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Physicochemical and functional characterization of pectin extracted from Moroccan citrus peels

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ABSTRACT

Three Moroccan citrus peels, such as orange, lemon, and bigarade, were chosen to extract the pectin using Box Behnken Design. At a temperature of 90 °C, pH = 1.5, and extraction time = 120 min, a high pectin yield was recorded with 28.57, 26.86, and 26.55% for lemon (LPP), bigarade (BPP), and orange (OPP) peel pectins respectively. The extracted pectins have a less branched linear structure with high GalA contents for BPP (86.0 mol%), LPP (82.0 mol%), and OPP (79.0 mol%), and a high degree of methyl esterification (>74%), but a low degree of acetylation (<4%). A narrow and homogeneous distribution was recorded with a higher average Mw for LPP (90.0 kDa) than OPP (88.0 kDa) and BPP (80.0 kDa). One gram of Moroccan citrus pectin could hold more than 23.0 g of water. The high emulsifying activity was recorded for the three citrus pectins (~50%), with higher emulsion stability for BPP pectin at 4 °C (94.42%). Furthermore, all extracted pectins exhibit a significantly non-Newtonian pseudoplastic behavior when the concentration increases. At 3% concentration, the apparent viscosity of BPP was higher than that of OPP and LPP with 40.26, 13.45, and 6.85 Pa s, respectively.

1. Introduction

Pectin is a class of complex and multifunctional polysaccharides. It represents 20–35% of the plant cell wall and is located mainly in the middle lamella and the primary barrier. Pectin is a hetero-polysaccharide, mainly composed of residues of galacturonic acids (GalA) linked in α-(1 → 4). It also includes a significant quantity of neutral sugars such as rhamnose, arabinose, and galactose (Schols & Voragen, 1996).

Depending on the molecular structure of the pectin, three main areas can be distinguished: homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II). The homogalacturonan region is composed of α-D-galacturonic acid. It can be found in an acidic form or esterified by methanol at C6 of the carboxyl groups and/or by acetic acid at O2 and/or O3 of the hydroxyl groups (Voragen, Coenen, Verhoef, & Schols, 2009). According to the esterification degree, pectins are characterized by a degree of methyl esterification (DM) and acetylation (DA). From a functional point of view, there are two categories of pectins: highly methylated pectins (DM > 50%), which can form a gel under acidic conditions in the presence of sugar, and low methylated pectins (DM < 50%), which can create a gel in the presence of divalent ions (mainly Ca^{2+}) and can be used for low-sugar products (Thakur, Singh, & Handa, 1997). In contrast, a high degree of acetylation in a pectin sample may hinder its gelling capacity but improve its emulsifying properties (Yapo, Robert, Etienne, Wathelet, & Paquot, 2007; Pilnik & Voragen, 1992). The gelling ability of pectin is also dependent on its average molecular weight, which impacts its viscosity. The higher the molecular weight of pectin is, the higher its viscosity and gelling capacity (Kar & Arslan, 1999).

Industrially, pectin is extracted from by-products of the fruit processing industry, mainly citrus peels, and less quantity from apple pomace (May, 1990). Various techniques are used to extract pectin from plant matrices, such as microwave and ultrasound-assisted extraction. However, the acidic extraction process is still the primary process used

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to recover those molecules. For acidic extraction, the plant material is treated with an acid (usually a mineral acid) at a high temperature, generally between 80 and 100 °C (Marić et al., 2018).

Worldwide, the annual consumption of pectin as an additive has achieved around 60,000 tonnes in 2018 (Industry experts, 2019). Furthermore, the global pectin market was expected to know a decent growth of 5.8% CAGR between 2018 and 2024 (Industry experts, 2019). Thanks to their gelling, thickening, stabilizing, and emulsifying capacity, pectins are used for several applications, mainly in food (making jams and stabilizing juices), pharmaceutical (drug encapsulation), medical (healing wounds, inducing apoptosis in the human prostate cancer cell, immunity stimulator) and cosmetic industries (Marić et al., 2018; Yamada, 1996).

The pectin content and related physicochemical and techno-functional properties are affected by several factors such as the source, the pedoclimatic conditions, and the used extraction techniques (Abid et al., 2017; Kaya, Sousa, Crépeau, Sørensen, & Ralet, 2014; Marić et al., 2018; Pérez, Rodríguez-Carvajal, & Doco, 2003). Moreover, raw material pretreatment also significantly impacts pectin extraction yield and quality (Dong et al., 2021; Kratchanova, Pavlova, & Panchev, 2004).

Moroccan citrus production has undergone a significant evolution over the last decade, 2 019 thousand tonnes in 2016 instead of 1 093 thousand tonnes in 2008 (FAO, 2017). This increase is due to its geographical position in the Mediterranean basin, the favorable pedoclimatic conditions, and the Green Morocco Plan adopted by the Minister of Agriculture to improve the agricultural sector. According to the Food and Agriculture Organization of the United Nations (FAO), orange and lemon production has achieved 1,019,000 tonnes and 39,000 tonnes, respectively in 2018. On the other hand, Morocco has an important agro-industrial platform which in 2016 processed around 73, 000 tonnes of citrus fruits, of which about 80% were oranges (FAO, 2017). Consequently, citrus processing generates thousands of tonnes of by-products each year, which are low valued despite their high content of substances with high added value, such as pectin (May, 1990; Methacanon, Krongsin, & Gamonpilas, 2014; Wang et al., 2016; Su et al., 2019; Dong et al., 2021; Tsuru, Umada, Noma, Demura, & Hayashi, 2021).

Furthermore, bigarade trees are planted in almost all Moroccan streets and on the roads for ornamental purposes. However, the fruits produced annually are only valued for a negligible quantity in traditional medicine. Therefore, the valorization of these vast amounts of by-products will have a significant socio-economic and environmental impact on the country.

This study is part of a project on the valorization of agro-industrial by-products in Morocco's Fez-Meknes region. There is no pectin production at the national scale. Instead, all pectins used are imported despite the abundance of raw material. Through this study, we expect to obtain citrus pectins with similar or even better properties compared to commercial pectins and compared to pectin extracted from the same matrices but cultivated under different pedoclimatic conditions. This work aims at optimizing the pectin extraction from three different Moroccan citrus matrices: orange, lemon, and bigarade peels. It also focuses on the physicochemical and functional characteristics of the three citrus pectins extracted under optimal conditions. A response surface methodology was used to evaluate each matrix's pectin content and investigate the influence of each factor on the extraction yield. The extracted pectins were subjected to various physicochemical characterizations to assess their monosaccharide composition, degree of methyl esterification (DM), degree of acetylation (DA), molecular weight (Mw) distribution, protein content, water holding capacity (WHC), oil holding capacity (OHC), emulsifying properties, and rheological behavior.

2. Materials and methods

2.1. Raw material and reagents

Fresh orange (*Citrus sinensis*) and lemon (*Citrus limon*) fruits were collected during the harvest season in "Gharb", situated in northwest Morocco, while bigarade (*Citrus aurantium*) fruits were collected randomly from trees planted in the streets. After washing and peeling the fruits, the peels were cut into small pieces and then blanched at 90 °C for 5 min to inactivate enzymatic reactions (Su et al., 2019). Next, the blanched peels were dried at 45 °C until a constant weight was achieved, then crushed in a blender, sifted through a 40 mesh sieve, and finally stored in plastic bags in a dry environment for further experiments.

Ethanol, hydrochloric acid, sulfuric acid, trifluoroacetic acid (TFA), sodium hydroxide, phenolphthalein, methanol, m-hydroxy-diphenyl, and standard monosaccharides, including galacturonic acid, rhamnose, arabinose, galactose, xylose, fucose, mannose, and glucose, were purchased from Sigma Chemicals and used as received. Pectin (0.6, 1.8, 5, 16, 50, and 150 kDa) from the Laboratory of Food Chemistry (Wageningen, The Netherlands) was used as a molecular weight standard.

2.2. Experimental design for pectin extraction

The one factor at a time (OFAT) method was used to determine the best liquid to solid ratio (LSR). For this purpose, we have varied the LSR (10, 20, 30, 40, 50, and 60 v/w) while the other factors are kept constant; pH at 2, the temperature at 80 °C, and extraction time at 120 min (Hosseini, Khodaiyan, & Yarmand, 2016b). This factor was considered constant in the experimental design after determining the best LSR for each vegetable matrix. Box Behnken Design (BBD) with three variables and levels (Table 1) was used to optimize the pectin extraction from three citrus peels (orange, lemon, and bigarade) and to show the effect of each factor (Temperature, pH, and extraction time) on pectin extraction yield.

2.3. Pectin extraction

Pectin was extracted from the different peel powders by a hot acidic extraction using a shaker water bath according to the method described by Kratchanova et al. (2004) with some modifications. First, 2g of dry powder was mixed with distilled water, and the pH was adjusted to the corresponding value (Table 1) with 0.5 M HCl. Then, the mixture was brought to the water bath at the chosen temperature, as indicated in Table 1. After extraction, the obtained solution was centrifuged at 3260×*g* for 20 min at room temperature, then vacuum filtered. Next, the filtrated supernatant was coagulated by adding an equal volume of ethanol (96%), then a slow agitation was applied for 10 min, and the mixture was stored overnight at 4 °C for precipitation. Afterward, the precipitated pectin was centrifuged and filtered, then washed twice with ethanol 80% and 96%, respectively. Finally, the extracted pectin was dried in an oven drier at 45 °C until a constant weight was achieved, then grounded and stored in airtight plastic bags for further analysis.

The extraction yield was calculated according to Eq. (1):

$$Yield\ (\%,\ dry\ basis) = \frac{weight\ of\ dried\ pectin\ (g)}{weight\ of\ dried\ powder\ (g)} \times 100 \quad (1)$$

2.4. Chemical characterization

2.4.1. Monosaccharide composition

The corresponding monosaccharide composition of orange, lemon, and bigarade peel pectins extracted at the optimum point was determined using an HPLC method (Broxterman, van Erven, & Schols, 2018) with a slight modification. The samples were prepared using methanolysis combined with TFA hydrolysis. First, the solubilized samples were heated for 16 h at 80 °C in 0.5 mL anhydrous 3 M HCl in absolute

Table 1
Box-Behnken design with experimental and predicted yields of pectin extracted from orange, lemon, and bigarade peel.

Factors	Unit	Actual Levels							
		- 1	0	+ 1					
Temperature X1	°C	70	80	90					
pH X2	-	1.5	2	2.5					
Time X3	min	90	120	150					
Run	X1	X2	X3	Orange		Lemon		Bigarade	
				[a]EY (%)	[a]PY(%)	[a]EY(%)	[a]PY (%)	[a]EY (%)	[a]PY (%)
1	70	1.5	120	19.72	19.04	18.84	19.66	19.66	19.57
2	90	1.5	120	26.55	26.36	28.57	28.69	26.86	27.71
3	70	2.5	120	8.79	8.97	5.52	5.39	4.09	3.03
4	90	2.5	120	12.74	13.41	13.51	12.68	10.87	11.17
5	70	2	90	13.82	14.45	14.14	13.82	10.83	11.31
6	90	2	90	20.38	20.51	19.91	20.29	18.63	17.96
7	70	2	150	16.41	16.27	15.45	15.07	10.98	11.66
8	90	2	150	22.61	21.98	24.60	24.92	21.77	21.29
9	80	1.5	90	20.81	20.86	23.31	22.80	22.03	21.93
10	80	2.5	90	9.92	9.11	7.27	7.71	5.10	5.39
11	80	1.5	150	21.45	22.26	26.24	25.79	24.44	23.77
12	80	2.5	150	11.04	10.99	10.09	10.60	6.76	7.23
13	80	2	120	15.18	15.49	16.15	16.03	12.63	12.89
14	80	2	120	15.73	15.49	15.87	16.03	13.04	12.89
15	80	2	120	15.57	15.49	16.08	16.03	13.00	12.89

[a] EY and PY are experimental yield and predicted yield, respectively.

methanol in a closed screw-cap, then hydrolyzed in 2 M TFA at 121 °C for 1 h. The hydrolyzed samples were analyzed using a High-performance anion exchange chromatography (HPAEC) equipped with an ICS3000 system coupled to an ICS5000 ED pulsed amperometric detector (Thermo Scientific). A PA-1 guard column (2 × 50 mm), a PA-1 column (2 × 250 mm), and a post-column (Thermo Scientific) were used. At a flow rate of 0.4 mL/min, pectin samples were eluted first with Milli-Q water for 27 min, then in 11 min to 0.17M NaOAc in 0.1M NaOH, in 5 min to 1 M NaOAc in 0.1M NaOH. The column was re-equilibrated with 0.1M NaOH for 7 min, then Milli-Q water for 15 min. Post column addition of 0.5M NaOH at 0.1 ml/min was performed between 0 and 35 min and 65 min. All Data were analyzed using The Chromeleon software 7.2.6 (Thermo Scientific).

Uronic acid (UA) content in the extracted pectin samples was determined after pre-hydrolysis with 72% (w/w) sulfuric acid at 30 °C for 1h, followed by hydrolysis with 1 M sulfuric acid at 100 °C for 3h (Seaman hydrolysis). For this purpose, an autoanalyzer (Skalar, Breda, the Netherlands) was used as described by Thibault (1979) using the colorimetric *m*-hydroxydiphenyl assay (Ahmed & Labavitch, 1977).

2.4.2. *Degree of methyl and acetyl esterification*

The degree of methyl esterification (DM) and acetylation (DA) of Moroccan citrus peel pectins extracted under optimal conditions are evaluated as described by Broxterman et al. (2018). First, 5 mg from each sample was saponified overnight in sealed vials with 0.1 M NaOH. Next, the methanol released was analyzed with GC-head space using TRACE™1300 Gas Chromatograph (Thermo Scientific™) GC headspace with ion trap J&W DB-WAXetr column (30 m, 0.25 mm, and 0.25 μm) (Agilent Technologies, USA). The obtained data were then analyzed with Xcalibur (Thermo Scientific).

The remaining saponified solutions were used to determine the degree of acetyl-esterification (DA). First, solutions were centrifuged at 18,000 g for 10 min and then analyzed with HPLC. The measurements were performed on an Ultimate 3000 system (Thermo Scientific), equipped with an Aminex HPX 87H column (300 mm × 7.8 mm) and a guard column (30 mm × 4.6 mm) (Bio-Rad, Hercules, USA). Samples were eluted at a flow rate of 0.6 mL/min with 50 mM H_2SO_4 and an oven temperature of 40 °C. A Shodex RI-101 detector (Showa Denko K.K.) was used to monitor the elution. Acetyl concentrations between 5.3 and 525 μg/mL were used as standard. The degree of methyl-esterification and acetylation were calculated as moles of methanol and acetic acid per 100 mol of galacturonic acid, respectively, and the data was analyzed by Chromeleon 7.2.6 (ThermoScientific).

2.4.3. *Molecular mass distribution*

Molar mass distribution of pectins extracted at optimum points was determined using high-performance size exclusion chromatography (HPSEC). Pectin solutions (2 mg/mL water, RT) were centrifuged at 18000×*g* for 10 min and analyzed on an Ultimate 3000 HPLC (Dionex, Sunnyvale, CA, USA) equipped with a Shodex RI-101 refractive index detector (Showa Denko, Tokyo, Japan). We have used three TSK-Gel columns connected in series (4000-3000-2500 SuperAW; 150 × 6 mm). These columns were preceded by a TSK Super AW-L guard column (35 × 4.6 mm), and all columns used were from Tosoh Bioscience (Tokyo, Japan). Ten μL samples were injected and eluted with a 0.6 mL/min flow rate at 55 °C with 0.2 M $NaNO_3$. Pectin (Laboratory of Food Chemistry, Wageningen, the Netherlands) molecular-mass standard was used to calibrate the system (Deckers, Olieman, Rombouts, & Pilnik, 1986).

2.5. *Water holding capacity (WHC) and oil holding capacity (OHC)*

The WHC and OHC of pectin samples extracted under optimal conditions were determined according to the modified method of Beuchat (1977). Samples (200 mg) were first mixed with 10 mL of distilled water or vegetable oil (density of 0.92 g/mL) in centrifuge tubs, then vortexed for 1 min and centrifuged at 3000×*g* for 30 min. Afterward, the supernatants were removed, and the remaining pellets were weighed. Tests were done in triplicate, and the results were presented as grams of water or oil held by 1g of pectin.

2.6. *Rheological measurement*

The steady flow behavior of pectin samples extracted at the optimum points from Moroccan citrus peels was measured using a rotational cylinder rheometer Physica MCR 501 (Anton Paar Co.) equipped with concentric cylinder geometry. Pectin samples at a concentration of 1%, 2%, and 3% were solubilized in deionized water at 50 °C for 7h, then cooled to room temperature and stored at 4 °C overnight before the analysis. The viscosity curves of samples at different shear rates were recorded. The tests were carried out with controlled shear rates of between 0.1 and 100 s^{-1} relatively at 25 °C. Before these tests, a transient viscosity was realized at different sharing points to establish the measuring point duration from which the viscosity reached its steady state.

2.7. Statistical analyses

All calculations and statistical analyzes of the experimental design (BBD) concerning the optimization of the extraction of pectin were carried out using the NemrodW software to see the effect of the physicochemical conditions of extraction on pectin yield. The Origin program was used to make the graphs and perform Tukey's test ($p < 0.05$).

3. Results and discussion

3.1. Optimization of extraction and result analysis

At first, the one factor at a time method was used to determine the best liquid to solid ratio (LSR) for pectin extraction from lemon, orange, and bigarade peels. Briefly, the LSR was varied, while the other factors (temperature, pH, and extraction time) were kept constant. The results show that the maximum yield was acquired at a LSR of 60, 50, and 40 v/w for pectin extraction from orange, bigarade, and lemon peels. Knowing that the raw materials have been treated in the same way, this difference is undoubtedly due to the nature of the plant matrix. Therefore, the optimal obtained ratio was considered for further optimization of pectin extraction through Box Behnken Design. As indicated in Table 1, a BBD experiment design with three variables and levels was used to optimize the other extraction factors (temperature, pH, and extraction time). Pectin extraction yield obtained was expressed as dry weight and was in the range of 8.79–26.55, 5.52–28.57, and 4.09–26.86% (w/w) for orange, lemon, and bigarade peel powder, respectively, while the maximum and minimum obtained yield corresponded to the run number 2 and 3, respectively, for all matrices.

The optimal conditions of 90 °C, pH of 1.5, and extraction time of 120 °C gave the best extraction yields with 28.57 ± 0.23, 26.86 ± 0.19, and 26.55 ± 0.27% for lemon, bigarade, and orange peel powder, respectively (Table 1). According to the other studies, the extraction yield obtained from Moroccan lemon peel is higher than that obtained from Iranian lemon peel using acidified date juice (11.21%) (Masmoudi et al., 2008). Moreover, it is also slightly higher than the yield obtained from citron peel using the same extraction technique (27.95%) (Pasandide, Khodaiyan, Mousavi, & Hosseini, 2018). In the meantime, the pectin yield recovered from orange is higher than that obtained from the same matrix after a microwave vacuum drying of the peel, with 21,94% (Dong et al., 2021). However, it is slightly lower than that obtained using phosphoric acid as an extracting agent (29.37%) at optimal conditions (Tovar, Godínez, Espejel, Ramírez-Zamora, & Robles, 2019). The strength of the used acid may explain this. Regarding the extraction technique, the hot acidic extraction used in this study allows for recovering more pectin from orange peel than using the ohmic heating extraction (18.32%) (Sabanci, Çevik, & Göksu, 2021). On the other hand, pectin extraction from bigarade peel has been studied frequently using several techniques. The previous studies displayed a low extraction yield (17.6%) using aqueous extraction (Hosseini, Khodaiyan, & Yarmand, 2016a) and a similar extraction yield using the advanced techniques as ultrasound-assisted extraction (26.6%) (Hosseini, Khodaiyan, Kazemi, & Najari, 2019) and microwave-assisted extraction (26.4%) (Hosseini et al., 2016b) compared to the present study. In comparison with other citrus sources, Moroccan citrus peels have a higher pectin yield than ripe mandarin orange peel (10.4%) (Tsuru et al., 2021) and pomelo peel (9.0%) using citric acid as extracting solvent (Van Hung, Anh, Hoa, & Phi, 2021).

The predicted yield mentioned in Table 1 was calculated based on the square polynomial equation obtained using NemrodW software. Table S1 (supplementary material) presents the coefficient values and the obtained statistical parameters for the model. For example, for orange peel pectin, the pH and extraction time variables' quadratic coefficient and interaction coefficients between all variables were not significant by F-test at a 95% confidence level. Only the interaction coefficient between pH and extraction time variables showed an insignificant effect on the extraction yield of pectin from lemon peel at a confidence level of 95%. In contrast, the corresponding response to bigarade pectin yield was not significantly affected by the interaction coefficients between temperature-pH and pH-extraction time at a confidence level of 95%.

After the exclusion of the corresponding coefficients that showed no significant effect on pectin extraction yield (Table S1), the square polynomial model for orange (Eq. (2)), lemon (Eq. (3)), and bigarade (Eq. (4)) peel were obtained.

$$\mathit{Orange\ Yield}\ (\%) = 15.493 + 2.943X_1 - 5.755X_2 + 0.822X_3 + 1.978X_1^2 \quad (2)$$

$$\mathit{Lemon\ Yield}\ (\%) = 16.033 + 4.080X_1 - 7.571X_2 + 1.469X_3 + 1.187X_1^2 - 0.610X_2^2 + 1.305X_3^2 - 0.435X_1X_2 + 0.845X_1X_3 \quad (3)$$

$$\mathit{Bigarade\ Yield}\ (\%) = 12.890 + 4.071X_1 - 8.271X_2 + 0.920X_3 + 1.725X_1^2 + 0.755X_2^2 + 0.938X_3^2 + 0.747X_1X_3 \quad (4)$$

X_1, X_2, and X_3 are the coded values for temperature, pH, and extraction time.

The results of the multiple regression analysis are shown in Table 2. The determination coefficient R^2 and the adjusted determination coefficient of the extraction yield of pectin from orange ($R^2 = 97.4\%$; Adj $R^2 = 96.4\%$), lemon ($R^2 = 99.5\%$; Adj $R^2 = 98.9\%$) and bigarade ($R^2 = 98.4$; Adj $R^2 = 97.7\%$) peels were high and indicate that the most variation of the responses could be explained and predicted by the obtained models. Besides, all models showed a significant p-value (<0.01) with a lack of fit, except for the orange model, which is well-fitted (p-

Table 2
Multiple regression analysis for Moroccan Citrus Peel Pectin extraction yield.

Matrix	Source	DF	Some of the squares	Mean square	F-value	*p*-value
Orange	Regression	4	353.923	88.481	95.12	< 0.01 ***
	Residual error	10	9.302	0.930		
	Lack-of-fit	8	9.142	1.143	14.28	6.70
	Pure error	2	0.160	0.080		
	Total	14	363.225			
	R^2			0.974		
	Adj R^2			0.964		
	Pred R^2			0.935		
Lemon	Regression	8	625.623	78.203	3683.03	< 0.01 ***
	Residual error	6	2.848	0.474		
	Lack-of-fit	4	2.805	0.701	33.03	2.96 *
	Pure error	2	0.042	0.021		
	Total	14	628.471			
	R^2			0.995		
	Adj R^2			0.989		
	Pred R^2			0.946		
Bigarade	Regression	4	696.287	174.071	3406.49	< 0.01 ***
	Residual error	10	11.394	1.139		
	Lack-of-fit	8	11.292	1.411	27.62	3.54 *
	Pure error	2	0.102	0.051		
	Total	14	707.681			
	R^2			0.984		
	Adj R^2			0.977		
	Pred R^2			0.963		

* significant at the level of 95%.
*** significant at the level of 99.9%.

value = 6.7). These results prove that all models were well suited to the response of the pectin extraction yield of all the matrices used.

3.2. *Effect of the extraction conditions on the yield*

Two-dimensional plots were generated by the experimental design software (NEMROD-W) to visualize the relationship between extraction factors and the obtained yield (Fig. 1). As shown, pectin yield increases by decreasing the extraction medium's pH (Fig. 1A, B, D, E, G, and H). Moreover, the slope coefficient of the pH variable was highly negative (−5.75, −7.57, and −8.27 for orange, lemon, and bigarade peel pectins, respectively), which indicates the predominant effect of this factor over the others. Levigne, Ralet, and Thibault (2002) found the same trends. This result is due to the ability of acidic pH to hydrolysis the plant cell wall, which improves the infusion of pectin into the extraction medium (Kazemi, Khodaiyan, Labbafi, Saeid Hosseini, & Hojjati, 2019). The temperature also significantly influences pectin extraction yield, as indicated in Fig. 1A, C, D, F, G, and I, but to a lesser extent than the effect of pH. However, the extraction yield increases with increasing temperature from 70 to 90 °C. High temperature increases the extraction yield of pectin from different plant matrices, which is related to the increased solubilization of the pectin and the fact that increased temperature facilitates the diffusion of pectin from plant tissues into the surrounding medium (Samavati, 2013). Compared to pH and temperature, the

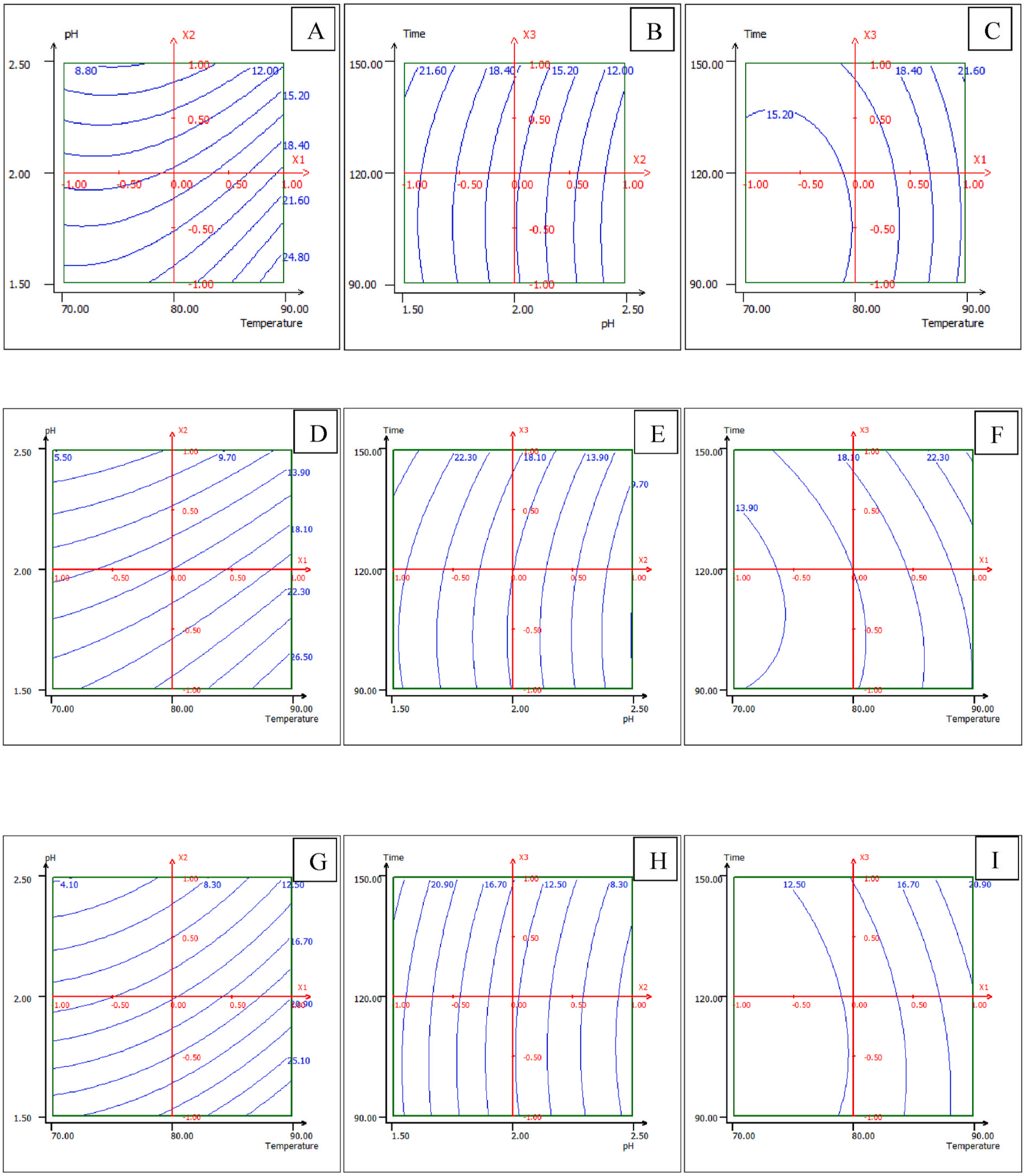

Fig. 1. Effect of the independent factors (Temperature, pH, and extraction time) on pectin extraction yield from orange (A, B, and C), lemon (D, E, and F), and bigarade (G, H, and I) peels. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

extraction time moderately affects the yield (Fig. 1B, C, E, F, H, and I). As can be seen, pectin extraction yield increases when the extraction time is increased from 90 to 150 min. This response provides more time for pectin molecules to be transferred into the solution (Samavati, 2013; Su et al., 2019).

3.3. *Structural characteristics*

3.3.1. *Monosaccharide composition*

The monosaccharide composition of pectin extracted under optimal conditions from orange (OPP), lemon (LPP), and bigarade (BPP) peels are presented in Table 3. GalA was the dominant sugar present in the three analyzed samples, with 79.0, 82.0, and 86.0 mol% in OPP, LPP, and BPP pectins, respectively. These values prove that the obtained molecules could be considered pectin (GalA content >65% of the whole molecule) according to the FAO regulations (JECFA, 2009). On the other hand, the amount of neutral sugars in all the analyzed samples was meager (Table 3). Galactose was the most important neutral sugar (11.0 mol %) in OPP, followed by arabinose, rhamnose, and xylose units with 4.0, 3.0, and 1.0 mol %, respectively. For LPP, galactose and arabinose had the same ratio (5.0 mol %), followed by rhamnose (3.0 mol %) and xylose (1.0 mol %). In contrast, the BPP pectin shows more galactose (6.0 mol %) than arabinose (2.0 mol %), rhamnose (2.0 mol %), and xylose (1.0 mol %). The predominance of galactose and the lower arabinose content in OPP and BPP pectin shows that the galactan side chains are rich. However, the same galactose and arabinose content in LPP pectin points out the presence of arabinan, galactan, or arabinogalactan side chains. Those results indicate the dominance of the homogalacturonan (HG) zone in all recovered pectin samples. The hot acidic conditions used to extract the pectin during this study have certainly led to the degradation of the arabinan, galactan, and arabinogalactan side chains, thus producing more linear pure molecules with a high level of GalA units (Colodel, Vriesmann, Teófilo, & de Oliveira Petkowicz, 2018). Similar tendencies were found in orange, lemon, lime, and grapefruit peel pectins extracted using oxalic acid, in which the GalA content was in the range of 81.70–85.14 mol %. In contrast, those pectins were characterized by the dominance of arabinose (10.20–12.88 mol %), and a low amount of galactose (2.10–3.85 mol %) as Kaya et al. (2014) reported it. Wang et al. (2016) reported that the ultrasound-assisted extraction of pectin from grapefruit peel decreases its GalA content and increases the amount of neutral sugars, which leads to a less linear structure than the conventional heating extraction. However, the amount of GalA in Moroccan citrus pectin molecules was higher than sour orange pectin extracted by ultrasound- and microwave-assisted extraction, with 65.3% and 71.0%, respectively (Hosseini et al., 2016b, 2019). The GalA values obtained were also higher than those obtained from fresh orange peel pectin extracted after a microwave pretreatment, with 66.4–70.1% (Kratchanova et al., 2004). On the other hand, pectin extracted from apple pomace had shown a similar GalA content (83.57 mol%) as Moroccan citrus pectins (Luo, Xu, & Fan, 2019). The presence of low glucose residues (Table 3) could be related to the co-extraction of hemicellulose and cellulose associated with pectin molecules (Wang et al., 2016).

Table 3
Chemical composition and functional properties of pectin extracted at the optimum point from orange (OPP), lemon (LPP), and bigarade (BPP) peels[a].

Chemical composition (%)[b]	OPP	LPP	BPP
Protein	1.07 ± 0.12^{a}	2.25 ± 0.22^{b}	4.65 ± 0.18^{c}
DE	79.83 ± 1.89^{a}	81.51 ± 2.26^{a}	80.33 ± 2.03^{a}
DM	74.0	77.0	77.0
DA	4.0	1.0	4.0
Mw (kDa)	88.0	90.0	80.0
Monosaccharide composition (% mol)[c]			
GalA	79.0	82.0	86.0
Gal	11.0	5.0	6.0
Rha	3.0	3.0	2.0
Ara	4.0	5.0	2.0
Xyl	1.0	1.0	1.0
Glu	2.0	4.0	3.0
Fuc	NF	NF	N.F
Man	NF	NF	N.F
Molar ratio[d]			
HG (%)	73.0	76.0	82.0
RG-I (%)	21.0	16.0	12.0
MR_1	3.47	4.75	6.83
MR_2	0.04	0.03	0.02
MR_3	5.0	3.33	4.0
Functional properties[e]			
WHC (g/g)	24.37 ± 1.18^{ab}	23.00 ± 0.87^{a}	25.50 ± 0.87^{b}
OHC (g/g)	1.55 ± 0.07^{a}	1.39 ± 0.08^{a}	1.36 ± 0.12^{a}
EA (%) At ~ 23 °C	52.83 ± 0.29^{a}	49.33 ± 1.15^{b}	53.17 ± 0.58^{a}
ES (%) After 1day at 4 °C	89.77 ± 0.68^{a}	77.00 ± 1.00^{b}	94.42 ± 0.52^{c}
After 1 day at ~ 23 °C	83.67 ± 0.29^{a}	74.73 ± 0.46^{b}	88.20 ± 1.31^{c}

[a] There were no significant differences between values followed by the same letters in the same row ($p < 0.05$).

[b] DE, DM, DA, and Mw are the degree of esterification, degree of methyl-esterification, degree of acetylation, and molecular weight, respectively.

[c] GalA, Gal, Rha, Ara, Xyl, Glu, Fuc, and Man are galacturonic acid, galactose, rhamnose, arabinose, xylose, glucose, fucose, and mannose, respectively, while NF is not found.

[d] HG, RG-I are homogalacturonan and rhamnogalacturonan I, respectively. HG = GalA – 2 x Rha; RG-I = 2Rha + Ara + Gal; MR_1 (pectin linearity) = HG/RGI = GalA/(2xRha + Ara + Gal + Xyl); MR_2 (contribution of RG regions in the structure) = Rha/GalA; MR_3 (the length of side chains) = (Ara + Gal)/Rha.

[e] WHC, OHC, EA, and ES are water holding capacity, oil holding capacity, emulsifying activity, and emulsion stability, respectively.

The molar ratios (MR) of pectin monosaccharides were calculated to provide information about pectin structure (Table 3). MR_1 represents the linearity of the pectin molecule. The MR_2 and MR_3 display the contribution of the rhamnogalacturonan (RG) region in the molecular structure and the length of the branched chains, respectively. As indicated in Table 3, MR_1 values obtained were 6.83, 4.75, and 3.47 for BPP, LPP, and OPP, respectively. Those values prove that the bigarade peel's obtained pectin was more linear than lemon and orange. This result is in line with the percentage of the HG region, which is higher in BPP (82.0%) pectin than LPP (76.0%) and OPP (73.0%). As reported by Schols and Voragen (1996), the variation of the molar ratio of Rha/GalA from 0.05 to 1 indicates that the rhamnogalacturonan I (RG-I) region is the main constituent of the pectin molecule, which is not the case in this study, where the MR_2 was less than 0.05 for all extracted pectin (Table 3). This tendency indicated low proportions of the RG-I domains and a predominance of the HG region, which is in good concordance with the calculated percentage of the RG-I region (12.0, 16.0, and 21.0%, respectively, for BPP, LPP, and OPP). Furthermore, the attached side chains to the RG-I region (MR_3) are longer in OPP pectin (5.0) compared to BPP (4.0) and LPP (3.33) pectins. According to the obtained results, BPP showed a less-branched linear structure than Iranian bigarade peel pectin extracted using ultrasound-assisted extraction (MR_1 = 1.91; MR_2 = 0.04; MR_3 = 10.22) (Hosseini et al., 2019). Otherwise, Moroccan citrus pectins are less linear than pomelo peel pectin extracted at pH 2, with a linearity MR_1 of around 7.75 (Methacanon et al., 2014).

3.3.2. *Degree of methyl and acetyl esterification*

The degree of methyl esterification (DM) and degree of acetylation (DA) of pectin extracted from the three matrices are presented in Table 3. The degree of methyl esterification is defined as the number of methyl-esterified GalA units on the total number of GalA. From the obtained results, BPP and LPP have the same DM with 77.0%, which is slightly higher than OPP (74.0%). These results confirm those obtained using the FTIR analysis (Fig. S1), which indicates that the extracted pectins were highly methyl-esterified. Based on these values, the extracted molecules can be classified as rapid set pectin and can form gels in the presence of sucrose and at low pH (Thakur et al., 1997). Furthermore, the obtained results were higher than those obtained from grapefruit peel pectin using both conventional heating (69.03 ± 2.62%) and ultrasound-assisted (58.78 ± 3.54%) extraction techniques (Wang et al., 2016). Moreover, a similar result was observed in Chinese orange pectin (DM = 74,51%) after vacuum microwave drying the peel (Dong et al., 2021).

The degree of acetylation (DA) is defined as the number of galacturonic acids esterified by an acetyl group on the total number of GalA. As indicated in Table 3, all extracted pectins can be classified as low acetylated molecules, with a DA of 1.0% for LPP and 4.0% for OPP and BPP. Moroccan citrus pectin presents a DA higher than ponkan pectin, with a DA of 0.1% (Colodel et al., 2018). At the same time, OPP and BPP show similar results to grapefruit pectin extracted by both conventional heating (3.65%) and ultrasound-assisted (3.98%) extraction techniques (Wang et al., 2016). The high degree of acetylation may hinder the gelling capacity of pectin, but in contrast, it could improve its emulsifying properties (Pilnik & Voragen, 1992). The emulsifying capacity of the extracted pectin (See the methodology in supplementary material) was found to be higher, with a slight difference between BPP (53.17%), OPP (52.83%), and LPP (49.33%). However, the emulsion made by BPP was more stable (94.42%) than that produced by OPP (89.77%) and LPP (77.00%), respectively (Table 3). The presence of acetyl groups was not an absolute requirement for pectin to act as an effective emulsifier and/or emulsion stabilizer. A high protein content could significantly improve pectin's emulsifying properties (Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003). As shown in Table 3, the protein content in the extracted pectins was found to be higher in BPP (4.65%) than in LPP (2.25%) and OPP (1.07%) (See the methodology in supplementary material).

3.3.3. Molecular mass distribution by HPSEC

The functional properties of pectin, such as emulsifying and rheological behavior, could significantly be affected by its molecular weight (Mw) distribution. The Mw distribution of OPP, LPP, and BPP pectins was determined by HPSEC (Fig. 2). The elution profile of the three samples analyzed showed a narrow and homogeneous distribution, with one significant population of high molecular weight and two minor populations of low molecular weight. The prominent peak exhibited the highest refractive index (RI) signal intensity at an elution time of 8.6, 8.7, and 8.8 min for the LPP, OPP, and BPP pectins. The other two peaks showed the lowest intensity of the RI signal and were at the same elution time for all pectins (13.8 and 14.8 min). Based on the amplitude of RI signals (Fig. 2), Moroccan citrus pectin samples are rich in high Mw molecules.

The Mw of pectin samples was determined and calculated according to the standard calibration curve obtained using charged pectin. According to the significant obtained peaks (Fig. 2), the molecular weight was 90.0, 88.0, and 80.0 kDa for LPP, OPP, and BPP, respectively (Table 3). The average Mw of Moroccan citrus pectin was smaller than commercialized orange pectin (146.7 kDa) and Chinese orange pectin extracted using conventional (137.1) and microwave-assisted (144.8) extraction techniques (Su et al., 2019). However, LPP and OPP show a higher Mw than ponkan pectin (80.65 kDa) extracted under reflux (Colodel et al., 2018). On the other hand, Moroccan citrus pectins showed a lower Mw than apple pomace pectin (119.57 kDa) extracted using acetic acid (Luo et al., 2019). This difference may be due to the different extraction and analysis techniques, sources of pectin, and the variety and pedoclimatic conditions in which they have grown. Depending on their higher molecular weight, Moroccan citrus pectins could be used as gelling and stabilizing agents. Deckers et al. (1986) have shown that the stability of drinkable yogurt increases with an increasing average molecular weight of pectin. In contrast, the higher molecular weight might slow down the solubility of pectin, but this could be overcome by its higher degree of methyl esterification (Thakur et al., 1997).

3.4. Water holding capacity (WHC) and oil holding capacity (OHC)

WHC and OHC are water or oil held by 1 g of pectin, respectively. Pectin with high WHC can improve the textural properties and resolve the syneresis problem in yogurt (May, 1990). The water and oil holding capacity of Moroccan citrus pectins are indicated in Table 3. For the WHC, the results show that all extracted pectins had a high water holding capacity, and 1 g of bigarade, orange, and lemon pectins can retain 25.50 ± 0.87, 24.37 ± 1.18 and 23.00 ± 0.87 g of water (w/w), respectively. Several factors could affect the WHC of pectins, such as the GalA content, the number of free hydroxyl groups, porosity, particle size, and molecular structure. The obtained values are remarkably higher than those obtained for bigarade pectin extracted using the ultrasound-assisted technique, with a WHC of 3.10 ± 0.12 g water/g pectin (Hosseini et al., 2019). They are also higher than pistachio green hull pectin, holding 4.11 ± 0.36 g water/g pectin (Kazemi et al., 2019). This significant difference is due to the high number of free hydroxyl groups in the HG region (Elleuch et al., 2011), as presented in Fig. S1 (see supplementary material), and to the low DA characteristics of Moroccan citrus pectin (Table 3), which increase its ability to bound more water.

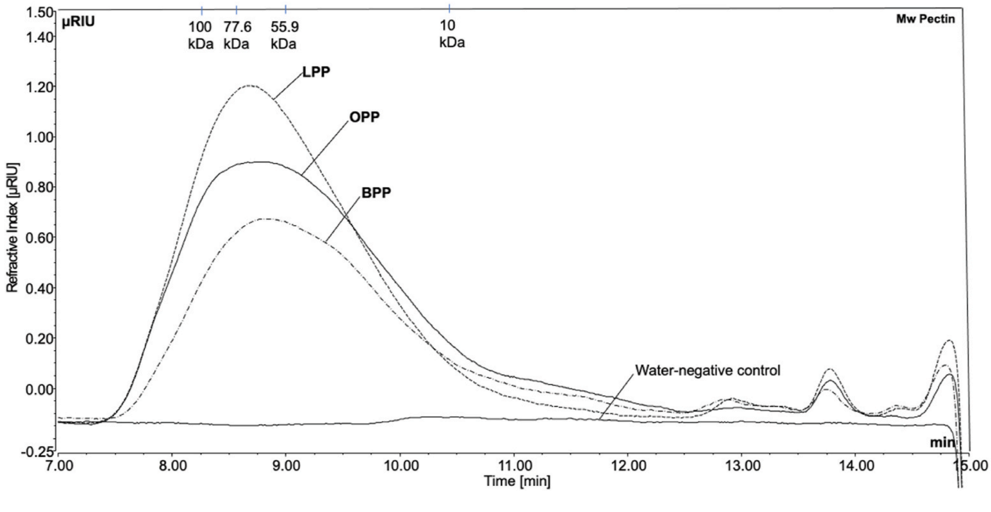

Fig. 2. High-Performance Size Exclusion Chromatography patterns of lemon (LPP), orange (OPP), and bigarade (BPP) pectins extracted at the optimum point. Pectin calibration is indicated as the second X-axis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Pectin with a high OHC can be an efficient stabilizer in emulsions and high-fat food products (Elleuch et al., 2011). Considering the sunflower oil density used in this experiment, the OHC capacity of Moroccan citrus pectins was low, with a slight difference between the three kinds of pectin (Table 3). The obtained results show that 1 g of OPP, LPP, and BPP pectin can hold 1.55 ± 0.07, 1.39 ± 0.08 and 1.36 ± 0.12 g of oil (w/w), respectively. These results are found to be higher than the commercial citrus pectin, with 0.93 ± 0.17 g oil/g pectin (Bayar, Friji, & Kammoun, 2018) and slightly higher than the ultrasound-assisted extracted pectin from bigarade, with 1.32 ± 0.21 g oil/g pectin (Hosseini et al., 2019). The OHC parameter could be influenced by several factors, such as the hydrophilic nature and the overall charge density of the surface (Elleuch et al., 2011).

3.5. Rheological measurement

Generally, pectin's physicochemical and techno-functional properties vary over a wide range according to the source of extraction. Hence, the steady flow behavior of Moroccan citrus pectins has been investigated (Fig. 3). As can be seen, the flow curves were well fitted to the power-law equation as follows (Eq. (5)),

$$\sigma = K.\gamma^{\,n} \tag{5}$$

where σ is the shear stress (Pa), γ is the shear rate (1/s), K is the consistency index (Pa.s), and n is the flow behavior index (unitless). The values of n and K are indicated in Table 4. As shown in Fig. 3, all the curves exhibit the shear-thinning behavior of a non-Newtonian fluid; this behavior becomes more evident as the concentration of the pectin solution rises. Correspondingly, the flow behavior indices of the nine pectin solutions were less than 1.0 (Table 4). However, at 1% concentration, OPP and LPP pectin solutions were close to Newton fluids since their viscosity varies slightly with the variation of the shear rate, and their n values were near 1.0. This behavior could be due to the weakness of the attraction forces between pectin molecules at low concentrations. The significant shear-thinning behavior obtained at high concentration is due to the disentanglement of the polymer network and the partial orientation of the chain in the shear flow direction with the relative increase in the shear rate (Pasandide et al., 2018). The higher viscosity recorded for the three citrus pectins is due to their high molecular weight and GalA content (Table 3), with the availability of a high amount of accessible OH groups (see Fig. S1 in the supplementary material), which led to tap a considerable amount of water inside the pectin structure. Furthermore, the higher DM (Table 3) increases the hydrophobic interactions between pectin molecules. All these structural characteristics enhance the viscosity of pectin in aqueous solutions. The difference in the apparent viscosity between the three pectins appears, even more when we get closer to the 0.1 [1/s] shear rate. A similar tendency was observed on grapefruit pectin (Wang et al., 2016) and apple pomace pectin (Luo et al., 2019), which showed that the viscosity decreased when the shear rate was increased. However, at a 3% concentration and a 0.1 [1/s] shear rate, the BPP pectin solution shows a higher apparent viscosity than OPP and LPP pectins with 40.26, 13.45, and 6.85 Pa s, respectively. This difference could be explained by the molecular weight, degree of methyl esterification, and the GalA content responsible for pectin-pectin interactions by hydrogen bonding (Kar & Arslan, 1999), as shown in Table 3. BPP appears to be the most suitable pectin as a texturizing agent because it has the highest viscosity and shows a shear-thinning behavior even at low concentrations (1%) compared to OPP and LPP pectins (Table 4). Food industries highly demand the shear-thinning behavior of some polymers like pectin because it enables liquid food to be easily pumped and gives food products a pleasant mouthfeel.

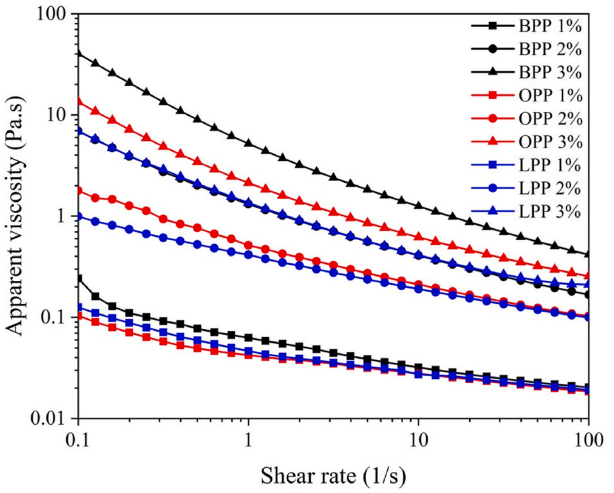

Fig. 3. Steady flow behavior of bigarade (BPP), orange (OPP), and lemon (LPP) pectin solutions at 1, 2, and 3%. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Pawer-law parameters of apparent viscosity of orange (OPP), lemon (LPP) and bigarade (BPP) peel pectins.

Pectin	Concentration (%)	[a]K (Pa.s^n)	[b]n	R^2
OPP	1.0	0.0415 ± 0.0005	0.8232 ± 0.0028	0.9999
	2.0	0.4426 ± 0.0118	0.6788 ± 0.0066	0.9988
	3.0	1.6878 ± 0.0804	0.5819 ± 0.0120	0.9940
LPP	1.0	0.0399 ± 0.0004	0.8409 ± 0.0021	0.9999
	2.0	0.3655 ± 0.0052	0.7153 ± 0.0035	0.9997
	3.0	0.8403 ± 0.0657	0.6852 ± 0.0193	0.9898
BPP	1.0	0.0497 ± 0.0013	0.8032 ± 0.0061	0.9994
	2.0	1.0805 ± 0.0399	0.5887 ± 0.0093	0.9965
	3.0	4.5256 ± 0.2356	0.4703 ± 0.0136	0.9860

[a] K consistency index.
[b] n flow behavior index.

4. Conclusion

The highest pectin extraction yield was obtained at a temperature of 90 °C, pH of 1.5, and extraction time of 120 min. Under these conditions, lemon, bigarade, and orange peels showed the highest pectin yield with 28.57, 26.86, and 26.55%, respectively. The extracted pectin presents a high amount of GalA (>79.0 mol%), indicating the high purity of the molecules. Furthermore, the HG region dominated all pectin structures, and they were more linear with low levels of ramifications. Based on the DM results, pectin samples could be considered high methoxy pectins with a rapid gel setting. The molecular weight distribution of all pectins gave a narrow and homogeneous distribution with a high average molecular weight of 90.0, 88.0, and 80 kDa for LPP than OPP and BPP. On the other hand, a high water holding capacity was recorded for three citrus pectin samples, while 1 g of these pectins can hold more than 23 g of water. The higher emulsion stability was attributed to those made by BPP pectins (ES = 94.42 ± 0.52%). The steady flow behavior of those molecules exhibits a share of thinning behaviors as a function of pectin concentration. Based on the results obtained, Moroccan citrus peels are rich in pectic substances and could be used as a raw material for industrial extraction. The pectins obtained are of high quality, similar to commercial molecules, and could be used as effective texturing and stabilizing agents.

CRediT authorship contribution statement

Noussaire El Fihry: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Formal analysis. **Khalil El Mabrouk:** Resources, Methodology, Supervision, Writing – review & editing. **Mia Eeckhout:** Resources, Supervision, Writing – review & editing. **Henk A. Schols:** Software, Resources, Data curation, Formal analysis, Methodology, Writing – review & editing. **Younes Filali-Zegzouti:** Software, Formal analysis. **Hassan Hajjaj:** Conceptualization, Funding acquisition, Investigation, Resources, Validation, Supervision, Project administration, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Noussaire El Fihry reports financial support, administrative support, equipment, drugs, or supplies, travel, and writing assistance were provided by Flemish Interuniversity Council.

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Appendix A. Supplementary data

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