Chapter

Extraction of Pectin from Orange Peel Wastes as an Ingredient for Edible Films Containing Kabog Millet Flour

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Abstract

Fossil-based plastic is a popular material for food packaging. It can cause negative environmental consequences due to its low biodegradability. To address this challenge, a pectin-based edible plastic with added nutritional value by incorporating whole-grain kabog millet flour was prepared. The pectin in the films was extracted by microwave-assisted and enzymatic procedures from orange peel wastes. The extracted pectin was tested for its degree of esterification using Fourier-transform infrared spectroscopy, its molecular weight and behavior in aqueous solutions using size-exclusion chromatography, and its monosaccharide composition using ion-exchange chromatography. Biodegradable and edible pectin films were produced and tested for their mechanical properties: maximum strain, maximum stress, and water contact angle. The results showed a significant increase in hydrophobicity of the film surface by adding whole-grain kabog millet flour. The maximum strain of the film, however, was reduced to around 80% upon the addition of the whole-grain kabog millet flour. Enzymatically-extracted pectin increased the film hydrophobicity. Hydrophobic surfaces have higher water resistance; thus, the enzymatically-extracted pectin can be developed for further applications. Due to the low elasticity of the films, a possible application would be as direct coating of fruits and vegetables incorporating antioxidants or antimicrobials.

Keywords: pectin, microwave-assisted, enzymatic extraction, edible films, kabog millet, fruit wastes

1. Introduction

1.1 Application of biodegradable films

In 2016, 335 million tons of plastics were produced globally, of which the majority were single-use plastics. Approximately 40% of the produced plastics were used for packaging [1]. This large volume of plastics leads to several problems: most oil-based plastics are highly resistant to biodegradation, which means if they enter the

environment, they can accumulate, and this leads to adverse environmental consequences [1]. These negative side effects could be reduced with biodegradable plastics. Since biodegradable plastics can be produced from fruit and vegetable wastes, the use of biodegradable plastics also helps valorize food wastes. Creating biodegradable plastics from food wastes could reduce the carbon footprint and the adverse environmental effects of oil-based plastics. Packaging is an essential step in maintaining food quality during manufacturing and shelf life. Depending on the packaging properties, it can also protect from microorganisms and moisture. With proper packaging, shelf life can be extended, and food waste can be reduced [2]. Due to the crucial role of packaging in this context, it is essential to develop new biodegradable materials with desired properties. An interesting application would be a direct coating of a product with a thin layer of edible plastic. The coating could be enriched with antimicrobial substances or antioxidants, which protect the product directly [3].

1.2 Pectin from fruit wastes as an ingredient of edible films

Some biopolymers, such as casein, alginate, and pectin, are increasingly gaining attention because of their inherent biodegradability. Biopolymers made from polysaccharides have a higher thermostability compared to biopolymers based on proteins, such as casein [4]. To improve the properties of the bioplastic, combinations of different polysaccharides or additives can be used. An interesting polysaccharide that could be taken advantage of to form bioplastic is pectin. Pectin is one of the main structural polysaccharides in dicot plants and consists mainly of galacturonic acid units as sugar backbone [3]. The carboxyl groups of the uronic acid residues can be present in different degrees of methylesterification, influencing the processing properties [5]. Pectin is classified as high methoxyl pectin if more than 50% of the hydroxyl groups are esterified, and low methoxyl pectin if less than 50% of the hydroxyl groups are esterified [6]. A significant percentage of pectin is found in fruit peels that are often discarded. It is possible to use the discarded peels to create edible films, which could be applied as coating for fruits and vegetables. Additionally, unused fruit peel wastes can be reused by creating biodegradable plastic [4]. Pectin edible films might be used for the protection of food with low moisture content due to their low resistance to humidity. They also provide an excellent barrier to aroma compounds and oxygen [7]. Their poor water vapor barrier properties could be explained by the hydrophilic nature of pectin [7]. A plasticizer and polyvalent cations are often added to edible pectin film formulations [3]. Plasticizers are used to reduce the brittleness; thus, improving the mechanical properties of the film [8]. Polyvalent cations such as calcium can be applied to crosslink the pectin chains and form a gel [3].

1.3 Extraction methods for pectin from fruit substrates

There are multiple methods for extracting pectin from fruit wastes: for example, enzymatic, acidic, and microwave-assisted extraction. These methods differ in the yield of resulting pectin and its properties. One possibility is the application of an enzymatic preparation like Celluclast® 1.5 L. The enzymes partly degrade the plant cell wall components and enable the subsequent extraction of pectin [9]. The advantage of this method is that no strong acids are used; thus, no acidic wastes are produced. Microwave-assisted extraction is another way to extract pectin, characterized by its short processing time and improved yields. The yield of the extracted pectin

varies with the microwave power used. The higher the applied microwave power, the higher the extraction yield [10].

1.4 Kabog millet flour as valuable ingredient of edible films

Kabog millet is an ecotype of *Panicum miliaceum* L. and grows only in the Philippines. The grain is rich in dietary fibers, proteins, and antioxidants, such as carotenoids and tocopherols [11]. Due to its high nutritional quality, the addition of whole-grain kabog millet flour to edible films is a promising option to improve their nutritional profile. In whole-grain kabog millet flour, lipids are also present, which may improve the hydrophobicity of the biopolymers [12]. Therefore, it may be a solution to one of the most common problems for bioplastics: low water resistance [13]. Whole-grain kabog millet flour has some promising properties that can be integrated into edible bioplastics formulations.

With these concepts in mind, the aims of this study were to 1) extract pectin from orange peel wastes by microwave-assisted extraction and enzymatic extraction; 2) characterize the extracted pectin by Fourier-transform infrared spectroscopy (FTIR) for the degree of esterification, by ion-exchange chromatography for the monosaccharide composition in pectin, and size-exclusion chromatography (SEC) for the polymer structure in water and its molecular weight; 3) create edible films containing kabog millet flour with the extracted and characterized pectin from different extraction methods; 4) characterize the produced films by their mechanical properties and their water contact angle; and 5) observe the effect of adding kabog millet flour on the properties of the films.

2. Materials and methods

2.1 Orange peel samples

The orange peel samples used to extract pectin were kindly provided by the Migros supermarket in Limmatplatz, Zürich (Switzerland) on February 07, 2022. The orange peels were produced as a by-product of orange juice production. They were frozen in plastic bags for 2 days at - 20°C and then freeze-dried for 2 days in a freeze dryer (Labconco FreeZone 4.5 Liter - 50°C Benchtop Freeze Dryer; Kansas City, US). Due to moisture after freeze-drying, the samples were cut into pieces with a diameter of 3 cm and were thoroughly dried in an oven at 70°C for 2 days. The dried orange peel pieces were shredded in a crusher (Durabase Migros; Zürich, Switzerland) and ground with a pestle and mortar. Prior to the pectin extractions, the moisture content was measured for 30 min at 120°C with a halogen moisture analyzer (Mettler Toledo HE53; Greifensee, Switzerland).

2.2 Chemicals

Hydrochloric acid (HCl) (≥37%), absolute ethanol (>99.8%), acetic acid (>99.8%), methanol (>99.9%), calcium chloride ($CaCl_2$) (≥ 97%, granulated), sodium acetate (NaOAc) (>99.0%), and Driselase (protein ≥10% from *Basidiomycetes* sp.) were purchased from Sigma-Aldrich GmbH (Steinheim, Germany). The Celluclast® 1.5 L enzymes were ordered from Novozymes (Bagsværd, Denmark). Glycerol (85%) was obtained from Hänsler AG (Herisau, Switzerland).

2.3 Microwave-assisted pectin extraction

The microwave-assisted extraction of pectin was performed according to Ref. [14] with a household microwave oven (Mio Star MW 01). Ten grams of sample powder was weighed in triplicates. Milli-Q water (pH 1.5) was added to the samples to reach an optimal liquid-to-solid ratio of 20 ml/g. The suspensions were microwaved at a power of 450 W and irradiation time of 20 min, and stirred every 5 min. The suspensions were then cooled down and transferred into 50-ml tubes. The tubes were centrifuged (Eppendorf 5810 R; Hamburg, Germany) at room temperature for 10 min at 4000 rpm and the supernatants were collected. The pectin was precipitated for 1.5 h at room temperature using an equal volume of 95% ethanol. The dispersion was filtered and washed three times with 95% ethanol at room temperature. The extracted pectin was dried at 50°C overnight.

2.4 Enzymatic extraction of pectin

The enzymatic extraction of pectin was performed according to [15], except that the duration was reduced from 18 h to 3 h. For extracting the pectin, 10 g of the orange peel powder was weighed in triplicates. To get a liquid-to-solid ratio of 15:1, 150 ml of Milli-Q water (pH 4.5) was used. For the suspension, 500 μl of Celluclast® 1.5 L enzymes was added (Novozymes, Bagsværd, Denmark) and the samples incubated for 3 h in a water bath at 50°C. The resulting solution was cooled to room temperature and transferred into 50-ml tubes. The tubes were centrifuged (Eppendorf 5810 R; Hamburg, Germany) at 4°C for 10 min at 4000 rpm. The supernatants were collected, and 96% ethanol was added at 4°C to a final ethanol concentration of 70%. The suspensions were precipitated for 1 h and transferred into 50-ml tubes, which were then centrifuged at 4000 rpm for 20 min at room temperature. The precipitate was washed with 70% ethanol and centrifuged again for 20 min under the same conditions. The combined precipitates were dried for 24 h at 60°C.

2.5 Characterization of the extracted pectins

2.5.1 Fourier-transform infrared spectroscopy (FTIR)

To determine the degree of esterification (DE) of the extracted pectin, FTIR analysis was performed according to Ref. [16], using an FTIR spectrometer (Varian 640 with golden gate-diamond ATR). To obtain a dense surface of pectin powder on the surface of the ATR diamond, three droplets of 100% methanol were added to the pectin powder to increase the density. After evaporation of the solvent, the absorption was measured several times to get the optimal spectrum with a definite pectin signal and a smaller signal of the remaining methanol. The DE could be calculated then with Eq. (1) using the absorbance (Abs) at the wavelengths at 1630 cm^{-1} and 1745 cm^{-1}, which are known as the fingerprint regions of pectin [16].

$$DE(\%) = \left[\mathrm{Abs}_{1745} / \left(\mathrm{Abs}_{1745} + \mathrm{Abs}_{1630} \right) \right] \times 100 \quad (1)$$

2.5.2 Ion-exchange chromatography

To analyze the pectin sample by ion-exchange chromatography, the pectin was digested with Driselase (Sigma-Aldrich) using a method of Ref. [17]. Five milligrams

of the pectin samples was weighed in triplicates. For ion-exchange chromatography, a Thermo Scientific™ Dionex™ ICS-5000 (Thermo Fisher Scientific™, U.S.) was used with a PA1 Dionex CarboPac™ BioLc™ 4 x 50 mm column. The sampler had a 1 ml/min rate, and the reference electrode was AgCl. The eluting solvents were A: 200 mM NaOH, B: 100 mM NaOH +1 M NaOAc, and C: water. The gradient system was 7.6% A and 92.4% C for 0–26 min running time: 50% A and 50% C for 26–33 min: 47.2% A, 6% B, and 46.8% C for 33–45 min: 35.2% A, 30% B, and 34.8% C for 45–78 min: 100% B for 78–91 min: 50% A and 50% C for 91–99 min: and 7.6% A and 92.4% C for 99–105 min.

Two different kits from Megazyme were used to determine the free glucose and fructose in the pectin powder. The glucose kit K-GLUC and the glucose and fructose kit K-FRUGL were purchased from Megazyme Ltd. (Bray, Ireland), and the analyses were performed according to the manufacturer's protocols.

2.5.3 Size-exclusion chromatography (SEC)

For the sample preparation, 5 mg of the pectin samples was weighed in triplicates and dissolved in 5 ml of Milli-Q water. The solutions were kept for 1 h at 80°C while stirring in a water bath and afterwards at room temperature overnight while stirring. The transparent solutions were filtered through a nylon filter with pores of 0.45 μm (13 mm Syringe Filter, Nylon 66, 0.45 μm; BGB (Böckten, Switzerland)) into vials followed by analysis by size-exclusion chromatography (OMNISEC, Malvern Panalytical Ltd., Malvern, United Kingdom). The system consisted of an OMNISEC RESOLVE chromatography compartment combined with a pump, an autosampler and two A6000M columns in series (8.0 × 300 mm, OMNISEC REVEAL VISCOTEK, Malvern Panalytical Ltd., Malvern, United Kingdom). The OMNISEC RESOLVE detector compartment was equipped with a low and right-angle laser light scattering detector (LALS/RALS), a refractive index (RI), and a viscometer. The mobile phase was composed of a solution of 0.1 M $NaNO_3$ and contained 0.02% of NaN_3. Both columns were kept at 25°C, and the flow rate was 8.83 ml/min. The injection volume was 100 μl. A polyethyleneoxide (PEO-24 K, VISCOTEK, Malvern Panalytical Ltd., Malvern, United Kingdom) standard and a dextran standard (Dextran-T68K, American Polymer Standards Corporation, Mentor, US) were used for calibration. All vials were measured with three injections. The molecular weight (M_w), the intrinsic viscosity ([η]), the hydrodynamic radius (R_h), and the average conformation of the polymer (α) were determined by the instrument software.

2.6 Film preparation

The pectin films were prepared according to Ref. [18]. Six different prototypes of films were produced (**Table 1**). They differed in the extraction method of the pectin, pectin content, and the presence of whole-grain kabog millet flour (Catmon Cebu, Philippines). The whole-grain kabog millet flour was prepared according to Ref. [11].

The pectin was added carefully to the Milli-Q water with stirring. Glycerol, $CaCl_2$, and the whole-grain kabog millet flour (if present in the film formulation) were added. The suspensions were heated to 70°C, and 25 ml was poured into plastic weighing boats (Sigma-Aldrich GmbH, diameter = 13.5 cm). The films were dried overnight at room temperature and then in an oven at 29°C with 29% humidity until they were dry.

Code	Pectin (EN) [g]	Pectin (Mi) [g]	70% glycerol [g]	Milli-Q water [g]	$CaCl_2$ [mg]	Whole-grain kabog millet flour [g]
Mi1.5No	—	1.5	1.5	97.3	15	—
Mi1.5Millet	—	1.5	1.5	97.3	15	2
Mi2.5No	—	2.5	2.5	95	25	—
Mi2.5Millet	—	2.5	2.5	95	25	2
EN1.5No	1.5	—	1.5	97.3	15	—
EN1.5Millet	1.5	—	1.5	97.3	15	2

Table 1.
Ingredient composition of produced film prototypes (EN = enzymatic extraction, Mi = microwave-assisted extraction; No = without whole-grain kabog millet flour, millet = with whole-grain kabog millet flour).

2.7 Characterization of the pectin films

2.7.1 Mechanical tests

Mechanical properties of the films were evaluated by measuring tensile length and elongation using a Z010 (ZwickRoell GmbH & Co. KG, Ulm, Germany) with a 10 N load cell. The experiments were performed at room temperature and in triplicates using film pieces of 0.5 mm × 2.5 cm. The maximum strain and the stress to break were calculated according to Eqs. (2) and (3) using the elongation (L_F) from starting point of 1 cm, the breaking force (F) and the cross-sectional area (A).

$$Maximum\ strain(\%) = \left[(L_F - 1\,cm)/1\,cm\right] \times 100 \quad (2)$$

$$Maximum\ stress(MPa) = F/A \quad (3)$$

2.7.2 Water contact angle measurement

To analyze the water contact angle of the different film prototypes, a Drop Shape Analyzer-DSA 100E (A.KRÜSS Optronic GmbH, Hamburg, Germany) was used. The contact angle of a 10 µl Milli-Q water droplet was determined by analyzing the drop shape. Measurements were done in triplicates at room temperature.

2.8 Statistical analysis

Data obtained from FTIR, the mechanical tests, and water contact angle measurements were evaluated with Microsoft Excel. For statistical analysis, an ANOVA was used followed by a post hoc Tukey's HSD ($p < 0.05$). The data obtained from ion-exchange chromatography, free sugar content analysis, and SEC were evaluated using IBM SPSS Statistics v. 28.0.0 (IBM, Armonk, United States) with a two-sided t-test, assuming homogenous variance ($p < 0.05$).

3. Results

3.1 Extraction yield

To compare the efficiency of the two different pectin extraction methods performed (enzymatic and microwave-assisted), the yields were calculated considering the moisture content (MC) of the orange peel powder used (MC_{Mi} = 5.85%, MC_{EN} = 6.36%). The yield of the microwave-assisted extraction (Mi) was 15.07 ± 0.91% and differed significantly from the enzymatic extraction yield (EN) (3.11 ± 0.40%, n = 3 and $p < 0.05$).

3.2 Degree of esterification (DE): FTIR

To characterize the different extracted pectin samples, three of them were tested on their degree of esterification: the two sample sets used for measuring extraction yield of the enzymatic and the microwave-assisted extraction methods, and pectin extracted by microwave in bulk amounts, used later for film production. EN pectin had a DE = 49.10 ± 2.66% and was significantly different from the Mi pectin (DE = 77.34 ± 0.88%) and exhibited a significant difference from the bulk Mi pectin (**Table 2**).

The Mi pectin was considered highly esterified in both cases since the degree of esterification was greater than 50%. The EN pectin could be described as moderately esterified because the resulted degree of esterification was located at the boundary of high- and low-esterified pectins.

Sample name	**DE [%]**
Mi pectin	77.34^{b} ± 0.88
EN pectin	49.10^{c} ± 2.66
Bulk Mi pectin	85.21^{a} ± 3.20

Data are presented as mean ± standard deviation (n = 3). Different subscript letters indicate significantly different values at $p < 0.05$ using Tukey's HSD.

Table 2.
DE of pectin extracted by different methods.

	Amount in Mi pectin [%]	**Amount in EN pectin [%]**	
L-Fucose	4.14 ± 0.44	3.35 ± 0.19	
L-Rhamnose	5.56 ± 0.45	3.60 ± 0.28	*
L-Arabinose	11.47 ± 0.23	7.32 ± 0.28	*
D-Galactose	9.86 ± 0.35	12.41 ± 0.40	*
D-Xylose	4.29 ± 0.44	5.37 ± 0.78	
D-Galacturonic acid	64.68 ± 1.65	67.94 ± 1.74	

Data are presented as mean ± standard deviation (n = 3). Asterisks show significant differences between the two extraction methods at $p < 0.05$ using a two-sided t-test with the same variance.

Table 3.
Monosaccharide composition of pectin differing in the extraction method without glucose.

3.3 Pectin composition: ion-exchange chromatography

To compare the monosaccharide compositions of the differently extracted pectin samples, ion-exchange chromatography was performed. Prior to this analysis, the pectin samples were digested with Driselase. The monosaccharide compositions of both pectin samples were comparable. For both extraction methods, the main monosaccharide was galacturonic acid, which did not differ significantly between the two extraction methods. Rhamnose, arabinose, and galactose differed significantly (**Table 3**).

The undigested pectin powders were also tested for their free glucose and fructose contents, using two different Megazyme kits. The K-GLUC kit measured only the free glucose and the K-FRUGL measured the free glucose as well as the free fructose. It showed that the pectin powder extracted from orange peel contained some free sugar such as fructose and glucose. The Mi pectin contained significantly more free sugars than EN pectin (**Table 4**).

3.4 Size-exclusion chromatography

The different pectin samples were analyzed by SEC to test the behavior of the molecules in aqueous solutions and to get information about their particle size. The molecular weight (M_w), the intrinsic viscosity ([η]), the hydrodynamic radius (R_h), and the average conformation of the polymer (α) were determined. The chromatograms of the two pectin samples each showed two populations of molecules, which were quantified. The two peaks were overlapping in the chromatogram, leading to approximate integration.

The two populations of molecules from the two pectin samples analyzed differed significantly in their molecular weight. The molecules extracted using the microwave-assisted procedure were characterized by higher molecular weight than those of the other pectin sample (**Table 5**). The chromatogram showed that Mi pectin had higher dispersity in molecules than EN pectin. The intrinsic viscosity of the different peaks varied significantly. The first peak of the EN pectin had the highest intrinsic viscosity (8.73 ± 0.39 dl/g). The intrinsic viscosity was higher at the first peaks, implying a more open structure and a higher hydrodynamic radius. The average conformation of both molecule populations of the EN pectin was significantly higher than that of the other pectin samples (**Table 5**).

3.5 Mechanical tests of the pectin films

The mechanical properties of the six pectin-containing film prototypes (for preparation see **Table 1**, Section **2.6**) were determined in triplicates. The parameters from these

	Free glucose [mg/l] (K-GLUC)	**Free glucose [mg/l] (K-FRUGL)**	**Free fructose [mg/l] (K-FRUGL)**
EN pectin	33.26 ± 3.70*	20.96 ± 2.03*	11.44 ± 3.77*
Mi pectin	92.85 ± 2.20*	34.77 ± 2.93*	28.73 ± 3.32*

Data are presented as mean ± standard deviation (n = 3). Asterisks show significant differences between the two extraction methods at $p < 0.05$ using a two-sided t-test with the same variance.

Table 4.
Free glucose and fructose measurement by two different Megazyme kits and UV/Vis spectroscopy.

	EN pectin	Mi pectin	
M_w Peak 1 [kDa]	211.10 ± 18.09	484.61 ± 15.84	*
M_w Peak 2 [kDa]	67.60 ± 2.87	90.55 ± 5.59	*
[η] Peak 1 [dl/g]	8.73 ± 0.39	6.46 ± 0.25	*
[η] Peak 2 [dl/g]	0.66 ± 0.10	0.98 ± 0.16	*
R_h Peak 1 [nm]	29.91 ± 1.31	35.35 ± 0.86	*
R_h Peak 2 [nm]	8.39 ± 0.47	11.07 ± 0.77	*
α Peak 1	0.983 ± 0.034	0.596 ± 0.020	*
α Peak 2	1.006 ± 0.227	N/C	

Data are presented as mean ± standard deviation (n = 9). Asterisks show significant differences between the two extraction methods at p < 0.05 using a two-sided t-test with same variance.

Table 5.
Molecular weight, intrinsic viscosity, hydrodynamic radius, and average conformation of the two pectin samples analyzed by OMNISEC with PEO-24 K as a calibration standard.

measurements, maximum strain and maximum stress, are shown in **Figures 1** and **2**, respectively. The maximum strain of the film Mi2.5No was 138.26 ± 13.97% and differed significantly from Mi1.5Millet, Mi2.5Millet, and EN1.5No, with considerably lower maximum strains than Mi2.5No. The films EN1.5Millet and Mi1.5No showed intermediate maximum strains and were not significantly different from the data of all other prototypes (**Figure 1**).

At the maximum stress, a significant difference between films produced with and without the whole-grain kabog millet flour was visible (**Figure 2**). The maximum stress of the film Mi2.5Millet was 3.50 ± 0.33 MPa, and the one from the film Mi2.5No was 8.38 ± 0.61 MPa. The only difference in the composition of these two films was the addition of 2 g whole-grain kabog millet flour to Mi2.5Millet, resulting in more brittle film properties. This observation was also significant between EN1.5No and EN1.5Millet and between Mi1.5No and Mi1.5Millet (**Figure 2**).

To determine the hydrophobicity of the created films, the static water contact angle was measured on the surface of the films. The films showed significant differences in

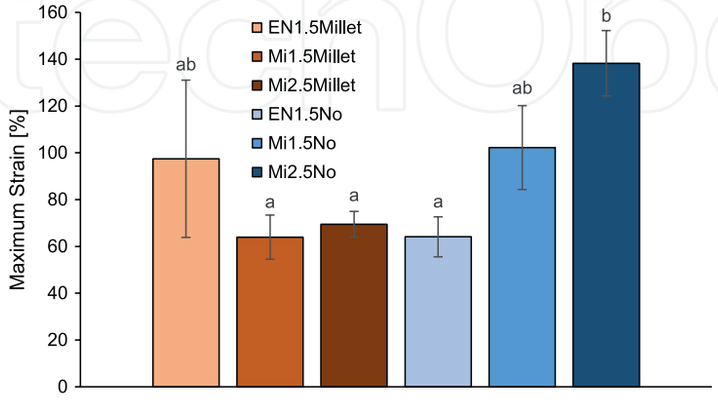

Figure 1.
The maximum strain of selected films. The different codes stand for different film prototypes compositions: EN = enzymatic extraction of pectin; Mi = microwave-assisted extraction of pectin; 1.5 = contains 1.5 g extracted pectin; 2.5 = contains 2.5 g extracted pectin; No = without whole-grain kabog millet flour; Millet = with whole-grain kabog millet flour. Data are presented as mean ± standard deviation (n = 3). Different subscript letters indicate significantly different values at p < 0.05 using Tukey's HSD.

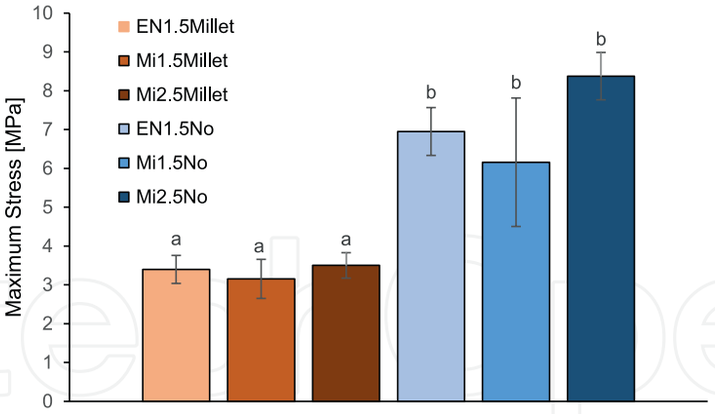

Figure 2.
The maximum stress of selected films. The different codes stand for different film prototypes compositions: EN = enzymatic extraction of pectin; Mi = microwave-assisted extraction of pectin; 1.5 = contains 1.5 g extracted pectin; 2.5 = contains 2.5 g extracted pectin; No = without whole-grain kabog millet flour; and Millet = with whole-grain kabog millet flour. Data are presented as mean ± standard deviation (n = 3). Different subscript letters indicate significantly different values at p < 0.05 using Tukey's HSD.

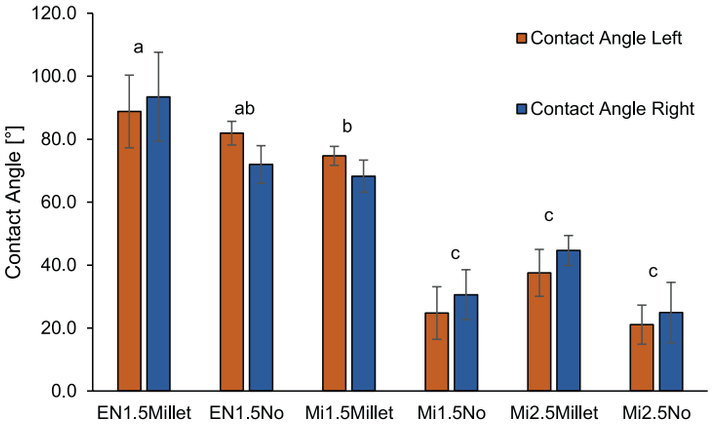

Figure 3.
Water contact angle left and right of selected films. The different codes stand for different film prototypes compositions: EN = enzymatic extraction of pectin; Mi = microwave-assisted extraction of pectin; 1.5 = contains 1.5 g extracted pectin; 2.5 = contains 2.5 g extracted pectin; No = without whole-grain kabog millet flour; and Millet = with whole-grain kabog millet flour. Data are presented as mean ± standard deviation (n = 3). Different subscript letters indicate significantly different values at p < 0.05 using Tukey's HSD.

contact angle as a function of the extraction method of pectin, the pectin concentration, and the presence of whole-grain kabog millet flour (**Figure 3**). Based solely on the extraction method of the pectin, it was observed that films produced with EN pectin showed significantly higher water contact angle than films produced from Mi pectin. This could be observed by comparing the mean contact angle of EN1.5No (76.94° ± 4.90) and Mi1.5No (27.68° ± 8.15). Biopolymers produced from EN pectin were characterized by higher hydrophobicity compared to biopolymers made from Mi pectin.

Comparing the difference in water contact angle between Mi2.5No/Millet and Mi1.5No/Millet, it could be observed that the change in the contact angle by adding the whole-grain kabog millet flour was decreased. With increasing pectin content, the effect of the whole-grain kabog millet flour on hydrophobicity was significantly reduced, and overall hydrophilicity was enhanced significantly. Comparing the water contact angle of Mi1.5Millet at 71.48° ± 4.05 and of Mi1.5No at 27.68 ± 8.15,

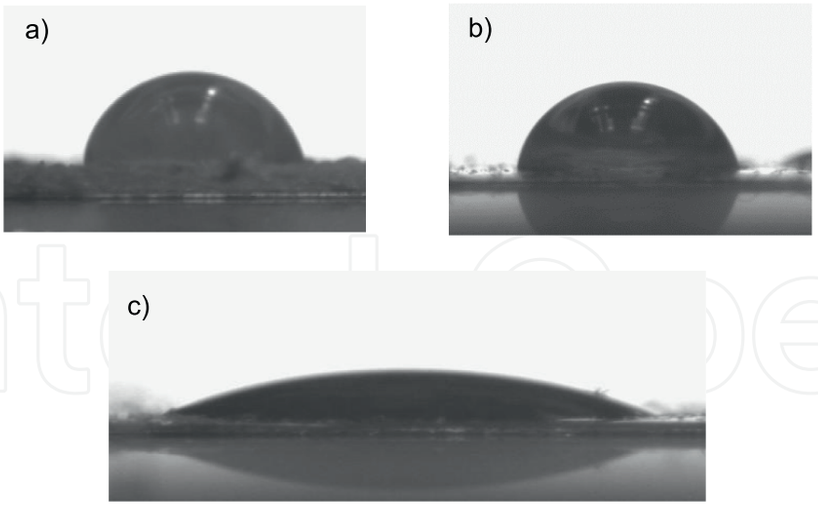

Figure 4.
Water droplet on the surface of a) Mi1.5Millet b) EN1.5No c) Mi1.5No. The different codes stand for different film prototypes compositions: EN = enzymatic extraction of pectin; Mi = microwave-assisted extraction of pectin; 1.5 = contains 1.5 g extracted pectin; No = without whole-grain kabog millet flour; Millet = with whole-grain kabog millet flour.

a significant increase in contact angle, and therefore in hydrophobicity could be observed with adding whole-grain kabog millet flour to the biopolymer. In comparison with the other two pairs of films, there was no significant effect observed from the addition of whole-grain kabog millet flour to the film. It is visible that the water contact angle was significantly lower for Mi1.5No compared to the films with enzymatically extracted pectin (EN1.5No) or added kabog millet flour (Mi1.5Millet) (**Figure 4a–c**).

4. Discussion

4.1 Extraction yield

The extraction yield differed significantly between the two extraction methods. The yield from enzymatic extraction using Celluclast® 1.5 L (~3%) was significantly lower than other studies using a similar protocol for apple pomace [19]. Orange peels contain 20.9% pectin [20] and apple pomace, 19–20% [21]. Therefore, the source of the pectin was not the main reason for the difference in yield. A possible explanation for the low yield in the current study is the incubation time of 3 h, which was shorter than 18 h used by Ref. [19]. It can be assumed that more cell wall components are digested with longer incubation time, and more pectin can be extracted [19]. Yield could be increased in further experiments by using longer incubation time or increasing the enzyme concentration. The yield from microwave-assisted extraction (~15%) was comparable to other studies [22]. The presence of free sugar, especially in the Mi pectin powder, can contribute to errors in the gravimetric measurement of the yield. A possible source of error could be also the precipitation of other polymers, which can bind to pectin, such as cellulose. Cellulose can also be extracted from plant material using microwave-assisted extraction and ethanol as solvent [23]. Therefore, it is possible that other polymers present in plant cell walls can precipitate and affect the gravimetric measurement of the pectin extraction yield.

4.2 Degree of esterification

The DE differed significantly between the two extraction methods. EN pectin can be described as moderately esterified because its DE was just at the boundary between high- and low-esterified. The obtained DE (~49%) was comparable to DE values using the same enzyme for extracting pectin [15]. It may be possible that only pectin with a lower degree of esterification can be released from the plant cell by enzymatic treatment. The DE of the microwave-assisted extracted pectin could be considered as highly esterified (~77%). The result was comparable to that of microwave-assisted extracted orange peel pectin (~71%) from [24], from lime peels (~71–92%) [25], and apple pomace pectin extracted by microwave (~74%) [9].

4.3 Pectin composition

As expected, the monosaccharide compositions of both pectin samples were comparable because the pectin was extracted from the same source and should therefore have a similar composition. The main component was galacturonic acid, which is the backbone molecule of pectin [4]. The galacturonic acid contents from both Mi pectin and EN pectin did not differ significantly. It was comparable to the galacturonic acid content in apple pectin [19] and orange peel pectin [24, 26]. The other monosaccharides except for rhamnose, galactose, and arabinose were also similar. The three significantly differing monosaccharides could be converted during extraction. The free glucose test with the K-GLUT assay was significantly higher in the Mi pectin powder than in the enzymatically extracted one. Due to the differences in the free glucose testing between the two applied kits, it can be assumed that the results had some uncertainties. These could be explained by the approximation of the reaction endpoint used in the K-FRUGL kit. Another reasonable explanation would be the presence of cellulose in the extracted pectin powder. (Nano) cellulose fibrils can be extracted using microwave-assisted methods [23]. Considering that orange peel has a cellulose content of approximately 50%, it is reasonable that the pectin powder also contained nanocellulose molecules [27]. Since microwave-assisted extraction is more vigorous and can increase the solubility of certain cellular compounds, it can be explained that more free glucose and nanocellulose fibrils were extracted from the cell wall and the glucose content, therefore, was higher in the Mi pectin sample than in the EN pectin powder.

4.4 Pectin molecular properties

The observed molecular weights of the first peak of each pectin sample from enzymatic extraction and microwave-assisted extraction were ~ 211 ± 18 kDa and ~ 485 ± 20 kDa, respectively. These values were comparable to the molecular weights of orange peel pectins, which had a molecular weight of 120 ± 10 kDa to 360 ± 20 kDa [28]. The dispersion of particle size and, therefore, of the molecular weight was broader in microwave-assisted extraction due to the rapid increase in temperature and internal pressure [16]. The second peaks could depict cleaved pieces of pectin or other dissolved cell components like cellulose. There are cellulose molecules or cellulose derivatives that would fit in the range of the molecular weight of the second peaks [29]. The intrinsic viscosity values from enzymatic extraction (~8.7 dl/g) and microwave-assisted extraction (~6.5 dl/g) fit within the range of 4.8–10.8 dl/g for the intrinsic viscosity of orange peel pectin [28]. The Mi pectin was characterized by a significantly lower intrinsic viscosity than the EN pectin. A lower intrinsic viscosity

describes a more condensed structure and higher molecular density [30]. In contrast to the intrinsic viscosity, the Mi pectin had a significantly higher hydrodynamic radius than the EN pectin, indicating a looser conformation. Due to this contradictory relationship, it can be assumed that the condensation of the pectin structures in water is similar. The average conformation of the two pectin samples differed. The EN pectin had values nearly twice as high as the Mi pectin. The Mi pectin had a conformation that can be described as a semi-flexible random coil-like structure due to its α value between 0.5 and 0.8 [30]. On the other hand, the EN pectin had both populations of particles with an average conformation higher than 0.8. They tend to be in a more rigid and rod-like structure.

4.5 Mechanical testing of the created films

The differences in maximum strain showed the trend that the elongation could be enhanced by increasing the pectin content. The maximum strain of Mi1.5No was 102.25 ± 17.93%, and if increasing the pectin content by 1 g/100 ml, the maximum strain reached 138.26 ± 13. 97% (Mi2.5No). The maximum stress was therefore increased by approximately 36%, which can be explained by increased film thickness and consequently, increased cross-sectional area; thus, more cross-linkages that need to be broken [18]. The film microstructure was altered with changing pectin content by analyzing with scanning electron microscopy [18]. With 2.5 g pectin per 100 ml film-forming solution, the film structure was the smoothest [18]. By increasing or decreasing the pectin content, the microstructure was disturbed. A significant effect of the different extraction procedures on the maximum strain of the film could not be observed. A significant change in maximum strain by adding whole-grain kabog millet flour could only be observed between the prototypes Mi2.5No (138.26 ± 13.97%) and Mi2.5Millet (69.45 ± 5.50%). The higher maximum strain of Mi2.5No cannot be explained by increasing the cross-sectional area because the films containing whole-grain kabog millet flour were characterized by the increased film thickness. It could be assumed that the internal arrangement was disturbed by adding the kabog millet flour; therefore, the elongation properties decreased. Compared to other biodegradable films based on polymers, the produced films tended to be rigid and inelastic. There were formulations for biodegradable films characterized by a maximum strain between 300% and 700% [13]. Regarding the maximum mechanical stress to break, films with kabog millet flour broke with the application of approximately half of the force per cross-sectional area than films without kabog millet flour. This leads to the conclusion that adding kabog millet flour changed the properties of the films toward higher brittleness. No significant differences between the maximum stress data of the films produced from different pectin samples could be observed. Therefore, the extraction method did not influence the mechanical properties of the produced pectin edible films.

4.6 Water contact angle

The water contact angles on the surface of the produced films provided information about the hydrophobic characteristics of the different film prototypes. A low water contact angle characterizes more hydrophilic properties of the edible pectin film [13]. In the application of pectin films for covering food products, a certain hydrophobicity would be desired to stabilize the protection layer against water. It could be observed that the films produced from EN pectin resulted in higher water

contact angles than films from the Mi pectin sample. This is contradictory to the results of the degree of esterification because the EN pectin showed a greater number of free carboxy groups due to its lower degree of esterification and, therefore, should be more hydrophilic [31]. A possible explanation for the lower contact angles observed for the Mi pectin could be its higher hydrodynamic radius. The higher hydrodynamic radius indicates higher hydrophilicity. Furthermore, the rod-like conformation of the EN pectin does not allow as many interactions with the surrounding water because only a part of the functional groups is exposed on the surface of the rod and therefore is characterized as more hydrophobic. The addition of the whole-grain kabog millet flour showed a significant increase in water contact angle between the films Mi1.5Millet and Mi1.5No, while between the other pairings, a difference could be observed but it was less pronounced. The addition of the kabog millet flour increased the hydrophobicity, which could be explained by its whole-grain character. In whole-grain products, lipids are present, including some lipophilic compounds such as carotenoids and tocopherols [11]. These lipophilic structures may shift and enhance the hydrophobic properties of the films. During the measurements of the water contact angle, some properties of the film were observed, which could be interesting for further research. The pectin films, especially the ones with kabog millet, showed volume expansion upon the addition of water, and the droplet of water turned white over time, so it can be assumed that some hydrophilic film compounds migrated into the water droplet.

5. Conclusion and outlook

The properties of the pectin obtained from enzymatic and microwave-assisted extraction differed in several of the tested properties. Enzymatically-extracted pectin was characterized as moderately esterified (49.1% DE) with a rod-like conformation in water and with a lower average molecular weight than the microwave-assisted extracted pectin. These pectin samples had a random coil-like conformation and were highly esterified (>75% DE). In addition, the yields of the two extraction methods were significantly different. While the microwave-assisted extraction had a yield comparable to literature, the enzymatic extraction resulted in low yield which may be improved in further experiments by increasing the enzyme concentration, incubation time, or other relevant parameters. The properties of films prepared from the extracted pectin material showed significant differences. Overall, the elasticity of the films was considerably lower than the data of comparable material described in the literature. The high amount of presumably cellulose (>70% glucose monomer units) present in the extracted material might play a role for the low elasticity observed. The enzymatically-extracted pectin is a promising material to produce edible pectin films due to the high contact angles determined for the films and thus enhanced hydrophobicity and stability to water. Adding whole-grain kabog millet flour increased the hydrophobic properties of the films but concomitantly, the brittleness of the pectin edible films also increased. It would need further research to adjust the protocol to produce pectin films containing whole-grain kabog millet flour without changing their elasticity. Furthermore, it would be interesting to evaluate the antioxidant properties of compounds present in the whole-grain kabog millet applied in a film or coating. This could pave the way for fruit waste valorization and the addition of nutritional components to edible, biodegradable films.

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Author contributions

Nils Rentsch: Conceptualization, data curation, formal analysis, investigation, validation, visualization, writing—original draft; Laura Nyström: Project administration, resources, software, writing—review and editing; Joan Oñate Narciso: Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, supervision, validation, writing—review and editing.

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Declaration of competing interest

None.

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