresh mucilage was also predominant in mucilage extracted from Mexican cladodes [33]. Ribeiro et al. [16] also found glucose, fructose, and galacturonic acid as the main sugars in cladode extracts of different ages, where fructose was absent in our samples. Glucose content was the highest in young cladode, while galacturonic acid was predominant in mature cladode [14]. In particular, the presence of galacturonic acid has a pivotal role in film-forming ability, such as viscosity, the capacity of water retention, and Ca^{2+} ion chelation ability, highlighting the different potential applications of our extracted mucilage. The difference in sugar composition reported in other studies may be due to the same factors mentioned above for proximate composition. The results of HPLC-RID were further confirmed by FTIR and NMR findings for in-depth structure elucidation.

Table 2. Sugar's composition of OFI liquid mucilage.

Monosaccharides	Content (mg/mL)
Galacturonic acid	0.083
Glucose	0.055
Xylose	0.103
Galactose	0.040
Rhamnose	0.037
Arabinose	0.031
Fucose	0.023
Total sugars by HPLC	0.375
Total sugars * by phenol-H_2SO_4	0.488
Unknown	RT at 13.811 min with a peak area of 1.657%.

* The method of phenol-H_2SO_4 was used to measure the total sugar of mucilage without modification [38].

3.2. Structural Characterization

3.2.1. FTIR

FTIR is used to determine the functional groups present in an extract and it is considered a useful technique to investigate the efficiency of extraction, to control its purity, and to reveal possible interactions with other compounds. The spectrum in Figure 3 shows a broad band at 3440 cm^{-1} attributed to the stretching vibration of the (–OH) group, corresponding to carboxylic acid and alcohol. The band of 2935 cm^{-1} can be assigned to the symmetric stretching vibrations of the –CH and polysaccharide, confirming that OFI has rich polysaccharide fractions. This band corresponds to the stretching vibration of hydroxy

methylene groups present both in the pyranose and furanose conformations of galactose, arabinose, and xylose, which have been identified as main monosaccharide in several OFI cladode varieties including the Egyptian one, agreeing well with related studies [23,31,32]. The absorption band of 1789 has been assigned to the galactosyl carbohydrate residues. The band of 1642 cm^{-1} indicates the extension vibrations of C=O stretching vibrations of uronic acids in polysaccharides, showing again the presence of polysaccharide fractions in OFI. The aromatic C=C stretching of lignin and their phenolic backbone was found at 1512^{-1} cm The band of 1428 cm^{-1} is due to the carboxylate group (COO-) asymmetric and symmetric stretching and again may be related to the presence of uronic acid and pectic substances. Moreover, it has been proven that the (COO-) of uronic acid reacts with water and cationic ions, such as calcium, which makes a great contribution to the viscosity of mucilage [23]. Bands of 1365 and 1162 cm^{-1} appear due to symmetric C–H bending and asymmetric C–O–C extension, which may be related to the presence of fibers. The peak band of 1255 cm^{-1} relates to the glycosidic linkage in the galactan domains and the presence of galacturonic acid. The tiny band at 980 cm^{-1} was recognized as xylans and arabinoxylans and is consistent with the sugar's composition in the hydrocolloid. The band of 841 represents the functional groups of polysaccharides include also the α-anomer in the pyranose form of sugars. This also might confirm that most of the sugar moieties in the heteropolysaccharides are in the β-configuration, with arabinose as the potential exception, agreeing with the HPLC results. The obtained results were in line with what was reported in a previous study [39]. The FTIR results confirmed the HPLC findings for polyphenols occurrence and have a strong link with XRD results, especially with calcium.
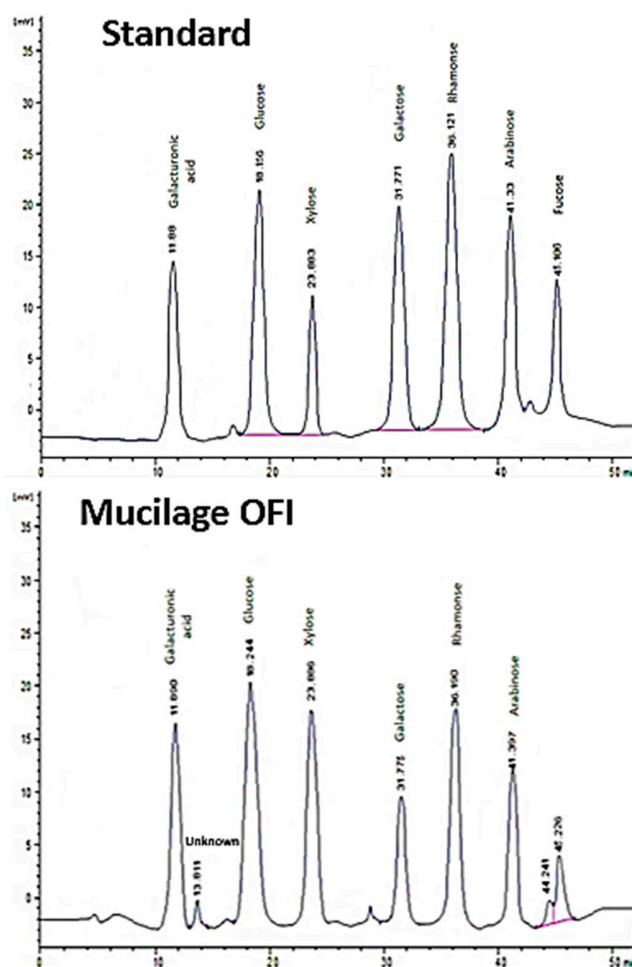

Figure 2. Mixed standard of monosaccharides (**above panel**) and sugar composition (**below panel**) of liquid mucilage extracted from OFI cladodes.
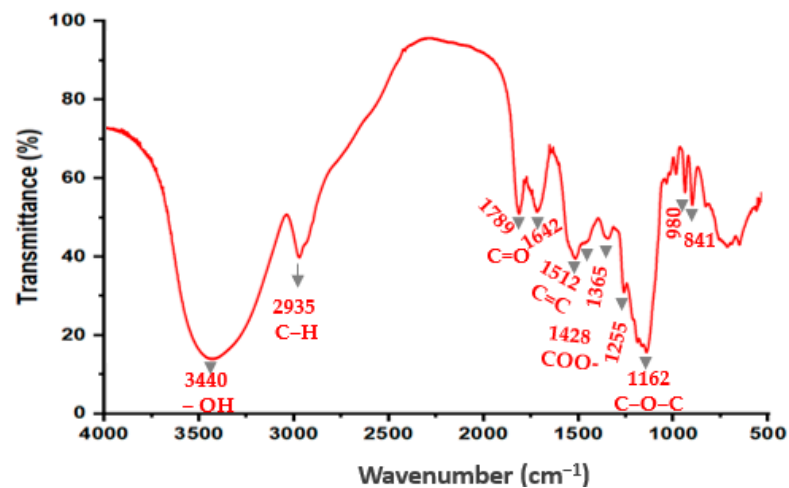
Figure 3. FT-IR spectrum of liquid mucilage extracted from fresh Egyptian OFI cladode.

3.2.2. XRD

XRD of liquid mucilage was performed to investigate its amorphous, semi-crystalline, or crystalline structure. The XRD pattern shows seven peaks at 2θ = 28.26, 29.42, 40.20, 50.03, 54.17, 61.27, and 73.60 (Figure 4). The major characteristic peaks in XRD patterns are at 29–30, 39–40, and 45–50°. The mentioned peaks indicate the presence of calcium carbonate, which appears in two crystalline forms, calcite, and calcium carbonate [$CaCO_3$]. Our results agreed with the results reported in [17–22]. Calcium occurrence by XRD analysis confirms results from FT-IR analysis. Calcium-magnesium bicarbonate [$CaMg(CO_3)_2$] and magnesium oxide [MgO] were also identified. Interestingly, our extracted mucilage showed good sources of calcium and magnesium, which have a bioavailability for humans, and this feature may increase the interest in mucilage utilization in the food industry [17]. Contreras-Padilla et al. [17] found oxalates in nopal powders (a form of druses) and recognized minerals as whewellite ($C_0CaO_4 \cdot H_2O$), calcite ($CaCO_3$), fairchildite ($K_2Ca(CO_3)_2$), potassium peroxydiphosphate ($K_4P_2O_8$), and sylvine (KCl).
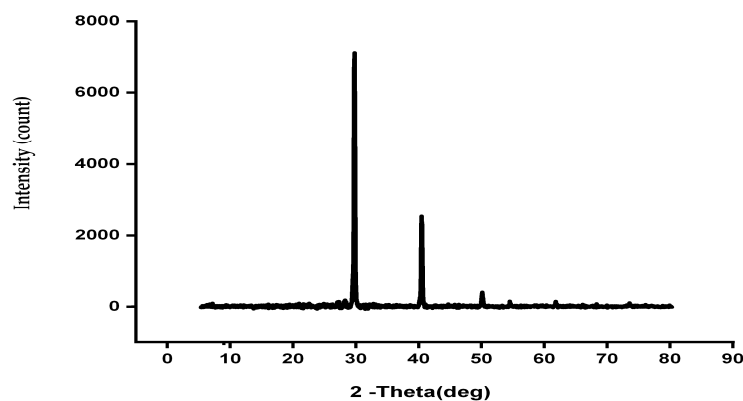

Figure 4. XRD patterns of liquid mucilage extracted from fresh OFI cladode.

3.2.3. NMR

NMR analysis was performed to illustrate the structural characterization of liquid mucilage extracted from fresh OFI cladode. The results of H^1 NMR and ^{13}C NMR are shown in Figure 5A,B, respectively. Data analysis of ^{13}C NMR spectra were divided into chemical shift regions: alkyl C (0–45 ppm), O-alkyl C (45–110 ppm), olefinic and aromatic C (110–160 ppm), phenolic C (140–160 ppm), and carbonyl C (160–220 ppm) [33]. The chemical shifts signals of ^{13}C NMR shown in Figure 5B were observed at 29.94 (C6), 31.92 (C5), 35.53 (C4), 40.70 (C3), 49.84 (C2), 55.78, 120.55 (C1), 128.38, and 138.62 ppm. The observed signals are at 120. 55 (C1), 128.38, and 138.62 are not specific to polysaccharides.

However, mentioned signals were attributed to aromatic and phenolic compounds. Our findings of the H[1] spectrum shown in Figure 5A were like Petera, et al. [40]. Signals were identified at 1.51 (H1), 3.63 (H2), 3.65 (H3), 3.79 (H6) 3.85 (H5), and 4.45 (H4) ppm which are attributed to (1 → 4) linked β-D Gal. The signal at 1.28 ppm was assigned to the methyl protons at position-6 of α-L-rhamnopyranosyl residues [41]. Moreover, some minor signals were also observed in [13]C (114–131 ppm) and [1]H (6–7 ppm) spectra. These signals are not specific to carbohydrates but are attributed to phenolic compounds [42]. Therefore, the extracted mucilage is a hydrocolloid exposing property spectra of a polysaccharide.
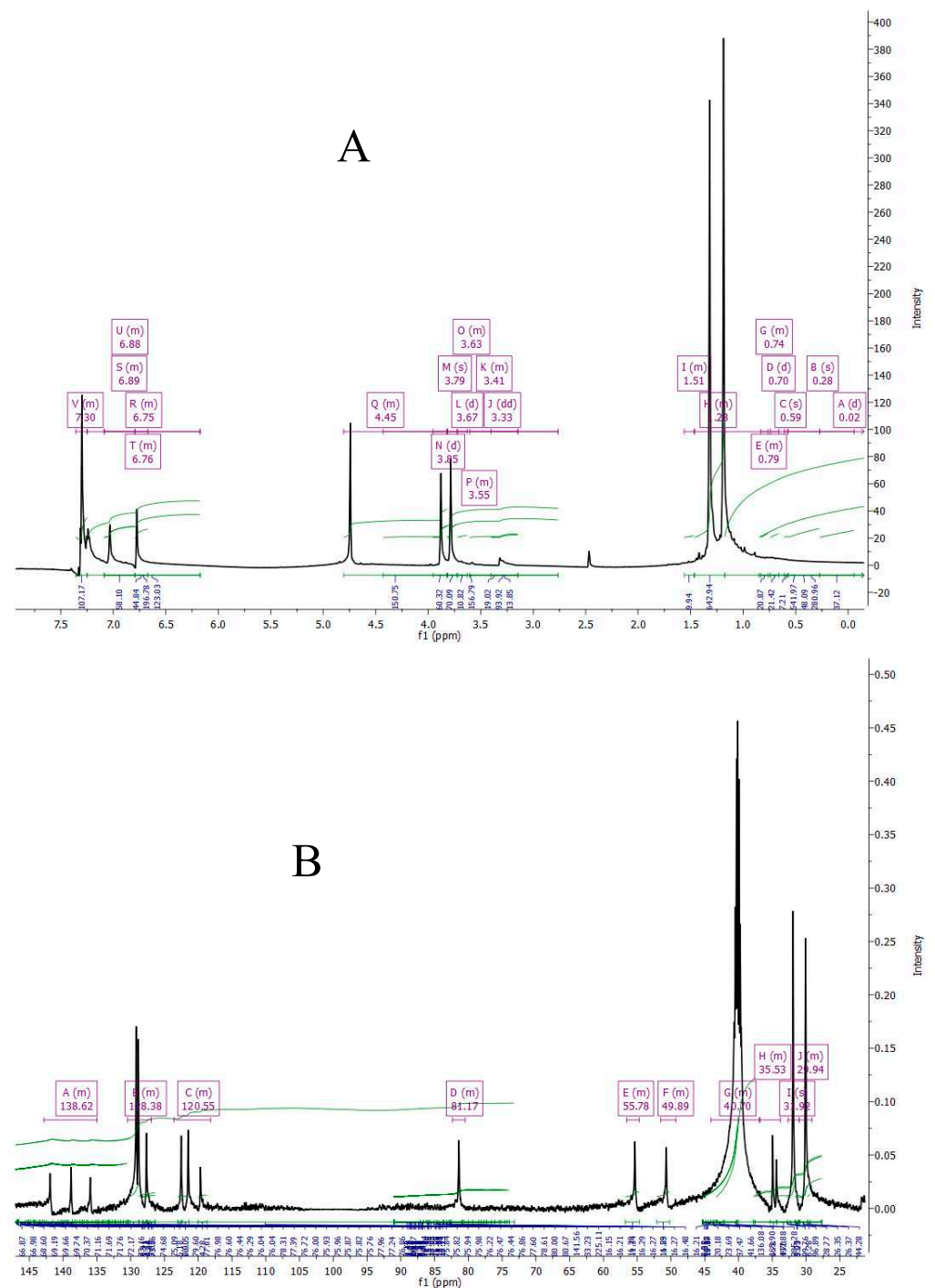
Figure 5. H[1]NMR spectrum (**A**) and ^{13}C NMR spectrum (**B**) of liquid mucilage extracted from OFI cladode.

3.3. Thermal Characterization

3.3.1. TGA

TGA was used to determine the changes in weight of the liquid mucilage extracted from fresh OFI cladode as a function of temperature properties. This technique is useful for determining sample decomposition patterns and stability to thermal exposure of polymers [23]. Figure 6 shows the thermograms of the liquid mucilage. The thermal stability of mucilage can be measured in two stages, possibly owing to the intricacy of mucilage from OFI cladodes and it comprised many different chemical components. The first mass change was 3.77% at temperatures of 38 °C. This rapid weight loss was attributed to the high loss of moisture corresponding to free water in mucilage particles [43]. The second decomposition stage was 26.53% at a temperature of 107 °C. Our results disagree with the previous studies [5,23,44] in the weight loss rates and temperatures, and this aspect is worthy of further investigation.
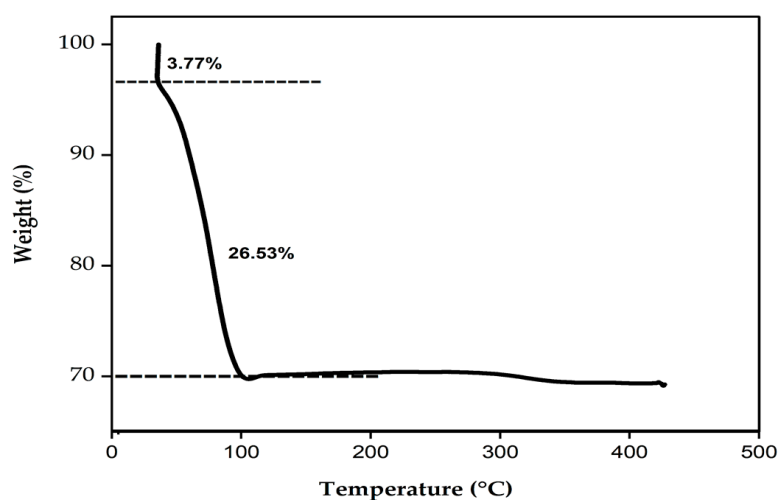

Figure 6. TGA of liquid mucilage extracted from fresh OFI cladode.

3.3.2. DSC

DSC was used to characterize the thermal properties of the liquid mucilage extracted from fresh OFI cladode. DSC was also used to detect physical and chemical or exothermic and endothermic changes that occur in the mucilage during thermal processing [45]. Figure 7 shows the DSC pattern of the liquid OFI mucilage with two heating curves. The first heating curve (endothermic) proved that the glass transition temperature (Tg) was observed at 85.9 °C, as it has been already observed [44] and this is attributed to the presence of compounds with lower molecular weights. Moreover, the second thermal event (exothermic) attributed to crystallization temperature (TC) was observed at 120.7 °C. As mentioned before, there are almost no studies on characterizing the liquid mucilage extracted from the cladodes of barbary figs, and therefore there is a severe lack of data and disagreement with the previous studies. In a study on long-chain fatty acids (LCFA), namely α-linolenic and linoleic acids, high-temperature resistance was noted in terms of thermal hydrolysis (up to 160 °C for 8 h) [46]. As *O. ficus indica* cladodes contain these LCFA, the thermal behavior of liquid mucilage can be attributed to this feature [42]. The results of the thermal behavior from TGA and DSC confirmed that the extracted mucilage had suitable thermal stability that could be valorized to prepare an edible coating/film with thermal stability during storage.
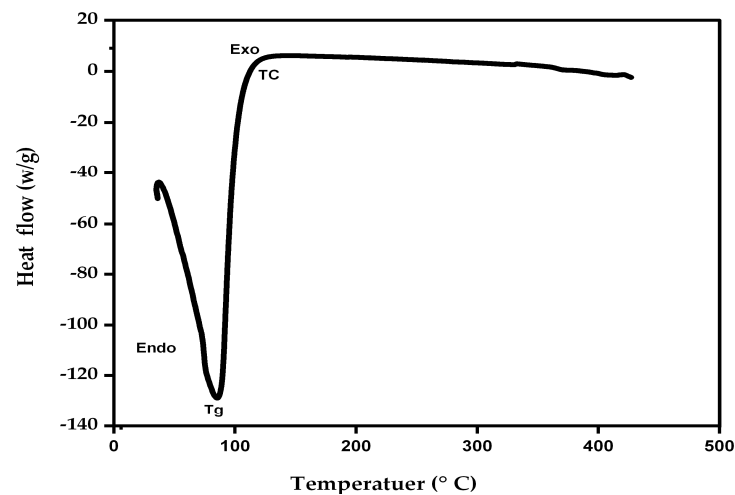
Figure 7. DSC of liquid mucilage extracted from OFI cladode.

4. Conclusions

Mucilage has promising properties and potential applications in food, pharmaceuticals, cosmetic, etc. For the first time, we aimed at extracting a liquid mucilage using a clean and environmental-based method from the key Egyptian OFI variety and characterizing it using different approaches, namely HPLC-RID, FTIR, XRD, NMR, DSC, TGA, chemical composition, TPC, TF, TFL, DPPH, ABTS, and ORAC assays. By measuring its chemical composition and physical properties, results found that about seven sugars were presented, where xylose, galacturonic acid, and glucose had peaked, orderly. XRD revealed that the extracted mucilage showed the incidence of minerals, especially calcite, and calcium carbonate, into the polysaccharide's matrix. The TGA thermogram specified the incidence of different chemical components by two stages of weight loss at 38 and 107 °C, whereas the DSC thermogram in the first heating curve (85.9 °C) noted the discharge of obstructed humidity and the incidence of polysaccharides with low molecular weight in OFI-mucilage with a good crystallization temperature. The extracted liquid mucilage also showed a potential antioxidant capacity with an adequate quantity of bioactive components represented as polyphenols, flavonoids, and flavonols. FTIR confirmed the chemical and physical characterization, where different functional groups belong to galactose, arabinose, xylose, uronic acid, galacturonic acid, fibers, and other polysaccharide fractions. The occurrence of pectins might be owing to the extraction assay we used, where we did not use ethanol precipitation. Overall, this is the first study about simply extracting mucilage from the Egyptian OFI and characterizing the liquid and fresh mucilage, which implied it is a successful, costless extraction, and effective for many edible applications. Given the wide range of uses that are attributed to mucilage, our findings illustrate the connection between its chemical components with thermal and structural behavior that might encourage the commercial cultivation of OFI in arid and semi-arid areas for different applications.

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