



Optimization of Extraction Condition and Characterization of Low Methoxy Pectin From Wild Plum

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Abstract

Edible films are the latest trend in food preservation. These films are prepared using natural biopolymers and food grade additives. Natural biopolymers such as carbohydrates, protein, lipids in combination with other additives are used to produce edible films. Among all the polymers, pectin is in high demand due to its flexibility and structural variability. In the present study, pectin from wild plum pomace was extracted and different conditions for the pectin extraction were optimized. Pectin extraction was carried out using hydrochloric acid, nitric acid and citric acid (0.5 N, pH 2.0) at 45 °C and 90 °C for 90 min. Biochemical characterization classified plum pectin as low methoxy (LM) pectin. Further characterization revealed that various parameters such as equivalent weight, methoxyl content, the degree of esterification and neutral sugar contents vary with different extraction protocol. The highest yield was obtained using HCl (13.26% dry basis) at 90 °C; whereas, highest equivalent weight was shown in pectin extracted using citric acid at 45 °C. Therefore, plum could be used as an efficient source of pectin.

Keywords Plum fiber · Pectin · Pectin oligosaccharide · Functional food

Introduction

Pectin is a complex mixture of high molecular weight, branched heteropolysaccharide that forms a matrix with celluloses and hemicelluloses. Pectin contains galacturonan segments and also other neutral sugars such as rhamnose (Rha), arabinose, galactose and xylose. Rhamnose (Rha) is a minor component of the pectin backbone and introduces a kink into the straight chain, whereas other neutral sugars are linked as the side chains [21]. Due to the presence of several sugars and partial methyl esterification of the main chain, pectic substances do not have a defined molecular weight like proteins, lipids and nucleic acids.

Pectin has a wide range of applications. In food sectors, pectin is used as emulsifier, gelling agent, thickener, stabilizer, and fat or sugar replacer in low-calorie foods [31]. In medicine, pectin is used mainly because of its anti-diarrhea property, for lowering of blood cholesterol levels and as a

natural prophylactic substance against poisoning with toxic cations. Pectin and pectin-derived oligosaccharide can also be used as functional foods [10]. In the recent years, increasing demand of fresh-cut fruits and vegetable has open a new market for edible films that are synthesized using natural biopolymers and food grade additives. These films reduce the exchange of moisture, gases, lipids and volatiles between food and environment, and also serve as protective barrier for microorganisms. Being biodegradable and recyclable, pectin films are preferred choice for the edible films. The degree of polymerisation (DP) and degree of methylesterification (DE) of pectin determine its application. The DP of the pectin molecule varies from a few hundred to 1000 saccharide units, which corresponds to molecular weights from about 50,000 to 150,000 (<http://www.herc.com/foodgums/pectin>).

According to the Ministry of Commerce (GoI), import of pectin to India has increased from \$4.84 million to \$10.14 in last few years. This trend is supposed to continue for next decades. Traditionally, citrus peel and apple pomace are considered as the commercial source of pectin due to their high pectin content and good colour properties. Most recently, pectin has also been extracted from other sources of pectin such as sugar beet, sunflower, guava, sun flower husk, etc. [1,

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4, 19]. It has been found that the extraction conditions vary with the nature of raw material and process [2] and therefore, the chemical characteristics and biological properties of pectin are influenced by the extraction conditions. Hence, it is important to select extraction conditions that allow high pectin yields without compromising the quality. Overall goal of this project is the extraction of pectin from wild plum and optimization of the variables within each extraction method to produce the highest yield of pectin without compromising the quality. Ultimately, this project will help to improve cost-efficiency and lower the environmental impact.

Plums are a diverse group of species which belong to the prunus genus of plants. These are produced in all the continents of world and India is the second largest producer of plum. In India, plums are mainly grown in the North-Western Indian States of Himachal Pradesh, Jammu and Kashmir, Uttar Pradesh and Utrakhhand. Annual production of plums in India is approx 12,000 ton. Plum fruits are eaten as fresh fruits, and also processed in plum jam, juice, squash and other recipes. Dried plums are called prunes. Fermented plum juice is used as plum wine in some of the European countries. During the processing of plum juice, pomace is produced as the waste product, which is used in animals feed. Plum pomace is a rich source of high-quality dietary fiber (up to 64.5%) and the good amount of polyphenols [16]. About 38–49% of the dietary fiber in plums pomace is pectin. Therefore, plum pomace can be considered as raw material for pectin.

$$\text{Methoxyl content (\%)} = \frac{\text{Vol of alkali (mL)} \times \text{Normality of alkali} \times 31 \times 100}{\text{Wt of sample (g)} \times 1000}$$

Materials and Methods

Sample Collection

Wild plums (*Prunus domestica*) were purchased from local market of Lucknow, UP(India), washed thoroughly, destoned and dried in tray drier for 5–6 h. Dried pulp was grinded to fine powder in grinder and used for pectin extraction.

Extraction of Pectin

To optimize the different parameters viz: type of acid, mild or strong (HCl, HNO₃ or citric acid) and temperature (45 and 90 °C) of the extraction, 5 g dry plum powder was added to 250-ml acidified water (pH 2.0). The mixture was then heated on water bath shaker with continuous stirring. After heating, the mixture was filtered using cheesecloth, and the filtrate was cooled down on ice. The filtrate was precipitated with an equal volume of 95% ethanol and left for 2 h at room temperature. The precipitate was filtered through Whatmann

No.1 filter paper, washed once with 70% ethanol containing 0.5% HCl, then with 70% ethanol to a neutral pH and finally with 96% ethanol. The resulting material was dried overnight at 55 °C in an air-forced oven.

Pectin Characterization

Determination of Equivalent Weight and Methoxyl Content (MeO)

All the methods were used as described by Ranganna [27]. For the determination of equivalent weight, pectin sample (0.5 g) was taken into a 250-ml conical flask and moist with ethanol (5 ml); then, 0.1 g of sodium chloride and 100 ml of distilled water were added. Few drops of phenol red indicator were added to the solution and titrated against 0.1-N NaOH. Appearance of purple color indicated titration end point.

$$\text{Equivalent weight} = \frac{\text{Wt of sample (g)} \times 1000}{\text{Vol of alkali (mL)} \times \text{Normality of alkali}}$$

The neutral solution obtained from determination of equivalent weight was mixed thoroughly with 25 ml of sodium hydroxide (0.25 N) and kept at room temperature for 30 min. After 30 min, mixture was acidified with 25 ml of 0.25-N hydrochloric acid was added and titrated against 0.1-N NaOH. Methoxyl content was calculated by following

Determination of Total Anhydrouronic Acid Content (AUA)

Total AUA of pectin was obtained using formula [19].

$$\% \text{ of AUA} = \frac{176 \times 0.1z \times 100}{w \times 1000} + \frac{176 \times 0.1y \times 100}{w \times 1000}$$

When molecular unit of AUA (1 unit) = 176 g, where z is the ml (titre) of NaOH from equivalent weight determination. y is the ml (titre) of NaOH from methoxyl content determination. w is the weight of sample.

Determination of Degree of Esterification (DE)

The DE of pectin was measured on the basis methoxyl and AUA content [24] and calculated by following formula.

$$\% \text{ DE} = \frac{176 \times \% \text{ MeO}}{31 \times \% \text{ AUA}} \times 100$$

Galacturonic Acid

The galacturonic acid content of the pectin samples was determined calorimetrically by *meta*-hydroxydiphenyl method [5]. Pectin samples (0.5 mg) were dissolved in 1-mL distilled water. To a 400- μ L pectin sample, 40 μ L of a 4.0-M sulfamic acid–potassium sulfamate solution (pH 1.6) was added, mixed thoroughly and kept on ice. To this mixture, 40- μ L H₂SO₄ containing 75-mm sodium tetraborate (2.4 mL) was added, and stirred vigorously on vortex mixer. The solution was incubated in a boiling water bath for 20 min. After cooling, 40 μ L of 0.15% (w/v) *m*-hydroxydiphenyl in NaOH 0.5% (w/v) was added and vortexed to mix properly. Appearance of pink colour in about 5–10 min indicated completion of reaction. Absorbance was read at 525 nm and GalA contents were calculated using a standard curve of GalA.

Determination of Neutral Sugars

Total neutral sugars were determined by Miller [17] method. Pectin powder (0.5 g) was dissolved in 10-ml distilled water and filter through Whatman filter paper. One ml filtrate was mixed with 3 ml of dinitrosalicylic acid (DNS) solution in a test tube and then heated on boiling water bath for 15 min. After cooling, absorbance was taken at 550 nm.

Individual sugar composition was determined according to Yapo et al [33]

Statistical Analysis

All experiments were carried out in triplicate. Results are expressed as mean values standard deviation.

FTIR Analysis

FTIR spectra of extracted pectin were determined by Fourier transform infrared (FTIR) spectrometry (Perkin Elmer, USA) as described earlier [8].

Result and Discussion

Pectin, a large heteropolysaccharide is found in almost all the plants. Commercially, pectin is extracted from apple and citrus fruits. Structure, molecular weight and chemical properties of pectin depend on its source, raw material and also on the extraction conditions. Pectin is generally extracted by hot acidified water. In this study, we have optimized the protocol for the extraction of pectin from plum fruits. For pectin extraction, different parameters such as acid (citric, nitric and hydrochloric acid), and extraction temperature (45 and 90 °C) were used. Results show that the yield of

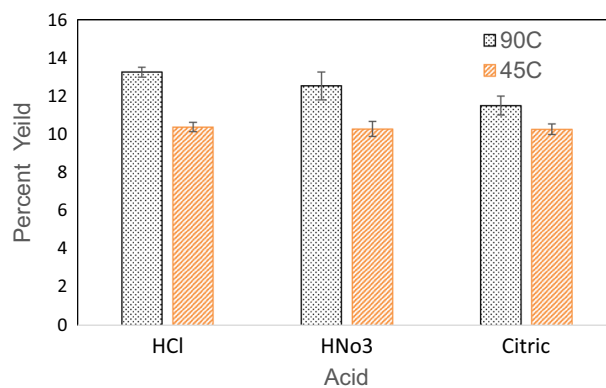


Fig. 1 Effect of different acid and extraction temperature on pectin yield

pectin varied from 10.67 to 13.26% when temperature varied from 45 to 90 °C using HCl, while nitric acid and citric acid yielded little less pectin. The yield of pectin was 10.13–12.98% using nitric acid and 10.08–11.47% using citric acid (Fig. 1). The lower yield obtained using mild acid at 45 °C indicated that ionic strength and extraction temperature both affect the yield of pectin and with increase in the temperature and strength of acid, yield tends to increase. The variance analysis confirmed the significant ($p < 0.05$) increase in yield with decreasing pH (or increasing acid strength). These results are consistent with the earlier studies [11, 33]. Castillo-Israel et al. [4] extracted pectin from Saba banana using hydrochloric acid (0.5 N, pH 1.5) and citric acid (0.5 N, pH 1.7) and obtained highest pectin yield using HCl (17.05% dry basis). Higher yield of pectin using strong acid could be due to the high ionic strength of HCl as compared to weak acids such as citric. High ionic strength of acids has higher affinity for cations which stabilizes the pectin molecule and thus enables better precipitation of pectin.

Total Anhydrouronic acid content (AUA%) in pectin is an essential parameter to evaluate the purity and degree of esterification. It also helps to determine the physical properties of a given pectin sample [29]. In this study, the AUA content ranges from 67.23 to 74.97% for pectin extracted using different acids at the different temperature. The highest AUA was found in pectin extracted with HCl at 45 °C. According to Food Chemical Codex [6], pectin containing AUA contents less than 65% is considered as contaminated with proteins, starch and sugars. These results confirmed the purity of plum pectin.

The percentage of galacturonic acid residues in the pectin backbone esterified with methanol is known as methoxy content or degree of esterification (DE) of pectin. Methoxy content of pectin is the measurements of its capacity to form gel and dispersibility in water. Pectin containing methoxy contents more than 7% is called as high methoxy pectin (HM). HM pectin dispersed easily in water and requires

sugar (> 65%), alcohols, or polyols and acidic environment for gelling. It is reported [30] that acidic environment restrains the dissociation of free carboxylic acid groups, and thus represses their electrostatic repulsion; and sugar molecules in solution help in stabilization of hydrophobic interactions between the methyl ester groups [3, 20, 28]. Hydrophobic interaction is essential to form and maintain gel [20].

Low methoxy (LM) pectin (containing less than 7.0% methoxyl content) forms gels with less concentration of sugars but in the presence of divalent cations such as calcium. Divalent ions are required to form bonds with adjacent pectin polymers and stabilize gel. Methoxy contents of pectin depend on the source and also mode of extraction of pectin. It may vary from 0.5 to 12% [1] in different pectin. Table 1 shows the methoxy contents of pectin extracted in this study. The results shows that pectin obtained from plum is low methoxy pectin and methoxy contents obtained from plum pectin are in agreement with the known pectin such as banana (7.03%), citrus (9.09%), peel of mango (7.33%), pomelo peel (8.57%), [12], passion (8.81–9.61%). Highest and lowest methoxy contents were found in pectin extracted using HCl at 45 and 90 °C, respectively.

To assess the chemical properties of plum pectin extract, the extracted samples were analyzed by Fourier Transform Infrared Spectroscopy (FTIR). It is a fast and non-destructive way to analyze the chemical composition and functional groups of a compound. The plum pectin spectrum was compared against commercially available apple pectin (Fig. 2a). The plum extract spectra showed similarities in its absorption pattern to that of commercially available pectin standards in the “fingerprint” region which suggest that the extract is effectively pectin. Furthermore, the higher absorbance at 1650 cm^{-1} than at 1750 cm^{-1} classified the extracted pectin as low methoxy pectin. FTIR spectrum of pectin extracted using different acids at two different temperatures also led to an interesting observation that strength of acid modified the nature of pectin. Pectin extracted with citric acid displayed higher absorbance at 1650 cm^{-1} and 1750 cm^{-1} (Fig. 2b), whereas low absorbance was observed in pectin extracted using strong acid at the same wave length (Fig. 2c, d), which

indicated that strong acids de-esterify the pectin and modify the pectin structure. It is observed in earlier studies also that extraction and processing of pectin alter these in situ ratios. Wai et al. [32] reported that pectin of varying methoxy contents can be obtained from the same sample by acid-, base- or enzyme-catalyzed de-esterification.

The long, heterogenous structure of pectin is prone to chemical, enzymatic, and even physical treatments; therefore, equivalent weight (MW) of extracted pectin may vary widely. The equivalent weight of plum pectin is displayed in Table 1. Lowest equivalent weight was observed in pectin extracted using HCl at 90 °C; whereas, pectin extracted using citric acid at 45 °C showed the highest equivalent weight. The variation in equivalent weight could be due to partial degradation of pectin at higher temperature or treatment with strong acid. Ramli and Asmawati [26] reasoned the presence of free acid in pectin for the increased or decreased equivalent weight.

The sugar composition of the extracted plum pectin is shown in Table 2. The galacturonic acid (GalA) content of pectin ranged from 61.4 to 79.2% as the extraction temperature was varied from 45 and 90 °C. The main neutral sugars were arabinose, rhamnose, galactose glucose, mannose and maltose. The presence of arabinose and rhamnose suggested that plum pectin contained higher amount of rhamnogalacturonic and/or arabinogalacturonic regions [14]. These values were consistent with [15, 23, 25] reported for chicory roots, carrots and sugar beet pectin. The difference in sugar value indicates the effect of different acids and temperature used for extraction. Hwang et al. [9] reported that different extraction procedures and plant source may affect the sugar contents of pectin. Georgiev et al. [7] reported variation in sugar contents from 15.45 to 19.40% in citrus pectin. These results indicate that the polysaccharide was mostly composed of galacturonic acid and a lower proportion of neutral sugars strongly suggesting that the extracted polysaccharide is pectin.

Biochemical studies and FTIR analysis classified the plum pectin as low methoxy pectin. LM pectin has vast application in food industry to give texture to yogurts and also used as food stabilizer in various fruit preparations.

Table 1 Physicochemical characteristics of plum pectin

Characteristics temperature	HCl		HNO ₃		Citric acid	
	90 °C	45 °C	90 °C	45 °C	90 °C	45 °C
Equivalent weight	625 ± 2.6	833.3 ± 4.3	694.44 ± 2.3	909.09 ± 3.3	943.39 ± 1.3	1020 ± 1.7
Methoxyl content (%)	5.32 ± 0.2	6.48 ± 0.1	5.55 ± 0.26	6.23 ± 0.16	6.49 ± 0.19	6.8 ± 0.9
Total Anhydrouronic acid content (AUA) (%)	71.1 ± 1.1	74.97 ± 1.5	73.92 ± 1	71.80 ± 2.3	69.88 ± 3.3	70.23 ± 3.6
Degree of esterification (DE) (%)	42.48 ± 0.5	49.06 ± 4.1	42.626 ± 2.1	49.27 ± 2.9	52.72 ± 4.3	54.9 ± 2.7
Ash content (%)	0.452 ± 0.1	0.483 ± 0.09	0.471 ± 0.05	0.574 ± 0.1	0.842 ± 0.04	0.917 ± 0.2

Mean ± SD (three determinations)

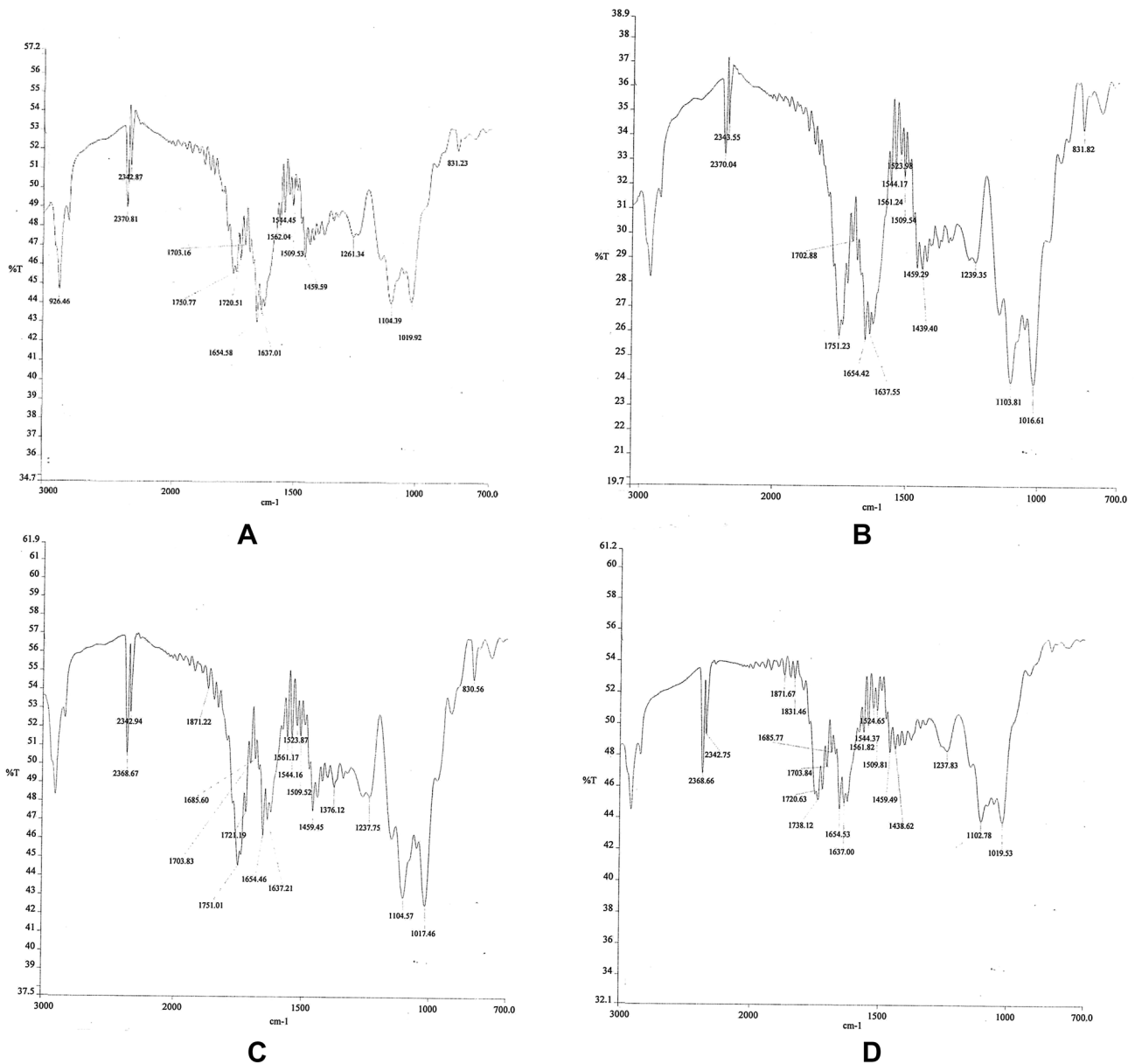


Fig. 2 FTIR spectrum of Apple pectin (A); plum pectin extracted using citric acid at 45 °C (B1) and 90 °C (B2); HCl, 45 °C (C1) and 90 °C (C2) and HNO₃ 45 °C (D1) and 90 °C (D2)

Recent research revealed an interesting and very important application of LM pectin in the preparation of edible films. Oms-Oliu et al. [22] observed that edible films coating made up of low methoxyl pectins, crosslinked with calcium chloride and sunflower oil prevented the dehydration onto fresh-cut melon improved and maintain the initial firmness during storage up to 15 days at 4 °C. Another study reported the extension of the shelf-life of avocados to over a month

at 10° C when coated with ML pectin films [13]. Similar results were reported by Moalemiyan et al. [18] in fresh-cut mangoes coated with pectin films. The study showed that film-coated fresh-cut mangoes remained in acceptable quality conditions for over 2 weeks. Bayarri et al. (2014) used lysozyme/LM pectin complexes to develop antimicrobial films and found that coating of these films was able to control pectinolytic bacterial infection on fruits.

Table 2 Sugar composition of plum pectin

Neutral sugars (%w/w) Temperature	HCl		HNO ₃		Citric acid	
	90 °C	45 °C	90 °C	45 °C	90 °C	45 °C
Rhamnose	4.68 ± 0.3	4.97 ± 0.2	4.01 ± 0.4	4.42 ± 0.1	4.36 ± 0.4	4.14 ± 0.2
Arabinose	2.76 ± 0.1	3.01 ± 0.16	3.21 ± 0.32	3.17 ± 0.09	2.46 ± 0.2	2.93 ± 0.16
Maltose	1.726 ± 0.1	1.54 ± 0.09	1.69 ± 0.1	1.73 ± 0.12	1.67 ± 0.3	1.701 ± 0.14
Mannose	3.54 ± 0.12	3.02 ± 0.1	3.28 ± 0.15	3.67 ± 0.2	3.27 ± 0.25	3.34 ± 0.3
Galactose	3.17 ± 0.31	3.95 ± 0.12	3.54 ± 0.2	3.42 ± 0.17	3.84 ± 0.54	3.26 ± 0.3
Xylose	0.06 ± 0.01	0.09 ± 0.02	0.12 ± 0.01	0.08 ± 0.03	0.06 ± 0.01	0.07 ± 0.03
Galacturonic acid	59.78 ± 0.6	61.25 ± 0.24	61.47 ± 0.43	62.53 ± 0.67	63.75 ± 0.37	63.86 ± 0.28

Results are expressed in g/100 g pectin; All results are obtained from triplicates. Mean ± SD (three determinations)

Therefore, from the present study, it can be concluded that plum pomace is a good source of LM pectin. Physical and chemical methods of extraction significantly affect the yield as well as the chemical composition of pectin. Further research is underway to study the functional properties of the extracted pectin and its application in edible films.

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