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Extraction of Pectin from Ethiopian Prickly Pear Fruit Peel and Its Potency for Preparing of Cellulose-Reinforced Biofilm

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Abstract:

The objective of this research was to extract and characterize the pectin from the fruit peels of Ethiopian prickly pears (EPP) (*Opuntia ficus-indica*) using microwave assisted method. Solution pH and microwave potential were optimized using different pH values (1, 3, and 4) and power (300, 400, and 500 W), respectively, to extract ameliorated pectin yield. The pectin yield for EPP varied between 2.3 and 10.0 percent. At a pH of 1.0 with 400 microwave intensity, the highest yield was seen (14.0 %). The extracted pectin from EPP had a 25.16 % ash content; however, the pectin sample contained less water and weighed less than the control sample. Amount

of methoxyl, anhydrouronic acid, and degrees of esterification were determined to be 2.28, 25.58, and 49.87, respectively. Further, transforming the acquired pectin from EPP into a bio-based film as well as evaluating its properties. As a result of this study, a new function was given to EPP waste, and biofilms were developed with cellulose-reinforced modification. Films were prepared using the film-casting method. The pectin-based biofilm (EPP-F) is plant-based biodegradable and has competitive mechanical properties compared with some of the commodity plastics. Therefore, with further improvements, EPP-F could be potentially used for nonstructural applications, such as a sustainable packaging material for the food industry.

Keywords: Green prickly pear, peels, extraction, pectin, biofilm, characterization

1. Introduction

Peel, core, unripe, and overripe fruit account for as much as 50 percent of the raw materials discarded by the fruit processing industry [1]. In addition to the high pectin content of the peel, these fruit wastes provide a low-cost raw material for animal feed. In this line, prickly pear peels can be used to lessen our reliance on landfills and increase the value of our agricultural by-products [2]. The cactus family includes the prickly pear (PP) (*Opuntia* spp.). In general, PP varieties thrive in arid, rocky, and cold environments. Consumers are interested in it because of the good health effects associated with its high nutritional value [3]. PP fruit can be found in different colors, such as green, yellow, purple, and orange. Their skins are thick, waxy, and spiky [4], [5]. Its structure allows for an exceptionally high concentration and distribution of nutrients such as B-group vitamins, calcium, magnesium, potassium, dietary fiber, copper, flavonoids, betalain, carotenoids, amino acids, and lipids [6]. The fruit's sweet acidic flavor comes from its high sugar content and low acidity, but its short shelf life of only about two to four weeks is a drawback [7], [8]. Diabetes, high cholesterol, and weakened immune systems are just some of the conditions that have been treated using PP fruit extracts and components [9], [10]. Ncibi et al. (2008) reported that between 40 and 60 percent of a human-edible fruits were made up of the pulp and seeds [11]. Usually, while processing fruit into pulp, wine, or juice, the peels are discarded as waste. The peel waste, which is rich in antioxidants and pectin they can be exploited to extract the valuable pectin, instead of purchasing commercial pectin [12].

Pectin is a 1,4-linked linear polysaccharide containing D-galacturonic acid [13]. It is a natural component of fruits and can be found in the intercellular spaces of the middle lamella [14]. Low methoxyl pectin has a degree of esterification (DE) of less than 50%, while high methoxyl pectin has a DE of more than 50%, and these two types of methoxyl pectin have different functional qualities [15]. The physicochemical features and uses of low and high methoxyl pectin are distinct due to their differences in DE [16]. The extraction step of pectin synthesis, which is commonly carried out via hot acid extraction, is crucial [17]. To get rid of the pectin on fruit peels, many people employ organic or mineral acids like nitric, sulphuric, or hydrochloric acid [18].

Most often, jams, jellies, confections, beverages, and acidified milk drinks all benefit from the addition of pectin towards stabilization, emulsifying, texturizing, and thickening properties [19]. Both the pectin source and the extraction method can have a significant impact on the final product's production and quality. Recent years have seen extensive studies devoted to discovering a high-quality pectin alternative to commercial pectin that can be extracted from plant materials, including the peels of passion fruit, pomelo, and mango [20]. However, the food sector continues to make insufficient use of pectin from these sources. Commercial pectin manufacturing could benefit from the utilization of indigenous fruits and their byproducts, especially in underdeveloped nations [21].

Pectin has been extracted from a wide variety of plants. Conventional pectin extraction, however, lowers the quality of the extracted substance by utilizing an acidic pH and a high temperature above 80 °C [22]. Researchers must therefore employ other recent extraction strategies, like the application of microwaves, to forestall the production of subpar pectin. In recent times, microwave extraction has become more popular than other methods since it requires less time and effort and uses less energy and solvent [23]. Studying the yield, physicochemical, and structural features of PP fruit, as well as extracting pectin from its peel, was one of the main focuses of this research.

In the present research, cellulose-reinforced pectin composites have been developed, for example, for tissue engineering applications and for food packaging applications from commercial sources [24], [25]. However, there is no study on directly using cellulosic fibres and pectin obtained from prickly pear waste to prepare a biofilm. This study investigated transforming pectin from prickly pear peel into a bio-based film as well as evaluating its properties. As a result,, a

fruitful function was given to EPP waste, and biofilms were developed without prior chemical modification of the pectin. Films were prepared using a film-casting method. The pectin-based bio-film (EPP-F) is plant-based and biodegradable and represents competitive mechanical properties with some of the commodity plastics. Therefore, with further improvements, EPP-F could be potentially used for nonstructural applications, for example, as a sustainable packaging material for the food industry.

2. Materials and methods

2.1 Raw materials

Green PP fruits were collected from the vegetable market province of Addis Ababa, Ethiopia. The exterior color index was used to determine that all of the chosen fruits had reached physiological maturity. After being washed with running water, all of the fruits were placed in cold storage at 5 ^{o}C.

2.2 Ethiopian Prickly pear peel (EPP) powder preparation

The skins and pulps of PP fruits were carefully removed using a knife and distilled water. The fruit was peeled, then thinly sliced (between 1 and 2 cm), and then divided into three piles on trays. A South African Eco Therm 278 Digital oven was used to dry the three batches of EPP peels at 60 °C for 72 h. The materials were dried, placed in a desiccator, milled (using a Polymix PX-MFC 90 D, Switzerland), and then sieved through an aperture sieve measuring 500 µm. The powder was stored in a desiccator after being vacuum-sealed in polyethylene bags [26].

2.3 Extraction process of pectin from EP

The extraction procedure involved the use of sulfuric acid. Five g of EPP peel powder and one hundred fifty mL of distilled water were placed in a 500-mL glass beaker, and the pH was brought down to 1, 2, 3, and 4 with 0.5 N sulphuric acid. The microwave was used to heat the solution at low (300 W), medium (400 W), and high power (500 W) for 10 minutes. Then, it was chilled and centrifuged at 3,000 rpm for 15 minutes (Universal 320 R; Herttich, Germany). Two hours were spent chilling the supernatant at 4 ^{o}C after it had been combined with ethanol (95 %) at a ratio of 1:2 (v/v). After the sample had coagulated, it was filtered and washed with 95% ethanol to remove the pectin. The wet pectin was kept at 35 ^{o}C for 24 h. By plugging the results into

equation (1), the % yield was calculated by weighing dried pectin using weighing balance (BPS51, BOECO Germany) [27].

$$\text{Pectin yield } (\%) = \frac{P}{\text{Bi}} \times 100 \qquad (1)$$

Where P is the amount (g) of the pectin extracted and B_i is the initial amount of EPP in dry peel basis (5 g).

2.4 Characterization of EPP pectin

2.4.1 Moisture content

The AOAC (2002) standard technique was used to calculate the moisture content. In this line, two g of pectin were placed on a clean, dry metal dish, and the dish was weighed. Overnight, the sample was dried at 105 ^{o}C, then allowed to cool using a desiccator before being weighed. Equation (2) was used to calculate the moisture level [28].

$$Moisture\ content\ (\%) = \frac{weight\ of\ dried\ sample}{weight\ of\ pectin} \times 100 \ldots\ldots\ldots (2)$$

2.4.2 Ash content

After dividing 2 g of pectin across 4 tared crucibles, the samples were heated for 4 h at 550 ^{o}C in a muffle furnace (Lasec type EMF 035). Using equation (3) from AOAC (2002), it can be calculated for the presence of ash content [29].

$$Ash\ content\ (\%) = \frac{weight\ of\ ash}{weight\ of\ pectin} \times 100 \ldots\ldots\ldots (3)$$

2.4.3 Equivalent weight

In a 250 mL conical flask, 0.5 g of pectin was mixed with 5 mL of ethanol (95 %). Then, one g of NaCl and 100 ml of distilled water were added, followed by 0.1 N NaOH and six drops of phenol red indicator. The titration point, indicated by the purple coloration, was reached. The neutralized solution was set aside for future methoxyl analysis. To determine the corresponding weight, using equation (4), the equivalent weight was deduced [30].

$$Equivalent\ weight\ (EW) = \frac{weight\ of\ samples\ (g)}{mL\ of\ alkali \times N\ of\ alkali} \times 100 \ldots\ldots (4)$$

2.4.4 Methoxyl content

In order to ascertain the methoxy content, the solution was neutralized by adding 25 mL of 0.25 N NaOH and then being agitated at room temperature for 30 min following the equivalent weight titration. As an end point, a purple color was achieved by adding 25 mL of a 0.25 N HCl solution. To get the methoxyl concentration, equation (5) was used as reported by Thi et al. (2021) [31]:

$$Methoxyl\ content\ (\%) = \frac{mL\ alkali \times N\ alkali \times 3.1}{weight\ of\ sample} \ldots\ldots. (5)$$

2.4.5 Total anhydrouronic acid (AUA)

An exact estimation of the AUA level is necessary for the extraction of pure pectin with the desired degree of esterification (DE) and physical properties. In compliance with this, equation (6) was used to determine AUA by plugging in values for the equivalent weight and methoxyl content [32].

$$AUA\ (\%) = \frac{176 \times 0.1z \times 100}{w \times 1{,}000} + \frac{176 \times 0.1y \times 100}{w \times 1{,}000} \ldots\ldots\ldots\ldots (6)$$

Where, w = sample weight (g), y = Methoxyl concentration in mL of NaOH, and z = mL of NaOH from equivalent weight.

2.4.6 Degree of Esterification (DE)

In this present study, DE was determined by adopting the procedure described by Ana et al. In this equation (7) was used to calculate the pectin DE [33].

$$DE = \frac{176 \times MeC\ (\%)}{31 \times AUA\ (\%)} \times 100 \ldots\ldots\ldots (7)$$

where %AUA = Anhydrouronic acid content and %MeC = Methoxyl content.

2.4.7 Structural properties and Flow behavior of EPP

The extracted EPP was analyzed by Fourier transform-infrared (FT-IR) and hydrogen-1 nuclear magnetic resonance (H-NMR) spectroscopy to confirm and investigate the structure of pectin. In addition, X-ray diffraction (XRD) spectroscopy was applied to obtain further information about the structure of pectin. The FT-IR spectrum of EPP pectin was collected in KBr pallets on a Bruker Tensor 27 FT-IR spectrometer (Billerica, MA, US), over a wavelength range

from 600 to 4000 cm^{-1}. NMR spectroscopy of pectin was accomplished on a Varian Unity Inova 500 NMR spectrometer (Varian, Inc.). The H-NMR spectrum was recorded at an internal temperature and acquisition time were 27 ^{o}C and 4.0 s, respectively. The XRD pattern of pectin was taken on a PHILIPS diffractometer. The diffraction angle (2θ) was in the range of 10–80^{o} with a step size of 0.05^{o} (2θ). The flow behavior of EPP aqueous solutions at concentrations of 0.1, 0.5, 1.0, 1.5, and 2.0 % (w/v) was measured using an LVDV-II Pro viscometer (Brookfield Engineering Inc., USA). The shear rate was ranged from 1 to 100 s^{-1} and the temperature was 25 ^{o}C. It is noteworthy that, three replicates were recorded for each experiment and the average results were reported.

2.5 Preparation of cellulose-reinforced Biofilm using EPP

A mixture of 2% (w/w) of cellulose powder (microcrystalline, CAS 9004-34-6, Merck, India) was prepared in 1% (w/v) citric acid solution under constant magnetic stirring while heating up to 70 °C. The acid solution also contained 7% (w/w) glycerol and 1 drop of organic antifoam/100 mL solution. The suspension was sieved through a metal sieve to eliminate nascent air bubbles before it was poured onto polytetrafluoroethylene plates and dried at 40 ^{o}C. Each plate contained 30 g of suspension and the diameter of the plates was 100 mm. The drying process was performed in a laboratory an incubator (New Brunswick, Scientific Excella, E24, USA). In the following, bio-films (EPP-Fs) were dried and peeled carefully. The properties of prepared EPP-Fs were examined for additional studies [34].

2.5.1 Mechanical Testing.

ISO 527 compliant tensile testing was performed for EPP-F using the Tinius Olsen H10KT universal tester in conjunction with the QMat software suite. At a test speed of 10 mm/min, a cross-head and a load cell measuring 250 N were used to rip apart the dumbbell-shaped samples. Elongation at break (percent), tensile strength (MPa), and elongation at max tensile strength (%) are presented as averages from triplicate tests on 22 mm by 4 mm gauge specimens (percent) [35].

2.5.2 Thermal Analyses

The decomposition characteristics of EPP-F were analyzed using thermogravimetric analysis (TGA; Q500 TA equipment; Waters LLC; USA). Each sample, ranging in weight from around 6 to 8 mg, was heated to 700 °C at a rate of 10 °C/min from room temperature. In the end, it all have

come down to analysis. A mean is presented after tests were performed in triplicate. Differential scanning calorimetry (DSC) was used to determine thermal properties (Q2000 TA Instruments, Waters LLC, USA). An EPP-F sample weighing between 6 and 8 mg was heated in an aluminium pan from 20 to 200 °C at a rate of 10 °C/min for both the initial and repeat scans. The analysis was done in a nitrogen atmosphere. Here, the average of three replicates of the experiments was provided. Using a tension film clamp, we conducted a DMTA (Q800, TA Instruments, Waters LLC, USA) in multifrequency strain mode to assess dynamic mechanical parameters. Before being subjected to a temperature gradient of 3 °C/min from 0 to 140 °C, a strain frequency of 1 Hz, and an amplitude of 15 m, the samples were cut to standard dimensions of 5.3 mm in width and 8-9 mm in length. The experiments were repeated three times [36].

2.5.3 X-ray Photoelectron Spectroscopy and UV transmission measurements

The film surface properties were characterized by XPS and transmission measurements. Hence, XPS Analysis (PHI Genesis Instruments, US) was carried out for deducing the chemical composition of the films' surface. Additionally, the UV 2450 Shimadzu Model Spectrophotometer was used to record the UV-Visible transmittance spectra. The spectrum was captured using film samples that were 0.055 ± 0.015 in thickness.

3. Results and Discussion

3.1 EPP Yield

In comparison to low (300 W) and high (600 W) power at pH levels 2, 3, and 4, pH 1 and a medium power level produced the highest pectin output ($p < 0.05$). Figure 1(a) displays the green EP utilized for extracting the pectin and Figure 1(b) and 1(c) show the extracted pectin and developed biofilm prepared from the pectin, respectively. Figure 2 shows the difference in pectin extraction yield from EPP using different pH values. Pectin yields from green EPP varied from 5.1 % to 14.0 %. Microwave power, pH, and their interactions all played a significant role in the final pectin output. At 400 W of microwave intensity and pH 1, EPP produced 14.0 % pectin, while at 400 W of power and pH 2 it produced 10.3 % pectin. However, pectin yields were not constant across all extraction scenarios. It's possible that variations in the fruit's cell wall thickness and the amount of polysaccharide contained in the intermediate lamella account for the observed differences in pectin output. According to Chen (2020) [37], pectin is a polysaccharide present in plant tissues, specifically the middle lamella, and its concentration varies with fruit maturity and

type. The high yield was traced back to the extraction technique, which involved the use of a solvent that was able to penetrate deeper into the purple peels' finer tissue, where insoluble pectic material in the cell wall was converted into soluble pectin [38].

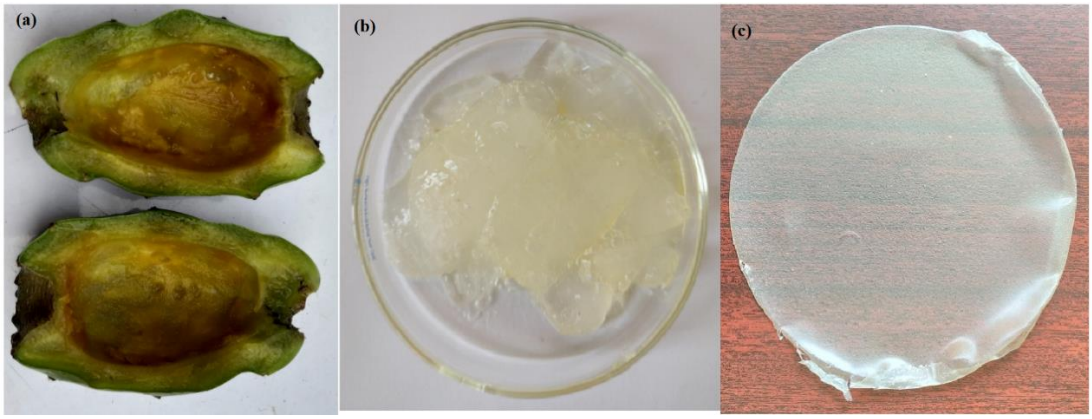

Figure. 1 (a) displays the green EP utilized for extracting the pectin and (b) extracted pectin (c) the biofilm prepared from the extracted pectin.

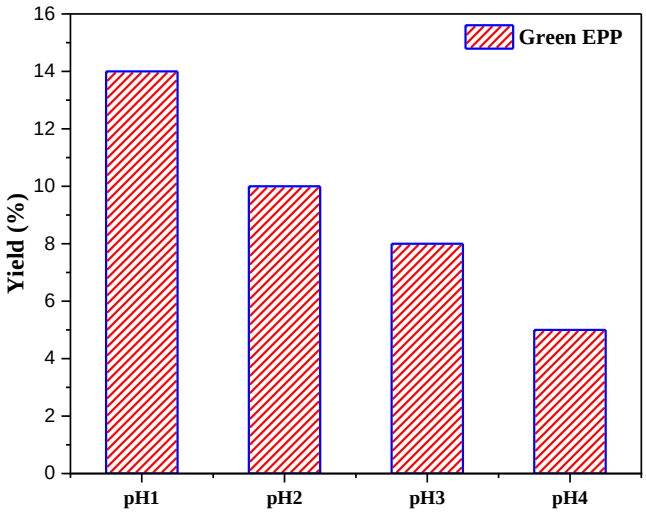

Figure 2: Pectin yield at 400 W microwave power at various pH levels extracted from EPP

The data also showed that pectin production went down when the pH went up. An increased pH causes a buildup of pectin, which slows down pectin release in plants. According to research by Shan et al., protopectin is created when cellulose and pectin molecules join forces [39]. Therefore, protopectin is separated during acid hydrolysis, yielding soluble pectin and cellulose.

Simultaneously, protopectin was transformed into soluble pectin as a result of the elimination of calcium and magnesium ions [40]. However, at a low pH, the abundance of hydrogen ions inhibits the hydrated carboxylate groups, transforming them into weakly hydrated carboxylic acid groups. Since the loss of charge decreased the repulsion between the polysaccharide molecules, the gelation characteristics of pectin were enhanced at lower pH values. Decreases in pH facilitate the release of pectin molecules from the peel by lessening their interaction with the hemicellulose fractions.

The findings are consistent with those of Yeoh et al. (2008), who found that orange peel pectin synthesis was increased by 4.5% when extracted using a microwave at pH 1 [41]. Acid-enhanced cell wall breakdown and consequent increases in pectin release are responsible for the increased pectin output [42]. Polysaccharides that could co-precipitate with pectin when ethanol was used to hydrolyze, and glucose release from starch hydrolysis increased as pH fell. Variations in EPP components across various kinds and extraction conditions were hypothesized to account for the significant standard deviations. However, caustic and potentially dangerous to human health, powerful acids like sulphuric acid were limited as described by Liew et al. [43]

3.2 Characteristics of extracted EP pectin

Table 1 lists some of EPP's physicochemical characteristics. The EPPs averaged 7.70 percent moisture. The moisture level of the isolated pectin utilized in this study was 7.70%, which is well within the pectin quality criterion range of 12% outlined by the Food Chemicals Codex (2016). Perez-Martnez et al. (2021) observed that the moisture level of purple, orange, and green prickly pear peel pectin was 7.55 percent, which is similar to what was found in a study on Opuntia cladode flour pectin [44]. However, Islam et al. (2012) backed up the results by saying that dragon fruit pectin typically has a moisture level of 11.3% [45].

Table 1: Physicochemical qualities of pectin extracted EPP

Characteristics	Observed value
Moisture content (%)	7.70 ± 2.00^{a}
Ash (%)	25.16 ± 0.69^{a}
Equivalent weight	119.73 ± 5.74^{a}
Methoxyl content (%)	3.86 ± 0.31^{b}

TAUA (%)	38.84 ± 2.29^{b}
Degree of esterification (%)	56.39 ± 1.60^{b}

Values are mean ± standard error of mean. In this case, the means that share a row are not statistically distinct from each other ($p < 0.05$). TAUA = Total Anhydrouronic Acid.

Due to its lower moisture content and dependence on drying techniques, pectin has a limited potential to absorb water. Pectin with a low moisture content hinders the growth of microorganisms that could affect its quality by producing pectinase enzymes, as noted by Castillo-Israel et al. [46]. EPP had a moisture percentage of 7.57–8.87 percent, while commercial pectin typically falls within a 7.0 percent moisture content range. Pectin extracted from golden delicious apple pomace has a moisture level of 8.80 percent, according to research by Jain et al. [47]. Since the green kind has a lower moisture content, the EPP it yields is more stable.

The amount of minerals in a food can be determined by calculating its ash content. The higher the mineral content of a food, the more ash it will have. Pectin extracted from green the EP variety had average ash percentages of 25.16 percent. Significantly ($p < 0.05$), it had less ash content. If the pectin was partially esterified under acidic (low pH) extraction conditions, the increased ash content could be attributed to higher concentrations of the carboxylic group of pectin and the counter ions. Hot acid-extracted pectin contains a sufficient amount of galacturonic acid to be regarded as a functional ingredient.

The majority of the ash comes from the high calcium level. Ash content, as noted by Islam et al. (2012), shifts depending on the extraction process and kind of fruit. The average ash level on cladode pectin found in this study is higher than the 16.65 percent found by Perez-Martnez et al. (2013). Islam et al. (2012) found that the ash content of dragon fruit pectin ranged from 6.9 to 11.6 %. Gel formation is affected differently by high ash pectin (10.69 percent) and low ash pectin (0.76 %) according to research by Rajni et al. [48]. However, ash content was generally higher than these estimates for EPP.

Unesterified galacturonic acid in pectin molecular chains accounts for the mass equivalence. The strength of the pectin gel can be determined by the relative weights of the ingredients. Pectin equivalent weight was found to be 119.73 g. The results showed that EPP was substantially (p 0.05) lighter. Hence, The EP fruit probably couldn't be selected until it was still immature. According to Tanje et al., the extraction method, country of origin, and ripeness of the fruit all play

a crucial role in the resulting comparable weight [49]. Pectin equivalent weights reported for orange, purple, and green prickly pear fruits were typically lower than those reported for lime and lemon. This low equivalent weight can be attributed to the use of sulphuric acid (a strong acid) as an extractant, which had a profound effect on the macromolecular and gelling properties of the pectin. This objective was reached by depolymerizing the galacturonan chain and lowering the free acid concentration. Gel formation, as a consequence of the pectin being significantly degraded, increases with a higher equivalent weight, while gel dissolution, as a result of a lower equivalent weight, decreases with a higher D-galacturonic acid (GalA) concentration [50]. In most cases, the corresponding weight of the extracted pectin from EPP types was smaller, indicating weaker gel formation.

The average amount of methoxyl present in EPP was 3.86 percent. Comparatively, the pectin from the EP had a greater methoxyl content than those from studies elsewhere. Shorter polygalacturonic acid chains resulted from the low pH and appropriate microwave power, which may explain the high methoxyl concentration observed. These results corroborate Islam et al. (2012) observation's that the methoxyl concentration of dragon fruit pectin was 2.98 - 4.34 percent. According to Salma et al. (2012), the methoxyl level of lemon peel pectin was 1.56% [51]. Depending on the pectin source and extraction process, the percentage of methoxyl in pectin can range from 0.2% to 13%, as reported by Aina et al. (2012). Researchers found that low methoxyl pectin (those with a methoxyl content of 7% or less) gel at lower sugar concentrations or in the absence of sugar. Despite having a methoxyl level of less than 7% in most cases, EPP is a superb source of pectin because of its low ester concentration.

The purity and DE are based on the AUA content. In addition, it assesses the extracted pectin's physical properties and recommends that it be at least 65% pure (Food Chemicals Codex 2016). There was an average AUA concentration of 38.84 in pectin isolated from EPP. The pectin was found to be pure because it has a lower ash percentage when extracted. In general, the AUA percentage recovered was less than 65%, suggesting that the pectin that was extracted may not be pure enough. In addition, galactose, arabinose, and rhamnose make up the bulk of EPP pectin's neutral sugar content. However, the extraction conditions affect how much GalA and neutral sugars are present in pectin. There is an adequate amount of GalA, a useful addition, in pectin extracted using hot acid. Partially esterified pectins have at least 10% organic components made

up of sugars like arabinose and galactose. Islam et al. (2012) found an AUA range for dragon fruit pectin of 45.3 - 52.2 %; however, the values found in the present research is much lower.

DE refers to the fraction of pectin's GalA groups that have been esterified. It plays a crucial role in determining the gel formation of pectin. Pectin DE was found to be 56.39 percent. The EPP's high DE values can be explained by its advanced age, specific origin and tissues, and unique extraction techniques. In studies elsewhere, Islam et al. (2012) reported DE values for dragon fruit ranging from 31.05 to 46.96%, and the values obtained are consistent with this range. Since green and orange pectin both have DE values greater than 50%, they qualify as high methoxyl pectin. High methoxyl pectin is more preferable as a thickener in soft drinks because it forms gels with lots of sugar with a low pH value. Because of these qualities, fruit juices and low-calorie or diet beverages can benefit from using pectin to provide a similar mouthfeel.

3.3 Molecular size distribution of Pectin extracted from EP

Isolated EPP was further characterized for emulsifying characteristics, antioxidant activity, and rheological qualities that may be drastically altered by its average molecular weight (Mw). As can be seen in Figure 3, there are two distinct populations within EPP: (i) a portion whose molecular weight distribution spans around 0.8–2.5 kDa, with an average Mw of 1.5 kDa; this is probably due to the presence of pectic oligosaccharides; and (ii) a subset having a median Mw of 615 and a range of Mw between 19.9 and 2000 kDa. Therefore, under ideal circumstances for extracting SLP pectin, a Mw of 615.8 kDa was achieved. However, EPP should be viewed as a heterogeneous natural polysaccharide due to its large Mw dispersion. When compared to sugar beet pulp pectin (70-355 kDa) and okra plant pectin (791-2693 kDa), EPP had a larger average Mw. The wide range of Mw could be due to factors like extraction technique or plant origin. Pectin's Mw can be lowered by increasing the microwave power and irradiation period, as shown by Yahui et al. [52].

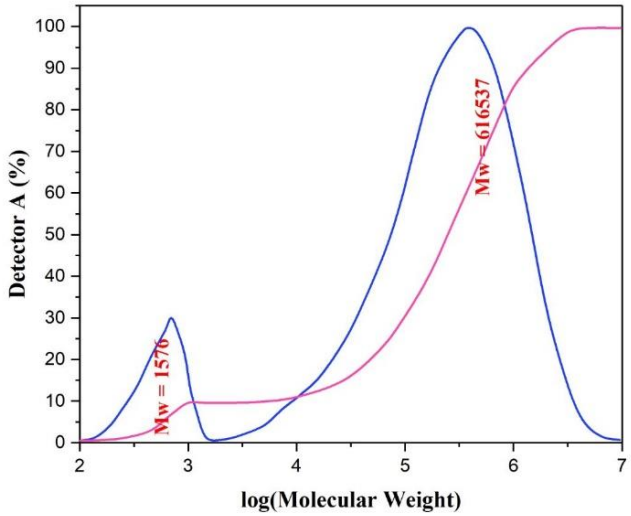

Figure 3. Molecule size distribution of Pectin from EP

3.4 Structural properties of EPP

Spectroscopic techniques like FT-IR and H-NMR [Figure 4(a) and 4(b)] were used to prove that pectin from EP, which was isolated under ideal condition possessed specific structural characteristics. As seen in Figure 4(a), the FT-IR spectrum of EP pectin showed the wide-ranging signals, measured between 3200 and 3700 cm^{-1}that are attributed to those locations. The C-H bonds of the CH, CH_2, and CH_3 groups were responsible for the recently observed signal at 3191.52 cm^{-1}. It was determined that the peaks between 1250 and 1500 cm^{-1} in GalA units' spectra were caused by their glycoside linkages (C-O-C) [53]. The GalA molecule's esterified and free carboxyl groups produced the 1993.85 and 1894.46 cm^{-1} signals, respectively. These two peaks, which indicate EP pectin's DE value, agreed with the result of the DE measurement. The esterified groups ($AOCH_3$) of GalA units are responsible for the strong signal at 3.67 ppm in the NMR spectra of EPP pectin [Figure. 4(b)]. Hydrogen-bonding signals were detected at 3.7, 3.9, 4.0, 4.8, and 5.1 ppm for C2, C3, C4, C1, and C5, respectively. The findings demonstrated that the extracted samples had a significant pectin structure. Pectin results were consistent with those seen in the literature.

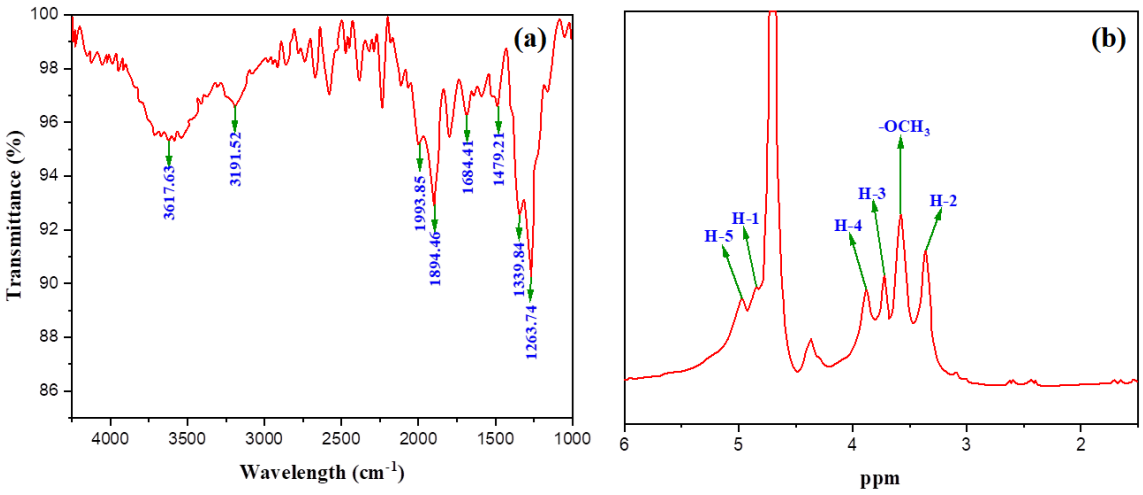

Figure 4. FT-IR and NMR spectra of extracted Green-EPP pectin

For a deeper structural investigation, Figure 5, which displays the XRD pattern of EPP. In this sense, an amorphous structure was inferred from the XRD pattern. The crystallinity of particular locations within the EPP pectin structure may account for the existence of strong signals at 12.61, 14.18, 15.23, 20.71, 21.81, 23.16, 33.26, and 37.66. Therefore, the XRD pattern produced indicated that EP pectin might have either a crystalline or an amorphous structure. Previous studies using various types of pectin have shown similar outcomes.

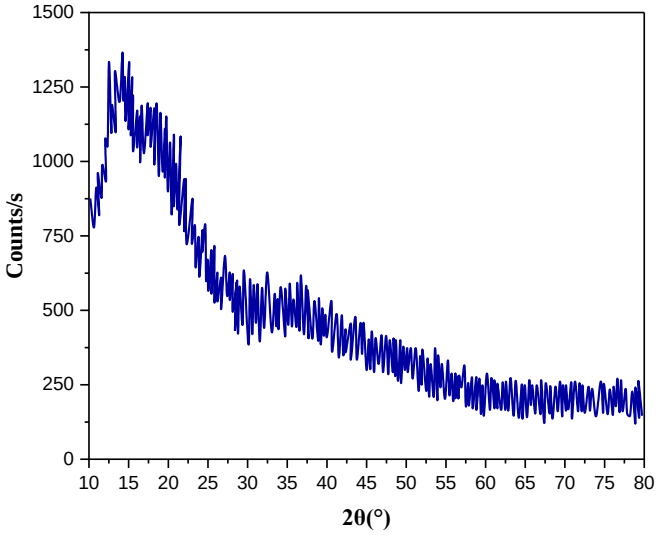

Figure 5. The Fig. XRD spectrum of Ethiopian Green Prickly peel pectin.

3.5 Flow behavior of EPP

In this study, the effect of concentration on the viscosity of EPP solution (Figure. 6) was discovered. It was found that the solution's apparent viscosity changed as a function of shear rate. The viscosity increased with the pectin concentration dose. Samples showed virtually Newtonian flow behavior at low concentrations (0.1, 0.5, and 1 % w/v), while pseudoplastic flow behavior took control at concentrations above 1 percent w/v. Additionally, increasing the shear rate to 10 s^{-1} caused a drastic decrease in viscosity at 1.5 and 2 % w/v. However, the rate at which viscosity declined beyond that shear rate was drastically reduced. The pseudoplastic behavior observed by Caian et al. [54] at 1 % w/v pectin concentration corroborates the solution's Newtonian behavior at lower concentrations. Pectin viscosity varies according to a number of structural and physicochemical factors in addition to molecular weight (Mw).

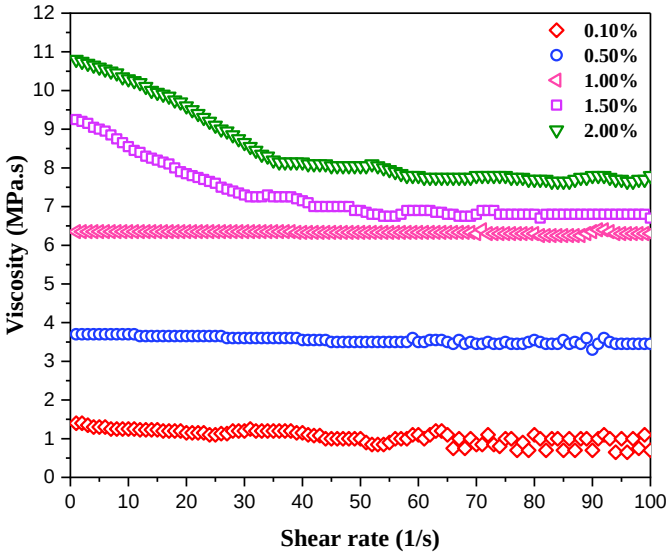

Figure 6. Different concentrations of EPP solution and their effects on flow behavior

3.6 Mechanical properties of EPP-F

The films created in this work using EPP (EPP-Fs) showed tensile strengths between 28 and 36 MPa (Figure 7). Cellulose-EPP composite was prepared by adjusting the ratio of EP pectin to cellulose by adding up to 8% w/w for better reinforcement property, as demonstrated by the work

of Yu et al. [50]. Unreinforced polymers were found to have tensile strengths between 16 and 32 MPa, and these values increased with reinforcing. High density polyethylene (HDPE), Low density polyethylene (LDPE), polytetrafluoroethylene (PTFE), polystyrene (PS), and polypropylene (PP) all have tensile strengths within the same range as EPP-Fs. In Figure 3, there was seen a contrast between the two. Except for PS (1.2-2.5%), the elongation of other common plastics was substantially more than that of EPP-F (LDPE 100-650 %; HDPE 10-1200 %; PTFE 200-400 %; PP 100-600 %).

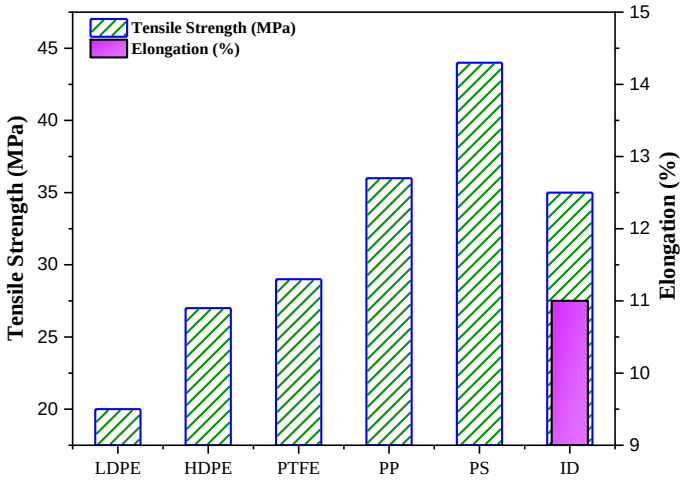

Figure 7. Analysis of Mechanical properties for Cellulose-reinforced EPP-F

3.7 Thermal Characteristics of EPP-F

Figure 8(a) display the results of the thermogravimetric analysis. The pattern was found to be depended on heat deterioration that occurred in three distinct phases. First thermal event was up to 3.95 % weight loss owing to evaporation of water in samples at an average temperature of 81.58 °C. Biopolymers and glycerol are both hygroscopic, meaning their moisture content shifts depending on the humidity of the air around them. The second thermal event, associated with the depolymerization of the pectin present in the film, occurred at 258.70 °C, with a breakdown ratio of 29.14 %. After heating, the film lost between 21.7 % of their mass to ash.

The glass transition temperature (T_g) of the films was determined by differential scanning calorimetry [Figure 8(b)]. For the EPP-F samples at first scanning, an endothermic transition was

observed approximately at 84.65 °C with onset temperature of 60.53 °C. When the sample was rescanned, the endothermic transition was no longer present. After rescanning the materials, the endothermic curves of biopolymers were no longer visible, as described by Aguilera et al. There was no melting point for EPP-Fs since thermograms showed no T_g. It follows that EPP-Fs are presumed to be amorphous due to the presence of fewer crystals [55].

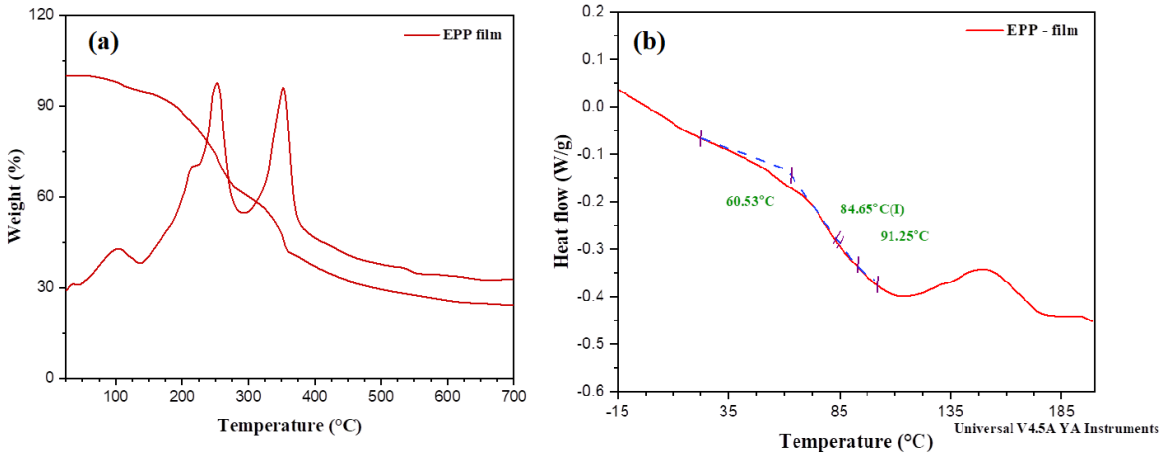

Figure 8. (a) TGA and (b) DSC thermogram displaying the EPP-film weight loss including derivative weight curves and an endothermic transition, respectively

3.8 UV transmission Characteristics and X-ray Photoelectron Spectroscopy

The UV-Vis absorbance spectrum of the developed film is presented in figure 9. The film has the low transparency, indicating that this film adsorbs more light. The low transparency is intimately related to the thickness [56]. The transparency (35.10 % mm^{-1}) is approximately similar to the transparency of polypropylene (38.20 % mm^{-1}) and higher than low-density polyethylene's transparency (between 15 and 20 % mm^{-1}) [57]. Similar to these results, Cazon and coworkers showed that poly (vinyl alcohol)/cellulose/Gly films have low transparency and absorb UV light as the Gly content is increased in the film. In their study, the highest transparency (89.38 % mm^{-1}) was achieved at 3.0 % wt/wt cellulose, 5.0 % wt/wt Gly, and 5.0 % wt/wt poly(vinyl alcohol) [58]. Tan and coworkers developed CHT films with grapefruit seed extract for bread packaging [59]. The light barrier protection rose from 58.84 to 93.14 % mm^{-1}) as the fruit extract concentration increased from 0 to 1.5 % wt/wt. Commercial packaging, including syndiotactic polypropylene

(89.1 % mm^{-1}), polyester (83.5% mm^{-1}), and poly(vinyl vinylidene chloride) (90.0 % mm^{-1}) have similar properties [41].

The chemical composition of the films' surfaces was analyzed by XPS. XPS (survey) spectrum is shown in Figure 10(a). XPS spectra show characteristic peaks assigned to oxygen (O1s, 533 eV) and carbon (C1s, 284–289 eV) [60]. These elements occur in the chemical structures of pectin, and glycerol. The film was characterized by the presence of C1s 68.2% and O1s between 27.0 % - 29.3 %. Pectin and glycerol that are mostly composed of carbon and oxygen. The high-resolution XPS spectra confirm the presence of characteristic chemical groups related to the polysaccharide structures and glycerol. The peaks assigned to –C–C and –C–H (283–286 eV), –C–O (285–288 eV), –C=O (286–289 eV), and –COOH (287–289 eV) [61]. The high-resolution XPS spectra (C1s) of the developed film present –C–O peaks with high intensities due to the presence of glycerol. This effect was evident with the 20 wt. % glycerol concentration.

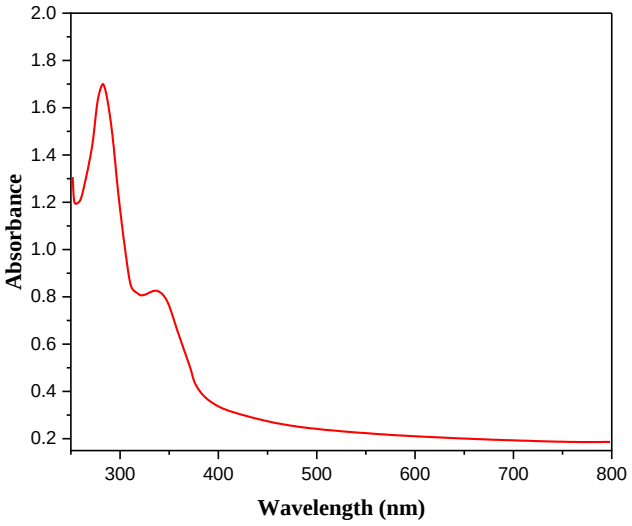

Figure 9. UV-Vis spectrum of the developed film.

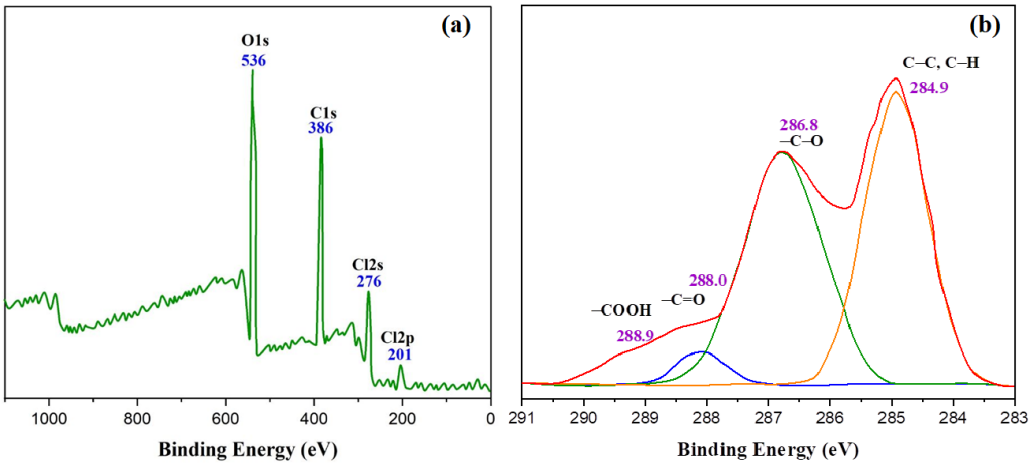

Figure 10. (a) Survey XPS spectrum and (b) High-resolution XPS spectrum of developed film

4. Conclusion

The quantity and type of pectin produced by Ethiopian green prickly pear (EP) fruit was affected by its pH, total soluble solids, titratable acidity, texture, and peel thickness, among other physicochemical characteristics. Pectin (EPP) was extracted most effectively at a pH of 1 and a microwave power setting in the sweet spot of the microwave intensity (medium power, 400W). The results of analyses for moisture, methoxyl, ash, equivalent weight, DE, and AUA were all within the acceptable limit for the EP pectin. FTIR spectroscopy confirmed that the isolated EP pectin, like conventional citrus pectin, had a wide range of functional groups. According to the results of the current study, EP can be a viable raw material for pectin production in the food processing industry. The food and pharmaceutical industries can all benefit from this citrus-based alternative to high-quality pectin. In addition, the cellulose-reinforced biofilm made from pectin from prickly pear fruit peel waste showed very good physical properties in terms of mechanical and thermal properties as compared with the some of the commodity plastics.

Declarations:

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Competing interest: Not applicable.

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Author Contributions:

Sugumar Mohanasundaram: Methodology, investigation, writing-original draft. **Barinderjit Singh**: Software application, review & editing. **Nitin Govindprabhu Suradkar**: Conceptualization, supervision. **Venkatesa Prabhu. S**: Methodology, Study Design, supervision and validation. **Gomadurai Chinnasamy**: Characterization and validation. **Mukesh Goel**: Data curation, and Formal analysis. **Jamal M. Khaled**: Project Administration, Editing-original Draft and Funding acquisition.

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